

# Cystatin C and Estimates of Renal Function: Searching for a Better Measure of Kidney Function in Diabetic Patients

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**Background:** Early identification of impairment in renal function is crucial in diabetic patients. Serum cystatin C may be the most sensitive indicator of glomerular filtration rate (GFR) in the clinical setting.

**Methods:** We compared cystatin C with creatinine, the Cockcroft-Gault (C-G) formula, and the Modification of Diet in Renal Disease (MDRD) study equation for the assessment of early decreased renal function in 288 diabetic patients (125 type 1, 163 type 2) with renal impairment [GFR:  $4\text{--}222\text{ mL}\cdot\text{min}^{-1}\cdot(1.73\text{ m}^2)^{-1}$ ]. Relationships of cystatin C, creatinine, and iohexol clearance were linearized by plotting their reciprocals in a simple regression model. Diagnostic efficiency was calculated from ROC curves.

**Results:** In this study population, cystatin C ( $P = 0.0013$ ) was better correlated with GFR ( $r = 0.857$ ) than were creatinine ( $r = 0.772$ ), C-G ( $r = 0.750$ ), and MDRD ( $r = 0.806$ ), a result replicated in patients with normal renal function ( $P = 0.023$ , type 1;  $P = 0.011$ , type 2), but not in those with decreased GFR. Mean cystatin C concentrations showed step-by-step statistically significant increases as GFR decreased, allowing very early detection of reduction in renal function. At  $90\text{ mL}\cdot\text{min}^{-1}\cdot(1.73\text{ m}^2)^{-1}$  and  $75\text{ mL}\cdot\text{min}^{-1}\cdot(1.73\text{ m}^2)^{-1}$  cut-points, diagnostic efficiencies of cystatin C (89% and 92%) were better than those of the other variables (79%–82% and 85%–86%, respectively;  $P = 0.01$ ).

**Conclusions:** All data supported the value of serum cystatin C compared with conventional estimates based on serum creatinine measurement for detecting very early reduction of renal function. Use of cystatin C to measure renal function will optimize early detection, prevention, and treatment strategies for diabetic nephropathy.

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Individuals with moderate (1) or mildly (2) decreased renal function are at increased risk for chronic kidney disease and cardiovascular disease. Adverse outcomes of renal failure can be prevented or delayed through early detection and treatment (3). Therefore the routine measurement of the urine albumin-to-creatinine (A/C)<sup>3</sup> ratio and estimation of glomerular filtration rate (GFR) are strongly recommended for patients at high risk for kidney failure and cardiovascular disease, such as diabetic patients (4).

Gold standard procedures for GFR measurement, based on the clearance of <sup>51</sup>Cr-EDTA or iohexol, are impractical in the clinical setting and for larger research studies. Otherwise, creatinine alone is unsatisfactory to estimate GFR, and leads to delays in detecting earlier stages of kidney failure (3). Indeed, in addition to renal function, serum creatinine depends on creatinine generation, extrarenal elimination, and tubular handling (5). By accounting for physiologic factors that affect creatinine, equations estimating GFR overcome some limitations. Creatinine clearance calculated by the Cockcroft-Gault

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<sup>3</sup> Nonstandard abbreviations: A/C, urinary albumin-to-creatinine ratio; GFR, glomerular filtration rate; C-G, Cockcroft-Gault formula; MDRD, Modification of Diet in Renal Disease study equation; iGFR, iohexol plasma clearance method; AUC, areas under the curve; CI, confidence interval; PPV, positive predictive values; NPV, negative predictive values; and BMI, body mass index.

(C-G) formula overestimates GFR as renal function declines and tubular secretion increases. The Modification of Diet in Renal Disease (MDRD) study equation (7) shows low accuracy at higher GFR (7).

Cystatin C has been identified as a new, promising, and easily measurable marker for prompt detection of early kidney failure (8,9). Cystatin C is produced at a constant rate by nucleated cells and released into the bloodstream with a half-life of ~2 h (10). Cystatin C is freely filtered and almost completely taken up and degraded, but not secreted, by proximal tubular cells. Several studies have used direct measures of GFR as the gold standard to compare cystatin C with creatinine and creatinine-derived estimates of GFR (11). Several studies have also been conducted in diabetic patients (12–21) in whom cystatin C seems to outperform creatinine-based estimations (12, 14–18, 20, 21). Nevertheless the utility of cystatin C remains uncertain (22).

Using iohexol plasma clearance as the reference GFR, we compared cystatin C with serum creatinine, C-G, and MDRD for estimating GFR in a large sample of patients with type 1 and type 2 diabetes.

### Patients and Methods

A total of 288 white diabetic patients were recruited at the Department of Endocrinology and Metabolism between January 2003 and December 2004. Our Ethics Committee approved the study that was conducted in accordance with the Helsinki Declaration. All study participants gave written informed consent.

Among type 1 diabetic patients ( $n = 125$ ), 31% had A/C ratios within the reference interval, 33% had microalbuminuria, and 36% had overt nephropathy. The respective values for type 2 diabetic patients ( $n = 163$ ) were 12%, 60%, and 28%. Hypertension, defined as blood pressure  $>140/90$  mmHg and/or ongoing treatment, occurred in 62% and 82% of patients, respectively. All patients with hypertension and/or increased albuminuria were on ACE-inhibitors and/or AT1-antagonists. In 73% of these patients, calcium channel blockers and/or diuretics or other antihypertensives were also employed.

