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Simple Cystatin C–Based Prediction Equations for Glomerular Filtration Rate Compared with the Modification of Diet in Renal Disease Prediction Equation for Adults and the Schwartz and the Counahan–Barratt Prediction Equations for Children

Anders Grubb,[1*] Ulf Nyman,[2] Jonas Björk,[3] Veronica Lindström,[1] Bengt Rippe,[4] Gunnar Sterner,[5] and Anders Christensson[5]

Background: **Serum creatinine is the most commonly used marker for estimation of glomerular filtration rate (GFR). To compensate for its drawbacks as a GFR marker, several prediction equations including several parameters are being used, with the Modification of Diet in Renal Disease (MDRD), Schwartz, and Counahan–Barratt equations being the ones most widely accepted for estimation of relative GFR in $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$. The present study analyzes whether these GFR prediction equations for adults and children might be replaced by simple prediction equations based on plasma concentrations of cystatin C.**

Methods: **Data from 536 patients (0.3–93 years), consecutively referred for determination of GFR by an invasive gold standard procedure, were used for the analysis. Calculations of bias (median percentage of error), correlation (adjusted R^2), and percentage of estimates within 30% and 50% of measured GFR were used in the comparisons.**

Results: **A cystatin C–based prediction equation using only concentration in mg/L and a prepubertal factor: GFR $[mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}]$ = 84.69 × cystatin C $(mg/L)^{-1.680}$ × 1.384 (if a child <14 years) assessed GFR equally well or better than the simplified MDRD, the Schwartz, and the Counahan–Barratt prediction equations for the adult (≥18 years) and juvenile groups of the investigated cohort. Age did not influence the cystatin C–based prediction equation for adults, whereas gender did, but with a factor close to unity (0.948 for females).**

Conclusion: **A GFR prediction equation based solely on cystatin C (in mg/L) and a prepubertal factor might replace the simplified MDRD prediction equation for adults and the Schwartz and Counahan–Barratt prediction equations for children.**

Knowledge of glomerular filtration rate (GFR)[6] is of crucial importance in the management of patients. Apart from a general evaluation of the kidney function, a more precise assessment is valuable on many occasions, e.g., to allow correct dosage of drugs cleared by the kidneys, to detect early impairment of renal function, to prevent further deterioration, to manage renal transplant patients, and for the use of potentially nephrotoxic radiographic contrast media. Determination of GFR with high accuracy requires the use of invasive techniques based on measuring the plasma clearance rate of injected substances that are exclusively excreted via glomerular filtration, e.g., inulin, ^{51}Cr-EDTA, ^{99m}Tc-diethylenetriaminepentaacetic

[1] Department of Clinical Chemistry, [3] Competence Centre for Clinical Research, and [4] Department of Nephrology, University Hospital, Lund, Sweden.
[2] Department of Radiology, Lasarettet, Trelleborg, Sweden.
[5] Department of Nephrology and Transplantation, University Hospital, Malmö, Sweden.
*Address correspondence to this author at: Department of Clinical Chemistry, University Hospital, S-22185 Lund, Sweden. Fax 46-46130064; e-mail anders.grubb@klinkem.lu.se.
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[6] Nonstandard abbreviations: GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; and CI, confidence interval.

acid, or radiographic contrast media such as ^{125}I-iothalamate and iohexol. These procedures are labor-intensive and not entirely free of risk for the patient. The plasma or serum concentrations of endogenous substances, particularly creatinine, have therefore been used as markers for GFR for more than a century. However, it has become evident that the creatinine concentration is far from ideal as a GFR marker because it is significantly influenced not only by GFR but also by factors such as muscle mass, diet, gender, age, and tubular secretion *(1–6)*. To compensate for the inadequacies of the creatinine concentration per se as a GFR marker, there have been several successful attempts at constructing GFR prediction equations including additional parameters *(5–11)*. The most widely accepted and used GFR prediction equations for adults are that proposed by Cockcroft and Gault *(7)*, which produces absolute GFR values in mL/min, and the Modification of Diet in Renal Disease (MDRD) equations, which produce relative GFR values in $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ *(8, 9)*. However, neither the MDRD nor the Cockcroft–Gault equation is suitable for estimation of GFR in children, for which other specialized prediction equations must be used, with the equations described by Schwartz et al. *(10)* or by Counahan et al. *(11)* as the most widely used. Plasma (or serum) cystatin C has been proposed as a marker for GFR *(12–15)*, and several studies using commercially available automated procedures for rapid determination of cystatin C *(16–20)*, as well as one metaanalysis *(21)*, have suggested that it is superior to serum creatinine for estimation of GFR. In the present study, we attempted to construct simple prediction equations for relative GFR values based on cystatin C mass concentration in mg/L and to compare the diagnostic performance of such equations with the performance of the simplified 4-parameter MDRD prediction equation for adults, which is based on creatinine mass concentration, age, gender, and race *(5, 6, 9)*, and with those of the Schwartz and Counahan–Barratt prediction equations for children, which are based on height and plasma creatinine *(10, 11)*.

Materials and Methods

PATIENT POPULATION AND SAMPLES

Plasma cystatin C was measured for all 536 patients (age range, 0.3–96 years; 262 females and 274 males) consecutively referred to the University Hospital of Lund for determination of GFR by iohexol clearance measurements during a period of 8 months (February 6, 2003, to October 14, 2003). At the same time, plasma creatinine as well as the additional variables required for prediction of GFR by the simplified 4-parameter MDRD, the Schwartz, or the Counahan–Barratt equations were measured. Common causes for referral of patients were manifest or suspected diabetic nephropathy, interstitial nephritis, glomerulonephritis, nephrotic syndrome, hematuria, proteinuria, reflux nephropathy, myeloma, vasculitis, consideration of initiation of hemodialysis, control after kidney transplantation, and determination of GFR in patients before start of treatment with drugs cleared by the kidneys. Table 1 shows the basic characteristics of the population studied. All procedures involving patients and data were in accordance with the Helsinki Declaration of 1975 concerning ethics principles for medical research involving human subjects. All blood samples were collected in lithium heparinate tubes (Vacutainer system; Becton Dickinson).

