Glomerular filtration rate estimated by cystatin C among different clinical presentations

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Glomerular filtration rate (GFR) estimates from serum creatinine has not been generalizable across all populations. Cystatin C has been proposed as an alternative marker for estimating GFR. The objective of this study was to compare cystatin C with serum creatinine for estimating GFR among different clinical presentations. Cystatin C and serum creatinine levels were obtained from adult patients ($n = 460$) during an evaluation that included a GFR measurement by iothalamate clearance. Medical records were abstracted for clinical presentation (healthy, native chronic kidney disease or transplant recipient) at the time of GFR measurement. GFR was modeled using the following variables: cystatin C (or serum creatinine), age, gender, and clinical presentation. The relationship between cystatin C and GFR differed across clinical presentations. At the same cystatin C level, GFR was 19% higher in transplant recipients than in patients with native kidney disease ($P < 0.001$). The association between cystatin C and GFR was stronger among native kidney disease patients than in healthy persons ($P < 0.001$ for statistical interaction). Thus, a cystatin C equation was derived using only patients with native kidney disease ($n = 204$). The correlation with GFR ($r^2 = 0.853$) was slightly higher than a serum creatinine equation using the same sample ($r^2 = 0.827$), the Modification of Diet in Renal Disease equation ($r^2 = 0.825$) or the Cockcroft–Gault equation ($r^2 = 0.796$). Averaged estimates between cystatin C and serum creatinine equations further improved correlation ($r^2 = 0.891$). Cystatin C should not be interpreted as purely a marker of GFR. Other factors, possibly inflammation or immunosuppression therapy, affect cystatin C levels. While recognizing this limitation, cystatin C may improve GFR estimates in chronic kidney disease patients.

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Ideally, glomerular filtration rate (GFR) should be determined with a method that is convenient, inexpensive, and accurate. GFR can be estimated from serum creatinine using the Modification of Diet in Renal Disease (MDRD) or Cockcroft–Gault equations.[1,2] However, these equations have not been generalizable across all clinical presentations. For example, the MDRD equation, derived with chronic kidney disease (CKD) patients, underestimated GFR in healthy persons by 29%.[3] This occurred in part because the strength of association between serum creatinine and GFR is much less among healthy persons compared to that of patients with CKD.[3–5] The MDRD equation also overestimates the strength of association between GFR and serum creatinine in type I diabetes pateints with normal serum creatinine levels.[6]

Studies have suggested that serum cystatin C may have advantages over serum creatinine for estimating GFR. Cystatin C is a low molecular weight basic protein (13 kDa) that is freely filtered and metabolized after tubular reabsorption.[7,8] Studies have reported that cystatin C is less influenced by age, gender and muscle mass than serum creatinine.[9–11] However, in at least one general population study, cystatin C was found to be influenced by several factors including age, gender, body size, cigarette smoking and C-reactive protein independent of creatinine clearance.[12] Another study suggested that cystatin C was not simply a marker of GFR because it predicted future cardiovascular events independent of estimated creatinine clearance.[13] A potential problem with these studies was the evaluation of cystatin C with serum creatinine-based equations or creatinine clearance instead of with measured GFR.

The primary objective of this study was to assess the relationship between cystatin C and GFR (iothalamate clearance) among clinical presentations that commonly lead to a GFR measurement. The secondary objective was to compare a cystatin C equation to a serum creatinine equation for estimating GFR.

RESULTS

Table 1 presents demographic characteristics and laboratory measurements, overall and by clinical presentation. GFR estimated by the abbreviated MDRD equation or the Cockcroft–Gault equation was similar to GFR measured by

Table 1 | Descriptive characteristics and laboratory measurements, overall and by clinical presentation

