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Generation of a New Cystatin C–Based Estimating Equation for Glomerular Filtration Rate by Use of 7 Assays Standardized to the International Calibrator

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BACKGROUND: Many different cystatin C–based equations exist for estimating glomerular filtration rate. Major reasons for this are the previous lack of an international cystatin C calibrator and the nonequivalence of results from different cystatin C assays.

METHODS: Use of the recently introduced certified reference material, ERM-DA471/IFCC, and further work to achieve high agreement and equivalence of 7 commercially available cystatin C assays allowed a substantial decrease of the CV of the assays, as defined by their performance in an external quality assessment for clinical laboratory investigations. By use of 2 of these assays and a population of 4690 subjects, with large subpopulations of children and Asian and Caucasian adults, with their GFR determined by either renal or plasma inulin clearance or plasma iohexol clearance, we attempted to produce a virtually assay-independent simple cystatin C–based equation for estimation of GFR.

RESULTS: We developed a simple cystatin C–based equation for estimation of GFR comprising only 2 variables, cystatin C concentration and age. No terms for race and sex are required for optimal diagnostic performance. The equation,

$$\text{eGFR} = 130 \times \text{cystatin C}^{-1.069} \times \text{age}^{-0.117} - 7,$$

is also biologically oriented, with 1 term for the theoretical renal clearance of small molecules and 1 constant for extrarenal clearance of cystatin C.

CONCLUSIONS: A virtually assay-independent simple cystatin C–based and biologically oriented equation for estimation of GFR, without terms for sex and race, was produced.

Knowledge of glomerular filtration rate (GFR)[18] is essential for the detection and monitoring of impairment of renal function, for safety in the use of potentially nephrotoxic pharmaceuticals and radiographic contrast media, and for administration of correct dosage of medicines cleared by the kidneys. Measurement of GFR in humans requires invasive techniques, and the plasma/serum concentration of endogenous substances, particularly creatinine, has therefore been used as marker for GFR for almost a century *(1)*. It has become evident, however, that the circulating creatinine concentration alone is far

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[18] Nonstandard abbreviations: GFR, glomerular filtration rate; eGFR, estimated GFR; JRC-IRMM, Joint Research Centre Institute for Reference Materials and Measurements; WG-SCC, Working Group for Production of an International Cystatin C Calibrator; CAPA, Caucasian, Asian, pediatric, and adult cohorts; LCS, Lund cystatin C standardization cohort; mGFR, measured GFR; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; P30, percentage of estimates within 30%; BMI, body mass index.

from ideal as a GFR marker because it is considerably influenced not only by GFR, but also by other factors including muscle mass, amount of ingested meat, and tubular secretion *(2–8)*. Creatinine-based GFR-estimating ($eGFR_{creatinine}$) equations that include demographic and anthropometric factors *(9–11)* can partially compensate for the influence of muscle mass.

Cystatin C was suggested as an alternative marker for GFR in 1979 *(12–14)*, and since the introduction of the first automated procedure for analysis of cystatin C in 1994 *(15)*, its use as marker for GFR has been extensively investigated. Circulating cystatin C concentrations are not influenced by ingestion of meat *(16, 17)* or tubular secretion, and the influence of muscle mass on cystatin C concentrations is substantially smaller than its influence on creatinine concentrations *(18–23)*. Cystatin C–based GFR-estimating ($eGFR_{cystatin\ C}$) equations, with simple formulations, therefore can often replace more complex $eGFR_{creatinine}$ equations, with vague and ill-defined anthropometric variables such as race *(2)*. Several $eGFR_{cystatin\ C}$ equations have been suggested. The main reason for these multiple equations has been the use of differing calibrators. To improve this situation, IFCC and the standardization authority, Joint Research Centre Institute for Reference Materials and Measurements (JRC-IRMM), of the European Commission have established and supported a Working Group for the Production of an International Cystatin C Calibrator (WG-SCC). This group recently produced such a calibrator (ERM-DA471/IFCC), which now is available to diagnostic companies and research groups *(24–26)*. Although the availability of a certified reference material allows different diagnostic companies to produce cystatin C assays with reduced interassay variability, further optimization of the individual assays is required to produce the best possible interassay agreement. The present work was undertaken in an effort to use the certified reference material coupled with further adjustments in the cystatin C-assays of 7 international diagnostic companies to achieve sufficiently good agreement between the assays that all can be used to develop virtually identical $eGFR_{cystatin\ C}$ equations from a cohort of persons with known GFR. An $eGFR_{cystatin\ C}$ equation that was virtually assay-independent was then developed by use of samples from Caucasian, Asian, pediatric, and adult (CAPA) cohorts, totaling 4960 individuals with varying GFRs as determined by renal or plasma clearance of inulin or plasma clearance of iohexol. The equation was tentatively called the CAPA equation.

