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Comparison of Serum Cystatin C, Serum Creatinine, Measured GFR, and Estimated GFR to Assess the Risk of Kidney Failure in American Indians With Diabetic Nephropathy

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Abstract

Background—We compared values of baseline serum cystatin C (SCysC), serum creatinine (SCr), and measured GFR (mGFR) for predicting end-stage renal disease (ESRD) in patients with type 2 diabetes and elevated albuminuria.

Study Design—Observational longitudinal study.

Setting & Participants—Pima Indians with type 2 diabetes and elevated albumin/creatinine ratio (ACR ≥ 30 mg/g).

Predictors—Baseline SCysC, SCr, and mGFR.

Outcomes & Measurements—Individuals were followed from their first diabetic examination with ACR ≥ 30 mg/g until December 2010, onset of ESRD, or death, whichever came first. Incidence rates adjusted for age, and sex were computed by Mantel-Haenszel stratification. The abilities of SCysC, SCr, and mGFR to predict ESRD were compared with receiver operating characteristic curves.

Results—Of 234 Pima Indians with a mean age of 42.8 years who were followed for a median of 10.7 (range, 0.6–21.3) years, 68 (29%) developed ESRD. The incidence of ESRD was significantly higher among patients in the lowest vs. highest tertile of 1/SCysC (incidence rate ratio, 2.43; 95% CI, 1.31–4.50). By contrast, mGFR and 1/SCr had J-shaped associations with ESRD. In unadjusted analyses, 1/SCysC had the highest area under the receiver operating characteristic curve (AUROC; 0.719±0.035) and mGFR the lowest (0.585±0.042; P<0.001); the AUROC for 1/SCr was intermediate (0.672±0.040; P of 0.1 and 0.03 vs 1/SCysC and mGFR, respectively). In analyses adjusted for age, sex, diabetes duration, height, weight, hemoglobin

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A1c, and ACR, 1/SCysC had the highest AUROC (0.845 ± 0.026). Models with mGFR or 1/SCr alone had similar AUROCs (0.815 ± 0.028) and both were lower than the model with 1/SCysC alone ($P=0.02$ and $P=0.03$, respectively).

Limitations—The predictive values of the filtration markers are limited to the extent that their precision is based on a single measurement.

Conclusions—SCysC was a better predictor of ESRD than mGFR or SCr in Pima Indians with type 2 diabetes and elevated albuminuria.

Diabetic kidney disease is characterized by a progressive decline in glomerular filtration rate (GFR) that often leads to kidney failure. This decline is frequently accompanied by an increase in urinary albumin excretion and together these factors are used to assess severity of diabetic kidney disease and predict progression to ESRD.¹ Whereas albuminuria is measured easily in a spot urine sample and is highly predictive of kidney disease outcome, GFR can only be measured (mGFR) indirectly in humans by infusion of filtration markers (e.g., inulin, iothalamate, iohexol, or ⁵¹Cr-EDTA) into the bloodstream and measurement of their disappearance from the blood or their appearance in the urine. To avoid this time-consuming procedure, GFR is generally estimated based on serum concentrations of creatinine (eGFR_{cr}), cystatin C (eGFR_{cys}), or both (eGFR_{cr-cys}), which are used in equations standardized for age, sex, and ethnicity. Whereas serum creatinine (SCr) is eliminated by glomerular filtration and proximal tubular secretion, serum cystatin C (SCysC) is an endogenous low molecular mass protein that is produced at a constant rate and virtually completely cleared from the circulation by glomerular filtration with subsequent proximal tubular uptake and degradation. For these reasons it has been studied extensively as an alternate marker of kidney function to SCr. At GFR levels ≥ 60 ml/min/1.73 m², SCysC appears to more accurately reflect kidney function than SCr.^{2–4}

Although each of these filtration markers may provide a reasonable estimate of kidney function in specific clinical settings, their value relative to mGFR in predicting ESRD in people with type 2 diabetes is less certain. In this study we compared the predictive value of SCysC, SCr, and mGFR for ESRD in subjects with type 2 diabetes and elevated albuminuria.

Methods

Study Population

Between 1965 and 2007, Pima Indians from the Gila River Indian Community participated in a longitudinal study of diabetes and its complications. As described previously,¹⁷ each member of this community at least 5 years old was invited to have a research examination approximately every 2 years. Diabetes was diagnosed by a 2-hour post-load plasma glucose concentration ≥ 200 mg/dl at these biennial examinations, or when the diagnosis was documented in the medical record. For the present study we selected participants from this longitudinal population-based study who also participated in longitudinal studies of glomerular function, had type 2 diabetes, and serial standardized measurements of GFR, SCysC, and SCr. Of the 267 who met these criteria, 234 had an ACR ≥ 30 mg/g following the diagnosis of type 2 diabetes. Their date of diabetes diagnosis was ascertained as part of the longitudinal population-based study; all other variables used in this analysis were measured during the kidney function studies. Baseline examinations for the present study included dates between April 1989 and June 2007. Analyses were limited to those with elevated albuminuria as they were the most likely participants to progress to ESRD during the study period. Among the 33 subjects not included because of persistent normoalbuminuria, only 2 progressed to ESRD.

