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Factors other than glomerular filtration rate affect serum cystatin C levels

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Cystatin C is an endogenous glomerular filtration marker hence its serum level is affected by the glomerular filtration rate (GFR). To study what other factors might affect it blood level we performed a cross-sectional analysis of 3418 patients which included a pooled dataset of clinical trial participants and a clinical population with chronic kidney disease. The serum cystatin C and creatinine levels were related to clinical and biochemical parameters and errors-in-variables models were used to account for errors in GFR measurements. The GFR was measured as the urinary clearance of ^{125}I-iothalamate and ^{51}Cr-EDTA. Cystatin C was determined at a single laboratory while creatinine was standardized to reference methods and these were 2.1 +/−1.1 mg/dL and 1.8 +/−0.8 mg/L, respectively. After adjustment for GFR, cystatin C was 4.3% lower for every 20 years of age, 9.2% lower for female gender but only 1.9% lower in blacks. Diabetes was associated with 8.5% higher levels of cystatin C and 3.9% lower levels of creatinine. Higher C-reactive protein and white blood cell count and lower serum albumin were associated with higher levels of cystatin C and lower levels of creatinine. Adjustment for age, gender and race had a greater effect on the association of factors with creatinine than cystatin C. Hence, we found that cystatin C is affected by factors other than GFR which should be considered when the GFR is estimated using serum levels of cystatin C.

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Estimates of glomerular filtration rate (GFR) are essential to the clinical assessment of kidney function and facilitate the detection, evaluation, and management of chronic kidney disease (CKD).[1] GFR-estimating equations are based on serum levels of endogenous filtration markers in combination with other variables; however, serum levels of these markers are affected by factors other than GFR. GFR-estimating equations based on serum creatinine, such as the Modification of Diet in Renal Disease (MDRD) Study equation, include the variables age, sex, and race as surrogates for creatinine generation by muscle.[2,3] However, these variables do not account for variation in creatinine generation due to diet, physiological, or clinical conditions that affect muscle mass. Consequently, GFR estimates based on serum creatinine may be inaccurate in healthy people with a high or low meat intake, building muscle, and in patients with illnesses complicated by malnutrition, inflammation, or deconditioning.

Cystatin C is an endogenous, 13 kDa protein filtered by the glomeruli and reabsorbed and catabolized by epithelial cells of the proximal tubule with only small amounts excreted in the urine. Cystatin C is being considered as a potential replacement for serum creatinine because it appears to be less affected by muscle mass.[4] However, recent reports have shown substantial variability in the relationship between GFR and cystatin C among populations, suggesting that there may be differences in generation, tubular reabsorption, or extra-renal elimination.[5] Such differences would affect the interpretation of GFR estimates based on cystatin C.

Using a large, pooled database from three research studies and one clinical population, we have previously reported that a GFR-estimating equation based on cystatin C was nearly as accurate as estimates based on creatinine, thus providing an alternative GFR estimate that is not linked to muscle mass. In this study, we examine the association of factors other than GFR to predict serum cystatin C and compare those associations to prediction of creatinine. Because GFR is measured with error, we used multivariable models that adjust for measured GFR and also incorporated estimates of

Table 1 | Study characteristics

Name	MDRD Study	AASK	CSG	NephroTest[a]
Type	RCT	RCT	RCT	CP
Location	USA	USA	USA	France
Center	MC	MC	MC	MC
N	1085	1205	266	438
Dates	1989–1992	1995–1998	1987–1992	2000–2004
Clearance method	Urinary	Urinary	Urinary	Urinary
Filtration marker	Iothalamate	Iothalamate	Iothalamate	EDTA

AASK, African American Study of Kidney Diseases and Hypertension; CP, clinical population; CSG, Collaborative Study Group: Captopril in Diabetic Nephropathy Study; EDTA, ethylenediaminetetraacetic acid. MC, multicenter; MDRD Study, Modification of Diet in Renal Disease Study; RCT, randomized clinical trial.
[a]The NephroTest initiative is a prospective hospital-based ongoing cohort that began in 2000, enrolling patients with all diagnoses of CKD stage 2–5 referred for extensive work-up by two nephrology departments. Data included in this study were collected between 2000 and 2004. These data are part of the data set presented in Froissart *et al.*[26].

GFR measurement error. These results will better inform us of the utility of cystatin C as an endogenous filtration marker.

RESULTS

Table 1 summarizes the study characteristics and Table 2 details the clinical characteristics of participants in each study and overall. Mean measured GFR (5th–95th percentile) was 48 (15–95) ml/min per 1.73 m^2 (0.80 (0.25–1.58) ml/s per 1.73 m^2). The mean standard deviation (s.d.) of serum cystatin C and creatinine was 1.8 (0.8) mg/l (135 (60) nmol/l) and 2.1 (1.1) mg/100 ml (186 (97) μmol/l), respectively. The mean age was 52 years. All patients were considered to have CKD.

In separate errors-in-variables models relating either log cystatin C or log serum creatinine to log GFR after adjusting for age, race, sex, and study, the coefficients for log GFR were –67.0% (95% confidence intervals −66.3, −67.7) and −70.5% (−69.8, −71.2), respectively. A coefficient of less than 100% signifies that a percent change in GFR is associated with smaller percent change in the serum levels of cystatin C and creatinine, indicating an association of the serum levels with factors other than GFR. A lower absolute level for the association with GFR of cystatin C than creatinine suggests factors other than GFR are more strongly associated with cystatin C than with creatinine.