Urinary albumin was measured by a nephelometric immunoassay on the BN<sup>TM</sup> II nephelometer (Dade/Behring) in at least 3 first-morning urine samples obtained in a 6-month period. Microalbuminuria was defined as an A/C ratio of 2.5 (3.5 in females) to 30 mg/mmol; clinical nephropathy as an A/C ratio  $>30$  mg/mmol and/or a serum creatinine  $\geq 133$   $\mu\text{mol/L}$  (1.5 mg/dL) in males or  $\geq 115$   $\mu\text{mol/L}$  (1.3 mg/dL) in females.

Serum and urinary creatinine were determined by a fully automated Jaffé kinetic method on a Roche/Hitachi 747 analyzer. Because the serum creatinine assay had not been recalibrated to be traceable to an isotope dilution mass spectrometry reference method (23), the original MDRD equation was employed for estimating GFR:  $[\text{GFR} (\text{mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}) = 186 \cdot (\text{creatinine})^{-1.154} \cdot (\text{age})^{-0.203} \cdot (0.742 \text{ if female})]$ . We also estimated GFR by

the C-G formula:  $\text{creatinine clearance} (\text{mL}/\text{min}) = (140 - \text{age}) \times \text{weight}/72 \times \text{creatinine} (\times 0.85 \text{ in females}) (3)$ .

Serum cystatin C was measured by a particle-enhanced nephelometric immunoassay (N Latex Cystatin C, Dade Behring Diagnostics) on the BN II nephelometer.

GFR was assessed by the iohexol plasma clearance (iGFR) method as previously described (24). An intravenous bolus of 5 mL of iohexol (Omnipaque 300; Nycomed) was injected. Blood samples were drawn at 5, 15, 60, 90, 180, 240, and 300 min. If creatinine was  $>176$   $\mu\text{mol/L}$  (2 mg/dL), samples were withdrawn also at 360 and 420 min after injection; if creatinine was  $>440$   $\mu\text{mol/L}$  (5 mg/dL) a further sample was taken at the 1440th minute.

For both the cystatin C assay and iGFR procedure, details are described in the online expanded Methods section [see the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol53/issue3>].

### STATISTICAL ANALYSIS

Results are presented as mean (SD) or median (range). To check gaussian distributions, data were evaluated by the Kolmogorov-Smirnov test, taking  $P < 0.001$  as significant. Cystatin C and creatinine were found not to have a gaussian distribution; their logarithms were employed in all statistical treatments. Differences in continuous variables were investigated by the one-way ANOVA followed by the Scheffé test and the unpaired Student *t*-test. Fisher exact test and  $\chi^2$  test were employed to analyze contingency tables. The limit of significance was  $P < 0.05$ . Reciprocals of cystatin C and creatinine allowed the linearization of the curvilinear relationship between iGFR and each serum marker. Correlations were investigated by the simple linear regression and by calculating the coefficient of regression.

To assess the diagnostic value of each marker, non-parametric ROC curves were generated by plotting the sensitivity vs 1-specificity. Areas under the curves (AUC), 95% confidence intervals (CI), and differences between ROC curves were calculated. In creating ROC curves, we used 3 cutoffs for iGFR: 60, 75, and 90  $\text{mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ . For each value we obtained the maximum diagnostic efficiency (the proportion of patients correctly classified at each cut-point), the cutoff limits at maximum efficiency, sensitivity and specificity, positive predictive values (PPV) and negative predictive values (NPV).

To investigate variables other than renal function affecting creatinine and cystatin C, multiple regression analyses were performed.

## Results

### DIABETIC PATIENTS

Patients with type 1 diabetes were younger and had longer diabetes duration than those with type 2 (Table 1). Body mass index (BMI) was lower and hemoglobin A1c higher in type 1 patients. Systolic pressure and rate of

**Table 1. General characteristics of patients with type 1 and type 2 diabetes, and comparison of renal function tests.**

	Type 1 diabetes (n =125)	Type 2 diabetes (n = 163)	P values
Gender, males/females	61/64	99/64	0.055 <sup>a</sup>
Age, years	37 (9)	60 (8)	<0.0001
Duration of diabetes, years	21 (9)	11 (10)	<0.0001
BMI, kg/m <sup>2</sup>	23.9 (2.8)	27.8 (4.0)	<0.0001
HbA1c, %	8.5 (1.5)	7.8 (1.3)	0.006
sBP, mmHg	128 (17)	135 (18)	0.011
dBP, mmHg	77 (9)	76 (10)	0.428
A/C ratio, mg/mmol	2.5 (0.28–319)	5.9 (0.40–203)	0.047 <sup>b</sup>
Diabetic nephropathy staging, % normoalbuminuria/microalbuminuria/overt nephropathy	30/32/38	12/70/18	<0.0001 <sup>c</sup>
Retinopathy staging, % absent/background/proliferative	8/22/70	48/28/24	<0.0001 <sup>c</sup>
Hypertension, % no/yes	37/63	26/74	0.042 <sup>a</sup>
Serum creatinine, μmol/L	114 (59) (53–466)	105 (67) (57–564)	0.048
	12.9 (6.7) mg/L (6.0–52.7)	11.9 (7.6) mg/L (6.5–63.8)	
Serum cystatin C, mg/L	1.17 (0.59) (0.31–3.18)	0.96 (0.46) (0.49–3.32)	0.009
Creatinine clearance estimated by the C-G formula, mL/min	78 (29) (21–141)	92 (37) (12–174)	0.002
GFR estimated by the abbreviated MDRD equation, mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>	69 (25) (13–128)	80 (29) (7–131)	0.001
iGFR, mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>	93 (41) (16–222)	87 (42) (4–192)	0.03