DETERMINATION OF GFR

GFR was determined by measurement of the plasma clearance of iohexol, a nonradioactive radiographic contrast medium commonly used for determination of GFR *(22–26)*. Each adult person was given 5 mL of iohexol solution (Omnipaque, 300 mg iodine/mL; Amersham Health AB) intravenously in an antecubital vein. Weight-adjusted doses were used for children. Clearance was calculated *(26)* from the iohexol concentration at least 4 h after the injection. Plasma iohexol concentrations were determined by HPLC *(22)*. The total CV of the method was 2.2% for a control sample with an assigned value of 32 mg/L and 1.9% for a control sample with an assigned value of 63 mg/L. These iohexol concentrations corresponded to GFR values of ~100 and 60 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$, respectively, for an adult with a typical body surface area. The plasma creatinine concentration was measured for each patient before injection of iohexol to determine whether more than 4 h had to pass before collection of samples for optimal calculation of iohexol clearance in patients with very low GFRs *(24, 25)*. GFR was expressed in relative values: $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$, using the DuBois–DuBois formula for calculation of body surface area *(27)*.

MEASUREMENT OF CREATININE AND PREDICTION OF GFR BY THE SIMPLIFIED 4-PARAMETER MDRD, THE SCHWARTZ, AND THE COUNAHAN–BARRATT EQUATIONS

Plasma concentrations of creatinine were measured by use of a creatininase enzyme-based procedure on a Hitachi Modular P analysis system. This method had a total CV of 3.0% at a creatinine concentration of 60 μmol/L and 1.4% at a creatinine concentration of 578 μmol/L. (Reference values used were 60–100 μmol/L for adult males and 50–90 μmol/L for adult females.) For each patient, the anthropometric characteristics (height, age, gender, and race) required for use of the simplified 4-parameter MDRD GFR prediction equation $\{186.3 \times [\text{serum creatinine } (\mu mol/L)/88.4]^{-1.154} \times \text{age (years)}^{-0.203} \times 0.742 \text{ (if female)} \times 1.212 \text{ (if African American)}\}$, the Schwartz GFR prediction equation $\{0.55 \times \text{height (cm)} \times [\text{plasma creatinine } (\mu mol/L)/88.4]^{-1}\}$, or the Counahan–Barratt prediction equation $\{0.43 \times \text{height (cm)} \times [\text{plasma creatinine } (\mu mol/L)/88.4]^{-1}\}$ were recorded *(5, 6, 8–11)*.

Table 1. Basic characteristics of the investigated population divided into adults (≥18 years) and children 14–17 and 0.3–13 years of age.[a]

Sex, n	Age, years	Total body weight, kg	Height, cm	Body surface area, m^2	Body mass index, kg/m^2	Plasma cystatin C, mg/L	Plasma creatinine, μmol/L	Iohexol clearance, $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$
Adults (≥18 years)								
Males (n = 226)	58 (27–83)	80 (56–118)	177 (162–190)	1.97 (1.66–2.33)	25 (18–37)	1.24 (0.80–3.44)	108 (45–470)	59 (11–127)
Females (n = 225)	58 (20–84)	66 (44–108)	164 (150–177)	1.72 (1.40–2.16)	24 (18–40)	1.14 (0.77–3.00)	78 (36–320)	69 (14–122)
Total (n = 451)	58 (24–83)	73 (46–115)	170 (151–189)	1.83 (1.45–2.31)	25 (18–39)	1.18 (0.79–3.07)	92 (39–400)	63 (11–124)
Children 14–17 years								
Males (n = 18)	15 (14–17)	59 (33–97)	171 (152–197)	1.70 (1.21–2.32)	21 (14–26)	0.98 (0.8–2.98)	70.5 (40–379)	90 (11–150)
Females (n = 14)	16 (14–17)	54 (43–99)	162 (148–177)	1.58 (1.36–2.05)	22 (16–36)	0.94 (0.75–1.40)	51 (40–101)	104 (57–133)
Total (n = 32)	16 (14–17)	58 (33–99)	167 (148–197)	1.64 (1.21–2.32)	21 (14–36)	0.95 (0.75–2.98)	56 (40–379)	98.5 (11–150)
Children 0.3–13 years								
Males (n = 30)	6.5 (0.3–13)	26 (6–81)	120 (66–182)	0.92 (0.32–2.02)	17 (15–25)	1.00 (0.77–2.23)	32.5 (14–136)	117.5 (37–240)
Females (n = 23)	9 (0.3–13)	25 (5–47)	126 (62–160)	0.94 (0.28–1.46)	16 (12–22)	1.00 (0.78–1.33)	34 (7–74)	110 (57–148)
Total (n = 53)	8 (0.3–13)	25 (5–81)	125 (62–182)	0.94 (0.28–2.02)	17 (12–25)	1.00 (0.77–2.23)	33 (7–136)	113 (37–240)

[a] Values are given as medians and 2.5th–97.5th percentiles (adults) or ranges (children).

MEASUREMENT OF CYSTATIN C

Plasma cystatin C was measured by an automated particle-enhanced immunoturbidimetric method *(16)* on a Hitachi Modular P analysis system with reagents (codes LX002, S2361, X0973, and X0974) obtained from DakoCytomation and according to the procedure recommended by the reagent producer. The total assay time is ~10 min. The procedure had a total CV of 2.1% at a cystatin C concentration of 1.0 mg/L and 1.7% at 4.0 mg/L (reference values used, 0.55–1.15 mg/L for persons 1–50 years of age and 0.63–1.44 mg/L for persons >50 years). All samples were analyzed within 1 day after collection or frozen at −20 °C until analyzed.

STATISTICAL ANALYSIS AND CONSTRUCTION OF CYSTATIN C–BASED GFR PREDICTION EQUATIONS

All statistical analyses were conducted with SPSS release 12.0.1 (SPSS Inc.). We regard $P < 0.05$ as statistically significant. Linear regression models based on log-transformed plasma clearance of iohexol [in $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$] and cystatin C (in mg/L) were built because these transformations implied regression residuals that were approximately symmetrically distributed with homogeneous variance. We established cystatin C–based prediction equations both with and without adjustment for gender; we also investigated the effect of age. We evaluated the cystatin C–based prediction equations and the simplified MDRD, Schwartz, and the Counahan–Barratt GFR prediction equations with respect to bias, correlation, and absolute percentage error *(28)*. When evaluating the simplified MDRD, Schwartz, and Counahan–Barratt GFR prediction equations, we established regression models between predicted and measured GFR on the log scale. Bias, defined as systematic deviation between the predicted and measured GFR, was determined as the median percentage error, i.e., the median of the differences between predicted and measured GFR in percentage of measured GFR. When appropriate, we also assessed the bias by fitting a regression model without intercept between predicted and measured GFR values on the log scale and testing whether the obtained slope coefficient differed significantly from unity. Correlation between predicted and measured GFR was expressed as adjusted R^2. We assessed the differences in adjusted R^2 values between the cystatin C–based prediction equations and the simplified MDRD, Schwartz, and Counahan–Barratt GFR prediction equations by comparing the squared unstandardized regression residuals, using the Wilcoxon signed-rank test. We measured the absolute percentage error, i.e., the absolute difference between predicted and measured GFRs, as a percentage of measured GFR, and used the McNemar test for correlated proportions to test systematic differences between the prediction equations, focusing on the proportion of the predicted GFR that differed no more than 30% and 50%, respectively, from the measured GFR. We also evaluated the diagnostic performance of the cystatin C–based and the MDRD

prediction equations by calculating their sensitivities and specificities in predicting GFR <60 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$. In addition, we calculated and compared *(29)* the areas under the ROC curves as overall measures of the abilities of plasma cystatin C and plasma creatinine to distinguish between GFR values above and below 60 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$.