	Overall	Healthy (potential donors)	Native kidney disease	Kidney transplant recipient	Other transplant recipient
Sample size	460	50	204	103	103
Female, *n* (%)	193 (42)	34 (68)	92 (45)	38 (37)	29 (28)
Caucasian, *n* (%)	446 (97)	50 (100)	197 (97)	100 (97)	99 (96)
Diabetic, *n* (%)	74 (16)	0 (0)	37 (18)	19 (18)	18 (17)
Age (years)	51±15	41±11	55±16	49±13	51±12
Height (cm)	171±10	168±9	171±10	171±10	171±10
Weight (kg)	84±20	80±17	86±23	83±20	84±17
Body mass index (kg/m^2)	29±6	28±6	29±7	28±5	28±6
Iothalamate GFR (ml/min/1.73 m^2)	57±29	101±16	51±29	52±18	54±22
MDRD GFR[a] (ml/min/1.73m^2)	55±24	86±13	50±25	51±15	57±21
C-G GFR[b] (ml/min/1.73 m^2)	55±25	87±19	49±26	50±15	55±19
Serum creatinine (mg/dl)	1.6±0.9	0.9±0.2	1.8±1.2	1.5±0.4	1.5±0.6
Cystatin C (mg/l)[b]	1.5±0.8	0.8±0.2	1.6±0.9	1.6±0.6	1.6±0.7

[a]MDRD GFR=$186 \times SCr^{-1.154} \times age^{-0.203}$ (0.742 if female) (1.21 if black).[1,14]
[b]C-G GFR=((140-age) weight(kg)/(SCr × 72)) (0.85 if female) (1.73/body surface area) × 0.84.[2,14]
Entries are mean±s.d. or count (percent) where appropriate.

iothalamate clearance except in the healthy group. Transplant recipients were classified by kidney graft alone ($n=103$, 50%), liver graft ($n=55$, 27%), heart graft ($n=30$, 15%), pancreas graft ($n=15$, 7%), and lung graft ($n=3$, 1%). Patients with non-kidney grafts could be further classified as those who had an iothalamate clearance as part of routine follow-up versus those who had it for CKD, but this distinction was not important in multivariable models.

The relationship between cystatin C and measured GFR (Figure 1a), and between serum creatinine and measured GFR (Figure 1b) is shown on a logarithmic scale. Natural logarithmic (ln) GFR was regressed on ln analyte for each clinical presentation. There was a stronger regression fit between ln GFR and ln cystatin C than between ln GFR and ln serum creatinine for the native kidney disease group ($r^2=0.853$ versus 0.770), the transplant recipient group ($r^2=0.768$ versus 0.671), and the healthy group ($r^2=0.382$ versus 0.123). There was an upward shift in the regression line between GFR and cystatin C among transplant recipients compared to those with native kidney disease (Figure 1a); this was not observed between GFR and serum creatinine (Figure 1b). The healthy group had a more gradual slope than the native kidney disease group when GFR was regressed on either cystatin C or serum creatinine.

Table 2 presents the effect of predictor variables on GFR in a multivariable model based on cystatin C versus a similar model based on serum creatinine. Both a 50% increase in cystatin C and a 50% increase in serum creatinine were associated with a 39% decrease in GFR. Age was a stronger predictor of GFR in a model based on serum creatinine than in a model based on cystatin C ($P<0.002$ for both). At the same serum creatinine level, female subjects had a 23% lower GFR than male subjects ($P<0.001$), but there was no difference at the same cystatin C level ($P=0.18$). At the same serum creatinine level, healthy persons had a 15% higher GFR than patients with native kidney disease ($P=0.002$), but there was no difference at the same cystatin C level ($P=0.28$). At the same cystatin C level, transplant recipients (kidney, liver, heart, pancreas, or lung) had a 19% higher GFR ($P<0.001$) than patients with native kidney

