Kidney Biomarkers and Decline in eGFR in Patients with Type 2 Diabetes

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Abstract

Background and objectives Biomarkers may improve identification of individuals at risk of eGFR decline who may benefit from intervention or dialysis planning. However, available biomarkers remain incompletely validated for risk stratification and prediction modeling.

Design, setting, participants, & measurements We examined serum cystatin C, urinary kidney injury molecule–1 (uKIM-1), and urinary neutrophil gelatinase-associated lipocalin (UNGAL) in 5367 individuals with type 2 diabetes mellitus and recent acute coronary syndromes enrolled in the Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) trial. Baseline concentrations and 6-month changes in biomarkers were also evaluated. Cox proportional regression was used to assess associations with a 50% decrease in eGFR, stage 5 CKD (eGFR<15 ml/min per 1.73 m^2), or dialysis.

Results eGFR decline occurred in 98 patients (1.8%) over a median of 1.5 years. All biomarkers individually were associated with higher risk of eGFR decline ($P<0.001$). However, when adjusting for baseline eGFR, proteinuria, and clinical factors, only baseline cystatin C (adjusted hazard ratio per 1 SD change, 1.66; 95% confidence interval, 1.41 to 1.96; $P<0.001$) and 6-month change in urinary neutrophil gelatinase-associated lipocalin (adjusted hazard ratio per 1 SD change, 1.07; 95% confidence interval, 1.02 to 1.12; $P=0.004$) independently associated with CKD progression. A base model for predicting kidney function decline with nine standard risk factors had strong discriminative ability (C-statistic 0.93). The addition of baseline cystatin C improved discrimination (C-statistic 0.94), but it failed to reclassify risk categories of individuals with and without eGFR decline.

Conclusions The addition of cystatin C or biomarkers of tubular injury did not meaningfully improve the prediction of eGFR decline beyond common clinical factors and routine laboratory data in a large cohort of patients with type 2 diabetes and recent acute coronary syndrome.

Clin J Am Soc Nephrol 13: 398–405, 2018. doi: https://doi.org/10.2215/CJN.05280517

Introduction

Landmark studies demonstrate slower declines in eGFR with angiotensin blockade (1) and strict hypertension control (1,2), but CKD and ESRD have nevertheless increased in recent years (3). It is estimated that approximately 9% of individuals with type 2 diabetes and 19% of individuals >65 years have an eGFR<45 ml/min per 1.73 m^2 (2,4,5), and clinical trials have substantial progression of CKD with annual declines in eGFR exceeding 2.5 ml/min per 1.73 m^2 despite the use of angiotensin blockade and optimal BP (1,6–8). Furthermore, the natural history of CKD is variable and the trajectory often confers dramatically different clinical outcomes (9,10). Traditional kidney biomarkers, such as serum creatinine and proteinuria, are nonspecific for the type of kidney injury, rise late with CKD progression, and lack reliability in predicting decline in kidney function (11,12).

Novel biomarkers have the potential to improve risk stratification and prediction of CKD progression (13–17). Improved identification of individuals at risk of accelerated progression would enable clinicians to identify patients likely to benefit from intensification of CKD preventative strategies and/or early dialysis planning. Serum cystatin C is a marker of glomerular filtration, whereas urinary kidney injury molecule–1 (uKIM-1) and urinary neutrophil gelatinase-associated lipocalin (uNGAL) are markers of tubular injury. These biomarkers have demonstrated promise as risk factors of kidney function decline (18–22). However, prior studies were of modest size, and tested associations without evaluating effects on the incremental utility for risk stratification and prediction of kidney function decline. Additionally, there are limited data on the value of repeated measurements over time.

The aim of this study was to analyze the prognostic significance and clinical utility of baseline serum cystatin C, uNGAL, and uKIM-1 as well as the change in uNGAL and uKIM-1 concentration over 6 months in predicting CKD progression in 5213 patients enrolled in the phase-4 EXAMINE (Examination of Cardiovascular Outcomes with Alogliptin versus

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Standard of Care; ClinicalTrials.gov Unique Identifier: NCT00968708) trial with type 2 diabetes and recent acute coronary syndromes.