To assess the diagnostic performance of the cystatin C–based prediction equation for all ages without gender as a factor, GFR $[mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}] = 84.69 \times$ cystatin C $(mg/L)^{-1.680} \times 1.384$ (if a child <14 years), beyond the patient set for which it was established, we used a cross-validation procedure *(30)* in which bias, correlation, and the absolute percentage error, as defined above, were assessed in all 100 randomly chosen test sets: (*a*) the data set was randomly split into 10 groups of approximately equal size; (*b*) the parameters of the cystatin C–based prediction equation were estimated while excluding group k ($k = 1, 2, \ldots, 10$) from the analyses; and (*c*) the group left out from parameter estimation was used as a test set. We repeated steps *a* through *c* above 10 times, which generated $10 \times 10 = 100$ sets of test sets, thus obtaining 100 estimates of bias, correlation, and absolute percentage error.

Results

The various MDRD prediction equations *(8, 9)* and the plasma cystatin C concentration *(13–21)* can be used for noninvasive estimation of the GFR of a patient. In an effort to compare these ways of estimating GFR, we determined plasma cystatin C and the values required for the simplified 4-parameter MDRD prediction equation (creatinine mass concentration, age, gender, and race) for all 536 patients consecutively referred to the hospital for determination of GFR by measurement of plasma clearance of iohexol during a period of 8 months. The basic characteristics of the population are displayed in Table 1. Because it turned out that the investigated population did not contain any African-American individuals, only creatinine mass concentration, age, and gender had to be used for application of the simplified MDRD prediction equation.

Cystatin C–based prediction equations for adults

Because the simplified MDRD prediction equation, in contrast to plasma cystatin C, directly produces an estimated GFR value [in $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$], GFR prediction equations based on plasma cystatin C had to be constructed to allow comparisons. To do so, we built linear regression models based on log-transformed plasma clearance of iohexol [in $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$] and cystatin C concentrations (in mg/L) because these transformations produced roughly symmetrically distributed regression residuals with homogeneous variance. Furthermore, because the MDRD prediction equation is based on data of an adult population (≥18 years), cystatin C prediction equations were initially constructed for only this cohort (451 patients) of the patient population investigated.

One cystatin C–based GFR prediction equation was constructed for all adult patients disregarding gender, and one with adjustment for gender. Gender turned out to be a statistically significant factor ($P = 0.017$), in contrast to age ($P = 0.119$). These efforts produced the following 2 equations:

$$\text{GFR}\ [mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}] = 83.93\ (81.70–86.23) \times \text{cystatin C}\ (mg/L)^{-1.676\ (-1.737\ \text{to}\ -1.615)}$$

where the values in parentheses are the 95% confidence intervals (95% CIs), and:

$$\text{GFR}\ [mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}] = 86.49\ (83.43–89.66) \times \text{cystatin C}\ (mg/L)^{-1.686\ (-1.747\ \text{to}\ -1.625)}$$

$$[\times 0.948\ (0.908–0.990)\ \text{if female}]$$

We then compared the diagnostic performance of the 2 cystatin C–based prediction equations with that of the simplified MDRD prediction equation. The MDRD prediction equation produced a statistically significant overestimation of GFR (slope coefficient, 0.97; 95% CI, 0.96–0.97), in contrast to the cystatin C–based prediction equations (Table 2). The correlations (measured as adjusted R^2 values) between the GFR estimates obtained by the cystatin C–based equations with or without gender and measured GFR did not differ significantly from that between GFR predicted by the MDRD equation and measured GFR ($P > 0.30$ in both cases). The proportions of patients with GFR estimates within 30% and 50% of measured GFR (Table 2) were significantly higher for the cystatin C–based equation without gender than the proportions obtained with the MDRD equation ($P = 0.001$ for 30% and $P < 0.001$ for 50%). We found a similar significant difference for the cystatin C–based equation with gender in comparison with the MDRD equation ($P < 0.001$ for both intervals). The individual percentage errors in GFR predicted by the cystatin C–based equation without gender and by the MDRD equation are shown in Fig. 1, A and C. However, the simplified MDRD equation was developed using creatinine concentrations measured with a modified Jaffe method *(8, 9)*, whereas the present study relied on creatinine concentrations measured by an enzyme-based method, which gave lower creatinine values and thus higher GFR estimates predicted by the simplified MDRD equation. This general overestimation also leads to a decrease in the proportion of GFR estimates within 30% and 50% of measured GFR produced by the MDRD equation. No reliable transformation of creatinine values obtained with the enzyme-based procedures into values produced by modified Jaffe procedures is known because modified Jaffe procedures determine pseudo-creatinine chromogens in addition to creatinine and because the amounts of pseudo-creatinine chromogens vary

Table 2. Bias, correlation, and percentage error of prediction equations to estimate relative GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] in adults (≥ 18 years).

Prediction equations to estimate relative GFR in mL · min^{-1} · (1.73 m^2)$^{-1}$	Bias, median percentage error	Correlation (adjusted R^2), %	Percentages of estimates	
			Within 30% of measured GFR	Within 50% of measured GFR
Cystatin C equations using				
Patients ≥18 years and				
GFR = 83.93 × cystatin C$^{-1.676}$				
All adults (n = 451)	1.95[a]	86.7	80.0	96.0
Males (n = 226)	−0.90		81.9	96.0
Females (n = 225)	4.35		78.2	96.0
GFR = 86.49 × cystatin C$^{-1.686}$ × 0.948 (if female)				
All adults (n = 451)	1.91[a]	86.8	82.3	96.7
Males (n = 226)	1.82		81.9	96.0
Females (n = 225)	1.97		82.7	97.3
All patients 0.3–93 years and juvenile factor[b]				
All adults (n = 451)	2.85	86.7	80.0	95.3
Males (n = 226)	−0.19		81.9	96.0
Females (n = 225)	5.25		78.2	94.7
All patients 0.3–93 years and gender and juvenile factors[c]				
All adults (n = 451)	2.57	86.8	81.8	96.5
Males (n = 226)	2.99		81.0	95.6
Females (n = 225)	2.48		82.7	97.3
Simplified MDRD[d]				
All adults (n = 451)	11.8	84.6	70.7	88.0
Males (n = 226)	13.3		69.5	86.7
Females (n = 225)	11.3		72.0	89.3
Simplified MDRD using mathematical bias correction[e]				
All adults (n = 451)	0.02[a]	84.6	79.2	93.1
Males (n = 226)	1.33		79.2	90.3
Females (n = 225)	−0.45		79.1	96.0