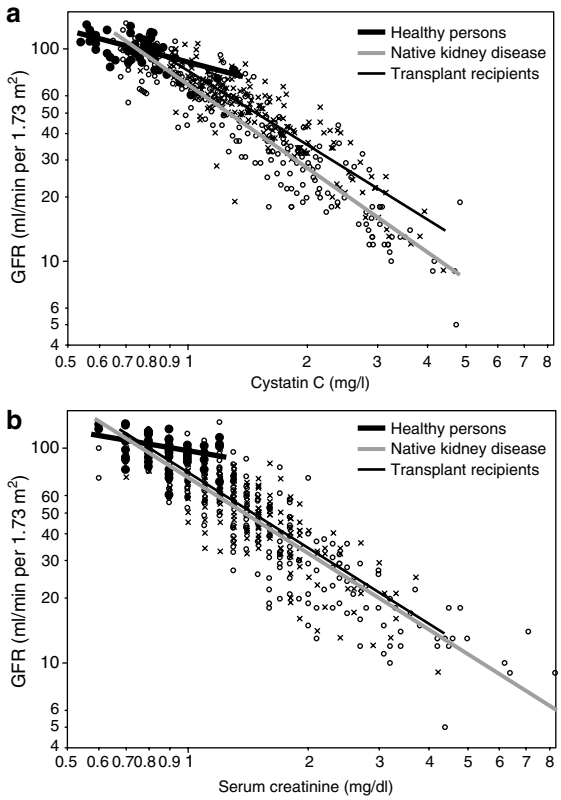

Figure 1 | Relationship between (a) cystatin C or (b) serum creatinine and measured GFR (iothalamate clearance) in 460 patients on a logarithmic scale. The closed circles represent healthy persons ($n=50$). The open circles represent patients with native kidney disease only ($n=204$). The crosses represent patients with solid organ transplants ($n=206$). The regression lines for all three patient groups (native kidney disease, transplant recipients, and healthy) are shown. For (**a**) cystatin C, the regression lines represent GFR prediction equations for patients with native kidney disease (equation (1)) and transplant recipients (equation (3)).

Table 2 | Effect of predictor variables on GFR in a model based on cystatin C versus a similar model based on serum creatinine (sample size=460)

	% Difference in measured GFR (95% confidence interval)	
	Cystatin C model	Serum creatinine model
Cystatin C (per 50% increase)	−38.8 (−40.2 to −37.3)	—
Serum creatinine (per 50% increase)	—	−39.0 (−40.5 to −37.6)
Age (per 50% increase)	−4.8 (−7.5 to −1.9)	−8.2 (−11.0 to −5.3)
Male (*n*=267)	Ref	Ref
Female (*n*=193)	−3.1 (−7.3 to 1.4)	−23.2 (−26.9 to −19.3)
Native kidney disease (*n*=204)	Ref	Ref
Healthy (*n*=50)	4.7 (−3.6 to 13.7)	14.8 (5.5 to 24.9)
Kidney recipient (*n*=103)	18.3 (11.8 to 13.7)	2.5 (−3.5 to 9.0)
Liver recipient (*n*=55)	19.1 (11.0 to 27.8)	−9.9 (−16.3 to −2.9)
Heart recipient (*n*=30)	25.5 (14.4 to 37.6)	3.8 (−5.9 to 14.5)
Other organ recipient[a] (*n*=18)	13.7 (1.4 to 27.3)	−2.9 (−14.0 to 9.6)
Model fit (r^2)[b]	0.852	0.832
Root mean square error (%)[c]	26.5	28.4

[a]Pancreas or lung.
[b]Model fit or coefficient of determination (r^2) is the proportion of between patient variability in measured GFR explained by the model.
[c]Root mean square error is an average error between measured GFR and estimated GFR given as a percentage.

Table 3 | Effect of predictor variables on GFR in cystatin C models compared to serum creatinine models by clinical presentation (native kidney disease, *n*=204; healthy, *n*=50)

	% Difference in measured GFR (95% confidence interval)			
	Cystatin C Models		Serum creatinine models	
Clinical presentation	Native kidney disease	Healthy	Native kidney disease	Healthy
Cystatin C (per 50% increase)	−40.3 (−42.1 to −38.4)	−20.2 (−26.0 to −14.0)	—	—
Serum creatinine (per 50% increase)	—	—	−39.0 (−41.0 to −36.9)	−18.4 (−29.3 to −5.8)
Age (per 50% increase)	−4.3 (−8.5 to 0.2)	−8.0 (−12.8 to −3.1)	−11.4 (−15.6 to −7.0)	−6.6 (−12.5 to −0.3)
Male	Ref	Ref	Ref	Ref
Female	1.4 (−5.7 to 9.1)	−4.9 (−12.0 to 2.8)	−26.2 (−32.1 to −19.7)	−10.9 (−21.8 to 1.6)
Model fit (r^2)[a]	0.856	0.493	0.827	0.237
Root mean square error (%)[b]	29.0	13.1	32.8	16.3

[a]Model fit or coefficient of determination (r^2) is the proportion of between patient variability in measured GFR explained by the model.
[b]Root mean square error is an average error between measured GFR and estimated GFR given as a percentage.

disease. At the same serum creatinine level, liver transplant recipients had a 10% lower GFR ($P=0.007$) than patients with native kidney disease, but there was no difference with non-liver transplant recipients ($P=0.36$).