Data are expressed as mean (SD), or as median (range), or as percentage.

HbA1c, hemoglobin A1C; sBP, systolic blood pressure; dBP, diastolic blood pressure.

Statistical tests performed: <sup>a</sup> Fisher exact test for a 2-by-2 contingency table; <sup>b</sup> Mann-Whitney U-test; <sup>c</sup>  $\chi^2$  test for a 2-by-3 contingency table. Unpaired Student t-test was employed to compare means between groups.

hypertension were higher in type 2 patients, whereas advanced complications, overt nephropathy, and proliferative retinopathy were more frequent in type 1 patients. These differences account, at least in part, for the differences in renal function between type 1 and type 2 diabetic patients. Both groups, however, share a wide and comparable range of explored renal function. Albuminuria correlated inversely with decreasing iGFR (n = 288,  $r = -0.439$ ,  $P < 0.0001$ ). This relation was stronger ( $P = 0.0002$ ) in type 1 patients ( $r = 0.668$ ,  $P < 0.0001$ ) than in type 2 patients ( $r = 0.341$ ,  $P < 0.0001$ ).

#### CORRELATIONS BETWEEN ENDOGENOUS PARAMETERS OF RENAL FUNCTION AND IGFR

Cystatin C correlated more strongly ( $P = 0.006$ ) with iGFR (n = 288,  $r = 0.857$ ,  $P < 0.0001$ ) than did creatinine ( $r = 0.772$ ,  $P < 0.0001$ ). Furthermore, cystatin C showed better correlation with iGFR ( $P < 0.001$ , and  $P < 0.05$ , respectively) than C-G ( $r = 0.750$ ,  $P < 0.0001$ ) and MDRD ( $r = 0.806$ ,  $P < 0.0001$ ). These correlations occurred in patients with type 1 and type 2 diabetes, but differences in correlation coefficients were stronger in type 1 than in type 2 patients (Table 2).

For both type 1 and type 2 diabetes, regressions were stronger for patients with reduced GFR [ $< 90$  mL · min<sup>-1</sup> · (1.73 m<sup>2</sup>)<sup>-1</sup>] than for those with normal GFR. For

patients with reduced GFR, both type 1 and type 2, all parameters had approximately the same correlation value with GFR ( $P < 0.0001$ ). For patients with normal GFR, cystatin C had a higher correlation value (type 1:  $r = 0.59$ ; type 2:  $r = 0.65$ ,  $P < 0.001$ ) than all the other variables (Table 3).

#### ENDOGENOUS PARAMETERS OF RENAL FUNCTION BY IGFR

GFR values were divided into 6 categories [ $< 45$ , 45–59.9, 60–74.9, 75–90, 90–120, and  $> 120$  mL · min<sup>-1</sup> · (1.73 m<sup>2</sup>)<sup>-1</sup>], incorporating the guidelines of the US National Kidney Foundation (3). Distributions of sexes ( $\chi^2 = 5.39$ ,  $P = 0.370$ ) and types of diabetes ( $\chi^2 = 7.88$ ,  $P = 0.162$ ) were similar in the 6 categories that did not differ in age ( $P = 0.496$ ) and only slightly in BMI ( $P = 0.006$ ), because of a lower BMI in patients with GFR  $< 45$  mL · min<sup>-1</sup> · (1.73 m<sup>2</sup>)<sup>-1</sup>. However, patients with iGFR  $> 120$  mL · min<sup>-1</sup> · (1.73 m<sup>2</sup>)<sup>-1</sup> were younger ( $P < 0.005$ ) and more frequently males ( $P = 0.027$ ).

In the study patient population as a whole (Fig. 1), as well as in type 1 and type 2 diabetic patients (data not shown), decreasing iGFR was associated with increasing cystatin C, increasing creatinine, and decreasing GFR as estimated by both C-G and MDRD. Among iGFR categories, however, a step-by-step statistically significant