[a] Zero median percentage error expected for this regression model because it was fitted based on all adults.
[b] GFR = 84.69 × cystatin C$^{-1.680}$ × 1.384 (if child <14 years).
[c] GFR = 87.62 × cystatin C$^{-1.693}$ × 1.376 (if child <14 years) × 0.940 (if female).
[d] GFR = 186.3 × [creatinine (μmol/L)/88.4]$^{-1.154}$ × age (years)$^{-0.203}$ × 0.742 (if female) × 1.212 (if African American).
[e] GFR = (1/1.118) × 186.3 × [creatinine (μmol/L)/88.4]$^{-1.154}$ × age (years)$^{-0.203}$ × 0.742 (if female) × 1.212 (if African American).

among samples. We therefore used a simple mathematic procedure to remove the general overestimation of GFR produced by the MDRD equation, using the creatinine values obtained by the enzyme-based procedure for this work. The transformation was done by multiplying all GFR values predicted by the MDRD equation, using a factor (1/1.118) based on the median bias (+11.8%) of the MDRD prediction equation (Table 2). The diagnostic performance of the MDRD prediction equation, obtained with this mathematic transformation, was then evaluated, and this procedure to remove bias increased the proportions of patients with MDRD-generated GFR estimates within 30% and 50% of measured GFR to percentages that were almost as high as those obtained with the 1-parameter cystatin C–based prediction equation (Table 2).

When we used the cystatin C–based prediction equation derived for the adult population, with gender included, to identify individuals in the adult population with GFR <60 mL · min^{-1} · (1.73 m^2)$^{-1}$, we obtained a sensitivity of 88.0% and a specificity of 90.6%. Similar sensitivity–specificity pairs were observed for the other cystatin C–based prediction equations in Table 2. The simplified MDRD equation yielded a lower sensitivity (81.5%) but a higher specificity (96.2%). The mathematically bias-corrected MDRD equation produced a sensitivity–specificity pair (sensitivity, 89.4%; specificity, 87.7%) not markedly different from that of the cystatin C–based equations. It should be noted, however, that the cystatin C–based and the MDRD prediction equations are constructed for optimizing the prediction of GFR at all GFR values and are not tailor-made for predicting whether the GFR of a patient is below a specific value, e.g., 60 mL · min^{-1} · (1.73 m^2)$^{-1}$. If plasma cystatin C and creatinine concentrations are to be compared concerning their capacities to identify individuals with GFR values below a certain diagnostically important cutoff, e.g., 60 mL · min^{-1} · (1.73 m^2)$^{-1}$, analysis of ROC curves might be more helpful than comparing general prediction equations. We therefore calculated and compared the areas under ROC curves as overall measures of the capacities of

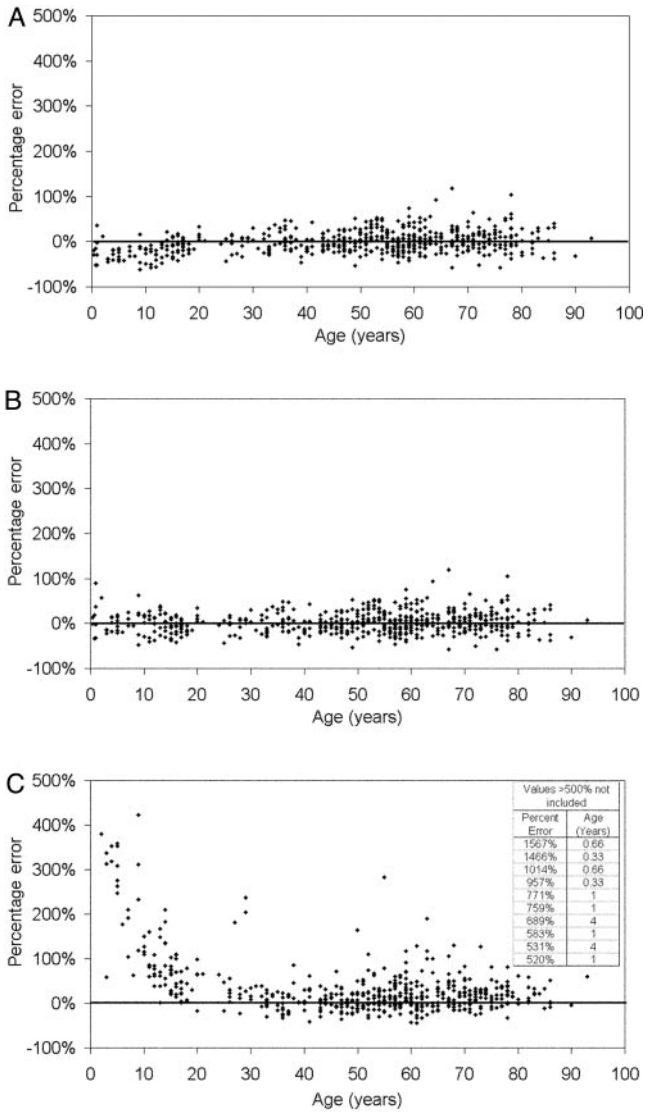

Fig. 1. Relationship between age and percentage error in predicted GFR.

(*A*). *percentage error* refers to the difference between GFR predicted by the equation GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = 83.93 × cystatin C (mg/L)$^{-1.676}$ and measured GFR, expressed as a percentage of measured GFR. (*B*), *percentage error* refers to the difference between GFR predicted by the equation GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = 84.69 × cystatin C (mg/L)$^{-1.680}$ × 1.384 (if child <14 years) and measured GFR, expressed as a percentage of measured GFR. (*C*), *percentage error* refers to the difference between GFR predicted by the simplified MDRD equation {GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = 186.3 × [serum creatinine (μmol/L)/88.4]$^{-1.154}$ × age (years)$^{-0.203}$ × 0.742 (if female) × 1.212 (if African American)} and measured GFR, expressed as a percentage of measured GFR.

plasma cystatin C and creatinine to distinguish between GFR values above and below 60 mL · min^{-1} · (1.73 m^2)$^{-1}$. The area under the curve for cystatin C (0.966) was significantly (P = 0.01) larger than that for creatinine (0.943). Gender contributed only marginally to the diagnostic performance of cystatin C–based prediction equations, which agrees with the observation that the sensitivity–specificity pairs generated by the cystatin C–based prediction equations when used for identifying GFR <60 mL · min^{-1} · (1.73 m^2)$^{-1}$ approximately corresponded to sensitivity–specificity pairs of the ROC curve for cystatin C.