Laboratory Measurements

All urine and serum samples were stored at -80°C until the day of assay, which for all measures, excepting SCysC, were performed within 30 days of the sample collection. SCr concentrations were measured by a modified Jaffé reaction with calibration traceable to an isotope dilution mass spectrometry (IDMS) measurement procedure.⁵ Urinary albumin was measured by nephelometric immunoassay and concentrations below the threshold detected by the assay (6.8 mg/l) were set to this value in the analyses. Urinary albumin excretion was estimated by computing the albumin-to-creatinine ratio (ACR) in units of mg/g. ACR was considered normal if <30 mg/g or elevated if $\text{ACR} \geq 30$ mg/g; macroalbuminuria was defined as $\text{ACR} \geq 300$ mg/g.

Urinary clearance of non-radioactively labeled iothalamate was estimated by the average of four timed urine collections, bracketed by the collection of blood samples, made at 20-min intervals after a water load and a 60-minute equilibration period. A high performance liquid chromatography system with a sensitive ultraviolet light detector was used to assay iothalamate at 236 nm (Instrumentation Shimadzu #6A, Shimadzu Corp).⁶ Serum SCysC was measured over an 8-month period on a BN Prospec System nephelometer (Dade Behring Inc., healthcare.siemens.com) using samples stored at -80°C since collection. The immunoassay was based on rabbit monospecific anti-human cystatin C anti-serum (Dade Behring Diagnostics, healthcare.siemens.com/laboratory-diagnostics). CysC values were calibrated according to the International Federation of Clinical Chemistry (IFCC) Working Group for Standardization of Serum Cystatin C⁷ by the following equation: $\text{IFCC_CYS} = (0.92532 \times \text{SCysC}) + 0.20346$. Intra-assay coefficients of variation for mGFR, SCr, and SCysC were 1.1%, 2.0%, and 2.5%, and inter-assay coefficients of variations were 2.9%, 4.3%, and 2.2%, respectively.

Body mass index was defined as weight divided by the square of height (kg/m^2). Mean arterial pressure was calculated as equaling $2/3$ diastolic arterial pressure + $1/3$ systolic arterial pressure.

The study was approved by the review board of the National Institute of Diabetes and Digestive and Kidney Diseases. Each participant gave informed consent.

Statistical analysis

Baseline clinical and demographic features are presented as means $\pm$ standard deviation or medians (range). Associations between baseline variables and SCysC were examined by Spearman correlations between continuous variables; Mantel-Haenszel χ^2 -tests for general association were used to examine relationships between SCysC tertiles and the categorical variables sex, hyperfiltration, hypertension or hypoglycemic treatment.

Participants were followed up from their first diabetic examination with $\text{ACR} \geq 30$ mg/g until December 31, 2010, onset of ESRD, or death, whichever came first. ESRD was defined as initiation of dialysis, kidney transplantation, or death from diabetic nephropathy if dialysis or transplantation was refused. Cause of kidney disease was determined by review of medical records. The incidence rate of diabetic ESRD was computed as the number of new cases of ESRD per 1,000 person-years at risk according to tertiles of mGFR and reciprocals of SCysC ($1/\text{SCysC}$ in mg/l) and SCr ($1/\text{SCr}$ in mg/dl). We used reciprocals of SCysC and SCr because these values changed in concert with changes in mGFR. Relationships between measures of filtration were examined by Spearman correlations.

The abilities of baseline measures of $1/\text{SCysC}$, $1/\text{SCr}$, and mGFR to predict ESRD were compared with receiver operating characteristic (ROC) curves calculated from Cox regression models that included the baseline variables age, sex, diabetes duration, height,

weight, hemoglobin A1c, ACR and the relevant filtration markers (per one standard deviation). All analyses used only baseline measurements of filtration markers because our primary interest was the clinically-relevant predictive value of filtration markers at a single time point for subsequent development of ESRD. Proportionality assumptions for individual predictors were assessed by inspection of Schoenfeld residuals. Product terms among predictor variables did not significantly improve the regression models and were not included. Baseline values of ACR were expressed as logarithm base 2 ($\log_2$) to reflect the association with ESRD corresponding to a 2-fold difference in ACR.