As seen graphically by the different slopes in Figure 1, there was a statistical interaction between serum analyte and healthy versus native kidney disease status in the prediction of GFR ($P<0.001$ for both cystatin C and serum creatinine). To understand the potential importance of these statistical interactions, ln GFR was modeled separately in the healthy group and the native kidney disease group (Table 3). In the healthy group, a difference in cystatin C or serum creatinine was less strongly associated with a difference in GFR compared to that in the native kidney disease group. Furthermore, in the healthy group compared to the native kidney disease group, age and gender were stronger predictors of GFR in a cystatin C model, but weaker predictors of GFR in a serum creatinine model. For both cystatin C and serum creatinine models, the root mean square error was approximately half for the healthy group compared to the native kidney disease group. Although subtle, there was also a statistical interaction between cystatin C and transplant recipient versus native kidney disease status in the prediction of GFR ($P=0.02$). A 50% increase in cystatin C was associated with a 37.1% decrease in GFR for the transplant recipient group compared to a 40.3% decrease in GFR for the native kidney disease group.

As shown in Figure 1a and Tables 2 and 3, the relationship between cystatin C and GFR varied across clinical presentation. Thus, we developed cystatin C and serum creatinine equations using only the native kidney disease group (equations (1) and (2), $n=204$). For the cystatin C model, gender was not statistically significant ($P=0.71$) and age was only borderline significant ($P=0.06$). Including these variables only increased the r^2 by 0.003. Thus, the final cystatin C equation did not include age or gender. A separate cystatin C equation was also developed for transplant recipients (equation (3), $n=206$). For convenience, Table 4 provides the corresponding cystatin C levels for each stage of CKD.[1]

Table 4 | Cystatin C levels for determining stage of CKD

Stage[a]	Description	GFR range[a] (ml/min/1.73 m^2)	Cystatin C level	
			Native kidney disease[b]	Transplant recipient[c]
1	Normal or increased GFR	⩾90	⩽0.80	⩽0.87
2	Mildly decreased GFR	60 to 89	0.80 to 1.09	0.87 to 1.23
3	Moderately decreased GFR	30 to 59	1.10 to 1.86	1.24 to 2.24
4	Severely decreased GFR	15 to 29	1.87 to 3.17	2.25 to 4.10
5	Kidney Failure	<15	>3.17	>4.10

[a]GFR estimates and CKD stage will be inaccurate if there is a calibration difference with the Dade-Behring BN II Nephelometer assay used in this study.
[b]Using the prediction equation: GFR=66.8 (cystatin C)$^{-1.30}$.
[c]Using the prediction equation: GFR=76.6 (cystatin C)$^{-1.16}$.

Native CKD

$$\text{GFR} = 66.8 \times (\text{cystatin C})^{-1.30} \quad (1)$$

Native CKD

$$\text{GFR} = 273 \times (\text{serum creatinine})^{-1.22} \times \text{age}^{-0.299} \times 0.738 \text{ (if female)} \quad (2)$$

Transplant recipient

$$\text{GFR} = 76.6 \times (\text{cystatin C})^{-1.16} \quad (3)$$

The correlation of the cystatin C equation (equation (1), $r^2 = 0.853$) was higher than the serum creatinine equation derived using the same sample (equation (2), $r^2 = 0.827$), although this did not reach statistical significance ($P = 0.15$).[15] It was also higher than the MDRD equation ($r^2 = 0.825$) and the Cockcroft–Gault equation ($r^2 = 0.796$). The cystatin C equation also appeared to perform slightly better than a serum creatinine equation when applied to independent datasets approximated with bootstrapping. The r^2 adjusted for optimism was 0.852 for the cystatin C equation (equation (1)) and was 0.821 for the serum creatinine equation (equation (2)). The cystatin C equation derived with transplant recipients (equation (3)) had an r^2 of 0.768 and an r^2 adjusted for optimism of 0.766.