CYSTATIN C–BASED PREDICTION EQUATIONS FOR ALL AGES

Serum and plasma concentrations of cystatin C have been described, in contrast to those of creatinine, to be virtually unaltered between 1 and 50 years of age *(31–33)*. Thus, from a theoretical point of view, it might be possible to use the cystatin C–based GFR prediction equations derived for adults for patients <18 years of age. In an effort to test this hypothesis, we used the cystatin C–based prediction equation for adults, without gender, to estimate GFR of all patients <18 years of age and studied the variation with age of the difference between predicted and measured GFR (Fig. 1A). The predicted GFR for children >13 years did not seem to deviate more from the measured GFR than did the predicted GFR for adults (Fig. 1A). Statistical analysis demonstrated that the percentages of predicted GFR within 30% and 50% of measured GFR for children 14–17 years of age did not differ significantly from those determined for adults (Table 2): the percentage of predicted GFR values within 30% was 81% (95% CI, 64%–93%), and the percentage within 50% was 100% (95% CI, 89%–100%).

When we used the cystatin C–based prediction equation for adults, without gender, to estimate the GFR of patients <14 years, however, we observed a systematic difference between predicted and measured GFR values (Fig. 1A). The difference, an underestimation of GFR of ~30%, seemed to be virtually constant for all ages <14 years (Fig. 1A). Statistical analysis showed no correlation between age and the difference between predicted and measured GFR (r = −0.05) for this age group, supporting the notion that age does not influence the difference between predicted and measured GFR for children <14 years. These observations prompted the construction of cystatin C–based GFR prediction equations containing a juvenile factor for children <14 years and using the data of the whole investigated population (536 persons; age range, 0.3–93 years). Two prediction equations for the entire age-span were thus constructed, one without and one with a gender factor:

$$\text{GFR [mL} \cdot \text{min}^{-1} \cdot (1.73\ \text{m}^2)^{-1}] = 84.69\ (82.52\text{–}86.83) \times \text{cystatin C (mg/L)}^{-1.680\ (-1.737\ \text{to}\ -1.622)}$$

$$[\times 1.384\ (1.292\text{–}1.483)\ \text{if child} < 14\ \text{years}]$$

and

$$\text{GFR [mL} \cdot \text{min}^{-1} \cdot (1.73\ \text{m}^2)^{-1}] = 87.62\ (84.69\text{–}90.56) \times \text{cystatin C (mg/L)}^{-1.693\ (-1.751\ \text{to}\ -1.635)}$$

$$[\times 1.376\ (1.284\text{–}1.473)\ \text{if child} < 14\ \text{years}]$$

$$[\times 0.940\ (0.902\text{–}0.979)\ \text{if female}]$$

The diagnostic performance of these 2 new cystatin C–based prediction equations for all ages compared with that of the MDRD equation for the prediction of GFR in adults is shown in Table 2 and in Fig. 1, B and C. As was the case for the cystatin C–based prediction equations constructed for adults only, the new equations did not overestimate GFR, in contrast to the MDRD prediction equation (Table 2). The proportions of patients with GFR estimates within 30% and 50% of measured GFR (Table 2) were significantly higher for the 2 new cystatin C–based equations for all ages (without and with gender factor) than the proportions obtained with the MDRD equation ($P = 0.001$ for GFR estimates within 30% and $P < 0.001$ for GFR estimates within 50%, irrespective of whether the gender factor was used in the cystatin C–based equation).

The diagnostic performance of the 2 new cystatin C–based prediction equations for all ages compared with those of the Schwartz and of Counahan–Barratt prediction equations for determining GFR in children is shown in Table 3 and Fig. 2. The Schwartz and Counahan–Barratt prediction equations produced overestimations of GFR [slope coefficients = 0.92 (95% CI, 0.90–0.93) and 0.96 (95% CI 0.95–0.98), respectively] in contrast to the cystatin C–based prediction equations for all ages (Table 3). The correlations (measured as adjusted R^2 values) between the GFR estimates obtained by the cystatin C–based equations for all ages and measured GFR did not differ significantly from those between GFR values predicted by either the Schwartz ($P > 0.30$ without gender and $P = 0.24$ with gender) or the Counahan–Barratt equation ($P > 0.30$ without gender and $P = 0.24$ with gender) and measured GFR. The proportions of patients with GFR estimates within 30% and 50% of measured GFR (Table 3) were higher for the 2 new cystatin C–based equations for all ages than the proportions obtained with the Schwartz (without and with gender, $P < 0.001$ for both intervals) and Counahan–Barratt (without gender, $P = 0.053$ for $\pm 30\%$ and $P = 0.001$ for $\pm 50\%$; with gender, $P = 0.006$ for $\pm 30\%$ and $P = 0.001$ for $\pm 50\%$) prediction equations. The individual percentage errors in GFR for persons <18 years predicted by the cystatin C–based equation for all ages without gender factor and by the Schwartz and Counahan–Barratt prediction equations are shown in Fig. 2. The simplified MDRD prediction equation is based on data from an adult population (≥18 years) and is not recommended for estimating the GFR of children *(8, 9)*. As shown in Fig. 1C, which displays the variation with age of the difference between MDRD equation–predicted GFR and measured GFR, the relationship for persons <18 years was complex.

DIAGNOSTIC PERFORMANCE OF THE CYSTATIN C–BASED PREDICTION EQUATIONS FOR ALL AGES IN THE STUDY POPULATION (0.3–93 YEARS)

The diagnostic performance of the 2 cystatin C–based prediction equations for all ages for the whole population studied, 536 persons (age range, 0.3–93 years), is given in Table 4. The variation with age of the difference between measured GFR and GFR predicted by the new cystatin

Table 3. Bias, correlation, and percentage error of prediction equations to estimate relative GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] in children (<18 years).

