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ORIGINAL ARTICLE

Plasma biomarkers improve prediction of diabetic kidney disease in adults with type 1 diabetes over a 12-year follow-up: CACTI study

Petter Bjornstad[1,2], Laura Pyle[1], David Z.I. Cherney[3], Richard J. Johnson[4], Rachel Sippl[2], Randy Wong[2], Marian Rewers[1,2] and Janet K. Snell-Bergeon[1,2]

[1]Department of Pediatric Endocrinology, University of Colorado School of Medicine, Aurora, CO, USA, [2]Barbara Davis Center for Diabetes, University of Colorado Denver, Aurora, CO, USA, [3]Department of Medicine, Division of Nephrology, and Department of Physiology, University of Toronto, ON, Canada and [4]Department of Nephrology, University of Colorado Denver, Aurora, CO, USA

Correspondence and offprint requests to: Petter Bjornstad; E-mail: Petter.Bjornstad@childrenscolorado.org

ABSTRACT

Background. The objective of the study was to determine whether plasma biomarkers of kidney injury improve the prediction of diabetic kidney disease (DKD) in adults with type 1 diabetes (T1D) over a period of 12 years.

Methods. Participants ($n = 527$, 53% females) in the Coronary Artery Calcification in T1D (CACTI) Study were examined during 2002–04, at a mean (± standard deviation) age of 39.6 ± 9.0 years with 24.8 years as the median duration of diabetes. Urine albumin-to-creatinine (ACR) and estimated glomerular filtration rate (eGFR) by CKD-EPI (chronic kidney disease epidemiology collaboration) creatinine were measured at the baseline and after mean follow-up of 12.1 ± 1.5 years. Albuminuria was defined as ACR ≥30 mg/g and impaired GFR as eGFR <60 mL/min/1.73 m^2. Kidney injury biomarkers (Meso Scale Diagnostics) were measured on stored baseline plasma samples. A principal component analysis (PCA) identified two components: (i) kidney injury molecule-1, calbindin, osteoactivin, trefoil factor 3 and vascular endothelial growth factor; and (ii) β-2 microglobulin, cystatin C, neutrophil gelatinase-associated lipocalin and osteopontin that were used in the multivariable regression analyses.

Results. Component 2 of the PCA was associated with increase in log modulus ACR [β ± standard error (SE): 0.16 ± 0.07, P = 0.02] and eGFR (β ± SE: −2.56 ± 0.97, P = 0.009) over a period of 12 years after adjusting for traditional risk factors (age, sex, HbA1c, low-density lipoprotein cholesterol and systolic blood pressure and baseline eGFR/baseline ACR). Only Component 2 of the PCA was associated with incident-impaired GFR (odds ratio 2.08, 95% confidence interval 1.18–3.67, P = 0.01), adjusting for traditional risk factors. The addition of Component 2 to traditional risk factors significantly improved C-statistics and net-reclassification improvement for incident-impaired GFR (ΔAUC: 0.02 ± 0.01, P = 0.049, and 29% non-events correctly reclassified, P < 0.0001).

Conclusions. Plasma kidney injury biomarkers can help predict development of DKD in T1D.

Keywords: albuminuria, biomarkers, diabetes mellitus, diabetic kidney disease, GFR

INTRODUCTION

Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease (ESRD) [1] in adults and carries a high risk of morbidity and mortality [2–4]. In fact, almost half of patients entering ESRD programs have diabetes mellitus [1]. The natural history of DKD is characterized by a long clinically silent period without overt signs or symptoms of nephropathy [3, 5]. Once overt DKD is present, ESRD can often be postponed, but rarely prevented, by effective antihypertensive treatment, blockade of the renin–angiotensin–aldosterone system and careful glycemic control [1]. Accordingly, an intensive search for biomarkers that predict future DKD risk leading to earlier intervention strategies has been ongoing for decades. Important scientific efforts, therefore, continue to focus on the discovery of biomarkers for the early recognition of renal injury.

The Kidney Injury Panels 3 and 5 from Meso Scale Diagnostics include assays for the quantitative determination of glutathione S-transferase alpha (αGST), calbindin, clusterin, kidney injury molecule-1 (KIM-1), osteoactivin, trefoil factor 3 (TFF3), vascular endothelial growth factor (VEGF) and β-2 microglobulin (B2M), cystatin C, neutrophil gelatinase-associated lipocalin (NGAL/Lipocalin-2), osteopontin (OPN) and uromodulin (UMOD). These kidney injury biomarkers

have been associated with acute and chronic tubular and glomerular injury in patients with diabetes [6]. In adults with type 2 diabetes, kidney injury biomarkers, including KIM-1 and B2M, were associated with rapid chronic kidney disease (CKD) progression [6]. However, few studies have looked at the association between these promising renal injury markers and development of DKD in adults with type 1 diabetes (T1D). Injury markers exhibit pleiotropic and synergistic effects, and it is therefore important to evaluate the combined influence of multiple markers in principal components analyses (PCA), which avoids overestimation of the association due to the potentially high correlation between the markers.