Materials and Methods

Study Population

There were 5367 individuals with type 2 diabetes and recent acute coronary syndrome from the EXAMINE trial analyzed. EXAMINE was a multicenter, placebo-controlled, randomized, double-blind trial comparing alogliptin, a dipeptidyl peptidase 4 inhibitor, with standard of care. The study design has been described previously (23). Key inclusion criteria included type 2 diabetes, recent admission for acute coronary syndrome, and glycated hemoglobin level between 6.5% and 11.0%, or 7.0% and 11.0% for insulin-dependent patients. Qualifying acute coronary syndromes could include myocardial infarction or unstable angina requiring hospitalization within 15–90 days before randomization. Patients with type 1 diabetes, unstable cardiac disorders, and dialysis within 14 days were excluded (23). Thirteen individuals of the EXAMINE cohort (n=5380) were not eligible due to missing eGFR or dialysis at baseline.

Independent Predictors

All biomarkers were measured at baseline. uKIM-1 and uNGAL were measured serially. Sufficient data for analysis of serial measurements were available at the 6-month interval only due to a high proportion missing data at other timepoints. Urinary biomarkers were stored at −80°C. All biomarker assays for cystatin C, uKIM-1, and uNGAL were batch analyzed at one time on the same reagent lot. Cystatin C was run on the Cobas analyzer (Roche Diagnostics) using the Randox assay with detection range of 0.4–10 mg/L (Bardane Industrial Park, Kearneysville, WV). Interassay coefficients of variation (CVs) 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.

NGAL was measured by enzyme immunoassay (R&D Systems): Analytic sensitivity was 0.16 ng/ml defined as the higher value of (*1*) the lowest nonzero standard or (*2*) the lowest concentration with accuracy between 80% and 120% and imprecision of ≤20%. The reference range was 0.40–72 ng/ml and 1.4–78.1 ng/mg creatinine (Cr) when normalized to Cr. Intra- and interassay CVs were 2.7%–3.2% and 0.0%–6.7%, respectively.

KIM-1 was measured by a microbead ELISA (R&D Systems). The analytic sensitivity of KIM-1 was 0.06 ng/ml and the detection range was 0.16–2.37 ng/ml with intra- and interassay CVs of 4.4% and 7.8%, respectively. Urinary biomarkers were indexed to serum creatinine as recommended by the National Institute for Diabetes and Digestive and Kidney Diseases CKD biomarkers consortium (19,21). Urinary creatinine was measured by a kinetic colorimetric assay on a Roche Automated Analyzer.

Outcome

The primary outcome of eGFR decline was defined as 50% decline in eGFR from baseline or ESRD. eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (24). Secondary outcomes were: (*1*) 30% decrease in eGFR, (*2*) new microalbuminuria (>30, ≤300 mg/g), and (*3*) new macroalbuminuria (>300 mg/g) from baseline to last visit.

Statistical Analyses

Variables are presented as mean±SD or n (%). Differences were compared using t test or two-way ANOVA for continuous variables and chi-squared tests for categoric variables. Baseline biomarker concentration and the change between baseline and 6 months for uNGAL and uKIM-1 were categorized into quartiles. Kaplan–Meier event rates were calculated per quartile and log-rank tests were used to compare incidence rates in the first and fourth quartiles. Nonparametric tests were used to assess trends across quartiles. Secondary analyses were conducted with and without standardization of urinary biomarker concentration by urinary creatinine concentration. Relationships between baseline microalbuminuria and macroalbuminuria with baseline biomarker concentrations were tested with Pearson correlations. Time-to-event analyses were used to test associations of baseline biomarkers and 6-month change with eGFR decline. Cox proportional hazards models were used to calculate hazard ratios (HR) and 95% confidence intervals (95% CIs), stratified by randomized therapy. The base model included nine standard predictors: age, sex, race/ethnicity, hypertension, systolic BP, hemoglobin A1c (HbA1c), CKD-EPI eGFR, and baseline proteinuria. The base model for associations between 6-month change in biomarkers and eGFR decline additionally included the baseline biomarker concentration. These models excluded individuals who reached the primary outcome within 6 months.

Biomarkers were standardized to a mean of 0 and SD of 1 to compare HR per 1-unit increase in each biomarker. Missing values of predictors were imputed: five data sets were created through the Markov Chain Monte Carlo method with age, sex, race/ethnicity, hypertension, baseline systolic BP, HbA1c, baseline eGFR, baseline proteinuria, and eGFR change from baseline to last visit (25). Findings were also analyzed by complete case approach that resulted in qualitatively similar findings (data not shown). Missing values of urinary biomarkers at baseline and 6 months were similarly imputed (25). Sensitivity analyses were conducted without biomarker standardization for urinary creatinine. The changes in uKIM-1/Cr and uNGAL/Cr were also analyzed as continuous variables using a quadratic term to evaluate nonlinearity.