A composite equation for the native kidney disease group based on cystatin C, serum creatinine, age, and gender had the best model fit ($r^2 = 0.892$, equation not shown). This was significantly higher than the serum creatinine equation ($P < 0.0001$).[15] However, a similar model fit ($r^2 = 0.891$) was obtained regressing measured GFR on the mean of the cystatin C equation (equation (1)) and the serum creatinine equation (equation (2)). In other words, when both cystatin C and serum creatinine are available, averaging the GFR estimates by each analyte improved correlation with measured GFR. As equation models predicted logarithmic GFR, the geometric mean was used:

$$\text{Composite estimated GFR (eGFR)} = \sqrt{(\text{cystatin C eGFR}) \times (\text{serum creatinine eGFR})} \quad (4)$$

DISCUSSION

This study characterized the relationship between cystatin C and measured GFR (iothalamate clearance) in a variety of clinical presentations. At the same cystatin C level, transplant recipients had a 19% higher GFR than native kidney disease patients. As expected, there was a much stronger association between cystatin C and GFR among native kidney disease patients ($r^2 = 0.853$) than among healthy persons ($r^2 = 0.382$). Because clinical presentation was an important predictor of GFR, a cystatin C equation was derived with native kidney disease patients only. Averaging the estimated GFR between a cystatin C equation and a serum creatinine equation improved the prediction of GFR over a serum creatinine equation alone in CKD patients.

These findings help clarify the relationship between cystatin C and GFR as compared to serum creatinine and GFR. The relationship between either serum analyte with GFR differed among clinical presentations. This, in part, may explain the discrepancy among prior studies that compared cystatin C with serum creatinine.[16] For this study, equations were only developed for patients with a clinical diagnosis of CKD. A diagnosis of CKD was based on an elevated serum creatinine level, other evidence of kidney damage (e.g., proteinuria) or a clinical presentation where most patients have reduction in GFR (transplant recipient). GFR can be estimated with a cystatin C equation for native kidney disease patients (equation (1)) or with a cystatin C equation for transplant recipients (equation (3)) as well as with the MDRD equation. Improved prediction may be obtained by averaging (geometric mean) the estimated GFR from the appropriate cystatin C equation with the MDRD equation.

When clinical presentation narrows the GFR distribution, this will decrease modeling error in the GFR estimate. This was demonstrated by the lower root mean square error for the healthy group compared to that for the native kidney disease groups (Table 3). Thus, it is important to have a diagnosis of CKD before applying these equations to estimate GFR.

Any GFR estimated by a serum analyte should still be interpreted with caution. Confounding factors associated with cystatin C (possibly inflammation or immunosuppression therapy) or serum creatinine (muscle mass or protein intake) may lead to inaccurate GFR estimates. This inherent limitation from factors that influence serum analyte levels independent of GFR is illustrated in Figure 2. With prediction equations, several analyte factors (production, tubular secretion, tubular reabsorption, and extra-renal clearance) are not directly measured. Instead they are assumed to

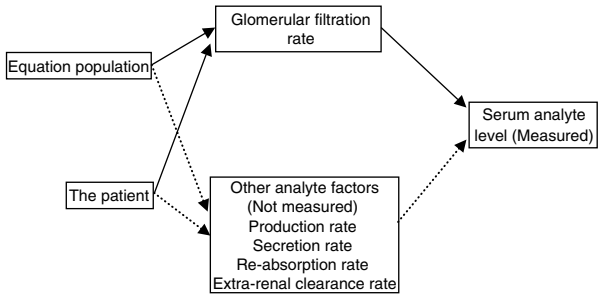

Figure 2 | Diagram that demonstrates an inherent problem with GFR prediction equations. An equation may not be accurate if unmeasured analyte factors (production, secretion, reabsorption, and extra-renal clearance) are modeled incorrectly for a particular patient (or patient group).

be constant or are modeled based on easily measured characteristics (e.g. age and gender) of the population used to derive the equation. Thus, an equation will not be accurate if the modeled effect of these analyte factors based on the equation population is incorrect for a particular patient (Figure 2).

For example, applying an equation derived in native kidney disease patients to a liver graft recipient will overestimate GFR by serum creatinine but underestimate GFR by cystatin C (Table 2). For serum creatinine, liver disease among liver graft recipients will decrease creatinine production leading to an overestimation of GFR.[17] For cystatin C, the inflammation or immunosuppression therapy associated with having a liver graft may increase cystatin C production leading to an underestimation of GFR.