Tables 3 and 4 show the regression coefficients and 95% confidence intervals relating serum cystatin C and creatinine to potential predictor variables, after controlling for GFR and study in models that adjust for measurement error in GFR and after adjustment for age, sex, and race. The coefficient represents the average percent difference in cystatin C or creatinine level for a difference between the 75th and 25th percentiles (the interquartile range, IQR) in the continuous variables (age, body mass index, and blood and urine levels) and for a difference between categories for dichotomous predictor variables (sex, race, and diabetes). The smaller the IQR, the larger the effect of a small change in the variable on serum levels of the markers. Within each column, the coefficients show the relative strength of association among variables.

Figure 1 compares the regression coefficients for cystatin C and creatinine that are displayed in Tables 3 and 4. Each plot character represents a predictor variable. Distance of the plot character from the zero on the horizontal and vertical axes indicates the strength of association of the predictor variable with level of cystatin C and creatinine, respectively. Panel A shows that after adjustment for GFR and GFR measurement error (but not age, race, and sex), older age and female sex were associated with lower cystatin C (by 4.3 and 9.2%, respectively) and lower creatinine (by 9.2 and 31.7%, respectively). Black race was not significantly associated with cystatin C (−1.9%) but was associated with higher creatinine levels (13.6%). Higher levels of height (4.8 and 21.8), weight (6.0 and 12.9), body mass index (4.2 and 3.5), and urine creatinine (4.1 and 19.2) were associated with higher levels of cystatin C and creatinine, respectively, and, except for body mass index, the magnitude of association was greater with creatinine than with cystatin C. A higher level of urine protein (on the log scale) was associated with a higher level of cystatin C (12.1%) and creatinine (10.0%). Diabetes was associated with higher levels of cystatin C (8.5%) and lower levels of creatinine (−3.0%). Similarly, higher C-reactive protein and white blood cell count and lower serum albumin were associated with higher levels of cystatin C (2.3, 3.1, and −1.9%, respectively) and lower levels of creatinine (−3.3, −3.2, and 5.9%, respectively). Higher urine urea nitrogen and urine phosphorus were associated with higher cystatin C (5.3 and 7.7%, respectively) and creatinine (10.1 and 12.6%, respectively).

Panel B of Figure 1 shows the same associations after adjustment for age, sex, and race. Adjustment for age, sex, and race markedly decreased the association of factors with serum creatinine. In contrast, this adjustment had little effect on the associations with serum cystatin C. After adjustment for age, sex, and race, the strongest associations with cystatin C were for proteinuria (10.8% on the log scale) and diabetes (8.0%). Stronger associations were seen for cystatin C than for serum creatinine for many variables (diabetes (8.0 vs 2.0%), systolic blood pressure (2.6 vs 0.8%), weight (5.2 vs 2.7%), body mass index (5.2 vs 2.5%), white blood cell count (3.0 vs 1.8%), hemoglobin (3.2 vs 0.6%), C-reactive protein (3.4 vs 0.9%), and urine protein (10.8 vs 5.1%)).

After accounting for GFR, its measurement error, as well as age, sex, and race, the percent change for log creatinine in predicting log cystatin C was 15.9% and the percent change for log cystatin C in predicting log creatinine was 16.1%. Serum urea nitrogen was also significantly associated with both creatinine and cystatin C after adjustment for the same factors.

DISCUSSION

Cystatin has been proposed as an alternative filtration marker to serum creatinine, and it clearly has promise to be so. The main findings of this study are that many factors other than

Table 2 | Patient characteristics: overall and by study

	Overall			MDRD			AASK			CSG			NephroTest[a]		
Variable	*N*	Mean/%	IQR	*N*	Mean/%	s.d.	*N*	Mean/%	s.d.	*N*	Mean/%	s.d.	*N*	Mean/%	s.d.
Age (years)	3418	53.2	19	1047	52	13	1647	54	10	287	34	8	438	59	15
Female	3418	32.		1047	39.1		1647	35.9		287	46.9		438	28.8	
Black	3418	53.5		1047	9.7		1647	100		287	7.7		438	8.5	
Diabetes	3418	13.9		1047	5.7		1647	0		287	100		438	21.9	
Hypertension	3417	66.7		1047	34.9		1646	99.7		287	34.6		438	40.0	
Systolic blood pressure (mm Hg)	3404	139	28	1047	132	18	1647	150	23	287	135.6	17.2	424	138.3	20.0
Diastolic blood pressure (mm Hg)	3239	88	19	1047	81	10	1647	96	14	287	82.9	10.2	259	78.0	11.1
BMI (kg/m^2)	3416	27.7	7	1046	27	4	1647	31	7	286	25.5	4.9	438	26.0	4.6
GFR (ml/min per 1.73 m^2)	3418	44.4	34	1047	33	14	1647	57	23	287	74.8	32.5	438	33.6	16.8
Serum creatinine (mg/100 ml)	3418	1.8	1	1047	2.34	1.09	1647	1.71	0.82	286	1.33	0.56	438	2.54	1.22
Blood urea nitrogen (mg/100 ml)	3224	25.0	18	1047	36.3	14.5	1647	22.6	11.4	287	25.6	15.7	244	43.6	20.4
Cystatin C (mg/l)	3418	1.6	1	1047	2.3	0.8	1647	1.5	0.7	287	1.4	0.7	438	2.2	0.8
Hemoglobin (g/100 ml)	2856	13.3	2	1014	13.0	1.9	1557	13.3	1.7	286	13.2	2.0	0	.	.
Potassium (mEq/l)	2968	4.2	1	1035	4.3	0.6	1647	4.2	0.6	287	4.3	0.5	0	.	.
Bicarbonate (mEq/l)	3335	25.0	4	1036	23	4	1647	25	3	285	26.1	3.3	368	25.5	3.2
Glucose (mg/100 ml)	2969	92.0	21	1036	93	26	1647	95	18	287	234	125	0	.	.
Calcium (mg/100 ml)	2962	9.1	1	1029	9.1	0.5	1647	9.2	0.5	287	9.0	0.6	0	.	.
Phosphate (mg/100 ml)	2974	3.6	1	1042	3.9	0.8	1647	3.5	0.7	286	3.7	0.7	0		
Albumin (g/100 ml)	3381	4.2	1	1047	4.0	0.4	1647	4.3	0.4	285	3.7	0.5	403	4.1	0.5
Total protein (g/100 ml)	2980	7.0	5	1047	2.1	0.8	1647	7.6	0.6	287	6.6	0.7	0	.	.
Total cholesterol (mg/100 ml)	2683	209.0	60	1041	216	46	1642	211	46	0	.	.	0	.	.
C-reactive protein (g/100 ml)	2997	0.4	1	1026	0.3	0.5	1643	0.4	0.6	0	.	.	328	2.3	5
Hemoglobin A1c (g/100 ml)	1032	5.6	0.9	1032	5.6	0.9	0	.	.	0	.	.	0	.	.
Urine creatinine (mg/day)	3008	1458	772	943	1406	420	1556	1679	659	247	1432	1262	262	1294	418
Urine urea nitrogen (g/day)	2896	8.7	5	943	9.4	2.9	1565	8.7	3.9	154	11.0	4.3	234	9.6	3.2
Urine protein (mg/day)	2755	165	986	943	330	1450	1565	71	353	247	1677.0	3133	0	.	.