**Table 2. Correlations between iGFR and reciprocal of serum cystatin C, reciprocal of serum creatinine, creatinine clearance calculated by the C-G formula, and GFR estimated by the abbreviated MDRD equation, respectively in type 1 and type 2 diabetic patients.**

	iGFR		
	All patients (n = 288)	Type 1 diabetes (n = 125)	Type 2 diabetes (n = 163)
1/serum cystatin C, mg/L	$r = 0.857^a$ ( $P < 0.0001$ )	$r = 0.912^b$ ( $P < 0.0001$ )	$r = 0.852^c$ ( $P < 0.0001$ )
1/serum creatinine, $\mu\text{mol/L}$	$r = 0.772$ ( $P < 0.0001$ )	$r = 0.729$ ( $P < 0.0001$ )	$r = 0.744$ ( $P < 0.0001$ )
Creatinine clearance calculated by the C-G formula, mL/min	$r = 0.750$ ( $P < 0.0001$ )	$r = 0.709$ ( $P < 0.0001$ )	$r = 0.756$ ( $P < 0.0001$ )
GFR estimated by the abbreviated MDRD equation, $\text{mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$	$r = 0.806$ ( $P < 0.0001$ )	$r = 0.756$ ( $P < 0.0001$ )	$r = 0.826$ ( $P < 0.0001$ )
Comparison between the 4 correlations	$= 0.001$ 3df	$< 0.0001$ 3df	$= 0.01$ 3df

Cystatin C and creatinine values were log-transformed.  
<sup>a</sup>  $P = 0.006$  vs correlation coefficient of 1/creatinine,  $P < 0.001$  vs correlation coefficient of C-G,  $P < 0.05$  vs correlation coefficient of MDRD.  
<sup>b</sup>  $P < 0.0002$  vs all other correlation coefficients.  
<sup>c</sup>  $P = 0.003$  vs correlation coefficient of 1/creatinine,  $P = 0.007$  vs correlation coefficient of C-G,  $P = 0.216$  vs correlation coefficient of MDRD.

change in the mean values was observed only for cystatin C (Fig. 1A). Thus cystatin C not only discloses early decreases in GFR [ $75\text{--}90 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ ] but also reflects changes within the reference interval [ $>90 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ ]. Creatinine (Fig. 1B) and C-G (Fig. 1C) did not reflect early decreases in renal function (differences in C-G within the GFR reference interval disappeared when corrected for age and sex), but showed significant decreases only when iGFR was  $75 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$  or lower. Use of MDRD seems to reflect changes even within the reference range (Fig. 1D), but renal function is estimated with low precision in individuals with a higher GFR. Indeed, the bias between

MDRD and iGFR tends to increase for GFR values  $< 45$  and  $> 90 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ . In particular, MDRD underestimated GFR by 19% and 29% in patients with iGFR values of  $90\text{--}120$  and  $> 120 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ , respectively (Fig. 2). Results were consistent irrespective of sex and type of diabetes.

#### NONPARAMETRIC ROC CURVES

ROC plots for cystatin C and creatinine (Table 4) demonstrated that the AUC of cystatin C was greater than that of creatinine at a cutoff level of 90 ( $P = 0.003$ ) and  $75 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$  ( $P = 0.0018$ ), but not at a cutoff threshold of  $60 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$  ( $P = 0.342$ ).

**Table 3. Correlations between iGFR and reciprocal of serum cystatin C, reciprocal of serum creatinine, creatinine clearance calculated by the C-G formula, and GFR estimated by the abbreviated MDRD equation, respectively in type 1 and type 2 diabetic patients with normal and reduced renal function.**

	iGFR			
	Type 1 diabetes (n = 125)		Type 2 diabetes (n = 163)	
	Reduced GFR $< 90$ (n = 61)	Normal GFR $> 90$ (n = 64)	Reduced GFR $< 90$ (n = 98)	Normal GFR $> 90$ (n = 65)
1/serum cystatin C, mg/L	$r = 0.872$ ( $P < 0.0001$ )	$r = 0.590^a$ ( $P = 0.0007$ )	$r = 0.758$ ( $P < 0.0001$ )	$r = 0.650^b$ ( $P < 0.01$ )
1/serum creatinine, $\mu\text{mol/L}$	$r = 0.754$ ( $P < 0.0001$ )	$r = 0.120$ ( $P = 0.375$ )	$r = 0.804$ ( $P < 0.0001$ )	$r = 0.310$ ( $P < 0.01$ )
Creatinine clearance calculated by the C-G formula, mL/min	$r = 0.683$ ( $P < 0.0001$ )	$r = 0.305$ ( $P = 0.021$ )	$r = 0.753$ ( $P < 0.0001$ )	$r = 0.382$ ( $P < 0.01$ )
GFR estimated by the abbreviated MDRD equation, $\text{mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$	$r = 0.758$ ( $P < 0.0001$ )	$r = 0.282$ ( $P = 0.0335$ )	$r = 0.811$ ( $P < 0.0001$ )	$r = 0.442$ ( $P < 0.01$ )
Comparison between the 4 correlations	NS 3df	0.0227 3df	NS 3df	0.0107 3df

Cystatin C and creatinine values were log-transformed.  
<sup>a</sup>  $P = 0.0013$  vs correlation coefficient of 1/creatinine,  $P = 0.025$  vs correlation coefficient of C-G,  $P = 0.0184$  vs correlation coefficient of MDRD.  
<sup>b</sup>  $p = 0.0009$  vs correlation coefficient of 1/creatinine,  $p = 0.005$  vs correlation coefficient of C-G,  $p = 0.019$  vs correlation coefficient of MDRD.