Materials and Methods

STUDY COHORT USED TO PRODUCE AN INTERNATIONAL CYSTATIN C–BASED ESTIMATING EQUATION FOR GFR IN ADULTS AND CHILDREN

We used plasma samples from 3495 Swedish [Lund cystatin C standardization cohort (LCS)] and 763 Japanese adults and from a mixed cohort of 262 Dutch and 440 Swedish children (LCS) to develop and validate internally an international $eGFR_{cystatin\ C}$ equation (Table 1). All procedures involving patients and data were in accordance with the Helsinki Declaration of 1975, revised in 2000, concerning ethical principles for medical research involving human subjects.

DETERMINATION OF GFR

We used plasma clearance of iohexol *(27, 28)* to measure GFR in the adult and pediatric Swedish population (see Supplemental Methods, which accompanies the online version of this article at http://www.clinchem.org/content/vol60/issue7). We used renal inulin clearance to measure GFR in the Japanese adult population, and single-injection plasma-disappearance of inulin in the Dutch pediatric population *(29, 30)*. These 3 methods produce comparable GFR values according to a recent metaanalysis *(31)*.

We used the Dubois–Dubois formula for calculation of body surface area *(32)*.

EFFORTS TO OPTIMIZE AGREEMENT BETWEEN 6 COMMERCIALLY AVAILABLE CYSTATIN C ASSAYS

The WG-SCC cooperated with 6 diagnostic companies (Abbott, Dako, Gentian, Roche, Sentinel, and Siemens), offering assays for cystatin C, to optimize assay agreement. The Abbott and Sentinel assays are identical. Five of these assays are particle-based immunoturbidimetric assays *(15)*, and one, Siemens, is a particle-based immunonephelometric assay *(33)* (see online Supplemental Methods for technical specifications).

To optimize assay agreement, we used plasma samples anticoagulated with Li-heparinate and by use of the Vacutainer system of Becton Dickinson, from 3495 adult (age $\geq$18 years) patients consecutively referred to the University Hospital of Lund for determination of GFR by iohexol clearance measurements. 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. In the Japanese cohort of adults, 21% had diabetes, 1.4% had received kidney transplants, and 1.4% were kidney donors. The Dutch

Table 1. Patient characteristics of the development and validation set in the Swedish adult cohort, Japanese adult cohort, and the mixed Dutch-Swedish cohort of children.[a]

Variable	Development sets			Validation sets		
	Swedish adults	Japanese adults	Swedish/Dutch children	Swedish adults	Japanese adults	Swedish/Dutch children
n	2295	413	456	1200	350	246
Female, %	47	38	37	47	41	41
Age, years	63 (21, 86)	55 (20, 78)	12.0 (2.0, 17.5)	63 (21, 85)	57 (22, 81)	11 (1, 17)
Body weight, kg	77 (49, 115)	60 (41, 88)	39 (11, 85)	77 (46, 116)	59 (51, 94)	40 (11, 85)
Height, cm	170 (152, 190)	163 (145, 178)	147 (83, 185)	170 (152, 190)	162 (145, 178)	148 (81, 185)
Body surface area, m^2	1.88 (1.49, 2.34)	1.64 (1.31, 2.04)	1.29 (0.49, 2.06)	1.89 (1.43, 2.34)	1.62 (1.30, 2.01)	1.30 (0.48, 2.01)
BMI, kg/m^2	26 (18, 38)	22 (17, 31)	18 (14, 29)	26 (18, 40)	23 (16, 32)	18 (14, 28)
Cystatin C, mg/L	1.52 (0.77, 4.49)	1.33 (0.67, 4.51)	0.97 (0.61, 3.15)	1.58 (0.77, 4.47)	1.32 (0.68, 4.78)	1.01 (0.60, 3.47)
mGFR, $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$, n (%)	53 (9, 116)	54 (8, 126)	103 (27, 200)	51 (10, 114)	55 (7, 129)	99 (22, 190)
<30	585 (26)	113 (27)	17 (4)	335 (28)	87 (25)	14 (6)
30–59	715 (31)	119 (29)	39 (9)	362 (30)	108 (31)	20 (8)
60–89	653 (28)	95 (23)	99 (22)	334 (28)	76 (22)	62 (25)
≥90	342 (15)	86 (21)	301 (66)	169 (14)	79 (23)	150 (61)