Area under the ROC curve and its standard error were calculated for each model by the generalized C statistic to account for variable follow-up times.⁸ Comparisons between areas for pairs of variables were assessed, with and without a Bonferroni correction, according to the formula of Hanley and McNeil.⁹ This method accounts for correlation among areas under the curve that occurs when ROC curves are derived from correlated variables measured in the same individuals; this correlation was estimated by a jackknife procedure.¹⁰ Area under the ROC (AUROC) can vary from 0.5 (no predictive value) to 1.0 (perfect prediction). Calculations were performed using SAS software version 9.3 (SAS Institute Inc, Cary, NC).

For additional comparisons, GFR was estimated based on equations (eGFR) derived from SCr and/or SCysC. The Bonferroni procedure was not used for these post-hoc comparisons. These equations report GFR indexed for body surface area, so they were compared with indexed mGFR (in ml/min/1.73 m²). For all other comparisons mGFR was not indexed for body surface area (in ml/min), because indexing for body surface area may significantly underestimate mGFR in overweight and obese individuals.¹¹ AUROCs for these estimates were computed from Cox regression models that included baseline variables age, sex, diabetes duration, height, weight, hemoglobin A1c, $\log_2$ (ACR), and eGFR. The equations in Box 1 were used to calculate eGFR. The multiplication factor of 1.212 for black race was not used when estimating GFR with the 4-variable MDRD Study equation ($eGFR_{cr(MDRD)}$), because in Pima Indians there is better agreement with mGFR if this term is excluded.¹⁵ Each model included quadratic terms for the filtration markers included in the model because of their non-linear relationships with the incidence of ESRD.

Results

The study included 234 participants (79 men, 155 women) with a mean age of 42.8 years who were followed up for a median of 10.7 (range, 0.6–21.3) years. During follow-up, 68 (29.0%) of the participants developed ESRD and 32 (13.6%) died without progressing to ESRD; 28 deaths were due to natural causes, of which 5 were cardiovascular. Baseline characteristics of the study cohort are shown in Table 1 according to tertiles of SCysC. Hyperfiltration, defined by an mGFR ≥ 154 ml/min, a value two standard deviations above the mean mGFR for Pima Indians with normal glucose tolerance, was present at baseline in 50% of the participants. Those with the lowest level of kidney function (highest tertile of SCysC) were older, had the highest body mass index and ACR, and the lowest hemoglobin A1c levels at baseline. They were also more likely to receive treatment with antihypertensive medicines. All other filtration markers correlated significantly with mGFR ($P < 0.001$), with correlations ranging from $r = 0.46$ for $1/SCr$ to $r = 0.76$ for $eGFR_{cr-cys}$; $r = 0.58$ for $1/SCysC$.

Unadjusted and age-sex-adjusted incidence rates of ESRD are shown in Table 2 by tertiles of $1/SCysC$, $1/SCr$, mGFR, and eGFR. Both the unadjusted and adjusted incidence rates were J-shaped for mGFR, $1/SCr$, and $eGFR_{cr(MDRD)}$, but were monotonic for $1/SCysC$,

eGFR_{cys}, and eGFR_{cr-cys}. Unadjusted incidence rates were monotonic for eGFR_{cr}(CKD-EPI) tertiles and were J-shaped when adjusted for age and sex.

Relationships between the various filtration markers and estimates of GFR with mGFR are shown in Figure 1. Trend lines in the figures illustrate the non-linearity of the relationships.

Unadjusted and adjusted AUROC estimates for the measured markers of filtration are compared in Table 3. Unadjusted AUROCs suggested that 1/SCysC and 1/SCr were significantly better discriminators of progression to ESRD than mGFR. After multivariable adjustment, models that included either mGFR or 1/SCr had lower AUROCs than the model with 1/SCysC ($P=0.03$ and $P=0.02$ for each respective pair). Furthermore, the AUROC from the model with 1/SCysC and covariates was significantly higher than the AUROC from the model with covariates alone (AUROC=0.808, $P=0.02$), whereas the AUROCs from the models with mGFR or 1/SCr and covariates did not differ from the AUROC for covariates alone ($P=0.3$ and 0.2 , respectively). These associations did not change when analysis was restricted to those with an ACR ≥ 300 mg/g at their first examination ($n=142$, of which 64 became ESRD cases). In the latter case, AUROCs from models including mGFR or 1/SCysC were higher than from the model with covariates alone ($P=0.02$ and $P=0.002$, respectively), whereas the AUROC from the model with SCr did not differ from the AUROC for covariates alone ($P=0.2$). When P -values for the three primary comparisons (mGFR, 1/SCr, and 1/SCysC) were adjusted using the Bonferroni procedure, both the unadjusted and covariate-adjusted models with 1/SCysC remained as significantly better discriminators of progression to ESRD than mGFR ($P<0.001$ and $P=0.05$, respectively).