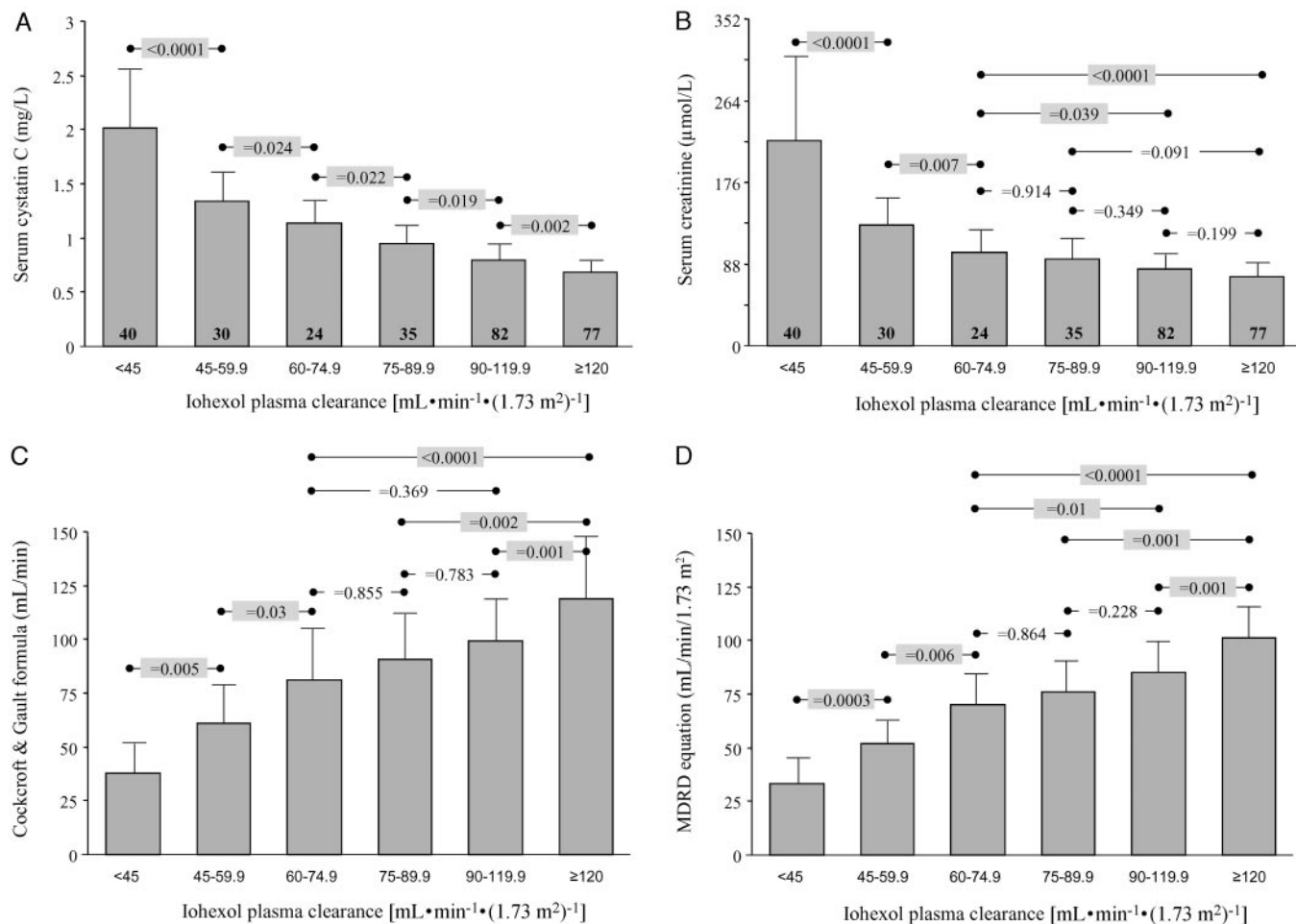


Fig. 1. Endogenous serum variables of renal function:(A) serum cystatin C; (B), serum creatinine; and estimated GFR from prediction equations (C), C-G formula; (D), MDRD equation; according to GFR assessed by iohexol plasma clearance.

For each variable, one-way ANOVA gives a *P* value <0.0001. Both cystatin C and creatinine were log-transformed before analysis (*P* values reported in the 4 panels were obtained by the Scheffé test).

Likewise, AUC for cystatin C was greater than those for C-G and MDRD at the cutoff levels of 90 (*P* = 0.0005 and *P* = 0.007, respectively) and 75 mL·min<sup>-1</sup>·(1.73 m<sup>2</sup>)<sup>-1</sup>

(*P* = 0.005 and *P* = 0.004, respectively), but not at the cutoff of 60 mL·min<sup>-1</sup>·(1.73 m<sup>2</sup>)<sup>-1</sup> (*P* = 0.192 and *P* = 0.461). All these results were reproduced in type 1 and type 2 diabetic patients (see Tables 1 and 2 in the online Data Supplement).