The base model was used to predict risk of eGFR decline. Improvement in risk prediction for each biomarker was assessed by adding each to the base model. Discrimination was evaluated using the C-statistic and net reclassification index (NRI) and integrated discrimination index (IDI) using predicted risk categories of <2% (low), 2%–5% (moderate), and >5% (high) risk of eGFR decline. Reclassification was considered appropriate when the model increased the risk category for individuals with eGFR decline (*e.g.*, low risk to moderate risk) or decreased the risk category of individuals without eGFR decline (*e.g.*, moderate risk to low risk). NRI and IDI confidence intervals were generated with the bootstrap method with 1000 replications (26). All analyses were conducted in SAS version 9.4 (SAS Institute Inc., Cary, NC).

Table 1. Baseline characteristics of Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) trial participants with and without eGFR decline

Characteristic, *n* (%)	Subjects with Decline in eGFR (*n*=98)[a]	Subjects without Decline in eGFR (*n*=5269)
Age, yr	63 (10)	61 (10)
Men	55 (56)	3588 (68)
Race		
White	66 (67)	3832 (73)
Asian	19 (19)	1069 (20)
Black	8 (8)	207 (4)
Body mass index (kg/m^2), mean±SD	31±6.6	30±5.6
Current smoker	6 (6)	723 (14)
Years of type 2 diabetes, mean±SD	15±9.9	9±8.1
HbA1c (%), mean±SD	8±1.0	8±1.1
Hypertension	90 (92)	4369 (83)
Congestive heart failure	50 (51)	1446 (27)
Percutaneous coronary intervention	51 (52)	3315 (63)
Coronary artery bypass grafting	27 (28)	660 (13)
Peripheral arterial disease	19 (19)	493 (9)
Cerebrovascular diseases	13 (13)	374 (7)
eGFR (ml/min per 1.73 m^2), mean±SD	40 (24)	75 (21)
Protein-to-creatinine ratio (mg/mg), mean±SD[b]	4.1±4.8	0.4±1.0
Microalbuminuria[c]	72 (90)	1707(56)
Macroalbuminuria[d]	57 (71)	444 (15)
Medications		
Antiplatelet agents	93 (95)	5122 (97)
β blockers	79 (81)	4325 (82)
Statins	81 (83)	4778 (91)
Calcium-channel blockers	38 (39)	1156 (22)
Diuretics	68 (69)	1879 (36)
Renin-angiotensin blockers	68 (69)	4284 (81)

eGFR, eGFR as measured by the Chronic Kidney Disease Epidemiology Collaboration equation; HbA1c, hemoglobin A1c.
[a]Among individuals with eGFR decline, 30 (31%) were attributed to a 50% decline in eGFR without reaching CKD stage 5 and 68 (69%) were attributed to ESRD.
[b]Baseline proteinuria measured by protein-to-creatinine ratio (mg/mg) was available in 83 of 98 individuals with eGFR decline and 3957 of 5269 individuals without eGFR decline.
[c]Albumin excretion was measured in 80 of 98 individuals with and 3048 of 5269 without eGFR decline. Microalbuminuria, >30–300 mg/g.
[d]Macroalbuminuria, ≥300 mg/g.

Results

Maximum follow-up time was 40 months and median was 18 months and there were 98 individuals who reached the composite outcome of eGFR decline (1.8%). Of these events, 30 (31%) were attributed to a 50% decline in eGFR without reaching CKD stage 5 and 68 (69%) were attributed to ESRD. In the latter group, 24 individuals (35%) had a decline in eGFR<15 ml/min per 1.73 m^2, 21 individuals (31%) required dialysis, and 23 (34%) individuals had both. Baseline eGFR in the group with ESRD was 29±18 ml/min per 1.73 m^2 with mean change in eGFR from baseline to last visit of −10.8±11.0 ml/min per 1.73 m^2. Most individuals reaching the composite end point had a baseline eGFR>20 ml/min per 1.73 m^2 (76% with CKD progression and 65% of individuals with ESRD) (Supplemental Table 1). There was no difference in the incidence of the composite outcome between treatment groups (Supplemental Table 2). Thirty-two subjects experienced events within 180 days of randomization.