Pair-wise comparisons between unadjusted AUROCs of models that included individual eGFRs with the AUROC for mGFR adjusted for body surface area are presented in Table 4. Unadjusted AUROCs were highest for eGFR_{cys} or eGFR_{cr-cys} and these were higher than the unadjusted AUROC for mGFR. Adjusted AUROCs were also highest for eGFR_{cys} or eGFR_{cr-cys}, and these were higher than the adjusted AUROC for mGFR. When compared with models that included baseline covariates only, eGFR_{cys} or eGFR_{cr-cys} and covariates each had a significantly higher AUROC, whereas both eGFR_{cr}(MDRD) and eGFR_{cr}(CKD-EPI) were similar to the model with covariates only.

Discussion

SCysC is a better early predictor of progression to ESRD in persons with type 2 diabetes and elevated albuminuria than mGFR or SCr. After adjusting for multiple demographic and clinical characteristics, the ability of SCysC to discriminate early on between those who progress to ESRD and those who do not progress remained superior to the other markers. After correction for multiple comparisons using the Bonferroni procedure, contrasts between SCysC and mGFR remained statistically significant, but this procedure may be overly stringent because it assumes independence among tests and these measures are highly correlated. Although each marker of kidney function was related to the outcome, the relationship between SCysC and incidence of ESRD was monotonic, whereas the relationships between mGFR or SCr and ESRD were J-shaped. This suggests that cystatin C potentially reflects non-GFR determinants of kidney disease. While these other factors remain to be defined and are therefore not adjusted for, they appear to improve the ability of SCysC to detect pathologic changes in kidney function.

Up to 70% of persons with diabetes have early hyperfiltration.¹⁶ In some, enhanced glomerular perfusion that leads to this hyperfiltration may simply represent a physiologic response to glycemic imbalance or high protein diet, whereas in others it may be a harbinger of progressive kidney damage.^{17–19} Although early hyperfiltration *per se* is not directly

related to nephron loss, the underlying metabolic disorder could determine progression of diabetic nephropathy.²⁰ In this context, SCysC concentration may provide additional information that differentiates purely physiologic changes in GFR from those attributable to nephron loss, leading ultimately to ESRD. This additional information could explain the monotonic relationship we observed between SCysC concentration and ESRD, as opposed to the J-shaped relationships seen for the other filtration markers.²¹ Factors that lead to better prediction by SCysC, however, are presently unknown. Nevertheless, a relative acceleration of tubular creatinine secretion in early diabetic kidney disease in association with the rise in single-nephron GFR that occurs with nephron loss could lead to the J-shaped relationship between 1/SCr and ESRD. Reported enhancement of tubular secretion of ¹²⁵I iothalamate²² in early diabetic kidney disease could have a similar effect on the relationship between mGFR and ESRD. It is noteworthy, however, that with our method of GFR determination from non-radioactively labeled iothalamate clearance, we have not detected any significant elevation above simultaneous inulin clearance to substantiate the phenomenon of iothalamate tubular secretion in humans.

Cystatin C is a sensitive marker of various pathologic processes associated with declining GFR, including cardiovascular disease and inflammation. This concept is supported by previous studies in which SCysC is strongly and monotonically associated with all-cause mortality and cardiovascular events.^{23–26} By contrast, SCr has a consistently J-shaped relationship with all-cause mortality and is not related to cardiovascular events.^{23–26} Among 4,637 participants in the Cardiovascular Health Study, for example, eGFR_{Cr} had a J-shaped association with all-cause mortality and only the lowest quintile was associated with cardiovascular events after multivariable adjustments.²⁵ In keeping with these findings, low SCr may reflect confounding from morbid conditions unrelated to kidney disease, whereas SCysC appears to discriminate the risk for ESRD or cardiovascular events in persons with preserved kidney function.

Previous studies have also compared the prognostic value of several endogenous filtration markers for ESRD and mortality in cohorts with data for mGFR. In both the Modification of Diet in Renal Disease (MDRD) Study²⁷ and the African American Study of Kidney Disease and Hypertension (AASK),²⁸ concentrations of SCr, SCysC, and β trace protein predicted progression to ESRD, independent of mGFR; SCysC and β trace protein were associated with the combined endpoint of ESRD and mortality in AASK, whereas higher SCr concentration and lower SCysC and β trace protein concentrations were associated with mortality in the MDRD Study. Each of these studies had restricted ranges of mGFR. Moreover, AASK enrolled no participants with diabetes and MDRD only a few, so the present study extends their findings for ESRD to a type 2 diabetic population with a wide range of mGFR, and examines the predictive value of various filtration markers relative to mGFR.