AASK, African American Study of Kidney Diseases and Hypertension; BMI, body mass index; CSG, Collaborative Study Group: Captopril in Diabetic Nephropathy Study; GFR, glomerular filtration rate; IQR, interquartile range; MDRD Study, Modification of Diet in Renal Disease Study; s.d., standard deviation.

[a]The NephroTest initiative is a prospective hospital-based ongoing cohort that began in 2000, enrolling patients with all diagnoses of CKD stage 2 to 5 referred for extensive work-up by two nephrology departments. Data included in this study were collected between 2000 and 2004. These data are part of the dataset presented in Froissart *et al.*[26]

To convert GFR from ml/min per 1.73 m^2 to ml/s per 1.73 m^2, multiply by 0.01667; to convert serum creatinine from mg/100 ml to μmol/l, multiply by 88.4; to convert blood urea nitrogen from mg/100 ml to mmol/l, multiply by 0.357; to convert cystatin C from mg/l to nmol/l, multiply by 74.9; to convert hemoglobin from g/100 ml to g/l, multiply by 10; to convert potassium from mEq/l to mmol/l, multiply by 1; to convert bicarbonate from mEq/l to mmol/l, multiply by 1; to convert glucose from mg/100 ml to mmol/l, multiply by 0.05551; to convert calcium from mg/100 ml to mmol/l, multiply by 0.2495; to convert phosphate from mg/100 ml to mmol/l, multiply by 0.3229; to convert albumin from g/100 ml to g/l, multiply by 10; to convert total protein from g/100 ml to g/l, multiply by 10; to convert total cholesterol from mg/100 ml to mmol/l, multiply by 0.02586; to convert C-reactive protein from g/100 ml to nmol/l, multiply by 95,240; to convert hemoglobin A1C from g/100 ml to g/l, multiply by 10; to convert urine creatinine from mg/day to g/day, divide by 1000; to convert urine protein from mg/day to g/day, divide by 1000.

GFR are associated with serum cystatin C, including key variables such as diabetes, measures of body size, and inflammation. These associations would lead to systematic bias of GFR estimates based on cystatin C in selected populations or clinical conditions as well as imprecision of GFR estimates in all populations. Clinicians can use these findings to aid in interpretation of serum levels and GFR estimates based on cystatin C.

Physiological processes other than glomerular filtration, such as tubular reabsorption or secretion, generation, and extra-renal elimination can affect the serum levels of endogenous filtration markers. Urinary excretion of the marker facilitates study of these processes. For example, the effect of medications on tubular secretion of creatinine was verified by comparing creatinine clearance to GFR measured using exogenous markers, and the relationship of muscle mass and diet to creatinine generation was established from studies of urinary excretion of creatinine. In addition, in clinical practice, clinicians can measure urinary creatinine excretion to assist in interpretation of unexpected values for GFR estimates based on creatinine. In contrast, the absence of urinary excretion of cystatin C makes it difficult to measure these physiological processes and to interpret GFR estimates from cystatin C in clinical practice. Instead, understanding of the determinants of cystatin C other than GFR in humans relies on epidemiological associations. Our study provides the first comprehensive investigation of associations with cystatin C to factors other than GFR.

We found a stronger association of serum creatinine than cystatin C with surrogates of muscle mass, including age, sex, race, and urine creatinine. This likely reflects smaller contribution of muscle to generation of cystatin C mass than creatinine. It is possible that GFR estimates based on cystatin C may be more accurate than estimates based on creatinine in patients with variation in creatinine generation due to diet or clinical conditions that affect muscle mass. This hypothesis has not been explicitly tested as such patients have not been systematically included in research studies.

The relationship of race with cystatin C levels, independent of GFR (to the best of our knowledge) has not been previously noted by others. Similar to the findings here, when we included age, sex, and race as coefficients in an equation to estimate GFR from serum cystatin C, the coefficients for these factors were significant but substantially smaller than in