Prediction equations to estimate relative GFR in mL · min^{-1} · (1.73 m^2)$^{-1}$	Bias, median percentage error	Correlation (adjusted R^2), %	Percentages of estimates	
			Within 30% of measured GFR	Within 50% of measured GFR
Cystatin C equations using				
All patients, juvenile factor[a]				
All children (n = 85)	−1.95	80.5	77.6	96.5
Males (n = 48)	−5.17		72.9	97.9
Females (n = 37)	9.22		83.8	94.6
All patients, juvenile and gender factors[b]				
All children (n = 85)	−0.60	81.1	82.4	96.5
Males (n = 48)	−1.82		79.2	97.9
Females (n = 37)	5.83		86.5	94.6
Schwartz equation[c]				
All children (n = 85)	50.9	76.1	24.7	49.4
Males (n = 48)	46.0		31.3	58.3
Females (n = 37)	59.2		16.2	37.8
Counahan–Barratt equation[d]				
All children (n = 85)	18.0	76.1	62.4	80.0
Males (n = 48)	14.1		66.7	83.3
Females (n = 37)	24.4		56.8	75.7

[a] GFR = 84.69 × cystatin C$^{-1.680}$ × 1.384 (if child <14 years).
[b] GFR = 87.62 × cystatin C$^{-1.693}$ × 1.376 (if child <14 years) × 0.940 (if female).
[c] GFR = 0.55 × height (cm) × [plasma creatinine (μmol/L)/88.4]$^{-1}$.
[d] GFR = 0.43 × height (cm) × [plasma creatinine (μmol/L)/88.4]$^{-1}$.

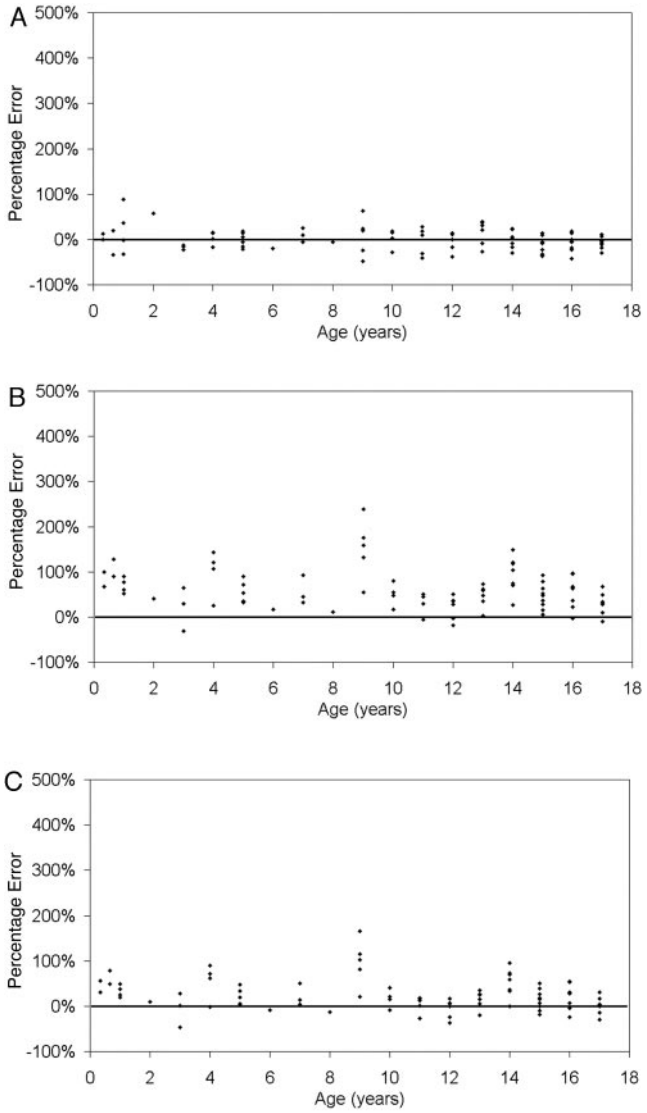

Fig. 2. Relationship between age and percentage error in predicted GFR.

(*A*), *percentage error* refers to the difference between GFR predicted by the equation GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = 84.69 × cystatin C (mg/L)$^{-1.680}$ × 1.384 (if child <14 years) and measured GFR, expressed as a percentage of measured GFR. (*B*), *percentage error* refers to the difference between GFR predicted by the Schwartz equation {GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = 0.55 × height (cm) × [plasma creatinine (μmol/L)/88.4]$^{-1}$} and measured GFR, expressed as a percentage of measured GFR. (*C*), *percentage error* refers to the difference between GFR predicted by the Counahan–Barratt equation {GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = 0.43 × height (cm) × [plasma creatinine (μmol/L)/88.4]$^{-1}$} and measured GFR, expressed as a percentage of measured GFR.

C–based equation for all ages without gender factor is shown in Fig. 1B.

CROSS-VALIDATION

Cross-validating the cystatin C–based GFR prediction equation for all ages, with a juvenile factor but without a gender factor in the equation, did not lead to great fluctuations of the diagnostic performance of this equation. The median percentage error in the 100 test sets was 2.22% (2.5th–97.5th percentiles, −6.37% to 7.69%), and the median adjusted correlation (R^2) was 87.0% (79.3%–92.4%). The median proportions of the estimates within 30% and 50% of measured GFR among the 100 test sets were 81.1% (69.8%–89.7%) and 96.0% (89.0%–100%), respectively.

PERCENTAGE ERROR OF PREDICTED GFR AS A FUNCTION OF MEASURED GFR

The difference between measured GFR in adults and GFR predicted by the 1-parameter cystatin C–based equation for adults without a gender factor or by the mathematically bias-corrected MDRD equation as functions of measured GFR are shown in Fig. 3. The 1-parameter cystatin C–based prediction equation had a tendency to overestimate the GFR of patients with a measured GFR <30 mL · min^{-1} · (1.73 m^2)$^{-1}$, and the median overestimation was 3 mL · min^{-1} · (1.73 m^2)$^{-1}$ in this GFR interval. The MDRD prediction equation also had a tendency for increased overestimation of GFR in this GFR interval.