Accordingly, the goal of this study was to examine the prospective association of these biomarkers of kidney injury in a PCA with incident albuminuria ($\geq$30 mg/g) and impaired glomerular filtration rate (GFR) ($<$60 mL/min/1.73 m^2) in adults with T1D over a period of 12 years in the Coronary Artery Calcification in T1D (CACTI) Study.

MATERIALS AND METHODS

Study design

Participants ($n = 527$, 53% females) in the prospective CACTI Study were examined during 2002–04, at a mean age of 39.6 $\pm$ 9.0 years with 24.8 years as the median duration of diabetes (Q1 = 19.1 years, Q3 = 32.1 years), and re-examined 12 years later. The study was approved by the Colorado Multiple Institutional Review Board and all participants provided informed consent. All clinical experimentation adheres to the Declaration of Helsinki.

Examination measurements

Physical examination measurements included height, weight, body mass index (BMI) and systolic and diastolic blood pressure. Hypertension was defined as systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg or treatment with antihypertensive medication. All subjects were given standardized questionnaires to obtain demographics, medical history and medication use.

Laboratory measurements and kidney injury biomarkers

After an overnight fast, blood was collected, centrifuged and separated. HbA1c, total plasma cholesterol and triglyceride (TG), high-density lipoprotein cholesterol and low-density lipoprotein cholesterol (LDL-C) levels were measured as previously described [7]. Kidney injury biomarkers (Kidney Injury Panels 3 and 5, Meso Scale Diagnostics) were measured on stored baseline plasma samples (samples stored at $-80\,^{\circ}$C). The assays were performed at the Barbara Davis Center for Diabetes (Aurora, CO, USA). The Meso Scale Discovery (MSD®) Human Kidney Injury Panels 3 and 5 Assay Kits (Gaithersburg, MD, USA) were used to measure the following biomarkers: αGST, calbindin, clusterin, kidney injury molecule-1 (KIM-1), osteoactivin, Trefoil factor 3 (TFF3), vascular endothelial growth factor (VEGF) and β2 microglobulin (B2M), cystatin C, epidermal growth factor (EGF), neutrophil gelatinase-associated lipocalin (NGAL)/Lipocalin-2, osteopontin (OPN) and uromodulin (UMOD). The Human Kidney Injury Panels use a chemiluminescence immunoassay technology with a broad detection range for each of these biomarkers from the lowest limits of detectability in picograms/milliliter (pg/mL) to $>$200 000 pg/mL. The assays were run according to the manufacturer's protocol on never-thawed samples. Average intra- and inter-assay precision for the biomarkers ranges between 3% and 8%, respectively. Whereas these kits were originally designed by MSD® to monitor drug-induced kidney toxicity, they have since been successfully applied to predict diabetic and non-DKD.

Renal measurements

Two urine samples were collected in duplicate and urine creatinine and albumin were measured [RIA, Siemens Medical Solutions Diagnostics (formerly Diagnostic Products Corp.), Los Angeles, CA, USA] and averaged to determine urinary albumin-to-creatinine ratio (ACR). Both urine ACR and estimated GFR (eGFR) by CKD-EPI creatinine were measured at the baseline and after mean follow-up of 12.1 $\pm$ 1.5 years. Albuminuria was defined as ACR $\geq$30 mg/g and impaired GFR as eGFR $<$60 mL/min/1.73 m^2.

Statistical analysis

Analyses were performed in SAS (version 9.4 for Windows; SAS Institute, Cary, NC, USA). Variables were checked for the distributional assumption of normality using normal plots. Variables that were positively skewed (e.g. KIM-1, TTF3, VEGF, B2M, cystatin C, NGAL and OPN) were natural log-transformed for the analyses. Differences in continuous parametric and log-transformed variables between participants with and without T1D were examined with the *t*-test. Parametric continuous data are presented as means $\pm$ standard deviation (SD). Nonparametric data are presented as the median and interquartile range, except for the log-transformed kidney injury markers, which are presented as the geometric mean and 95% confidence interval (CI). Categorical data are presented as the number of subjects and the percent. Statistical testing to detect differences between groups included the *t*-test for parametric continuous data, the Wilcoxon rank-sum test for nonparametric data and the χ^2-test for categorical data.