Table 3 | Percent change in level of cystatin C

		Not adjusted			Adjusted for GFR			Adjusted for GFR measurement error (0.015)			Adjusted for GFR measurement error (0.015), age, sex, and race		
Variable	IQR	Coeff	L	H	Coeff	L	H	Coeff	L	H	Coeff	L	H
Age* (years)	19.38	5.5	2.0	9.2	−3.8	−5.2	−2.5	−4.3	−5.7	−2.9			
Female*	1	−0.7	−5.2	3.9	−8.8	−10.6	−7.0	−9.2	−11.0	−7.4			
Black	1	−36.1	−38.7	−33.4	−4.1	−6.1	−2.0	−1.9	−4.0	0.3			
Diabetes	1	−14.5	−20.3	−8.2	7.3	3.9	10.9	8.5	5.0	12.2	8.0	4.2	12.1
Hypertension	1	−23.8	−27.2	−20.3	−1.2	−3.2	0.8	0.1	−2.0	2.2	2.6	−0.2	5.5
Height* (cm)	14.4	−2.1	−5.3	1.2	4.5	3.0	6.0	4.8	3.3	6.3	−0.5	−2.4	1.4
Weight* (kg)	25.2	−7.6	−10.2	−5.0	5.4	4.0	6.7	6.0	4.7	7.4	5.2	3.7	6.6
BMI* (kg/m^2)	7.29	−7.0	−9.3	−4.5	3.6	2.4	4.9	4.2	2.9	5.5	5.2	3.9	6.6
Systolic blood pressure* (mm Hg)	28	−2.5	−5.1	0.2	1.3	0.0	2.6	1.5	0.2	2.8	2.6	1.2	4.0
Diastolic blood pressure (mm Hg)	19	−15.4	−17.9	−12.8	0.0	−1.3	1.5	0.9	−0.5	2.3	0.4	−1.2	1.9
Log serum creatinine* (mg/100 ml)	0.65	123.5	120.2	126.8	35.0	31.0	39.1	20.4	15.8	25.2	15.9	14.2	17.6
Log SUN* (mg/100 ml)	0.69	95.3	91.0	99.7	14.8	12.0	17.5	7.7	4.9	10.5	6.5	−0.1	13.5
Hemoglobin* (g/100 ml)	2.4	−22.3	−24.7	−19.9	3.1	1.6	4.7	4.9	3.3	6.6	3.2	0.5	5.9
Log WBC* (cells/μl)	0.4	8.0	4.5	11.5	3.3	1.7	5.0	3.1	1.5	4.8	3.0	1.4	4.5
Sodium (mEq/l)	4	−1.1	−3.6	1.4	0.4	−0.6	1.5	0.5	−0.5	1.6	0.8	−0.8	2.4
Potassium (mEq/l)	0.7	17.1	13.0	21.3	1.3	−0.1	2.8	0.5	−0.9	2.0	0.0	−1.1	1.0
Bicarbonate (mEq/l)	4	−26.4	−28.0	−24.7	−2.8	−4.0	−1.5	−1.2	−2.5	0.2	−0.9	−2.2	0.5
Log glucose (mg/100 ml)	0.22	−7.0	−8.4	−5.7	0.9	0.1	1.7	1.4	0.6	2.2	1.1	0.3	1.9
Albumin* (g/100 ml)	0.5	−13.3	−15.6	−10.9	−2.5	−3.7	−1.3	−1.9	−3.1	−0.7	−2.2	−3.5	−0.9
Calcium (mg/100 ml)	0.6	−9.9	−12.5	−7.2	0.1	−1.0	1.3	0.7	−0.5	1.9	1.2	0.0	2.4
Phosphate (mg/100 ml)	0.8	32.9	27.9	38.1	1.6	0.3	2.9	−0.1	−1.4	1.3	−0.1	−1.4	1.3
Total cholesterol (mg/100 ml)	60	−0.3	−3.5	2.9	−1.5	−2.9	−0.1	−1.6	−2.9	−0.2	−0.4	−1.7	1.0
Log CRP* (g/100 ml)	1.75	10.1	7.0	13.3	2.6	1.3	4.0	2.3	0.9	3.7	3.4	1.9	4.9
Urine creatinine* (mg/day)	0.77	−13.8	−18.5	−8.9	3.1	1.6	4.6	4.1	2.4	5.8	1.7	0.4	3.0
Urine phosphate* (mg/day)	0.38	−14.6	−18.4	−10.7	6.4	4.1	8.7	7.7	5.4	10.1	5.8	3.4	8.3
Urine urea nitrogen* (g/day)	4.69	−11.6	−14.2	−9.0	4.4	2.9	5.8	5.3	3.9	6.8	4.0	2.5	5.5
Log urine protein* (mg/day)	3.15	63.1	57.0	69.5	14.5	12.1	17.0	12.1	9.7	14.6	10.8	8.3	13.4

BMI, body mass index; Coeff, coefficient; CRP, C-reactive protein; GFR, glomerular filtration rate; H, higher confidence limit; IQR, interquartile range; L, lower confidence limit; WBC, white blood cell.

Each row shows different models based on the variable. Continuous variables are expressed as interquartile range, which is the difference between the 25th and 75th percentiles. The model in column 1 includes the variable adjusted for study. Column 2 includes the model adjusted for variable, study terms, GFR, and the interaction of GFR and study. Column 3 includes the model adjusted for variable, study terms, GFR, GFR measurement error, and the interaction of GFR and study. Column 4 includes the model adjusted for variable, study terms, GFR, GFR measurement error, the interaction of GFR and study, age, sex, and race. The coefficient is expressed as $100 \times (e^{coeff} - 1)$, which can be interpreted as a geometric mean percent change in the filtration marker for a change of two quartiles in the variable.

*P-value < 0.0001 for the interaction of study $\times$ variable in a model that includes variable, study terms, GFR, GFR measurement error, age, sex, and race, and interaction of study by GFR and by variable.