As a potent physiological modulator of inflammation and connective tissue repair, SCysC plays an important role in vascular remodeling and stability of atherosclerotic lesions, conditions typically developing early during progression of chronic kidney disease.^{29–31} Biomarkers of inflammation involved in atherosclerosis, such as TNF- α , IL-6, and leukocytes, also predict kidney function decline and ESRD in patients with either type 1 or type 2 diabetes,^{32–35} supporting the notion that inflammation mediates progression of both vascular and kidney pathology.³⁶ Because SCysC appears at the interface of these mechanisms, it may impart additional predictive information about kidney disease that explains some of the improved risk prediction beyond the gold-standard mGFR.^{21,37}

Several factors may limit the predictive value of other filtration markers for health outcomes. The increase in SCr that occurs in the presence of kidney damage is a relatively

late finding, and confounding factors such as muscle mass, tubular secretion, and extrarenal elimination may further reduce its value as a predictor of ESRD. Moreover, greater imprecision at lower concentrations of SCr compounds the difficulty of interpreting SCr levels in those with normal or high-normal GFR³⁸ who might experience the largest subsequent reductions in kidney function. On the other hand, because SCysC concentration is not as influenced by these factors,² changes in SCysC concentration may reflect changes in kidney function across a wider range of GFR.^{39–41}

High doses of corticosteroids and thyroid dysfunction alter cystatin production and may be relevant to using cystatin as a marker of GFR or as a predictor of health outcomes. Glucocorticoids and hyperthyroidism increase the production of SCysC, whereas hypothyroidism decreases cystatin levels to a greater extent than GFR. Serum creatinine concentration increases in hypothyroidism because of reduced tubular secretion and increases transiently with glucocorticoid treatment. In the present study, one patient was diagnosed with hypothyroidism and was not taking her prescribed levothyroxine at the baseline examination. Removal of this participant from the analysis had no effect on the conclusions.

Absolute measurements of GFR were used in this analysis for the comparisons with SCysC and SCr, because the study cohort included overweight and obese subjects and indexing for body surface area may significantly underestimate their GFR, particularly among the most obese.¹¹ Furthermore, it is unclear whether body surface area standardizes filtration rate for total circulating fluid volume, metabolically reactive tissue, kidney size, or other factors that might relate to kidney function. Therefore, the necessity for such indexing remains vague.^{42,43} Including body surface area or height and weight as covariates in a regression model may be less prone to bias than using the traditional body surface area standardized GFR computed as a ratio.⁴⁴ For comparison with eGFR, however, body surface area adjusted mGFR was used for comparability with the estimates.

Elevated glycemia is generally believed to promote initiation of diabetic kidney disease, but has less impact on its progression, whereas blood pressure promotes both its initiation and progression. Therefore, in this analysis, we assumed that hemoglobin A1c confounded the relationship between the filtration markers and ESRD, whereas blood pressure was potentially both a confounder and an intermediate variable in the causal pathway between the level of filtration and ESRD. Accordingly, we adjusted for the effect of hemoglobin A1c and not for blood pressure because doing so could bias the estimated effect of the filtration marker. Conclusions were unchanged if we included mean arterial pressure in the model. If we assumed that hemoglobin A1c was an intermediate variable and did not control for it, SCysC was no longer a better predictor of progression to ESRD in those with microalbuminuria, but remained a significantly better predictor in those with macroalbuminuria (data not shown).

In summary, SCysC is a better predictor of progression to ESRD than mGFR or SCr among persons with type 2 diabetes and elevated albuminuria. In subjects with normal or high-normal GFR, the predictive ability of SCysC remained superior to the other filtration markers, suggesting that measurement of SCysC may allow earlier risk stratification of patients at high risk for progression to kidney failure. The predictive value of SCysC for ESRD in patients with type 2 diabetes may be enhanced beyond the gold-standard measurement of GFR because of additional renal and non-renal information SCysC may impart.

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Figure 1. Scatterplot of baseline mGFR as a function of baseline filtration markers (1/SCysC, 1/SCr) and baseline eGFR (eGFR_{cr}(CKD-EPI), eGFR_{cr}(MDRD), eGFR_{cys}, eGFR_{cr-cys}). Filled circles represent participants who developed ESRD and open circles represent those who did not. The vertical dashed lines reflect the cutoffs for tertiles of the variable on the x-axis and the horizontal dashed lines the cutoffs for tertiles of mGFR. Trend lines illustrate the non-linearity of the relationships.

Table 1

Baseline demographic and clinical characteristics according to SCysC tertile.