Baseline Characteristics

Individuals with eGFR decline were 2.2 years older, more frequently female, and had a higher burden of comorbidities than individuals without progression (Table 1). Baseline proteinuria was nearly ten-fold higher in individuals with eGFR decline compared with individuals without. The proportions of individuals with microalbuminuria and macroalbuminuria were also higher in the group with eGFR decline. Additionally, individuals with the composite end point had significantly lower baseline eGFR compared with those who did not reach the end point (40 ml/min per 1.73 m^2 versus 75 ml/min per 1.73 m^2) (Table 1). No baseline demographics or clinical characteristics were missing except HbA1c (0.0%, 1 of 5348 missing). Baseline urine protein-to-creatinine was available in 85% (83 of 98) of individuals with eGFR decline and 75% (3957of 5269) of individuals without. It was missing in 25% of the total cohort (1329 of 5348).

Associations of Baseline and Short-Term Changes in Biomarkers with eGFR Decline

Baseline cystatin C was available in 5231 (97%), uKIM-1/Cr in 4875 (91%), and uNGAL/Cr in 4962 (93%) of the cohort (percent missing at baseline cystatin C, 3%; KIM-1/Cr, 9%; and uNGAL/Cr, 7%). Serial measurements at 6 months of uKIM-1/Cr were available in 3865 (72%) and of uNGAL/Cr in 3982 (74%). Quartiles of baseline biomarkers did not differ between alogliptin and placebo groups (Supplemental Tables 3 and 4).

Table 2. Event rates according to baseline biomarkers and change in biomarker from 0 to 6 mo in participants of the Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) trial

Variable	Cystatin C Baseline	uKIM-1–to-Creatinine Baseline	uNGAL-to-Creatinine Baseline	Change in uKIM-1–to-Creatinine[a]	Change in uNGAL-to-Creatinine[a]
Decline in eGFR[b]					
Q1	0.2% (2 of 1270)	0.6% (7 of 1199)	0.6% (7 of 1249)	1.5% (16 of 1050)	2.8% (30 of 1066)
Q2	0.2% (3 of 1310)	1.4% (17 of 1232)	0.9% (11 of 1236)	1.1% (11 of 10,385)	0.4% (4 of 1049)
Q3	0.9% (12 of 1334)	1.5% (18 of 1216)	1.4% (17 of 1233)	1.3% (14 of 1058)	0.8% (9 of 1079)
Q4	6.0% (78 of 1290)	3.7% (45 of 1222)	4.4% (54 of 1237)	1.6% (17 of 1050)	1.4% (15 of 1066)
P Q1 versus Q4	<0.001	<0.001	<0.001	0.86	0.02
P trend	<0.001	<0.001	<0.001	0.74	0.02
30% decline in eGFR, ml/min per 1.73 m^2/1.73 m^2					
Q1	1.2% (15 of 1272)	1.7% (20 of 1199)	1.5% (19 of 1250)	5.1% (54 of 1050)	6.9% (74 of 1066)
Q2	1.5% (19 of 1313)	4.6% (56 of 1232)	2.7% (33 of 1237)	3.9% (40 of 1039)	2.7% (28 of 1049)
Q3	4.9% (66 of 1337)	5.0% (61 of 1216)	4.3% (53 of 1237)	3.7% (39 of 1061)	3.3% (36 of 1082)
Q4	10.3% (133 of 1291)	8.0% (98 of 1222)	8.8% (109 of 1238)	6.1% (64 of 1050)	5.6% (60 of 1068)
P Q1 versus Q4	<0.001	<0.001	<0.001	0.34	0.21
P trend	<0.001	<0.001	<0.001	0.36	0.24
New-onset microalbuminuria[c]					
Q1	32.2% (68 of 211)	34.0% (82 of 241)	29.7% (71 of 239)	48.5% (100 of 206)	46.6% (76 of 163)
Q2	29.1% (68 of 234)	27.0% (65 of 241)	32.7% (72 of 220)	32.7% (51 of 156)	35.2% (70 of 199)
Q3	37.7% (93 of 247)	38.9% (72 of 185)	37.1% (85 of 229)	31.5% (57 of 181)	27.9% (58 of 208)
Q4	39.0% (69 of 177)	41.6% (69 of 166)	42.6% (66 of 155)	25.5% (42 of 165)	33.6% (49 of 146)
P Q1 versus Q4	0.17	0.12	0.01	<0.001	0.02
P trend	0.05	0.03	0.01	<0.001	0.004
New-onset macroalbuminuria[d]					
Q1	7.9% (33 of 418)	13.1% (58 of 444)	13.2% (56 of 423)	18.8% (82 of 436)	23.3% (96 of 412)
Q2	8.0% (36 of 448)	13.5% (67 of 496)	11.0 (52 of 473)	16.6% (62 of 374)	11.9% (48 of 403)
Q3	13.0% (72 of 554)	13.6% (63 of 462)	110% (55 of 500)	10.0% (39 of 391)	8.2% (33 of 403)
Q4	22.2% (116 of 522)	12.4% (58 of 469)	17.5% (85 of 487)	11.6% (47 of 405)	13.2% (53 of 402)
P Q1 versus Q4	<0.001	0.75	0.08	0.004	<0.001
P trend	<0.001	0.77	0.06	<0.001	<0.001