To convert serum creatinine from mg/100 ml to μmol/l, multiply by 88.4; to convert SUN mg/100 ml to mmol/l, multiply by 0.357; to convert hemoglobin from g/100 ml to g/l, multiply by 10; to convert WBC from 10^3/μl to 10^9/l, multiply by 1; to convert sodium from mEq/l to mmol/l, multiply by 1; to convert potassium from mEq/l to mmol/l, multiply by 1; to convert bicarbonate from mEq/l to mmol/l, multiply by 1; to convert glucose from mg/100 ml to mmol/l, multiply by 0.05551; to convert albumin from g/100 ml to g/l, multiply by 10; to convert calcium from mg/100 ml to mmol/l, multiply by 0.2495; to convert phosphate from mg/100 ml to mmol/l, multiply by 0.3229; to convert total cholesterol from mg/100 ml to mmol/l, multiply by 0.02586l; to convert C-reactive protein from g/100 ml to g/l, multiply by 10; to convert urine creatinine from mg/day to g/day, divide by 1000; to convert urine phosphate from mg/day to g/day, divide by 1000; to convert urine protein from mg/day to g/day, divide by 1000.

equations to estimate GFR from serum creatinine.[4] The association with race varies by modeling strategy and weakened by adjustment for measurement error in GFR in this paper compared to the GFR-estimating equation. In contrast, in analyses from the National Health and Nutrition Examination Survey, we previously reported differences in serum levels of cystatin C among races, even among young healthy individuals in whom GFR is presumably normal.[6] Possibly, this could not only reflect true differences in GFR among race groups, such as hyperfiltration among African Americans compared to whites, but also reflect variation in other characteristics among the race groups in the National Health and Nutrition Examination Survey. GFR measurements in a representative multiethnic population will be necessary to determine whether the cause of variation in cystatin C levels reflects variation in measured GFR or in factors affecting cystatin C other than GFR.

We also observed stronger magnitude of associations of body mass index and weight with cystatin C than with creatinine, which may indicate an association of cystatin C with fat mass. In this context, the association of higher cystatin C with diabetes may, in part, also reflect the association with fat mass. These are important considerations for use of cystatin C in clinical practice, given the high and increasing prevalence of obesity and diabetes.[7] The association of proteinuria with higher cystatin C may reflect the association of diabetes with proteinuria in our data set but could also reflect tubular damage. The association of higher urine urea nitrogen and urine phosphate with higher serum levels of both markers after

Table 4 | Percent change in serum creatinine

		Not adjusted			Adjusted for GFR			Adjusted for GFR measurement error (0.015)			Adjusted for GFR measurement error (0.015), age, sex, and race		
Variable (unit)	IQR	Coeff	L	H	Coeff	L	H	Coeff	L	H	Coeff	L	H
Age* (years)	19.38	0.5	−3.2	4.3	−9.0	−10.3	−7.2	−9.2	−10.7	−7.7			
Female*	1	−25	−28.6	−21.2	−31.6	−32.8	−29.9	−31.7	−33.1	−30.2			
Black	1	−28.3	−31.5	−24.8	12.5	8.1	13.8	13.6	10.7	16.7			
Diabetes	1	−24.7	−30.0	−18.9	−4.4	−8.3	−1.6	−3.9	−7.3	−0.4	2.0	−2.0	6.2
Hypertension	1	−18.7	−22.7	−14.5	7.3	3.8	9.2	7.9	5.2	10.7	0.4	−2.3	3.1
Height* (cm)	14.4	13.4	9.5	17.4	21.6	19.4	23.4	21.8	19.8	23.8	0.5	−1.5	2.5
Weight* (kg)	25.2	−2.3	−5.1	0.6	12.6	10.4	13.8	12.9	11.1	14.6	2.7	1.3	4.2
BMI* (kg/m^2)	7.29	−7.8	−10.3	−5.2	3.3	1.4	4.5	3.5	2.0	5.1	2.5	1.2	3.9
Systolic blood pressure* (mm Hg)	28	−3.2	−6.0	−0.4	0.8	−0.9	2.3	0.9	−0.7	2.5	−0.8	−2.1	0.6
Diastolic blood pressure (mm Hg)	19	−10.7	−13.5	−7.8	6.9	4.5	8.1	7.2	5.4	9.1	−1.1	−2.7	0.6
Log serum cystatin C(mg/l)	0.65	151.3	146.2	156.5	45.9	51.0	66.3	36.2	27.7	45.1	16.1	8.7	24.1
Log SUN* (mg/100 ml)	0.69	104.1	99.1	109.3	18.6	19.4	26.5	15.7	12.1	19.3	16.1	12.8	19.4
Hemoglobin* (g/100 ml)	2.4	−20.3	−22.9	−17.5	7.9	4.9	8.6	8.6	6.7	10.6	0.6	−1.1	2.3
Log WBC* (cells/μl)	0.4	1.4	−1.9	4.8	−3.1	−4.7	−1.2	−3.2	−5.0	−1.4	−1.8	−3.3	−0.2
Sodium (mg/100 ml)	4	0.8	−2.0	3.6	2.4	1.1	3.6	2.5	1.2	3.7	0.7	−0.4	1.8
Potassium (mEq/l)	0.7	19.4	14.9	24.0	2.8	1.4	5.1	2.5	0.7	4.3	0.9	−0.5	2.3
Bicarbonate (mEq/l)	4	−26.7	−28.6	−24.8	−1.6	−4.2	−1.0	−0.9	−2.6	0.8	−0.7	−2.1	0.8
Log glucose (mg/100 ml)	0.22	−8.7	−10.0	−7.4	−0.7	−1.7	−0.2	−0.5	−1.3	0.3	0.2	−0.6	1.1
Albumin* (g/100 ml)	0.5	−6.9	−9.5	−4.2	5.6	3.8	6.7	5.9	4.4	7.4	2.3	1.0	3.6
Calcium (mg/100 ml)	0.6	−11.9	−14.6	−9.0	−1.7	−3.5	−0.5	−1.5	−3.0	0.0	−0.2	−1.4	1.1
Phosphate (mg/100 ml)	0.8	35	30.1	40.0	2.2	1.6	4.9	1.5	−0.2	3.3	3.2	1.9	4.7
Total cholesterol (mg/100 ml)	60	−3.4	−6.7	0.1	−4.5	−6.2	−2.8	−4.6	−6.3	−2.8	0.4	−1.0	1.8
Log CRP* (g/100 ml)	1.75	4.3	1.2	7.6	−3.1	−4.4	−1.4	−3.3	−4.8	−1.7	0.4	−0.9	1.8
Urine creatinine* (mg/day)	0.77	−2.7	−5.1	−0.3	18.7	11.1	25.3	19.2	11.8	27.1	7.8	4.4	11.3
Urine phosphate* (mg/day)	0.38	−14.3	−18.6	−9.8	11.9	8.0	13.9	12.6	9.5	15.7	5.9	3.1	8.7
Urine urea nitrogen* (g/day)	4.69	−8.1	−10.8	−5.2	9.7	7.4	10.9	10.1	8.4	11.9	6.1	4.5	7.7
Log urine protein* (mg/day)	3.15	61.0	54.4	67.9	10.9	9.6	15.2	10.0	7.2	12.8	5.1	2.7	7.5