Discussion

To reduce the drawbacks associated with the use of serum creatinine as a GFR marker, at least 14 GFR prediction equations have been developed that include several parameters in addition to the creatinine concentration *(5–11)*. Careful studies *(5, 6)* have indicated that the prediction equations for adults proposed by Cockcroft and Gault *(7)* (giving absolute GFR values in mL/min) and by Levey et al. *(8, 9)* [giving relative GFR values in mL · min^{-1} · (1.73 m^2)$^{-1}$] are particularly useful, and these equations are also the most widely accepted and used. The prediction equations for children (<18 years) proposed by Schwartz et al. *(10)* and by Counahan et al. *(11)* are also generally accepted and widely used *(5, 6)*. The authors of several studies have suggested that plasma cystatin C might be used as a GFR marker, citing several advantages to the use of cystatin C compared with serum creatinine *(12–21)*; however, to our knowledge, no studies suggesting prediction equations for relative GFR based on cystatin C and comparing the diagnostic performance of such equations with the performance of the equations proposed by Levey et al. *(8, 9)*, Schwartz et al. *(10)*, and Counahan et al. *(11)* have been published. We therefore measured plasma cystatin C for all 536 patients consecutively referred for determination of GFR by measuring the plasma clearance of iohexol during a period of 8 months. These data were used in an effort to produce such cystatin C–based prediction equations and to compare their diagnostic performance with the performance of the simplified 4-parameter MDRD prediction equation for adults *(9)* and the Schwartz *(10)* and Counahan–Barratt *(11)* prediction equations for children <18 years. We chose the simplified 4-parameter MDRD prediction equation for adults for the comparisons because careful studies have shown that it displays diagnostic performance as good as the performance of the more complicated MDRD equations using more variables *(5, 6)*. The initial efforts to produce cystatin C–based prediction equations for rela-

Table 4. Bias, correlation, and percentage error of cystatin C-based prediction equations to estimate relative GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] in all patients investigated (children and adults, 0.3–93 years).

Prediction equations to estimate relative GFR in mL · min^{-1} · (1.73 m^2)$^{-1}$	Bias, median percentage error	Correlation (adjusted R^2), %	Percentages of estimates	
			Within 30% of measured GFR	Within 50% of measured GFR
Cystatin C equations using				
All patients 0.3–93 years, juvenile factor[a]				
All patients (n = 536)	1.97[b]	87.2	79.7	95.5
Children 0.3–13 years (n = 53)				
Children 14–17 years (n = 32)				
Males (n = 274)	−1.26		80.3	96.4
Females (n = 262)	5.52		79.0	94.7
All patients 0.3–93 years, gender and juvenile factors[c]				
All patients (n = 536)	1.80[b]	87.4	81.9	96.5
Children 0.3–13 years (n = 53)				
Children 14–17 years (n = 32)				
Males (n = 274)	1.55		80.7	96.0
Females (n = 262)	2.63		83.2	96.9

[a] GFR = 84.69 × cystatin C$^{-1.680}$ × 1.384 (if child <14 years).
[b] Zero median percentage error expected for this regression model because it was fitted based on all patients.
[c] GFR = 87.62 × cystatin C$^{-1.693}$ × 1.376 (if child <14 years) × 0.940 (if female).

tive GFR used only the adult (≥18 years) persons of the population studied because the diagnostic performance of the cystatin C–based prediction equations was to be compared with that of the MDRD prediction equation for adults. We constructed a single prediction equation for all adult patients, using only the cystatin C concentration as the independent variable, because statistical analysis demonstrated that age did not improve prediction performance and that the statistically significant gender factor (0.948 for females) was close to unity.

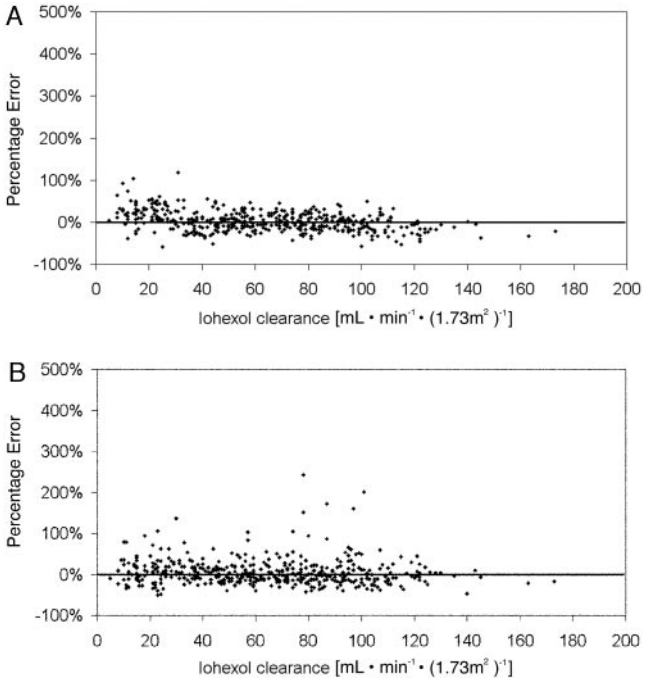

Fig. 3. Percentage error in predicted GFR of adults as a function of measured GFR.

(*A*), *percentage error* refers to the difference between GFR predicted by the equation GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = 83.93 × cystatin C (mg/L)$^{-1.676}$ and measured GFR, expressed as a percentage of measured GFR. (*B*), *percentage error* refers to the difference between GFR predicted by the mathematically bias-corrected simplified MDRD equation {GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = (1/1.118) × 186.3 × [serum creatinine (μmol/L)/88.4]$^{-1.154}$ × age (years)$^{-0.203}$ × 0.742 (if female) × 1.212 (if African American)} and measured GFR, expressed as a percentage of measured GFR.

The resulting 1-parameter cystatin C–based prediction equation, GFR [mL · min^{-1} · (1.73 m^2)$^{-1}$] = 83.93 × cystatin C (mg/L)$^{-1.676}$, placed a significantly higher proportion of patients with GFR estimates within 30% and 50% of the measured GFR than did the simplified MDRD equation (Table 2), although the correlations between the GFR estimates obtained by the 2 prediction equations and measured GFR did not differ significantly (Table 2). The MDRD equation overestimated GFR significantly, in contrast to the cystatin C–based equation (Table 2), but the simplified MDRD equation was developed with creatinine concentrations measured by a modified Jaffe method *(8, 9)*, whereas the present study relied on creatinine concentrations measured by an enzyme-based method, which gives lower creatinine values and thus higher GFR estimates predicted by the simplified MDRD equation. Use of a simple mathematical procedure to remove this general overestimation of GFR produced by the MDRD equation increased the proportions of patients with MDRD-generated GFR estimates within 30% and 50% of measured GFR to values that were almost as high as those obtained with the 1-parameter cystatin C–based prediction equation. Although the improvement in diagnostic performance observed in the present study for the 1-parameter cystatin C–based prediction equation compared with that of the MDRD-equation thus cannot be considered as very large, an interesting observation is that the

cystatin C–based prediction equation requires just 1 variable, the cystatin C concentration, to achieve diagnostic performance at least as good as the MDRD equation based on 4 variables.

It has been clearly demonstrated that the selection of persons used for constructing prediction equations for GFR significantly influences the prediction equations obtained *(34)*. The MDRD equation was derived from data from a cohort of persons with chronic kidney disease and did not include healthy persons *(34)*. This might impair its diagnostic performance for populations including a high proportion of healthy persons, e.g., the population studied in the present investigation.