PCA

Injury markers exhibit pleiotropic and synergistic effects. Evaluating single independent markers would likely underestimate these effects; thus, a method that considers the combined influence of multiple markers, and therefore the overall injury burden, would be preferable. However, combining markers into a composite measure needs to avoid overestimating the association due to the potentially high correlation between markers. Accordingly, we used PCA with orthogonal rotation to derive uncorrelated linear transformations of the biomarkers. We considered the eigenvalues ($\geq$1), scree plots and interpretability (variables with factor loads $\geq$0.40) of the final solution in determining the minimum number of components to use for further analysis. Markers with factor loads $\geq$0.40 on multiple

components (αGST) were dropped, and the PCA was run again. In the final PCA, the first two components (Component 1: KIM-1, calbindin, osteoactivin, TFF-3 and VEGF; Component 2: B2M, cystatin C, NGAL and OPN) explained 72% of the total variance and were used in the multivariable regression analyses. UMOD did not meaningfully load on either component and was examined separately.

Kidney injury composite scores

To test a measure of the combined effects (kidney injury burden), a composite score was constructed using the component interpretation from the PCA. Participants were assigned a score of 1 for each biomarker with a value exceeding the 75th percentile. The scores were added, and a composite score could, therefore, range from 0 (in which none of the markers exceeded the 75th percentile) to 4 (in which all markers exceeded the 75th percentile).

For Component 1, participants were stratified into three groups: those with a composite score of 0–1 ($n = 363$), those with a score of 2–3 ($n = 103$) and those with a score of 4–5 ($n = 61$). For Component 2, participants were stratified into three groups: those with a composite score of 0 ($n = 268$), those with a score 1–2 ($n = 172$) and those with a score 3–4 ($n = 87$). A composite score was also created with all biomarkers: those with a composite score of 0–1 ($n = 219$), those with a score 2–3 ($n = 144$), those with a score of 4–6 ($n = 107$) and those with a score 7–10 ($n = 57$).

Multivariable modeling

Multivariable logistic regression models were employed to determine the independent effect of the principal components and the composite score on the incident albuminuria and impaired GFR. Potential confounding variables were considered for inclusion in the models based on *a priori* criteria: significance in previous work, significant contribution to the model fit (P-value of the Wald $\chi^2 < 0.05$) or confounding the association between the main variable of interest and the outcome by >10%. Covariates included in the multivariable models were age, sex, Hb**A**1c, systolic **b**lood pressure and LDL **c**holesterol (ABC risk factors), in addition to baseline log ACR for incident albuminuria and baseline eGFR for incident-impaired GFR. We also examined change in ACR and eGFR over a period of 12 years as a continuous variable in multivariable linear regression models. Change in ACR was log-modulus transformed [$y' = \text{sign}(y)*\log(1 + |y|)$] due to positive and negative values with a positively skewed distribution.

Prediction performance analyses

Prediction metrics for incident albuminuria and incident-impaired GFR were examined with C-statistics, integrated discrimination index (IDI) and continuous net reclassification improvement (NRI). Participants without data at baseline and follow-up were excluded from the analyses. Because C-statistics have been criticized for being insensitive to changes in clinical decisions yielded for information gained [8–10], we also utilized IDI and continuous NRI. The NRI estimates correct changes in clinical classification across risk thresholds [8–10], and IDI use probability differences instead of categories [8–11]. Event reclassification describes the percentage of events (i.e. impaired GFR) correctly reclassified by the addition of Component 1 or Component 2 to Model 1 [age, sex, HbA1c, SBP, LDL-C (ABC risk factors), baseline log ACR or baseline eGFR]. Similarly, non-event reclassification reports the percentage of non-events (i.e. no impaired GFR) correctly reclassified by the addition of Component 1 or Component 2 to Model 1. Significance was based on α-level of 0.05.

RESULTS

Participants' characteristics stratified by incident DKD status are presented in Table 1. Participants with T1D who developed DKD over a period of 12 years were older with longer-standing T1D, and greater prevalence of statin and ARB use compared with their peers who did not develop DKD. Table S1 in Supplementary data summarizes the distributions for the kidney injury markers stratified by incident DKD status in adults with T1D. Participants with T1D, who developed DKD, had higher NGAL, KIM-1, cystatin C, B2M and αGST, and lower UMOD compared with the participants with T1D who did not experience DKD. The other markers were not significantly different between the two groups.