BMI, body mass index; Coeff, coefficient; CRP, C-reactive protein; GFR, glomerular filtration rate; H, higher confidence limit; IQR, interquartile range; L, lower confidence limit; WBC, white blood cell.

Each row shows different models based on the variable. Continuous variables are expressed as interquartile range, which is the difference between the 25th and 75th percentiles. The model in column 1 includes the variable adjusted for study. Column 2 includes the model adjusted for variable, study terms, GFR, and the interaction of GFR and study. Column 3 includes the model adjusted for variable, study terms, GFR, GFR measurement error, and the interaction of GFR and study. Column 4 includes the model adjusted for variable, study terms, GFR, GFR measurement error, the interaction of GFR and study, age, sex, and race. The coefficient is expressed as $100 \times (e^{coeff}-1)$, which can be interpreted as a geometric mean percent change in the filtration marker for a change of two quartiles in the variable.

*P-value < 0.0001 for the interaction of study $\times$ variable in a model that includes variable, study terms, GFR, GFR measurement error, age, sex, and race, and interaction of study by GFR and by variable.

To convert serum creatinine from mg/100 ml to μmol/l, multiply by 88.4; to convert SUN mg/100 ml to mmol/l, multiply by 0.357; to convert hemoglobin from g/100 ml to g/l, multiply by 10; to convert WBC from 10^3/μl to 10^9/l, multiply by 1; to convert sodium from mEq/l to mmol/l, multiply by 1; to convert potassium from mEq/l to mmol/l, multiply by 1; to convert bicarbonate from mEq/l to mmol/l, multiply by 1; to convert glucose from mg/100 ml to mmol/l, multiply by 0.05551; to convert albumin from g/100 ml to g/l, multiply by 10; to convert calcium from mg/100 ml to mmol/l, multiply by 0.2495; to convert phosphate from mg/100 ml to mmol/l, multiply by 0.3229; to convert total cholesterol from mg/100 ml to mmol/l, multiply by 0.02586; to convert C-reactive protein from g/100 ml to g/l, multiply by 10; to convert urine creatinine from mg/day to g/day, divide by 1000; to convert urine phosphate from mg/day to g/day, divide by 1000; to convert urine protein from mg/day to g/day, divide by 1000.

adjustment for GFR suggests that diet may also be a determinant of cystatin C.

Our findings of associations of cystatin C with body mass index, as well as inflammation, and proteinuria are consistent with previous reports.[8–13] Recent studies have shown that in studies of preadipocyte cell cultures, there is increased cystatin C production during preadipocyte differentiation.[14] As obesity is now recognized as an inflammatory state, the findings of both inflammation and obesity are informative. Other studies have also shown the association of cystatin C with thyroid hormone levels.[15] We were not able to verify these data as thyroid hormone levels were not measured in the current studies.

Many studies have shown stronger association of serum cystatin C with mortality and cardiovascular disease than serum creatinine, particularly in studies of older adults and we have previously demonstrated higher levels of cystatin C in older adults in the National Health and Nutrition Examination Survey.[6,16–19] In part, these findings may reflect greater accuracy of cystatin C than creatinine as a filtration marker in this population. Another possible explanation, as is suggested by this study, is differential effects of factors other than GFR on levels of serum cystatin C and creatinine that are more prevalent in older adults.[20] In this study, diabetes, higher C-reactive protein, higher white blood cell count, and lower serum albumin (all risk factors for mortality) were associated with a higher serum cystatin C and lower serum creatinine. The opposite direction of the relationships of these factors to the filtration markers would confound the comparison of the filtration markers in their prediction of risk. These studies adjusted for many of the factors that we identified, and therefore the findings in these studies may