uKIM-1, urinary kidney injury molecule–1; uNGAL, urinary neutrophil gelatinase-associated lipocalin; Q, quartile.
[a]Subjects with eGFR decline that occurred within 180 d (6 mo) are excluded for the calculations of biomarker change over 6 mo (n=32). No subjects are excluded for the baseline calculations.
[b]eGFR decline was defined as 50% decrease in eGFR from baseline or ESRD with stage 5 CKD eGFR<15 ml/min per 1.73 m^2 dialysis, or both.
[c]Incident microalbuminuria, >30–300 mg/g albumin-to-creatinine.
[d]Incident macroalbuminuria, ≥300 mg/g albumin-to-creatinine. Urine albumin-to-creatinine ratio (mg/g) was calculated as urine albumin (mg/dl)-to-urine creatinine (mg/dl)×1000.

Rates of eGFR decline were greater in quartile (Q)4 compared with Q1 for all baseline biomarkers (P<0.001), with a stepped increase by quartile (P<0.001). Results were qualitatively similar when biomarker concentration at baseline and 6-month change were examined without standardization for urinary creatinine (data not shown). The secondary outcome of 30% decline in eGFR followed a similar pattern. Baseline microalbuminuria and macroalbuminuria were correlated with baseline cystatin C (Pearson correlation coefficient 0.13, P<0.001) and cystatin C, uKIM-1/Cr, and uNGAL/Cr (<0.001), respectively (Supplemental Table 5). There was a trend toward decreasing incidence of microalbuminuria across quartiles of the 6-month change in biomarker. Incident macroalbuminuria showed similar associations with baseline cystatin C and the change in urinary biomarkers but not with baseline urinary biomarkers (Table 2).

After exclusion of 32 individuals who reached the primary end point within 6 months, rates of eGFR decline did not differ by quartile of 6-month change in uKIM-1. However, change in uNGAL was inversely associated with eGFR decline (P Q1 versus Q4=0.02, P trend=0.02) (Supplemental Table 3, Table 2). These relationships were qualitatively similar in analyses without standardization for urinary creatinine (data not shown). Both the 6-month change in uKIM-1 and uNGAL were inversely associated with protein excretion (Table 2).

Crude and Adjusted Time-to-Event Analyses

In time-to-event analyses, a 1 SD increase in each biomarker at baseline was associated with eGFR decline in unadjusted models. The largest association was observed with cystatin C (HR, 2.37; SD±0.38; 95% CI, 2.16 to 2.60; P<0.001). Cystatin C was independently predictive (HR, 1.66; 95% CI, 1.41 to 1.96; P<0.001) of the outcome but uKIM-1 (SD±0.90) and uNGAL (SD±157.50) were not. Both the 6-month change in uKIM-1 and uNGAL were associated with eGFR decline in crude models (Table 3). An increase in uNGAL (SD±263.71) but not uKIM-1 (SD±1.25) at 6 months was independently associated with a higher risk of eGFR decline. A 1 SD increase in the change of uNGAL from baseline was associated with 7% higher risk of eGFR decline using standardization (HR, 1.07; 95% CI, 1.02 to 1.12; P=0.004). Results were qualitatively similar

Table 3. Crude and adjusted association of baseline biomarker or change at 6 mo with eGFR decline in participants of the Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) trial