Table S2 in the Supplementary data reports the kidney injury biomarker patterns derived from the PCA. Values for KIM-1,

Table 1. Participants' characteristics stratified by T1D status

Variables	Incident DKD[a]		P-value
	Yes ($n = 37$)	No ($n = 208$)	
Age at baseline (years)	43 ± 9	39 ± 9	0.005
Sex (female, %)	51	55	0.70
T1D duration at baseline (years)	29 ± 10	25 ± 9	0.02
Hemoglobin A1c (HbA1c) (%) at baseline	8.0 ± 1.5	7.5 ± 1.0	0.06
HbA1c (mmol/mol) at baseline	64 ± 16	58 ± 11	0.06
Systolic blood pressure (SBP) at baseline (mmHg)	114 ± 11	110 ± 12	0.07
Diastolic blood pressure (DBP) at baseline (mmHg)	74 ± 8	75 ± 10	0.30
low density lipoprotein cholesterol (LDL-C) at baseline (mg/dL)	96 ± 27	99 ± 27	0.52
BMI at baseline (kg/m^2)	26 ± 4	26 ± 5	0.46
angiotensin-converting-enzyme inhibitor (ACE inhibitor) at baseline (yes, %)	43	29	0.07
angiotensin II receptor antagonist (ARB) at baseline (yes, %)	26	5	<0.0001
Statins at baseline (yes, %)	46	26	0.02
estimated glomerular filtration rate (eGFR) at baseline (mL/min/1.73 m^2)	86 ± 21	107 ± 14	<0.0001
Albumin-to-creatinine ratio (ACR) at baseline (mg/g)[b]	26 (14–49)	6 (56)	<0.0001
eGFR at 12-year follow-up (mL/min/1.73 m^2)	53 ± 24	90 ± 14	<0.0001
ACR at 12-year follow-up (mg/g)[b]	53 (28–101)	4 (4–5)	<0.0001

Data are presented as means ± SD, unless otherwise specified.
[a]Only participants with complete renal data at baseline and 12-year follow-up included in analyses.
[b]Geometric means and 95% CI.

Table 2. Multivariable logistic models examining relationships between kidney injury markers and development of DKD over 12 years in adults with T1D