At the cut-point of 90 mL·min<sup>-1</sup>·(1.73 m<sup>2</sup>)<sup>-1</sup>, the maximum diagnostic efficiency of cystatin C (89%) was higher than those of creatinine (82%, *P* = 0.04), C-G (79%, *P* = 0.004) and MDRD (80%, *P* = 0.02). The cutoff limit of 0.98 mg/L for cystatin C corresponds to a PPV of 93% and a NPV of 87%. The respective features for the cutoff limit of 98 μmol/L (1.11 mg/dL) for creatinine were PPV 86% and NPV 80%. At the cutoff point of 75 mL·min<sup>-1</sup>·(1.73 m<sup>2</sup>)<sup>-1</sup>, the maximum diagnostic efficiency was 92% for cystatin C, 85% (*P* = 0.02) for creatinine, 86% for C-G (*P* = 0.04), and 86% for MDRD (*P* = 0.04). The cutoff limit of 1.13 mg/L for cystatin C corresponds to a PPV of 93% and to a NPV of 91%. The respective features for the cutoff limit of 110 μmol/L (1.25 mg/dL) for creatinine are PPV 81% and NPV 87%. No differences between the param-

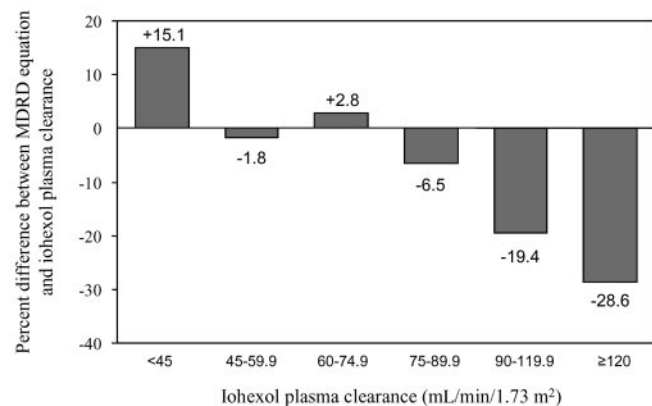


Fig. 2. Percentage difference between GFR estimated by the MDRD equation and the iohexol plasma clearance according to GFR assessed by iohexol plasma clearance.

**Table 4. ROC analysis for serum cystatin C, serum creatinine, creatinine clearance calculated by the C-G formula, and GFR estimated by the abbreviated MDRD equation in the whole group of diabetic patients.**

	ROC Area	95%CI	Diagnostic efficiency, %	Cutoff point, %	Sensitivity, %	Specificity, %	PPV, %	NPV, %
iGFR cutoff level: 90 mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>								
Cystatin C	<b>0.94</b>	0.90–0.97	<b>89</b>	0.98	82	95	93	87
Creatinine	<b>0.86</b>	0.81–0.91	<b>82</b>	98 (1.11)	72	90	86	80
C-G	<b>0.84</b>	0.79–0.89	<b>79</b>	82	74	82	77	79
MDRD	<b>0.87</b>	0.83–0.92	<b>80</b>	68	70	89	84	78
	<i>P</i> = 0.003 vs creatinine		$\chi^2 = 11.2$					
	<i>P</i> = 0.0005 vs C-G		3 <i>df</i>					
	<i>P</i> = 0.007 vs MDRD		<i>P</i> = 0.01					
iGFR cutoff level: 75 mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>								
Cystatin C	<b>0.97</b>	0.94–0.99	<b>92</b>	1.13	79	97	93	91
Creatinine	<b>0.89</b>	0.85–0.94	<b>85</b>	110 (1.25)	71	92	81	87
C-G	<b>0.89</b>	0.84–0.94	<b>86</b>	70	70	94	84	87
MDRD	<b>0.90</b>	0.86–0.95	<b>86</b>	61	73	94	85	88
	<i>P</i> = 0.0018 vs creatinine		$\chi^2 = 11.2$					
	<i>P</i> = 0.0047 vs C-G		3 <i>df</i>					
	<i>P</i> = 0.0044 vs MDRD		<i>P</i> = 0.01					
iGFR cutoff level: 60 mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>								
Cystatin C	<b>0.97</b>	0.95–0.99	<b>92</b>	1.23	77	97	89	93
Creatinine	<b>0.95</b>	0.92–0.98	<b>91</b>	124 (1.40)	72	97	88	91
C-G	<b>0.94</b>	0.90–0.98	<b>90</b>	65	79	93	79	93
MDRD	<b>0.96</b>	0.93–0.99	<b>92</b>	55	79	96	87	94
	<i>P</i> = 0.342 vs creatinine		$\chi^2 = 1.04$					
	<i>P</i> = 0.192 vs C-G		3 <i>df</i>					
	<i>P</i> = 0.461 vs MDRD		<i>P</i> = 0.79					

Cystatin C expressed in mg/L; creatinine expressed in mg/dL; C-G expressed in mL/min; MDRD expressed in mL · min<sup>-1</sup> · (1.73 m<sup>2</sup>)<sup>-1</sup>. Cystatin C and creatinine values were log-transformed before ROC analysis. Bold font indicates the variables for which statistical analysis was performed.

Data for type 1 and type 2 diabetic patients separately were also produced (see Table 1 and Table 2, respectively, in the online Data Supplement).

ters estimating GFR were observed when the cut-point was at 60 mL · min<sup>-1</sup> · (1.73 m<sup>2</sup>)<sup>-1</sup> (Table 4).