	Overall	SCysC tertile			Correlation with SCysC
		Lowest	Middle	Highest	
SCysC ^{***} (mg/L)	0.76 (0.51–2.23)	0.64 (0.51–0.71)	0.76 (0.71–0.82)	0.95 (0.83–2.23)	
n (% male)	234 (34)	76 (28)	81 (35)	77 (39)	P=0.3 [†]
Age (y)	42.8 ± 10.5	37.1 ± 8.6	43.0 ± 10.1	48.1 ± 9.9	r=0.47, P<0.001
Diabetes duration (y)	12.3 ± 6.2	10.7 ± 5.4	12.0 ± 5.9	14.3 ± 6.6	r=0.23, P<0.001
mGFR (ml/min)	156 ± 52	189 ± 44	156 ± 43	123 ± 46	r=-0.58, P<0.001
mGFR (ml/min/1.73 m ²)	136 ± 42	170 ± 36	135 ± 30	104 ± 33	r=-0.70, P<0.001
Hyperfiltration [*]	116 (50)	57 (49)	40 (34)	19 (16)	P<0.001 [†]
SCr ^{***} (mg/dl)	0.66 ± 0.25	0.54 ± 0.12	0.62 ± 0.15	0.82 ± 0.34	r=0.53, P<0.001
ACR (mg/g)	76 (30–8603)	41 (30–1071)	76 (30–2537)	89 (33–8603)	r=0.20, P=0.002
Hemoglobin A1c (%)	9.7 ± 2.2	10.5 ± 2.1	9.3 ± 2.0	9.3 ± 2.2	r=-0.23, P<0.001
Body Mass Index (kg/m ²)	34.7 ± 8.5	33.0 ± 7.0	35.1 ± 9.0	36.1 ± 9.2	r=0.11, P=0.08
hsCRP [‡]	6.2 (0.4–361.0)	6.1 (0.4–29.7)	6.4 (0.8–41.9)	7.5 (0.4–361.0)	r=0.18, P=0.02
Mean arterial pressure (mm Hg)	92 ± 10	90 ± 9	91 ± 10	96 ± 11	r=0.20, P=0.002
Hypertension treatment	(12)	(7)	(7)	(23)	P=0.002 [†]
Hypoglycemic treatment	(68)	(63)	(62)	(81)	P=0.02 [†]

Note: Data for Pima Indians with type 2 diabetes and elevated ACR (≥ 30 mg/g) at baseline; Spearman correlations between SCysC as a continuous variable and the other continuous variables are also shown. Unless otherwise indicated, values for categorical variables are given as number (percentage) or (percentage); values for continuous variables are given as mean ± standard deviation or median (range).

Conversion factor for serum creatinine in mg/dL to μmol/L, x 88.4

[†]P-value from χ^2 -test.

[‡]184 participants with hsCRP at the baseline examination.

^{*}Hyperfiltration is defined by an mGFR ≥ 154 ml/min, a value two standard deviations above the mean mGFR for Pima Indians with normal glucose tolerance.

^{**}SCysC values are International Federation of Clinical Chemistry standardized.

^{***}SCr values are isotope dilution mass spectrometry standardized.

Table 2
Unadjusted and age-sex-adjusted incidence rate of diabetic ESRD by kidney marker tertile

Unadjusted incidence of ESRD						
	Lowest tertile		Middle tertile		Highest tertile	
	Cases/person-y	Incidence rate**	Cases/person-y	Incidence rate**	Cases/person-y	Incidence rate**
mGFR (ml/min)	31/877.1	35.34	16/821.6	19.47	21/871.8	24.09
mGFR (ml/min/1.73m ²)	32/878.8	36.41	16/839.0	19.07	20/852.6	23.46
1/SCysC	35/868.1	40.32	19/843.0	22.54	14/859.5	16.29
1/SCr	36/833.0	43.22	8/693.3	11.54	24/1044.2	22.98
eGFR _{cys}	35/862.7	40.57	19/840.0	22.62	14/867.9	16.13
eGFR _{cr-cys}	38/897.4	41.34	15/820.8	18.27	15/852.2	17.60
eGFR _{cr}						
CKD-EPI	35/893.6	39.17	17/790.3	21.51	16/886.6	18.05
MDRD	35/864.0	40.51	15/842.9	17.80	18/863.6	20.84