	Albuminuria (≥30 mg/g)		Impaired GFR (<60 mL/min/1.73 m^2)	
	Crude	Adjusted[a]	Crude	Adjusted[b]
Individual biomarkers of Component 1				
KIM-1 [per 1 SD (0.42)]	1.15 (0.70–1.87), P = 0.59	0.60 (0.30–1.21), P = 0.15	1.71 (1.25–2.35), P = 0.0008	1.63 (1.06–2.53), P = 0.03
Calbindin [per 1 SD (132.3)]	0.76 (0.36–1.59), P = 0.47	0.20 (0.04–1.07), P = 0.06	0.91 (0.59–1.39), P = 0.65	0.88 (0.51–1.50), P = 0.63
Osteoactivin [per 1 SD (675.9)]	0.79 (0.45–1.38), P = 0.40	0.48 (0.24–0.98), P = 0.045	1.41 (1.02–1.94), P = 0.04	0.85 (0.52–1.40), P = 0.53
TFF-3 [per 1 SD (0.347)]	1.07 (0.64–1.77), P = 0.80	0.74 (0.36–1.52), P = 0.41	1.39 (0.99–1.95), P = 0.06	1.16 (0.68–1.99), P = 0.59
VEGF [per 1 SD (0.369)]	1.02 (0.61–1.71), P = 0.95	0.73 (0.40–1.33), P = 0.30	1.14 (0.80–1.64), P = 0.46	1.17 (0.73–1.87), P = 0.52
Individual biomarkers of Component 2				
B2M [per 1 SD (0.324)]	2.29 (1.39–3.77), P = 0.001	1.70 (0.95–3.06), P = 0.08	2.60 (1.72–3.94), P < 0.0001	1.68 (0.98–2.89), P = 0.06
Cystatin C [per 1 SD (0.287)]	2.20 (1.42–3.41), P = 0.002	1.46 (0.85–2.52), P = 0.17	2.44 (1.72–3.46), P < 0.0001	2.18 (1.31–3.63), P = 0.003
NGAL [per 1 SD (0.305)]	1.71 (1.06–2.76), P = 0.03	1.22 (0.71–2.10), P = 0.46	1.75 (1.23–2.49), P = 0.002	1.44 (0.89–2.32), P = 0.14
OPN [per 1 SD (0.574)]	1.71 (1.01–2.90), P = 0.04	1.74 (0.92–3.27), P = 0.09	1.29 (0.88–1.89), P = 0.20	1.49 (0.97–2.31), P = 0.07
Markers not part of Components 1 and 2				
UMOD [per 1 SD (138.7 µg/mg)]	0.26 (0.12–0.60), P = 0.001	0.37 (0.16–0.86), P = 0.02	0.49 (0.30–0.79), P = 0.004	0.46 (0.25–0.86), P = 0.02
αGST [per 1 SD (0.663)]	1.31 (0.81–2.11), P = 0.28	1.34 (0.76–2.39), P = 0.31	1.28 (0.90–1.82), P = 0.17	1.32 (0.81–2.14), P = 0.27
Principal components				
Component 1	0.70 (0.32–1.56), P = 0.39	0.28 (0.08–1.02), P = 0.053	1.23 (0.81–1.85), P = 0.34	1.06 (0.58–1.95), P = 0.84
Component 2	2.64 (1.55–4.51), P = 0.0004	1.82 (0.95–3.48), P = 0.07	2.83 (1.85–4.35), P < 0.0001	2.08 (1.18–3.67), P = 0.01
Composite score for Component 1				
0–1[c]	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
2–3	0.35 (0.04–2.75), P = 0.32	0.24 (0.02–2.38), P = 0.22	2.48 (1.01–6.10), P = 0.048	1.45 (0.46–4.55), P = 0.53
4–5	0.49 (0.06–3.86), P = 0.50	0.00 (0.00–999.99), P = 0.97	2.02 (0.70–5.81), P = 0.19	1.44 (0.39–5.33), P = 0.59
Composite score for Component 2				
0[c]	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1–2	2.88 (0.79–10.52), P = 0.11	3.65 (0.76–17.59), P = 0.11	1.23 (0.46–3.32), P = 0.68	2.12 (0.64–7.01), P = 0.22
3–4	9.74 (2.40–39.44), P = 0.001	5.14 (0.82–32.26), P = 0.08	8.16 (3.24–20.53), P < 0.0001	5.66 (1.56–20.57), P = 0.008
Composite score for all biomarkers				
0–1[c]	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
2–3	2.07 (0.54–7.95), P = 0.29	3.90 (0.73–20.66), P = 0.11	2.66 (0.85-8.36), P = 0.09	2.81 (0.70–11.30), P = 0.15
4–6	2.98 (0.71–12.45), P = 0.14	1.84 (0.29–11.73), P = 0.52	5.70 (1.87–17.35), P = 0.002	4.47 (1.12–17.90), P = 0.03
7–10	4.58 (0.76–27.45), P = 0.10	0.63 (0.04–9.81), P = 0.74	9.16 (2.58–32.54), P = 0.0006	5.98 (1.17–30.54), P = 0.03

[a]Adjusted for age, sex, HbA1c, LDL-C, SBP and baseline log ACR.
[b]Adjusted for age, sex, HbA1c, LDL-C, SBP and baseline eGFR.
[c]Reference group.
Significant results are highlighted in yellow.

calbindin, osteoactivin, TFF3 and VEGF were positively correlated with Component 1; that is, participants with higher scores on this component would have higher values of these kidney injury markers (factor loads of 0.87, 0.93, 0.71, 0.85 and 0.85, respectively). Similarly, scores on Component 2 were correlated with the values of B2M, cystatin C, NGAL and OPN (factor loads of 0.86, 0.86, 0.86 and 0.50, respectively).

PCA and multivariable logistic regression models

To determine the associations of the kidney injury markers on the development of albuminuria and impaired GFR, univariable and multivariable logistic regression models were fit for the individual biomarkers, principal components and the composite score as a measure of collective kidney injury burden. The results are presented in Table 2. Only Component 2 of the PCA was associated with incident impaired GFR [odds ratio (OR) 2.08, 95% CI 1.18–3.67, P = 0.01], adjusting for age, sex, HbA1c, LDL-C, systolic blood pressure and baseline eGFR. Neither component was associated with incident albuminuria in fully adjusted models (Table 2). Higher UMOD concentrations at baseline predicted lower odds of incident albuminuria (OR 0.37, 95% CI 0.16–0.86, P = 0.02) and impaired GFR (OR 0.46, 95% CI 0.25–0.86, P = 0.02), per 1 SD (138.7 µg/mg), in the multivariable models.