The methods used in this study were chosen to minimize bias in the comparison of two different serum analytes. Sampling or measurement bias can occur when applying previously derived statistical models (e.g., MDRD equation and Cockcroft–Gault equation) to new data. Thus, comparisons were made between statistical models specifically derived for this study that varied either the serum analyte or the sampled population. This improved internal validity for the comparison between cystatin C and serum creatinine (as measured in this study) for predicting GFR (as measured in this study) among different populations (as sampled in this study). Finally, samples were not identified by measured GFR, because measured GFR was the dependent variable in the regression models.

The results of this study are consistent with previously published studies. The 19% higher GFR at the same cystatin C level among transplant recipients compared to native kidney disease patients is consistent with reports by other investigators.[18,19] The mechanism for this finding is not understood, but plausible hypotheses include increased cystatin C production from systemic inflammation[12,13,20] or use of immunosuppresion therapy[21,22] among transplant recipients. In this study, the regression fit between ln GFR and ln cystatin C was stronger than between ln GFR and ln serum creatinine for each clinical presentation. This was consistent with a meta-analysis that found cystatin C to be either superior or equivalent to serum creatinine in the correlation with GFR.[16] Another study also found that a cystatin C (Dade–Behring assay) equation ($r^2 = 0.91$) had a better model fit than a serum creatinine equation ($r^2 = 0.84$), but effects of clinical presentation, body size, age, and sex were not considered in the comparison. The equation for that study, GFR (ml/min) $= 77.2 \times$ cystatin $C^{-1.26}$, gives GFR estimates 15–20% higher than the native kidney disease equation (equation (1)) of this study.[23]

There were several potential limitations to this study. First, patients were classified into clinical presentations based on a medical chart review. To decrease misclassification bias, patients were only grouped by levels of easily identifiable clinical characteristics. Second, among native kidney disease patients, only those with an increased severity of illness, such that a nephrologist would measure the patient's GFR, were represented. Thus, patients with microalbuminuria and a normal serum creatinine level were inadequately represented in the native kidney disease group. Third, the generalizability of the cystatin C equation needs to be tested in other centers with more diverse racial groups and different mixtures of CKD etiologies. Finally, any calibration differences between the Dade-Behring BN II Nephelometer used in this study and other cystatin C assays can lead to inaccurate GFR estimates, a well-described problem with serum creatinine equations.[24]

In conclusion, the relationship between cystatin C and GFR can depend on the clinical presentation. In patients with CKD, a cystatin C equation is complementary to the MDRD equation or other serum creatinine equations for improving GFR estimates. Depending on the clinical setting, estimated GFR with a cystatin C equation can be averaged with a serum creatinine equation or used in place of the serum creatinine equation. For example, among patients with very high or very low muscle mass, a cystatin C equation alone may be preferential. A better understanding of the factors that effect cystatin C levels independent of GFR could potentially improve Cystatin C equations and determine the best settings for their application.

MATERIALS AND METHODS

Study subjects

A previously reported series of consecutive patients ($n = 502$) had an outpatient GFR measurement by iothalamate clearance, between 27 October 1999 and 3 March 2000 and agreed to participate in the study.[3] Medical records were abstracted for demographic and clinical characteristics present at the time of the iothalamate clearance. Clinical presentation was grouped into three mutually exclusive categories: native CKD, solid-organ transplant recipient (with or without known CKD), and healthy (potential kidney donor). Among the native kidney disease patients, the suspected etiology was hypertension or unknown (36%), diabetes mellitus (13%), other glomerulopathy (26%), and other non-glomerulopathy (25%).

Transplant recipients were further divided into those with a kidney graft alone and those with other organ grafts (liver, heart, pancreas, or lung) with or without a kidney graft. Transplant recipients typically have GFR measurements during routine

outpatient follow-up when serum creatinine levels are stable. Patients who did not fit into the above categories were excluded due to inadequate sample size and heterogeneity ($n = 32$). This included protocol evaluation for potential non-kidney transplant recipients (pancreas, $n = 5$; heart, $n = 6$; or lung, $n = 9$), spinal cord injury patients with neurogenic bladders being screened for kidney disease ($n = 8$), a cancer patient who needed chemotherapy dosing ($n = 1$), and reason for GFR measurement indeterminate ($n = 3$). Children (ages < 17 years) were also excluded ($n = 10$).