Age-sex-adjusted incidence rate of ESRD**, a			
	Lowest tertile	Middle tertile	Highest tertile
mGFR (ml/min)	49.33 (26.19–72.47)	18.39 (9.11–27.67)	35.01 (17.47–52.56)
mGFR (ml/min/1.73m ²)	43.00 (23.88–62.12)	18.85 (9.05–28.64)	31.65 (15.92–47.39)
1/SCysC *	47.88 (29.45–66.31)	25.62 (12.95–38.28)	16.93 (7.29–26.57)
1/SCr	43.72 (25.37–62.10)	12.94 (2.93–23.00)	†
eGFR _{cys}	63.85 (34.96–92.73)	27.87 (14.17–41.58)	14.56 (6.58–22.54)
eGFR _{cr-cys}	51.76 (31.64–71.88)	20.41 (9.27–31.56)	†
eGFR _{cr}			
CKD-EPI	45.12 (25.77–64.46)	23.33 (11.93–34.74)	32.40 (0.61–64.20)
MDRD	43.43 (26.89–59.98)	18.30 (7.92–28.68)	30.92 (10.98–50.85)

Note: Data for Pima Indians with type 2 diabetes and elevated albuminuria (ACR ≥ 30 mg/g). SCysC values are International Federation of Clinical Chemistry standardized. SCr values are isotope dilution mass spectrometry standardized.

** Cases per 1,000 person-years.

^aValues in parentheses are 95% confidence intervals.

^{*}P for trend <0.05;

[†]There were less than 10 person-years of follow-up for those aged 55 years, resulting in an unreliably high adjusted rate. All other strata had at least 30 person-years of follow-up. ESRD, end-stage renal disease; mGFR, measured glomerular filtration rate; SCr, serum creatinine; SCysC, serum cystatin C; CI, confidence interval; eGFR_{cr}, estimated glomerular filtration rate based on SCysC; eGFR_{cr-cys}, estimated glomerular filtration rate based on SCr and SCysC; eGFR_{cr}, estimated glomerular filtration rate based on SCr; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; MDRD, Modification of Diet in Renal Disease

Table 3

Pair-wise comparisons of AUROCs for kidney markers.

Measured Kidney Marker	AUROC ± SE	P for pair-wise comparisons of AUROC			
		1/SCr	1/SCysC	mGFR **	Covariates only*
1/SCysC					
Unadjusted	0.719±0.035	0.1	–	<0.001	
Adjusted for covariates*	0.845±0.026	0.03	–	0.02	0.02
1/SCr					
Unadjusted	0.672±0.040	–	0.13	0.03	
Adjusted for covariates	0.815±0.028	–	0.03	0.9	0.2
mGFR **					
Unadjusted	0.585±0.042	0.03	<0.001	–	
Adjusted for covariates	0.815±0.028	0.9	0.02	–	0.3
Covariates only	0.808±0.027	0.2	0.02	0.3	–

Note: The unadjusted models include a squared term of the respective filtration marker. Each of the adjusted models includes the individual filtration marker, the squared term for this marker and baseline age, sex, diabetes duration, hemoglobin A1c, height, weight, log2(ACR). The right column shows pair-wise comparisons of AUROCs generated by multivariable adjusted Cox regression models with AUROCs from models that include the covariates only (i.e., without the kidney marker). Filtration markers are ordered by descending unadjusted AUROC.

** Expressed in mL/min (not adjusted for body surface area).

AUROC=area under the receiver operating characteristic curve; SCr, serum creatinine; SCysC, serum cystatin C; SE, standard error; mGFR, measured glomerular filtration rate; ACR, albumin-creatinine ratio;

Table 4

Pair-wise comparisons of AUROCs for selected eGFRs and BSA-adjusted mGFR

	AUROC $\pm$ SE	P for pair-wise comparisons of AUROC	
		mGFR	Covariates only*
eGFRcr-cys			
Unadjusted	0.698 $\pm$ 0.037	0.007	
Adjusted for covariates	0.832 $\pm$ 0.026	0.04	0.03
eGFRcys			
Unadjusted	0.695 $\pm$ 0.035	0.005	
Adjusted for covariates	0.845 $\pm$ 0.026	0.04	0.02
eGFRcr(CKD-EPI)			
Unadjusted	0.667 $\pm$ 0.040	0.3	
Adjusted for covariates	0.816 $\pm$ 0.027	0.9	0.1
eGFRcr(MDRD)			
Unadjusted	0.655 $\pm$ 0.040	0.4	
Adjusted for covariates	0.813 $\pm$ 0.028	0.7	0.4
mGFR			
Unadjusted	0.629 $\pm$ 0.039	–	
Adjusted for covariates	0.816 $\pm$ 0.028	–	0.2
Covariates only	0.808 $\pm$ 0.027	0.2	–

Note: eGFRs and mGFRs expressed in ml/min/1.73m². The unadjusted models include a squared term of the respective estimate of kidney function. Each of the adjusted models includes the individual kidney function estimate, the squared term for this estimate and baseline age, sex, diabetes duration, hemoglobin A1c, height, weight, log₂(ACR). The right column shows pair-wise comparisons of AUCs generated by multivariable adjusted Cox regression models with AUCs from models that include the covariates only (i.e., without the estimate of GFR). Markers are ordered by descending unadjusted AUC.