Composite scores and multivariable logistic regression models

Participants with 4–5 of the Component 1 biomarkers >75th percentile did not demonstrate greater risk of albuminuria or impaired GFR compared with those with 0–1 biomarkers >75th percentile in unadjusted or adjusted models (Table 2). In contrast, participants with 3–4 of the Component 2 biomarkers >75th percentile had greater odds of impaired GFR (OR 5.66, 95% CI 1.56–20.57, P = 0.008) compared with those with 0 biomarkers >75th percentile, in adjusted models (Table 2).

Participants with 7–10 (OR 5.98, 95% CI 1.17–30.54, P = 0.03) and 4–6 (OR 4.47, 95% CI 1.12–17.90, P = 0.03) of all biomarkers >75th percentile had greater risk of developing impaired GFR compared with those with 0–1 biomarkers >75th percentile, in adjusted models (Table 2). Similar relationships were not observed with incident albuminuria (Table 2).

PCA and multivariable linear regression models

Component 2 was associated with an increase in ACR over a period of 12 years [β ± standard error (SE): 0.16 ± 0.07, P = 0.02] after adjusting for age, sex, HbA1c, systolic blood pressure, LDL-C and baseline log ACR. Similarly, Component 2 was associated with a decrease in eGFR over a period of

Table 3. Prediction performance analyses for Component 1 and Component 2 (C-statistics and IDI)

Model 1 (age, sex, HbA1c, SBP and LDL-C) versus Model 1 + Component 1 or Component 2			
Models	AUC	ΔAUC (±SE)	IDI (±SE)
Incident CKD			
Model 1 (Model 1: age, sex, HbA1c, SBP, LDL-C and baseline eGFR)	0.89	–	–
Model 1 + Component 1	0.89	–	–
(Component 1 + Model 1) versus Model 1	–	0.00 ± 0.00, P = 0.82	0.00 ± 0.00, P = 0.71
Model 1 + Component 2	0.92	–	–
(Component 2 + Model 1) versus Model 1	–	0.02 ± 0.01, P = 0.049	0.03 ± 0.02, P = 0.15
Incident albuminuria			
Model 1 (Model 1: age, sex, HbA1c, SBP and LDL-C)	0.89	–	–
Model 1 + Component 1	0.92	–	–
(Component 1 + Model 1) versus Model 1		0.02 ± 0.02, P = 0.19	0.05 ± 0.03, P = 0.13
Model 1 + Component 2	0.92		–
(Component 2 + Model 1) versus Model 1		0.03 ± 0.02, P = 0.16	0.04 ± 0.03, P = 0.21

Significant results are highlighted in yellow.

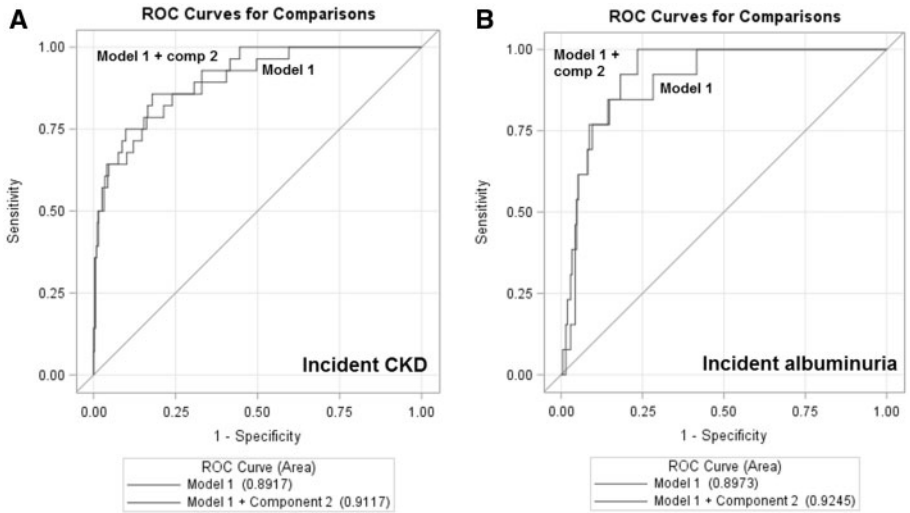

FIGURE 1: ROC curves for comparisons between Model 1 versus Model 1 + Component 2 in patients with (**A**) incident CKD and (**B**) incident albuminuria.