[a] Data are median (2.5th, 97.5th percentile) unless noted otherwise.

cohort of children comprised 49% oncology patients and 51% patients with glomerulonephritis/glomerulopathy. The corresponding numbers for the Swedish cohorts were not available.

Efforts were made to optimize several cystatin C assays produced by Japanese manufacturers to maximal agreement by use of the certified reference material. One of these assays, a sol particle homogeneous immunoassay for measuring serum cystatin C *(34)* produced by Alfresa, was used to determine serum cystatin C in the Japanese cohort. We therefore investigated the agreement between this assay and the assays used for the Swedish and Dutch cohorts.

DEVELOPMENT AND INTERNAL VALIDATION OF NEW CYSTATIN C–BASED GFR-ESTIMATING EQUATIONS

All statistical analyses were conducted by use of SPSS Statistics release 20.0.0 (IBM Corp.) and Microsoft Excel. From the complete data set, comprising 4960 GFR and cystatin C determinations for adults and children, we established a development set with 2295 randomly chosen Swedish adults, 413 Japanese adults, and 456 Swedish and Dutch children. The remaining 1796 patients formed 3 internal validation sets with 1200 Swedish adults, 350 Japanese adults, collected April to July 2007, and 246 Swedish and Dutch children. There were no important differences with respect to general characteristics between the development and the corresponding internal validation sets (Table 1).

All regression analyses in the development set were conducted by use of statistical weights (see online Supplemental Methods). The purpose was to reweight all regression analyses to give equal importance to the Swedish and Japanese adult populations, despite their unequal sample sizes (2295 and 413 individuals, respectively), when estimating the regression coefficients. First, the extrarenal elimination, *E,* was estimated as the intercept in a linear regression model with measured GFR (mGFR) as the dependent variable and 1/cystatin C as the independent variable *(35)*. We then estimated the regression coefficients (*a*, *b*, and *c*) of the following equation:

$$\ln(\text{mGFR} + E) = \ln(a) + b \times \ln(\text{cystatin C}) + c \times \ln(\text{age}), \quad \text{(Eq. 1)}$$

where ln denotes the natural logarithm, cystatin C concentration is in milligrams per liter, and age is in years. Linear regression with 1/mGFR as regression weights was used to increase the importance of patients with low GFR *(11)*. The regression equation above corresponds to an eGFR equation of the following form:

$$\text{eGFR} = a \times \text{cystatin C}^{b} \times \text{age}^{c} - E. \quad \text{(Eq. 2)}$$

We also assessed the effect of adding sex (0 = male, 1 = female) as an additional covariate to the regression equation.

The equations, with coefficients established on the basis of the development set, were validated in the 3 internal validation sets separately in comparison with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) cystatin C equation *(36)*. The validation of the equations focused on bias and accuracy *(37)*. Bias was defined as the median of the individual differences between eGFR and mGFR (eGFR – mGFR), expressed in $\text{mL} \cdot \text{min}^{-1} \cdot (1.73\ \text{m}^2)^{-1}$. Accuracy was expressed as the median of the absolute percentage differences between eGFR and mGFR relative to mGFR. The accuracy was further reflected by P30, i.e., the percentage of estimates within 30% of mGFR. At least 75% of eGFR should be within 30% of mGFR to be considered "sufficient for good clinical decision-making" *(38)*. Nonparametric and asymptotic 95% CIs were calculated as measures of the statistical uncertainty in medians and proportions (P30), respectively, for overall results. To facilitate comparisons, validation results among children were presented also for the CKD-EPI cystatin C equation, although this equation is intended for GFR estimation only among adults.