Biomarker	Unadjusted HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
Baseline				
Cystatin C	2.37 (2.17 to 2.60)	<0.001	1.66 (1.41 to 1.96)	<0.001
uKIM-1/Cr	1.22 (1.14 to 1.31)	<0.001	0.97 (0.85 to 1.10)	0.61
uNGAL/Cr	1.28 (1.21 to 1.34)	<0.001	1.03 (0.94 to 1.14)	0.55
Change from baseline to 6 mo				
uKIM-1/Cr	1.08 (1.00 to 1.16)	0.05	1.05 (0.98 to 1.12)	0.15
uNGAL/Cr	1.06 (1.01 to 1.11)	0.01	1.07 (1.02 to 1.12)	0.004

eGFR decline was defined as 50% decrease in eGFR from baseline, stage 5 CKD eGFR<15 ml/min per 1.73 m^2, dialysis, or both eGFR<15 ml/min per 1.73 m^2 and dialysis. Time from randomization to eGFR decline was fit using the Cox proportional hazards model with stratification per treatment and with adjustment for nine predictors: age, sex, race, ethnicity, historical hypertension, systolic BP, hemoglobin A1c, Chronic Kidney Disease Epidemiology Collaboration eGFR, and baseline proteinuria. All missing values of predictors were estimated through the Markov Chain Monte Carlo method with imputation. Subjects with eGFR decline within 180 d (6 mo) are excluded for the models of biomarker change over 6 mo (n=32). The base model for the change in biomarkers includes the nine traditional predictors plus the baseline biomarker concentration. SDs for baseline biomarkers: Cystatin C±0.38, uKIM-1/Cr±0.90, uNGAL/Cr±157.50. SD for change from baseline to 6 mo: uKIM-1/Cr±1.25 and uNGAL/Cr±263.71. HR, hazard ratio; 95% CI, 95% confidence interval; uKIM-1/Cr, urinary kidney injury molecule–1–to-creatinine; uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin–to-creatinine.

when the predictors were examined continuously with quadratic terms to account for nonlinear effects (Table 3).

Risk Prediction Modeling

In the base model, the C-statistic was 0.93 for predicting eGFR decline. Addition of baseline cystatin C slightly improved the C-statistic (0.94), but the change was NS (P=0.16) (Table 4). Addition of baseline or 6-month change in urinary biomarkers did not influence the model. Furthermore, there was no improvement in NRI with any of the three biomarkers at baseline and only cystatin C improved the IDI (0.016; 95% CI, 0.005 to 0.033). Neither the NRI nor IDI improved significantly when the changes in biomarkers were added to the base model.

Discussion

We used a large, well defined patient population with type 2 diabetes and recent acute coronary syndrome from the EXAMINE trial to evaluate the predictive utility of baseline cystatin C, uKIM-1, and uNGAL and short-term changes of urinary biomarkers in identifying patients at risk of eGFR decline. Baseline cystatin C and change in uNGAL were associated with eGFR decline, but the addition of urinary biomarkers did not improve risk prediction, discrimination, or reclassification. Cystatin C marginally improved risk prediction although the difference was not statistically significant. These results support the growing body of evidence that static measurements of urinary tubular injury markers do not improve prediction of CKD progression beyond standard clinical factors and laboratory measures (27,28). We uniquely demonstrate that monitoring for changes in these biomarkers did not improve clinical prediction of eGFR decline either. This may reflect an absence of clinical utility but also reflects the excellent predictive ability of traditional risk factors including demographics, comorbidities, and baseline eGFR which left little room for meaningful improvement in discrimination and calibration.

Evidence on the utility of kidney biomarkers in predicting eGFR decline is evolving. An early study of 96 patients in 2009 reported that uNGAL was an independent risk marker for CKD progression with modest sensitivity and specificity (20). Similarly, uNGAL and uKIM-1 were higher in a study of 303 patients with diabetes with rapid progression of CKD (defined as >25% annual decrease in eGFR) compared with individuals without rapid progression (18). These initial findings evaluated association only and did not assess improvement in predictive modeling. More recent studies include a matched case-control study of 135 patients with advanced CKD (18), a cohort study of 260 Native Americans (21), and an observational study of >3000 participants with preexisting CKD enrolled in the Chronic Renal Insufficiency Cohort Study (CRIC) (19). Like our study, each uniformly failed to demonstrate useful improvement in clinical prediction models that included demographic factors, eGFR, and proteinuria. Similarly, CRIC also reported a high C-statistic for a base model alone (19). The results of these studies varied with respect to independent associations of biomarker concentrations with CKD progression. Limitations of these studies include small sample sizes and heterogeneous populations with diverse causes of CKD. Additionally, only single biomarker concentrations rather than changes over time or multimarker models were analyzed.

This analysis builds on prior results by providing data from the largest cohort of individuals with diabetes and acute coronary syndrome studied to date. In contrast to CRIC, we analyzed individuals with and without preexisting CKD. Baseline Cystatin C was independently associated with CKD. Although the degree of improvement was marginal, it supports the clinical utility of novel glomerular filtration markers in predicting eGFR decline when serum creatinine alone is unreliable, and suggests that glomerular filtration markers may be more helpful than tubular injury markers in predicting kidney function decline. This is biologically plausible because a low GFR at baseline may reasonably be expected to be a better discriminator of further GFR loss than concomitant tubular injury.