The MDRD prediction equations for relative GFR in $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ and the Cockcroft–Gault prediction equation for absolute GFR in mL/min were established for adult populations ≥18 years and are considered less suitable for estimation of GFR in children *(5–9)*. However, because the plasma cystatin C concentration, in contrast to that of serum creatinine, has been described as virtually constant among healthy individuals 1–50 years of age *(31–33)*, we used the 1-parameter cystatin C prediction equation, without gender factor and based solely on data from adults, for prediction of GFR of all 536 patients (0.3–93 years of age) investigated in the present study. We then studied the variation with age of the difference between predicted and measured GFR (Fig. 1A) and found that the diagnostic performance of the prediction equation did not differ for adults (≥18 years) and for children 14–17 years of age; however, the prediction equation produced a consistent underestimation of ~30% of the measured GFR for children <14 years (Fig. 1A). On the other hand, the difference between predicted and measured GFR for children 0.3–13 years of age did not vary with age, agreeing with the situation for the adult population. This observation allowed a simple modification of the cystatin C–based prediction equation to permit its use in all age groups by use of data from all 536 patients and addition of a juvenile constant of 1.384 for children <14 years. Interestingly, the diagnostic performance for adults ≥18 years of this new cystatin C–based prediction equation for use in all age groups was not significantly different from that of the 1-parameter cystatin C–based prediction equation constructed with data solely from the adult cohort (Table 2). Introduction of a gender factor into the cystatin C–based prediction equation for use in all age groups only marginally improved its diagnostic performance, agreeing with the situation for the cystatin C–based prediction equation based solely on data from adults. We do not know why the cystatin C prediction equation based solely on data from adults consistently underestimated GFR by ~30% compared with the measured values for children <14 years, but one might speculate that the production of cystatin C per 1.73 m^2 of body surface area differs between prepubertal and older individuals, being higher in prepurbetal persons. It is probably not a coincidence that the improvement in diagnostic performance achieved by the use of a juvenile factor seem to be maximal if the factor was applied for children <14 years of age rather than for children <18 years of age. The 13–14 years limit represents the start of puberty, a significant biological event, whereas the 17–18 years limit does not represent such a clear biological event. It cannot be excluded that use of the 17–18 years age limit in many connections is associated with its legal status rather than with its biological significance. It might thus be suitable to specify the biological context of the juvenile factor in the cystatin C–based prediction equation by calling it a prepubertal factor. Another possible explanation for the requirement of a prepubertal factor to achieve optimal diagnostic performance of cystatin C–based prediction equations for children might be that the DuBois–DuBois equation *(27)*, used to calculate body surface area in the present study, is based only on data for adult individuals and thus might overestimate the body surface area of prepubertal children. However, recalculation of the body surface area for patients <14 years in the present study, using the equation proposed by Haycock et al. *(35)*, validated for use in children, rather than the DuBois–DuBois equation did not change the extent of underestimation of GFR in children by cystatin C–based prediction equations without a prepubertal factor.

It should be observed that, although GFR was determined for some 0.3-year-old children in the present investigation, still younger children have been reported to have higher plasma concentrations of cystatin C *(32)*. The cystatin C–based prediction equation for all ages, constructed with data for patients 0.3–93 years of age in the present study, should therefore be applied with great caution for the estimation of GFR in children younger than 0.3 years.

In an effort to evaluate the diagnostic performance of the simplified MDRD prediction equation for the children included in the present study, we investigated the variation with age of the difference between measured GFR and GFR predicted by the MDRD equation (Fig. 1C). As expected *(5, 6, 8, 9)*, the MDRD equation was not ideal for prediction of the relative GFR in children, considering the complex relationship between age and the difference between predicted and measured GFR for children <18 years and the large deviations observed for several children (Fig. 1C). However, several other creatinine-based prediction equations have been suggested for children <18 years, such as those of Schwartz et al. *(10)* and Counahan et al. *(11)*, which are among the most widely used and recommended equations *(5, 6)*. We compared the diagnostic performance of these prediction equations for the 85 children in the present patient cohort with the performance of the 2 cystatin C prediction equations for all ages constructed with data for all 536 persons in the studied cohort. The prediction equations of Schwartz et al. *(10)* and Counahan et al. *(11)* overestimated GFR, in contrast to the cystatin C–based prediction equations, and

the proportions of patients with GFR estimates within 30% and 50% of measured GFR were higher for the cystatin C–based equations (Table 3). It should be noted, however, that the prediction equations of Schwartz et al. *(10)* and Counahan et al. *(11)*, like the simplified MDRD equation, were developed with creatinine concentrations measured by modified Jaffe methods, whereas in the present investigation, we relied on creatinine concentrations measured by an enzyme-based method, which gave lower creatinine values and thus produced higher GFR estimates predicted by the Schwartz and Counahan–Barratt equations. Although this might have caused at least part of the overestimation of GFR by these prediction equations in the present study as well as accounting for a portion of the decreases in the proportions of GFR estimates within 30% and 50% of measured GFR, it is not likely that this difference in measured creatinine values can fully account for the considerable differences in diagnostic performance observed. A study by Filler and Lepage *(36)* also suggested that the Schwartz prediction equation overestimates GFR and that cystatin C–based prediction equations might be more suitable for children. Filler and Lepage *(36)* and Bokenkamp et al. *(31)* have also suggested cystatin C–based prediction equations for GFR in children.

Several commercial systems for measuring serum and plasma cystatin C are available *(16, 37)*. The use of different assay systems and calibrators has probably contributed to the differences in the reference values reported for plasma cystatin C *(38–40)*, which could also cause problems when cystatin C–based prediction equations for GFR are to be used because the different assay systems will have to use slightly different prediction equations to achieve maximal diagnostic performance. Availability of an international calibrator for cystatin C might reduce such problems, and the IFCC is endorsing the establishment of a working group for the production of such a calibrator.

Although the results of the present investigation suggest that simple cystatin C–based prediction equations for all ages might offer advantages compared with creatinine-based prediction equations, it should be emphasized that cystatin C–based prediction equations cannot replace the use of gold standard procedures for determination of GFR because the diagnostic performance of cystatin C–based prediction equations is not perfect, particularly in some clinical situations, e.g., patients treated with large doses of corticosteroids *(41)* or patients with thyroid dysfunction *(42)*, and also because the diagnostic performance of cystatin C–based equations has not been tested in all relevant clinical situations. However, the use of cystatin C–based prediction equations may reduce the need to perform invasive determinations of GFR and may allow a more precise selection of patients requiring such gold standard procedures.

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