For the Swedish adults and children and for the Dutch children, mean values of 2 available cystatin C measurements from 2 different assays were used to increase statistical precision in the estimated regression coefficients. However, the validation of the equations was based on a single cystatin C measurement to mimic the expected accuracy in clinical practice. The coefficients of the final equation presented were obtained by use of the total data set (n = 4960), with the same weighting scheme (equal weights for the Swedish and Japanese adult populations) as for the development set, to increase statistical precision further.

Results

OPTIMIZATION OF AGREEMENT BETWEEN 6 COMMERCIALLY AVAILABLE CYSTATIN C ASSAYS

In a joint effort, the WG-SCC and the 6 diagnostic companies, Dako, Siemens, Gentian, Roche, Abbott, and Sentinel, worked to improve the agreement between the cystatin C assays provided by the companies. Several of the factors known to influence the performance of assays of cystatin C (see online Supplemental Table 1) were varied to increase the agreement between assays, but only 2 were systematically studied for all assays. These concerned use of the same total volume and consistent volume of antiserum in all standards and samples *(39)* and selection of positions of the calibrator points used to construct the dose–response curves, including most

Table 2. Deming regression analysis between each of 5 cystatin C assays (Siemens, Gentian, Roche, and Sentinel/Abbott) adjusted for maximal agreement and expressed as linear equations (y = slope × x + intercept) in relation to the Dako cystatin C assay (x; mg/L).[a]

Test	Samples, n	Slope (95% CI)	Intercept (95% CI)	R^2
Siemens	979	0.984 (0.974–0.994)	−0.0124 (−0.0242–−0.000547)	0.993
Gentian	988	1.058 (1.050–1.066)	−0.0697 (−0.0799–−0.0594)	0.998
Roche	992	1.012 (1.007–1.018)	−0.0250 (−0.0320–−0.0180)	0.998
Sentinel/Abbott	806	0.932 (0.928–0.935)	0.0388 (0.0325–0.0450)	0.998
Quintuple tests of plasma pools[b]				
Alfresa (vs Dako)	10	1.048	−0.1105	0.999
Alfresa (vs Gentian)	10	1.049	−0.0343	1.000

[a] Deming regression allows for errors in both the independent and dependent variables.
[b] For comparison, regression analysis of quintuple tests of 10 plasma pools with varying concentrations of cystatin C are presented for the Alfresa assay vs the Dako and Gentian cystatin C assays. A delta value of 1 was used in the analysis.

important assay intervals (see online Supplemental Results, Supplemental Figs. 1 and 2).

Linear equations and correlation coefficients for the 6 assays adjusted for maximal agreement are given in Table 2 with the values produced by the Dako assay as the independent variable. Table 2 also shows the same data for the Alfresa assay by use of quintuple tests of 10 plasma pools with varying concentrations of cystatin C.

The equations of Table 2 were then used to assess the achievable increase in agreement between the assays with results from the Swedish provider of external quality assessment for clinical laboratory investigations, Equalis (www.equalis.se/en/start.aspx). Equalis has monitored the quality of cystatin C assays for 10 years by distributing plasma samples with cystatin C concentrations unknown to the laboratories running cystatin C. During the last 3 years, 35 laboratories have participated in the quality assessments. The CVs for the determined cystatin C concentrations during the 17 different test periods during 2010–2012, by use of the mean cystatin C values for each type of assay to calculate CV, varied from 6% to 14%, with a mean

Table 3. Overall bias, precision, and accuracy of the CAPA and CKD-EPI cystatin C equations [Inker et al. *(36)*] in the validation sets of Swedish adults (n = 1200), Japanese adults (n = 350) and the mixed cohort of Dutch and Swedish children (n = 246).