Laboratory measurements

All patients had a non-radiolabeled iothalamate clearance using a previously described standard laboratory method.[25] Briefly, after oral hydration with 4–6 glasses of water, patients received a subcutaneous injection of non-radiolabeled iothalamate (Conray, Mallinckrodt Medical, St Louis, MO, USA). Following a 1-h equilibrium period, the patient voided, the first serum sample was drawn and a timed urine collection was begun. A sonographic scanner assessed bladder emptying and a bladder catheter was placed in patients with urinary retention. Following the timed urine collection (approximately 45–60 min), a second serum sample was obtained. GFR was calculated by the clearance equation ($U_{Io}V/P_{Io}$ where U_{Io} and P_{Io} are the urine and plasma concentrations of iothalamate, and V is the urine flow) using the mean of two serum samples and one urine sample assayed for iothalamate via capillary electrophoresis. The between-day assay coefficient of variation was 4.3%. All GFR measurements were standardized for body surface area (per 1.73 m^2) by multiplying by 1.73 and dividing by body surface area.[26,27]

Cystatin C and creatinine levels were assayed from the first serum sample obtained during the iothalamate clearance test. Cystatin C was assayed by particle-enhanced immuno-nephelometry (Dade-Behring BN II Nephelometer). The between-day assay coefficient of variation was 3.5%. The assay showed linearity based on serial dilutions (measured $= 0.996 \times$ expected $+ 0.015$; $n = 16$) over a range of 0.16 to 2.61 mg/l. Samples showed stability at room temperature (up to 7 days), when frozen at $-20^{\circ}C$ (up to 2 years) and through three freeze–thaw cycles. Serum creatinine was measured by the uncompensated rate-Jaffe reaction (Hitachi 911 auto-analyzer). The between-day assay coefficient of variation was 4.7% at 1.1 mg/dl and 1.8% at 5.6 mg/dl. GFR was estimated using the abbreviated MDRD equation[14,28] and the Cockcroft–Gault equation (standardized for body surface area and adjusted to predict GFR in the same native kidney disease population used to derive the MDRD equation).[2,14] The original Cockcroft–Gault equation predicts creatinine clearance and because of tubular creatinine secretion, it overestimates GFR in CKD patients.

Statistical analysis

Univariate statistics (frequency, mean, standard deviation) were assessed overall and stratified by clinical presentation. For each clinical presentation group, ln GFR was regressed on ln analyte (cystatin C or serum creatinine). GFR was ln transformed for constant variability. Cystatin C and serum creatinine levels were ln transformed for linearity within groups. Multiple linear regression was used to predict ln GFR with the covariates: ln analyte, ln age, female (0–1 indicator variable) and clinical presentation (0–1 indicator variables with native kidney disease as the reference group). Age was ln transformed to allow easier comparison with the MDRD equation, but findings were similar when age was not ln transformed. Height and weight were not included as predictors in models, because GFR had been standardized for body surface area using a formula based on height and weight.[27] Interaction terms between the analyte and clinical presentation were included in additional models.

As the relationship between cystatin C and GFR differed among clinical presentations, a cystatin C equation was derived using only patients with native kidney disease. The model fit (r^2) of this equation was compared to a serum creatinine equation derived with the same data, the MDRD equation and the Cockcroft–Gault equation. The root mean square error between different models was compared. Statistical significance for the difference in r^2 between equations was determined using the method of Meng *et al.*[15] An additional cystatin C equation was developed for transplant recipients.

To assess performance on independent data sets, new equations were internally validated with bootstrapping.[29] Each equation was evaluated using 500 random bootstrap samples from the full set of data used to derive the equation. Stepwise selection was used to add the serum analyte, age and gender to each model. Model optimism (including stepwise selection) was determined from the mean difference between the r^2 of each bootstrapped sample and the r^2 when applying that bootstrapped sample's parameters to the original data set. r^2 adjusted for optimism was determined from the original data r^2 minus model optimism. Statistics were performed with JMP 5.1 and with SAS 8.2 (SAS Institute, Cary, NC, USA).

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