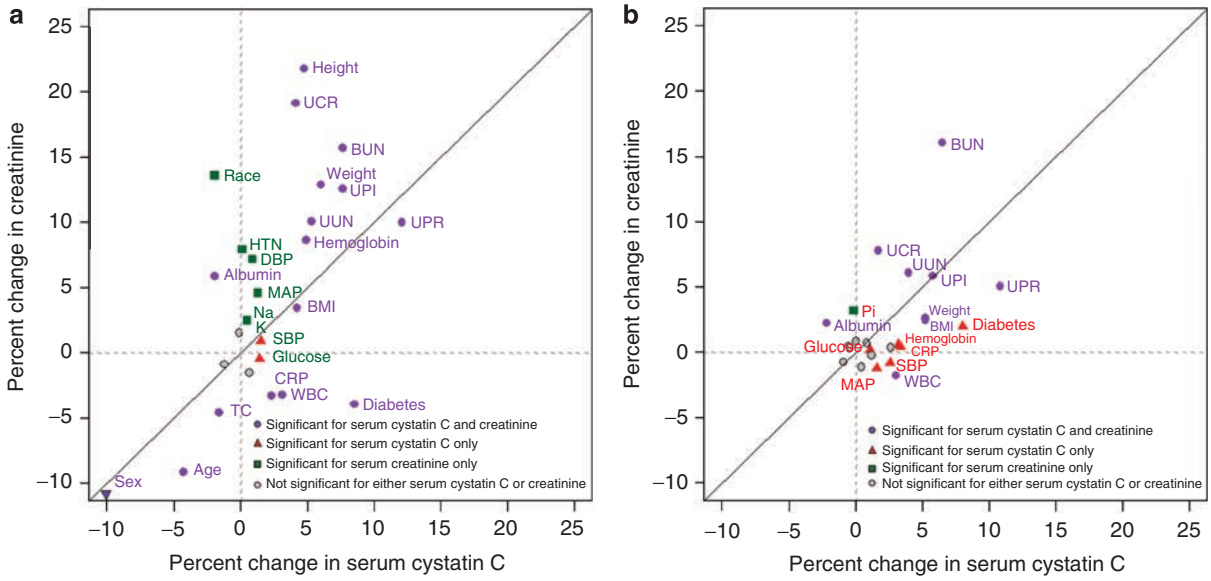

Figure 1 | Comparison of coefficients of variables predicting log cystatin and log creatinine. Solid diagonal line is the line of identity. For continuous predictor variables the coefficients are expressed as the percent differences in cystatin or creatinine associated with a difference of one interquartile range in the predictor variable (i.e., a change from the 25th to 75th percentile) after adjusting for GFR in models that incorporate measurement error in the GFR assay. For dichotomous predictor variables the coefficients indicate the percent differences in cystatin or creatinine associated with the presence vs the absence of the factor. Variables that fall along the line of identity have a similar relationship to serum creatinine and cystatin C. Points away from the line of identity represent variables with a different magnitude of association with cystatin C and creatinine. Variables near the origin have a weak relationship with the filtration marker. The plot character colors indicate significance of the relationships between the predictor variable to cystatin C, creatinine, neither or both. Gray dots indicate variables that were not significantly associated with either cystatin C or creatinine. For all variables, the coefficients for cystatin C and creatinine were significantly different from one other ($P<0.001$). HTN, hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; WBC, white blood cell count; Na, sodium; K, potassium; Pi, phosphate; Ca, calcium; HCO_3, bicarbonate; TC, total cholesterol; alb, albumin; gluc, glucose; UUN, urine urea nitrogen; UCR, urine creatinine; UPI, urine phosphate; UPR, urine protein. (**a**) After adjustment for GFR and GFR measurement error. Variables that were not significantly associated with either variable (indicated by gray dots) include serum bicarbonate, total calcium, and phosphate. Sex is indicated on the margins of the figure as a downward arrow, as the coefficients are bigger than the scale. (**b**) After adjustment for GFR and GFR measurement error, age, sex, and race. Variables that were not significantly associated with either cystatin C or creatinine (indicated by gray dots) include hypertension, height, diastolic blood pressure, sodium, bicarbonate, total calcium, and total cholesterol.

reflect residual confounding due to errors in measurements of these factors or confounding by other unmeasured and unknown factors.

The associations of cystatin C with non-GFR determinants that we report in this study, although are significant, are relatively small. The observed effect sizes reflect the average levels within the current study population and are likely to be larger in individual patients or in populations with selected clinical conditions, such as the obese, the chronically ill, or those with high levels of inflammation. Larger-than-average effects of non-GFR determinants could lead to important errors in GFR estimation from serum cystatin C in individual patients and systematic bias in selected populations. Rule *et al.*[21] showed that bias of cystatin C-based estimating equation differed among patients with native kidney disease, kidney transplant recipients, and potential kidney donors, consistent with systematic differences in non-GFR determinants of cystatin C among these populations. Similarly, variation in non-GFR determinants would explain the observed imprecision of cystatin C-based estimating equations even in relatively homogenous populations with known CKD.[4]

Clinicians can use knowledge of non-GFR determinants of cystatin C to assist in interpretation of serum levels and GFR estimates based on cystatin C. This is analogous to the interpretation of GFR estimates based on knowledge of non-GFR determinants of serum creatinine. For example, because the relationship between serum creatinine and muscle mass is understood, an attentive clinician can interpret the level of the serum creatinine or the estimated GFR differently in a patient with vs without muscle wasting, even though there is no term for muscle wasting in the GFR-estimating equation. As such, our data suggest that the value of a cystatin C level should be interpreted with knowledge of several factors, such as obesity, inflammation, and diabetes.

Strengths of the study include the large study population composed of 3418 patients with CKD in three research studies and one clinical population, measurement of cystatin C in a single laboratory, calibration of the creatinine assays in each study to standardized values, careful measurement of GFR using urinary clearances of exogenous filtration markers, use of analytical techniques that incorporated measurement error in GFR, and the large number of potential predictors available.

There are also several limitations. First, we have shown results of associations of single variables, adjusted only for GFR, age, sex, and race, rather than a full multivariable adjustment. Second, incomplete adjustment for measurement error and biological variation in GFR may lead to residual confounding between variables associated with GFR and cystatin C, which could explain the association of serum cystatin C and creatinine with each other and with serum urea nitrogen even after adjustment for GFR. We estimated the level of measurement error in only two of the studies: MDRD Study and the African American Study of Kidney Disease and Hypertension (AASK). Possibly, the measurement error may be different in the other two studies included in the pooled database. Third, the study population was restricted to patients with native kidney disease and without serious comorbid conditions that would exclude them from participating in clinical trials. Fourth, study participants were likely selected in part on the basis of previous creatinine values, which can lead to a bias in the estimated regression coefficients for creatinine and cystatin, as cystatin C remained associated with creatinine after controlling for GFR. Nevertheless, all studies were of CKD populations and previous studies have suggested that for creatinine-based estimating equations differences among subgroups based on demographic characteristics are minimal for populations with native kidney disease.[1,22] Finally, the data are pooled from multiple studies and there is variation among studies in some of the observed relationships. This variation may be due to population differences or differences in how the covariates were ascertained or measured.