	Swedish adults	Japanese adults	Swedish/Dutch children[a]
Bias, median difference, $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ (95% CI)			
CAPA	−5.2 (−5.9 to −4.8)	0.5 (−0.5 to 1.7)	−8.8 (−11.2 to −6.2)
CKD-EPI	−5.7 (−6.2 to −5.2)	1.0 (−0.6 to 1.9)	−10.6 (−13.5 to −7.4)
Precision, interquartile range of differences between eGFR and mGFR, $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ (95% CI)			
CAPA	12.0 (11.2 to 12.8)	14.8 (13.0 to 18.1)	27.9 (22.8 to 32.1)
CKD-EPI	12.5 (11.5 to 13.2)	14.8 (12.3 to 17.8)	25.1 (20.7 to 29.5)
Accuracy, median absolute percentage difference (95% CI)			
CAPA	15.5 (13.5 to 17.5)	16.1 (12.2 to 20.0)	16.4 (11.8 to 21.0)
CKD-EPI	16.4 (14.3 to 18.5)	16.6 (12.7 to 20.5)	14.9 (10.5 to 19.3)
P30, % (95% CI)[b]			
CAPA	82.8 (80.7 to 84.9)	77.7 (73.3 to 82.1)	80.1 (75.1 to 85.1)
CKD-EPI	80.0 (77.7 to 82.3)	78.9 (74.6 to 83.2)	80.9 (76.0 to 85.8)

[a] The CKD-EPI equation is not intended for use among children. Results presented only for comparison.
[b] P30 refers to percentage of GFR estimates within 30%, respectively, of mGFR.

Table 4. Comparison of the CAPA and CKD-EPI cystatin C equations regarding bias and accuracy stratified by mGFR, age, and BMI intervals in the validation sets of Swedish adults (n = 1200) and Japanese adults (n = 350). Median mGFRs are given for the various GFR and age intervals.

	mGFR interval, mL · min^{-1} · (1.73 m^2)$^{-1}$			
Variable	**<30**	**30–59**	**60–89**	**≥90**
Number in the Swedish/Japanese cohorts	335/87	362/108	334/76	169/79
Median mGFR, mL · min^{-1} · (1.73 m^2)$^{-1}$ in the Swedish/Japanese cohorts	18/19	44/43	73/76	102/101
Bias, mL · min^{-1} · (1.73 m^2)$^{-1}$				
Swedish adults				
CAPA	−1.1	−5.7	−10.2	−15.0
CKD-EPI	−1.6	−7.4	−9.8	−9.8
Japanese adults				
CAPA	2.3	1.0	−2.8	−11.1
CKD-EPI	2.0	−0.8	−1.5	−3.5
Accuracy, P30, %[a]				
Swedish adults				
CAPA	75.8	84.0	86.5	86.4
CKD-EPI	77.6	76.2	81.7	89.3
Japanese adults				
CAPA	60.9	81.5	85.5	83.5
CKD-EPI	65.5	79.6	84.2	87.3

	Age intervals, years			
Variable	**18–39**	**40–59**	**60–79**	**≥80**
Number in the Swedish/Japanese cohorts	161/90	351/118	570/131	118/11
Median mGFR, mL · min^{-1} · (1.73 m^2)$^{-1}$ in the Swedish/Japanese cohorts	82/88	61/53	45/44	24/29
Bias, mL · min^{-1} · (1.73 m^2)$^{-1}$				
Swedish adults				
CAPA	−5.9	−5.8	−4.9	−4.6
CKD-EPI	−3.5	−5.7	−6.1	−5.9
Japanese adults				
CAPA	−2.8	0.7	1.6	0.3
CKD-EPI	0.6	1.4	0.6	1.0
Accuracy, P30, %				
Swedish adults				
CAPA	85.7	85.2	83.3	68.6
CKD-EPI	85.7	84.9	79.1	61.9
Japanese adults				
CAPA	81.1	79.7	74.0	72.7
CKD-EPI	83.3	80.5	74.0	81.8

Continued on page xx

Table 4. Comparison of the CAPA and CKD-EPI cystatin C equations regarding bias and accuracy stratified by mGFR, age, and BMI intervals in the validation sets of Swedish adults (n = 1200) and Japanese adults (n = 350). Median mGFRs are given for the various GFR and age intervals. (*Continued from page xx*)