Cystatin C values are International Federation of Clinical Chemistry standardized. Serum creatinine values are isotope dilution mass spectrometry standardized.

AUROC=area under the receiver operating characteristic curve; BSA, body surface area; eGFR, estimated glomerular filtration rate; SE, standard error; mGFR, measured glomerular filtration rate; eGFRcys, estimated glomerular filtration rate based on SCysC; eGFRcr-cys, estimated glomerular filtration rate based on SCr and SCysC; eGFRcys, estimated glomerular filtration rate based on SCysC; eGFRcr(CKD-EPI) estimated glomerular filtration rate based on SCr and calculated using the Chronic Kidney Disease Epidemiology Collaboration equation; eGFRcr(MDRD), estimated glomerular filtration rate based on SCr and calculated using the Modification of Diet in Renal Disease Study equation.

Box 1

Equations used to calculate eGFR.

CKD-EPI Cystatin C Equation (2012)¹²

$$133 \times (\text{SCysC}/0.8)^{-0.499} \times 0.996^{\text{Age}} \times 0.932 \text{ if female [if SCysC} \leq 0.8]$$

$$133 \times (\text{SCysC}/0.8)^{-1.328} \times 0.996^{\text{Age}} \times 0.932 \text{ if female [if SCysC} > 0.8]$$

CKD-EPI Creatinine-Cystatin C Equation (2012)¹²

$$130 \times (\text{SCr}/0.7)^{-0.248} \times (\text{SCysC}/0.8)^{-0.375} \times 0.995^{\text{Age}} \text{ [if female, SCr} \leq 0.7, \text{ and SCysC} \leq 0.8]$$

$$130 \times (\text{SCr}/0.7)^{-0.248} \times (\text{SCysC}/0.8)^{-0.711} \times 0.995^{\text{Age}} \text{ [if female, SCr} \leq 0.7, \text{ and SCysC} > 0.8]$$

$$130 \times (\text{SCr}/0.7)^{-0.601} \times (\text{SCysC}/0.8)^{-0.375} \times 0.995^{\text{Age}} \text{ [if female, SCr} > 0.7, \text{ and SCysC} \leq 0.8]$$

$$130 \times (\text{SCr}/0.7)^{-0.601} \times (\text{SCysC}/0.8)^{-0.711} \times 0.995^{\text{Age}} \text{ [if female, SCr} > 0.7, \text{ and SCysC} > 0.8]$$

$$135 \times (\text{SCr}/0.9)^{-0.207} \times (\text{SCysC}/0.8)^{-0.375} \times 0.995^{\text{Age}} \text{ [if male, SCr} \leq 0.9, \text{ and SCysC} \leq 0.8]$$

$$135 \times (\text{SCr}/0.9)^{-0.207} \times (\text{SCysC}/0.8)^{-0.711} \times 0.995^{\text{Age}} \text{ [if male, SCr} \leq 0.9, \text{ and SCysC} > 0.8]$$

$$135 \times (\text{SCr}/0.9)^{-0.601} \times (\text{SCysC}/0.8)^{-0.375} \times 0.995^{\text{Age}} \text{ [if male, SCr} > 0.9, \text{ and SCysC} \leq 0.8]$$

$$135 \times (\text{SCr}/0.9)^{-0.601} \times (\text{SCysC}/0.8)^{-0.711} \times 0.995^{\text{Age}} \text{ [if male, SCr} > 0.9, \text{ and SCysC} > 0.8]$$

CKD-EPI Creatinine Equation (2009)¹³

$$144 \times (\text{SCr}/0.7)^{-0.329} \times (0.993)^{\text{Age}} \text{ [if female and SCr} \leq 0.7]$$

$$144 \times (\text{SCr}/0.7)^{-1.209} \times (0.993)^{\text{Age}} \text{ [if female and SCr} > 0.7]$$

$$141 \times (\text{SCr}/0.9)^{-0.411} \times (0.993)^{\text{Age}} \text{ [if male and SCr} \leq 0.9]$$

$$141 \times (\text{SCr}/0.9)^{-1.209} \times (0.993)^{\text{Age}} \text{ [if male and SCr} > 0.9]$$

4-Variable MDRD Study Equation^{14, *}

$$186 \times (\text{SCr})^{-1.154} \times (\text{age})^{-0.203} \times [0.742 \text{ if female}]$$

* Not shown is the black race term, which was not used for any participant.

MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; SCr, serum creatinine; SCysC, serum cystatin C; eGFR, estimated glomerular filtration rate.