In summary, although cystatin has promise as an alternative filtration marker to creatinine, like creatinine, cystatin C is affected by factors other than GFR that must be considered in interpretation of its serum level in clinical practice. The best GFR estimate may be the combination or sequential use of both filtration markers, with the expectation that the use of both markers minimizes the impact of physiological processes other than GFR that affect each marker. Further research is required to better understand the non-GFR determinants of cystatin C across a broader range of populations and to define the use of both creatinine and cystatin C in GFR estimation.

MATERIALS AND METHODS

Sources of data

Chronic Kidney Disease Epidemiology Collaboration is a research group formed to develop and validate improved estimating equations for GFR by pooling data from research studies and clinical populations (hereafter referred to as 'studies').[4] The current analysis is based on a pooled data set of individual patient data from four studies where frozen samples were available for assay of cystatin C: MDRD Study, AASK, Collaborative Study Group (CSG) Study,[2,3,5,23–25] and the NephroTest cohort, a clinical population in Paris, France[26] (Table 1). Data from the baseline examination for these studies were used.

Measurements

GFR was measured as four period urinary clearances of ^{125}I-iothalamate in the MDRD Study, AASK and CSG, and as five period urinary clearances of ^{51}Cr-EDTA in NephroTest and is reported adjusted for body surface area (Table 1). Comparisons of ^{125}I-iothalamate and ^{51}Cr-EDTA clearances to urinary clearance of inulin, the reference standard for GFR measurements, demonstrated high correlation.[26–28] Samples were assayed for cystatin C with a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dade Behring, IL, USA) in samples stored at −80 °C. The inter- and intraassay coefficients of variation for cystatin C were 3.2–4.4 and 2.0–3.0%, respectively. Stability in serum stored at −80 °C has been demonstrated.[29] Serum creatinine assays were calibrated to standardized serum creatinine values at the Cleveland Clinic Research Laboratory.[1,22] The results of the calibration procedures have been previously described.[4,30]

Variables

A potential list of variables that are hypothesized to affect serum cystatin C or creatinine by mechanisms other than GFR (non-GFR determinants) was developed from review of the literature and from physiological and clinical considerations. Variables in the analysis included measures of muscle mass and body size (age, sex, race, height, weight, body mass index, urine creatinine), cardiovascular disease risk factors (hypertension, diabetes, systolic blood pressure, diastolic blood pressure, glucose, total cholesterol), measures related to severity of kidney disease (hemoglobin, serum levels of sodium, potassium, bicarbonate, calcium, phosphate, and urine protein), measures of inflammation (albumin, C-reactive protein, white blood cell count), and measures of dietary intake (urine phosphate, urine urea nitrogen). Other endogenous filtration markers were also considered as covariates (serum cystatin C, creatinine, and urea nitrogen). Measurement methods and definitions for each of the categorical variables have been described in the individual reports of these studies.[2,3,23–26,31]

Statistical analyses

Summary statistics and scatter plots were used in initial exploratory analyses to investigate the relationships between candidate variables and the levels of serum cystatin C and serum creatinine in the overall data set. Continuous variables were transformed so as to create a linear relationship with log-transformed cystatin C and creatinine in bivariate analyses. Sex and race were expressed as binary factors indicating presence or absence of female sex and black race, respectively. Diabetes and hypertension were expressed as present or absent.

The relationships of cystatin C and creatinine with the predictor variables were investigated by first performing separate linear regressions to relate log-transformed cystatin C and creatinine to each individual predictor variable after controlling for log-transformed GFR, study, and the interaction between GFR and study. We repeated these analyses using errors-in-variables regression analysis to incorporate measurement in GFR into these models.[32] A measurement error variance of 0.015 was assumed for log-transformed GFR based on analyses of the longitudinal variability in log-transformed baseline GFR measurements spaced an average of approximately 3 months apart in the MDRD Study and 0.6 months apart in the AASK Study.[33] Sensitivity analyses were performed with different levels of measurement error ranging from 0 to 0.020. Results were consistent for measurement error variance ranging from 0.010 to 0.020, which covers the plausible range. This errors-in-variables regression was repeated after adding terms for age, female sex, and black race to the model for each predictor variable.

The relative strengths of relationships of the predictor variables with log cystatin C and log creatinine were compared and graphically displayed in scatter plots. For continuous variables, regression coefficients were standardized to indicate the geometric mean percent difference in either serum cystatin C or creatinine associated with a 1.0 IQR higher value for the predictor variable; for dichotomous predictor variables the regression coefficients are expressed as the geometric mean percent difference in the response variable associated with presence vs absence of the predictor. The statistical significance of the difference between the coefficients for the predictor variables with log cystatin C and log serum creatinine was determined by applying the sign test to compare 800 bootstrap samples with P-value of <0.001 indicating a significant difference between the coefficients.

Analyses were computed using R (version 2; Free Software Foundation Inc., Boston, MA, USA) and SAS software (version, 9.1; SAS Institute Inc., Cary, NC, USA).

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DISCLOSURE

All the authors declared no competing interests.

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Collaborative Study Group: Captopril in Diabetic Nephropathy Study: Roger Rodby, Richard Rohde.
African American Study of Kidney Disease and Hypertension: Gabriel Contreras, Julia B. Lewis.
NephroTest: Jerôme Rossert, Marc Froissart.
Statistical evaluation: Christopher H. Schmid, Tom Greene, Liang Li, Marshall Joffe.

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