	BMI intervals, kg/m^2			
Variable	<20	20–24	25–29	≥30
Number in Swedish/Japanese cohorts	69/59	348/181	492/90	291/20
Median mGFR, mL · min^{-1} · (1.73 m^2)$^{-1}$ in the Swedish/Japanese cohorts	57/75	60/52	52/53	42/45
Bias, mL · min^{-1} · (1.73 m^2)$^{-1}$				
Swedish adults				
CAPA	−2.4	−5.0	−5.1	−6.1
CKD-EPI	−3.3	−5.2	−5.6	−7.1
Japanese adults				
CAPA	0.4	−0.1	1.4	0.0
CKD-EPI	4.3	0.6	1.3	1.4
Accuracy, P30, %				
Swedish adults				
CAPA	82.6	86.5	83.3	77.3
CKD-EPI	76.8	82.2	81.1	76.3
Japanese adults				
CAPA	72.9	75.7	82.2	90.0
CKD-EPI	76.3	76.8	82.2	90.0

[a] P30 refers to percentage of GFR estimates within 30% of mGFR.

CV of 9.7% (see online Supplemental Fig. 3A). If the equations of Table 2 are applied to these results, the corresponding CV is decreased to between 1% and 5%, with a mean CV of 2.8% (see online Supplemental Fig. 3B). If the cystatin C results are used to estimate GFR according to the cystatin C–based GFR estimation equation developed in this work (see below), a corresponding increase in agreement is seen (see online Supplemental Fig. 4). The CV for eGFR varies between 9% and 19%, with a mean CV of 13.9%, and is reduced to between 2.0% and 7.1%, with a mean CV of 3.7% after applying the equations of Table 2. Online Supplemental Fig. 5 demonstrates the practical consequences of this for the full GFR spectrum.

Ten plasma pools with varying concentrations of cystatin C were analyzed by the Alfresa assay and 2 of the adjusted assays described above by use of quintuple tests. The agreement between the 2 adjusted cystatin C assays (Dako and Gentian) on one hand, and the Alfresa assay on the other hand, was excellent. Table 2 shows the results.

EVALUATION OF EQUATIONS IN THE DEVELOPMENT SET

The eGFR equation without a sex factor produced similar negative bias among both females and males [−2.6 and −4.0 mL · min^{-1} · (1.73 m^2)$^{-1}$ in median] when evaluated in the development set (n = 3164) with statistical weights. The overall accuracy was also similar (P30 = 79% for both sexes). Among children, most of them with a GFR within reference intervals, there was a sex difference in bias [median bias −8.5 and +0.8 mL · min^{-1} · (1.73 m^2)$^{-1}$ for boys and girls, respectively]. However, adding sex as a covariate in the eGFR equation yielded a correction factor of 0.99 for females, implying that the eGFR equations with and without a sex factor would yield similar estimates. Internal validation results below are therefore presented for the eGFR equation from the development set without sex only (labeled "CAPA" in the tables).

INTERNAL VALIDATION

Overall accuracy of the CAPA equation was satisfactory in the 3 validation cohorts (P30 = 83%, 78%, and 80% among Swedish adults, Japanese adults, and children, respectively) and comparable with the CKD-EPI cystatin C equation (Table 3). Both the CAPA and the CKD-EPI equation exhibited some negative bias among Swedish adults [−5 and −6 mL · min^{-1} · (1.73 m^2)$^{-1}$ in median] and among children [−9 and −11 mL · min^{-1} · (1.73 m^2)$^{-1}$ in median].

Bias and accuracy of the 2 equations were generally similar among males and females in the 3 validation cohorts. Among Swedish adults, P30 for males and fe-

males was 81% and 84%, respectively, for the CAPA equation and 81% and 78% for the CKD-EPI equation (not in tables). The corresponding figures among Japanese adults were 77% and 78% for CAPA and 77% and 81% for CKD-EPI. The CKD-EPI equation was less accurate among girls (P30 = 74%) than among boys (P30 = 86%), whereas the CAPA equation had similar performance for both sexes (P30 = 81% and 79% for boys and girls).

STRATIFICATION FOR GFR IN ADULTS

In the Swedish cohort, both the CAPA and CKD-EPI equation demonstrated an increasing negative bias with increasing mGFR measurements, reaching about −15 and −9 mL · min^{-1} · (1.73 m^2)$^{-1}$, respectively, at mGFR ≥90 mL · min^{-1} · (1.73 m^2)$^{-1}$ (Table 4). At the same time, accuracy in terms of P30 increased with increasing mGFR (Table 4). In the Japanese cohort, no marked bias was noted except for the negative bias [11 mL · min^{-1} · (1.73 m^2)$^{-1}$ in median] for the CAPA equation at mGFR ≥90 mL · min^{-1} · (1.73 m^2)$^{-1}$. Substandard P30-values were noted for both equations at mGFR <30 mL · min^{-1} · (1.73 m^2)$^{-1}$ in the adult Japanese population.