#### EXTRARENAL FACTORS AFFECTING SERUM CONCENTRATIONS OF RENAL FUNCTION VARIABLES

In patients with normal GFR [ $>90$  mL · min<sup>-1</sup> · (1.73 m<sup>2</sup>)<sup>-1</sup>], mean (SD) creatinine was higher ( $P < 0.05$ ) in males than in females [80 (14)  $\mu$ mol/L, 0.91 (0.16) mg/dL, vs 73 (10)  $\mu$ mol/L, 0.83 (0.11) mg/dL, respectively], but no differences ( $P = 0.16$ ) were observed for cystatin C [0.76 (0.15) vs 0.71 (0.08) mg/L]. In patients with decreased GFR, no sex differences were observed for either creatinine or cystatin C.

Age was weakly related to creatinine in type 1 ( $r = 0.20$ ,  $P = 0.02$ ) and type 2 diabetic patients ( $r = 0.21$ ,  $P = 0.007$ ) as a whole, but not in patients with normal GFR. Age was also related to cystatin C in type 1 ( $r = 0.32$ ,  $P = 0.006$ ) and in type 2 diabetic patients ( $r = 0.46$ ,  $P < 0.001$ ). This correlation persisted in patients with normal GFR ( $r = 0.45$ ,  $P < 0.001$ ), particularly in those with type 2 diabetes ( $r = 0.59$ ,  $P < 0.001$ ).

An inverse correlation was observed between BMI and creatinine ( $r = -0.17$ ,  $P = 0.004$ ). This correlation was also present in those with normal GFR ( $r = -0.18$ ,  $P = 0.02$ ) and therefore was not driven by the lower BMI of patients with severe renal failure. A correlation between BMI and

cystatin C ( $r = -0.19$ ,  $P = 0.006$ ) was also observed, but was lost in patients with normal GFR.

Multiple regression analysis indicated that for the whole patient group, extrarenal factors (including hypertension and smoking) affected both creatinine (sex, BMI) and cystatin C (age, hypertension) independently of renal function, but explained variations increased only very slightly, by 3%–4%. In the subgroup with normal GFR, the same extrarenal factors affected to some degree the explained variation of both creatinine (by 12%, from 12%–24%) and cystatin C (by 13%, from 36%–49%). Thus, half of the explained variation of creatinine was attributable to extrarenal factors, whereas extrarenal factors account only for approximately one-fourth of explained variation of cystatin C.

#### Discussion

The production of cystatin C has been extensively reported to be independent of and unaffected by sex, age, height, weight, and muscle mass (10). In our study, cystatin C, unlike creatinine, was unaffected by sex and BMI, but was correlated with age independently of GFR. These effects, negligible when the entire range of renal function was considered, became significant in patients with normal GFR. Compared with creatinine, however,

the proportion of variations in cystatin C attributable to extrarenal factors is considerably lower (50% vs 26%).

Two large studies (25, 26) with consistent results found that cystatin C is influenced by many variables (age, sex, body mass, smoking, hypertension, coronary heart disease, C-reactive protein) other than renal function alone, even after adjustment for kidney function. Both studies excluded patients with moderate and severe renal failure, and in both cystatin C was highly correlated with age ( $r \sim 0.40$ ).

Creatinine tends to be increased in patients with hypothyroidism and decreased in those with hyperthyroidism. The production of cystatin C is differently influenced by thyroid hormone, so that concentrations of cystatin C are low in hypothyroidism (27), even in mild forms (28), and increased in hyperthyroidism (27). Thyroid dysfunction is increased in frequency in the diabetic population (29). In our study, thyroid status was known in all participants, and patients with no euthyroidism were excluded.

Even if nonrenal influences, including polymorphisms in the promoter and exon 1 of the cystatin C gene (30, 31), make cystatin C not completely reliable as a measure of renal function, most studies have found cystatin C to be a better marker of GFR than creatinine. In a recent meta-analysis (9), approximations of GFR performed with cystatin C compared with creatinine had higher correlation coefficients (0.816 vs 0.742;  $P < 0.001$ ) and ROC-AUC (0.926 vs 0.837;  $P < 0.001$ ).

Previous studies have compared creatinine and creatinine-derived formulas with cystatin C in patients with diabetes, employing an acknowledged gold standard as the reference method for GFR (12–15, 17, 19–21), but most of them included small numbers of patients. Overall, 115 type 1 diabetic patients were included in 4 studies (13, 15, 19, 21) and 355 type 2 patients were enrolled in 7 studies (12–14, 17, 19–21). As far as the gold standard for GFR is concerned,  $^{51}\text{Cr-EDTA}$  (12–14, 19, 21), iohexol (15), or  $^{99\text{m}}\text{Tc-DTPA}$  (20) plasma clearance techniques, or  $^{125}\text{I}$ -iothalamate urinary clearance (17) were employed. In all these studies, with one relevant exception (14), only a few blood samples were obtained after tracer injection, and results were analyzed with a unicompartamental model. To increase accuracy, we adopted a multiple sampling protocol to measure iGFR after iohexol injection, with longer time intervals between the injection and sampling points as creatinine increased (24).