12 years (β ± SE: −2.56 ± 0.97, P = 0.009), after adjusting for age, sex, HbA1c, systolic blood pressure, LDL-C and baseline eGFR. In contrast, Component 1 did not significantly associate with change in ACR or change in eGFR over a period of 12 years (data not shown).

Prediction performance analyses

Of all the variables in the fully adjusted models, Component 2 (C-statistic: 0.77, 95% CI 0.68–0.86) was the strongest single predictors of incident-impaired GFR. Similarly, for incident albuminuria, Component 2 (C-statistic: 0.74, 95% CI 0.62–0.85) was the strongest predictor. C-statistics for models including age, sex and ABC risk factors (Model 1) with and without Component 1 or Component 2 are shown in Table 3. The addition of Component 1 to Model 1 did not improve C-statistics or IDI for incident CKD or incident albuminuria (Table 3). In contrast, the addition of Component 2 to Model 1 improved C-statistics (ΔAUC: 0.02 ± 0.01, P = 0.049) for incident CKD (Table 3, Figure 1). For incident albuminuria, the addition of Component 1 or Component 2 did not improve C-statistics or IDI (Table 3, Figure 1). For category-free NRI, 29% of non-events were correctly reclassified by the addition of Component 2 to Model 1 for incident-impaired GFR (Table 4). The addition of Component 2 to Model 1 did not improve reclassification of events or non-events for incident albuminuria (Table 4).

In sensitivity analyses, the addition of UMOD to Component 2 + Model 1 (age, sex and ABC risk factors) did not further improve C-statistics for incident CKD (ΔAUC: 0.02 ± 0.03, P = 0.50) or albuminuria (ΔAUC: 0.02 ± 0.04, P = 0.66).

DISCUSSION

Our data demonstrate that biomarkers of kidney injury are prospectively associated with development of albuminuria and impaired GFR over a period of 12 years in adult with T1D. The second principal component (B2M, cystatin C, NGAL and OPN) was strongly associated with greater risk of impaired GFR, and UMOD conferred protection from DKD development. A composite score of the biomarkers of the second principal component (measuring kidney injury burden) was also strongly associated with the development of impaired GFR.

Table 4. Prediction performance analyses for Component 1 and Component 2 (category-free NRI)

Model 1 versus Model 1 + Component 1	
Incident CKD component (Model 1: age, sex, HbA1c, SBP, LDL-C and baseline eGFR)	
Category-free NRI	0.04 (95% CI − 0.34 to 0.42)
• Percentage of events correctly reclassified	14 (P = 0.45)
• Percentage of non-events correctly reclassified	18 (P = 0.0009)
Incident albuminuria (Model 1: age, sex, HbA1c, SBP, LDL-C and baseline log ACR)	
Category-free NRI	0.46 (95% CI − 0.09 to 1.00)
• Percentage of events correctly reclassified	23 (P = 0.41)
• Percentage of non-events correctly reclassified	22 (P = 0.001)
Model 1 versus Model 1 + Component 2	
Incident CKD component (Model 1: age, sex, HbA1c, SBP, LDL-C and baseline eGFR)	
Category-free NRI	0.50 (95% CI 0.13 to 0.88)
• Percentage of events correctly reclassified	21 (P = 0.26)
• Percentage of non-events correctly reclassified	29 (P < 0.0001)
Incident albuminuria (Model 1: age, sex, HbA1c, SBP, LDL-C and baseline log ACR)	
Category-free NRI	−0.02 (95% CI − 0.58 to 0.53)
• Percentage of events correctly reclassified	8 (P = 0.78)
• Percentage of non-events correctly reclassified	5 (P = 0.45)

Significant results are highlighted in yellow.

Finally, the addition of Component 2 to American Diabetes Association's ABC risk factors improved the prediction metrics of incident impaired GFR. Collectively, these results suggest that kidney injury biomarkers can help predict development of DKD in adults with T1D.