Our study is unique in measuring longitudinal changes of urinary biomarkers. The apparent decrease in biomarker

Table 4. Risk prediction in models of eGFR decline with and without baseline biomarker concentration and change over 6 mo in participants of the Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) trial

Variable	C-Statistic	Goodness of Fit Chi Squared	Net Reclassification Index (95% CI Bootstrap)	Integrated Discrimination Index (95% CI Bootstrap)
Base model[a]	0.93	5.13		
Plus baseline biomarker				
uKIM-1/Cr	0.93	5.14	−0.04 (−0.11 to 0.00)	−0.00 (−0.00 to 0.00)
uNGAL/Cr	0.93	3.55	0.0215 (0.00 to 0.07)	0.00 (−0.00 to 0.01)
Cystatin- C	0.94	5.31	−0.04 (−0.16 to 0.07)	0.07 (0.03 to 0.13)
Base model	0.97	3.87		
Plus change in biomarker over 6 mo				
uKIM-1/Cr	0.97	3.76	0.00 (−0.00 to 0.00)	0.00 (−0.00 to 0.01)
uNGAL/Cr	0.97	3.76	0.00 (−0.00 to 0.00)	0.00 (−0.01 to 0.03)

The base model included nine predictors: age, sex, race, ethnicity, history of hypertension, systolic BP, hemoglobin A1c, Chronic Kidney Disease Epidemiology Collaboration eGFR, and baseline proteinuria. All predictors with missing values were estimated through the Markov Chain Monte Carlo method with five-time imputation. Time from randomization to end point was fit using the Cox proportional hazards model with stratification per treatment and adjustment on the baseline variables listed above. Subjects with eGFR decline within 180 d (6 mo) were excluded for the models of biomarker change over 6 mo (n=32). 95% CI, 95% confidence interval; uKIM-1/Cr, urinary kidney injury molecule–1–to-creatinine; uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin–to-creatinine.
[a]The base model for the change in biomarkers included the nine traditional predictors plus the baseline biomarker concentrations.

concentration over time must be interpreted cautiously given that only 75% of subjects had measurements at both baseline and 6 months. This decline could represent recovery of kidney function after hemodynamic insult and contrast during the initial hospitalization. However, assay drift is unlikely given that biomarkers were batch analyzed.

Higher uNGAL and uKIM-1 over 6 months were positively associated with an increase in the risk of eGFR decline in crude models, but only the change in uNGAL at 6 months was independently associated with progression. These findings are consistent with a small study of critically ill patients with severe AKI requiring RRT in whom a decline in uNGAL was associated with improved risk prediction and increased odds of kidney recovery (28). Previous studies have not reported on serial measurements; thus, it is unknown whether a 6-month change in urinary biomarker concentration is a sufficiently long interval to assess for associations with eGFR decline. Additional studies are needed to expand upon these findings.

The lower proportion of incident micro- and macroalbuminuria detected in individuals with greater increases in uNGAL and uKIM-1 was unexpected. This may be attributable to the positive correlation with higher baseline urinary albumin excretion in individuals with higher baseline biomarker concentrations diminishing the relative change across quartiles. Many confounding factors influence changes in proteinuria and the ability to accurately capture these changes over time. It is well known that albuminuria fluctuates randomly or in association with hemodynamic changes, kidney insults, and medication management. These points are especially relevant in this cohort of patients with diabetes with recent hospitalization for acute coronary syndrome (29). Additionally, a high proportion of individuals were on renin-angiotensin inhibitors at baseline. These agents were continued in most subjects throughout follow-up (30) and are therefore less likely to influence the results. Lastly, these results should be interpreted cautiously given the high percentage of missing data on urinary albumin excretion.