Using 4 methods of evaluation (correlations with GFR, mean values of each variable in patients stratified by GFR values, ROC curves, and diagnostic efficiency) we showed that cystatin C is more sensitive for detecting early renal function impairment than creatinine and creatinine-derived formulas.

The correlation of cystatin C with GFR was stronger than the correlation with creatinine, C-G, or MDRD. As reported in type 2 diabetes (14), and also observed in our study in both type 1 and type 2 patients, the correlations between GFR and creatinine or cystatin C were higher in

patients with decreased than in those with normal GFR. The regressions with GFR were superimposable in the subgroup with reduced GFR, whereas in patients with normal renal function the relationship between cystatin C and GFR was stronger than between GFR and the other 3 variables. This different behavior is due not only to the wider range of GFR values of patients with reduced renal function but also to the role played by different pathophysiological factors. In patients with reduced GFR, both cystatin C and creatinine are strongly and comparably influenced by renal impairment. Furthermore, cystatin C has been recently reported to have significant nonrenal clearance that, as for creatinine, has a relatively greater impact in patients with severe renal failure (32). Thus cystatin C and creatinine are strongly influenced by common factors and closely correlated in patients with reduced GFR, whereas the correlation is lower in patients with normal GFR as a consequence of the different weight of different factors that affect serum concentrations. The lower variance and the relatively greater importance of extrarenal covariates in affecting serum creatinine largely account for its insensitivity for detecting small decreases in GFR, in the so-called creatinine-blind GFR area.

Comparison of the mean values of the 4 variables in groups with different GFRs clearly revealed the performance of cystatin C when we focused on differences within the creatinine-blind GFR range, when creatinine and C-G are normal or do not change significantly despite declining renal function. With MDRD calculations it seems possible to detect changes in renal function even within the GFR reference range, but at the price of an unacceptable underestimation of GFR. Furthermore, the large sample of patients with high GFR [ $>120 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ ] included in our study showed significantly reduced cystatin C, suggesting that low cystatin C could be a useful marker for hyperfiltration. Therefore, cystatin C might be employed in large prospective studies for exploring hyperfiltration, a condition whose prognostic role in the history of diabetic nephropathy has been debated for decades (33).

The diagnostic efficiency of cystatin C, as shown by the AUCs of the ROC curves is higher than those of the other indexes. The GFR reference threshold chosen, below which GFR is defined as impaired, influences the diagnostic efficiency of the methods under investigation. At both 90 and 75  $\text{mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$  GFR, efficiency was higher for cystatin C and comparable for the other parameters. The lower the GFR limit chosen, the more the ROC curves for MDRD, C-G, creatinine, and cystatin C approach each other. Our ROC curves confirmed that cystatin C is a better diagnostic tool than creatinine, C-G, and MDRD both for identifying diabetic patients with normal ( $>90$ ) or near-normal [ $>75 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ ] GFR and for detecting patients with early ( $<75$ ) or very early [ $<90 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ ] impairment of GFR.

Previous studies on the role of cystatin C in detecting early renal failure in diabetic patients were contradictory. Some authors showed that cystatin C was more effective than creatinine in detecting initial reduction of GFR in type 2 (12, 14, 17, 20, 21) as well as in type 1 diabetes (15, 21). Two further studies, which did not employ a gold standard method for GFR, confirmed that cystatin C better differentiates GFR values among type 1 (18) and type 2 (16, 18) diabetic patients. Two studies (13, 19), on the other hand, showed that cystatin C is not more sensitive than creatinine for detecting early renal failure. Such discrepancies may be attributable at least in part to intraassay variations for creatinine and cystatin C measurements related to differences in assay techniques. We employed a nephelometric assay for cystatin C, a method claimed to perform with higher accuracy (34). Discrepancies may also rise from different and often arbitrarily chosen cut-points for the definition of abnormalities in renal function. In our study, the cut-points for GFR stratification incorporate the guidelines of the US National Kidney Foundation (3). Finally, our study includes the greatest number of type 1 and type 2 patients evaluated so far to explore the value of cystatin C.

More recently, some studies have successfully investigated the possibility of introducing cystatin C-based formulas without anthropometric variables to replace creatinine-based equations in predicting GFR (35). Furthermore, in a cross-sectional study of 251 patients, composed mainly of type 2 diabetic patients (82%), a GFR estimated from cystatin C had a predictive performance towards GFR equal to commonly used creatinine-based estimates (36). On the other hand, trends in the reciprocal of cystatin C concentrations during a 4-year follow-up correlated more closely to changes in urinary iothalamate clearance than C-G and MDRD in type 2 diabetic patients with normal or increased GFR (37).

In conclusion, although multiple factors in addition to renal function may influence cystatin C, our study provides convincing evidence that cystatin C may be more useful for detecting early renal impairment in both type 1 and type 2 diabetic patients than are creatinine and commonly employed creatinine-derived formulas. These results are remarkable in light of data suggesting that cystatin C is a useful indicator of the association of mild kidney dysfunction with increased risk for cardiovascular events (38), peripheral arterial disease, heart failure (39), and death (38). Furthermore, recent studies (37, 40) suggest that very early renal failure, instead of or in addition to microalbuminuria, may be considered the early marker of the underlying progressive kidney damage associated with diabetes.

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