The kidney injury markers that loaded strongly on Component 2 and were associated with DKD development were B2M, cystatin C, NGAL and OPN. Because cystatin C is an endogenous filtration marker, we ran sensitivity analyses removing cystatin C from Component 2. The output of these sensitivity analyses demonstrated that the predictive value of Component 2 remained in the absence of cystatin C. Of the variables driving the predictive component in Component 2, three factors, B2M, OPN and NGAL, represent tubular injury markers [12]. Elevated concentrations of B2M and cystatin C are also associated with glomerular damage [13]. NGAL is known to be elevated in adolescents and adults with T1D with or without albuminuria indicating tubular damage [14, 15]. OPN plays a pro-inflammatory role [16], is overexpressed in nephritis and plays a prominent role with NGAL [17]. Collectively, the markers of Component 2 represent tubular and glomerular injury. In contrast, the kidney injury markers that loaded on Component 1 (KIM-1, calbindin, osteoactivin, TFF-3 and VEGF) did not individually or collectively associate with DKD development. KIM-1 and calbindin are recognized biomarkers for acute kidney injury [18–20], and osteoactivin is upregulated in the tubular epithelium in response to renal injury and has been implicated in renal interstitial fibrosis [20–22]. TFF3 expression is markedly reduced in response to renal tubular injury and VEGF concentrations increase during impaired renal function following hypoxia or other acute and chronic injuries [23]. αGST was not associated with development of DKD in adults with T1D. The literature has suggested that elevated concentrations of αGST may indicate proximal tubular function and injury [24]. Except for KIM-1 and calbindin, the markers of Component 1 are thought to contribute to the pathogenesis of glomerular and tubular damage rather than reflecting the actual injury.

UMOD did not meaningfully load on either component and was examined separately. Greater concentrations of UMOD at baseline conferred lower odds of developing albuminuria and impaired GFR over a period of 12 years. UMOD, also known as Tamm–Horsfall protein [25, 26], has historically been considered a protein that functions primarily as an antibacterial, but has recently received attention for its strong association with CKD. Mutations in UMOD, for example, are the cause of a hereditary renal disease, familial juvenile hyperuricemic nephropathy and polymorphisms in UMOD are risk factors for renal disease progression in Genome-wide association studies. Increased expression of UMOD can cause it to leak into the renal interstitium and cause inflammation and kidney damage [25–27]. It is produced by the epithelial cells of the thick ascending loop of Henle and serves as a protective role against stone formation and urinary tract infection [26]. Studies also indicate that UMOD decreases in the setting of acute tubular injury, possibly playing a protective role in the downregulation of cytokines and other mediators of inflammation [28, 29]. Consistent with these reports, our data suggest a protective role of higher UMOD concentrations in DKD development. This is in contrast to the report by Torffvit *et al.* who did not find a significant difference between 58 participants with T1D and DKD and 76 controls [30].

To our knowledge, only a limited number of studies have examined the longitudinal relationships between kidney injury markers and development of DKD in adults with T1D. Our data suggest that both glomerular and tubular injury markers predict DKD. Diabetic tubular nephropathy is associated with basement membrane thickening, tubular hypertrophy, epithelial-mesenchymal transition, glycogen accumulation and interstitial inflammation [31]. While diabetic glomerulopathy has received significant attention from researchers, diabetic tubular injury is known to associate better with renal function than glomerular injury [32, 33]. Furthermore, tubular damage may be induced earlier than glomerular injury in the course of DKD [34]. A histologic study in adult diabetic patients with albuminuria demonstrated that only 29% had histological

evidence of glomerulopathy, whereas 42% had evidence of tubulopathy [35].

Our study does have important limitations worth mentioning. Not all potentially influential kidney injury biomarkers were measured, and due to the limited number of participants with incident DKD, we are unable to dissect which biomarkers are associated with early versus advanced DKD. However, owing to the common biologic pathways of many kidney injury markers, we decided to combine markers into a composite score, which could strengthen a single measure with multiple possible pathways. We only had data on the kidney biomarkers at baseline and could not evaluate changes in biomarkers over time and how these changes relate to development of DKD. Another limitation includes lack of time-to-event data for DKD. Strengths of our study include the sample size, 12-year prospective data, PCA, sensitivity analyses and comprehensive prediction performance analyses with C-statistics, IDI and NRI.

In conclusion, kidney injury biomarkers were associated with DKD risk over a period of 12 years. These kidney injury panels may help in risk stratification and predict the future development of DKD. Further research is needed to better understand the underlying pathophysiologic mechanisms responsible for changes in these biomarkers over time and to assess the impact of existing and novel therapies on biomarker levels, thereby guiding early intervention strategies.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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AUTHORS' CONTRIBUTIONS

P.B. researched, wrote, contributed to discussion, analyzed data and reviewed/edited the article; M.R. designed the CACTI Study, researched, contributed to the discussion and reviewed/edited the article; D.Z.I.C., R.S., R.W. and R.J.J. contributed to the discussion and reviewed/edited the article; J.K.S.-B. and L.P. researched, wrote and analyzed data, contributed to the discussion and reviewed/edited the article. P.B. and J.K.S.-B are guarantors of the analyses and work.

CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this article have not been published previously in whole or part, except in abstract format.

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