Strengths of this study include the large population of high-risk individuals with type 2 diabetes and recent acute coronary syndrome in which it is reasonable to expect a sufficient proportion to experience eGFR decline. Additionally, the majority of individuals had baseline eGFR>20 ml/min per 1.73 m^2. At this level of eGFR baseline biomarkers and their change over time should be predictive of eGFR decline. Several limitations should also be noted. Only 98 individuals (1.8%) had CKD progression and the median follow-up was 1.5 years. A small proportion of subjects that reached the end point had baseline eGFR<20 ml/min per 1.73 m^2. Although measurement error could have contributed to these end points, this is unlikely given that the majority required dialysis. Similarly, an even smaller subgroup of subjects with a baseline eGFR<15 ml/min per 1.73 m^2 reached the end point due to the initiation of dialysis alone. The initiation of chronic dialysis is a clinical decision influenced by symptoms of uremia, comorbidities, and patient preferences. The inherent subjectivity of this end point relative to the more objective end point of achieving an eGFR<15 ml/min per 1.73 m^2 or 50% decline in eGFR is an additional limitation that may have limited the potential of biomarkers to predict the outcome. Previous studies of biomarkers reported event rates as high as 32% (20). However, this study was in a small cohort of white Europeans with severe baseline CKD and a high proportion of GN who were followed for >1.5 years (20), whereas event rates were 20% after 3.2 years of follow up in CRIC (19). Although we identified nearly 100 events, the relatively low event rate suggests that most individuals had relatively stable nephropathy without significant baseline kidney disease. Our findings are thus best interpreted within the context of similarly low-risk populations of individuals with and without CKD and may not be generalizable to populations at high risk of eGFR decline.

Both the severity and cause of CKD have been shown to influence baseline and change in biomarkers (11). Biomarkers, especially uNGAL, may be differentially elevated

in IgA nephropathy, autosomal dominant polycystic kidney disease, and diabetic nephropathy (11). Although our study population was uniformly diagnosed with diabetes, whether diabetes was responsible for eGFR decline in all cases is uncertain. Lastly, data on proteinuria was missing in a high proportion and biomarker concentrations were available at 6 months in only 75%. Although missing values were imputed and results should be unbiased, this procedure could have introduced error.

In summary, this study of individuals with type 2 diabetes and recent acute coronary syndrome provides further evidence that clinical prediction models of standard clinical risk factors, eGFR, and proteinuria robustly predict eGFR decline. Although novel biomarkers may provide clinical utility in prediction of decline in eGFR in other settings such as AKI or glomerular disease, our data suggest that single or repeated measures of novel kidney biomarkers of glomerular filtration and tubular injury offer at most minimal incremental prognostic value for the short-term prediction of eGFR decline in type 2 diabetes. Additional data from diverse populations on serial measures of kidney biomarkers over time are needed to further assess utility in risk stratification and prediction modeling.

Acknowledgments

The authors had unrestricted access to the data and were responsible for the current analyses.

We thank the individuals involved in the Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) study which was sponsored by Takeda.

Disclosures

K.G.G. is supported by the National Heart, Lung, and Blood Institute (NHLBI) Training in Academic Nephrology postdoctoral grant (T32 DK007527). W.B.W. is chair of the Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) steering committee and has received fees from Takeda. G.L.B. is on the Steering Committee of Clinical Trials for Janssen, AbbVie, and Bayer. He is also a consultant at Merck, Relypsa, and KBP BioSciences. F.Z. has served on boards or steering committees for Actelion, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim (BI), Boston Scientific, CEVA, CVRx, Vifor Fresenius, GE Healthcare, J&J, KBP BioSciences, Livanova, Novartis, NovoNordisk, Pfizer, Quantum Genomics, Relypsa, Resmed, Roche, Takeda, and ZS Pharma. C.A.W. is employed by Takeda. S.K. was previously employed by Takeda. M.V. is supported by the NHLBI T32 postdoctoral training grant (T32HL007604). D.A.M. reports collaboration with Abbott Laboratories, Amgen, AstraZeneca, Daiichi Sankyo, Eisai, GlaxoSmithKline, Merck, Novartis, Roche, and Takeda; and fees from Abbott Laboratories, Aralez Pharmaceuticals, GlaxoSmithKline, Merck, Peloton, Roche, and Verseon. C.P.C. reports grants from Amgen, Arisaph, BI, Bristol-Myers Squibb (BMS), Daiichi Sankyo, Janssen, Merck, and Takeda; and fees from Alnylam, Amgen, Arisaph, Astra Zeneca, BI, BMS, GlaxoSmithKline, Kowa, Lipimedix, Merck, Pfizer, Regeneron, Sanofi, and Takeda. D.M.C. reports fees from BI and Ingelheim/Eli Lilly and funding from Janssen.

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Received: May 17, 2017 **Accepted:** December 4, 2017

Published online ahead of print. Publication date available at www.cjasn.org.

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.05280517/-/DCSupplemental.