STRATIFICATION FOR AGE IN ADULTS

In the Swedish cohort, both the CAPA and CKD-EPI equation demonstrated a negative bias [4 to 6 mL · min^{-1} · (1.73 m^2)$^{-1}$] at all age intervals, whereas both equations were virtually unbiased in the Japanese cohort irrespective of age (Table 4). Substandard P30 among the elderly (>80 years old) was noted for both equations in the Swedish cohort, but only for the CAPA equation in the Japanese cohort. The pattern with substandard P30 among those >80 years old was similar among males and females (not in tables).

STRATIFICATION FOR BODY MASS INDEX IN ADULTS

In the Swedish cohort, both the CAPA and CKD-EPI equation demonstrated a negative bias [3 to 7 mL · min^{-1} · (1.73 m^2)$^{-1}$] at all body mass index (BMI) intervals, whereas both equations were generally unbiased in the Japanese cohort at all BMI intervals except for the CKD-EPI equation at BMI <20 kg/m^2 [+4 mL · min^{-1} · (1.73 m^2)$^{-1}$] (Table 4). The equations demonstrated P30 >75% at all BMI intervals in both cohorts.

STRATIFICATION FOR GLOMERULAR FILTRATION RATE IN CHILDREN

Both the CAPA and CKD-EPI equation demonstrated a negative bias [2 to 6 mL · min^{-1} · (1.73 m^2)$^{-1}$] at GFR measurements <120 mL · min^{-1} · (1.73 m^2)$^{-1}$, whereas the negative bias above this level was marked, exceeding 25 mL · min^{-1} · (1.73 m^2)$^{-1}$ for both equations (Table 5).

Table 5. Comparison of the CAPA and CKD-EPI cystatin C equations regarding bias and accuracy stratified by mGFR and age intervals in an internal validation set of a mixed cohort of Dutch and Swedish children (n = 246). Median mGFRs are given for the various GFR and age intervals.[a]

	mGFR interval, mL · min^{-1} · (1.73 m^2)$^{-1}$		
Variable	<60	60–119	≥120
Number of children	34	138	74
Median mGFR, mL · min^{-1} · (1.73 m^2)$^{-1}$	41	92	143
Bias, mL · min^{-1} · (1.73 m^2)$^{-1}$			
CAPA	−2.5	−6.3	−27.9
CKD-EPI	−5.0	−6.2	−26.4
Accuracy, P30, %[b]			
CAPA	82.4	81.2	77.0
CKD-EPI	79.4	82.6	78.4
	Age intervals, years		
Variable	1–6	7–12	13–17
Median mGFR, mL · min^{-1} · (1.73 m^2)$^{-1}$	117	102	89
Number of children	54	84	108
Bias, mL · min^{-1} · (1.73 m^2)$^{-1}$			
CAPA	5.2	−7.5	−13.4
CKD-EPI	−4.9	−9.4	−12.1
Accuracy, P30, %			
CAPA	79.6	85.5	76.9
CKD-EPI	85.2	84.5	75.9

[a] The CKD-EPI equation is not intended for use among children. Results presented only for comparison.
[b] P30 refers to percentage of GFR estimates within 30% of mGFR.

Still, P30 ranged between 77% and 83% at all GFR measurements for both equations.

STRATIFICATION FOR AGE IN CHILDREN

Both the CAPA and CKD-EPI equations demonstrated an increased negative bias with increasing age reaching between −12 and −13 mL · min^{-1} · (1.73 m^2)$^{-1}$ at age >12 years (Table 5). Still, P30 ranged between 76 and 86% at all levels for both equations at all age intervals.

FINAL EQUATION

The final CAPA equation, with coefficients estimated from the complete data set, is

$$\text{eGFR} = 130 \times \text{cystatin C}^{-1.069} \times \text{age}^{-0.117} - 7. \quad \text{(Eq. 3)}$$

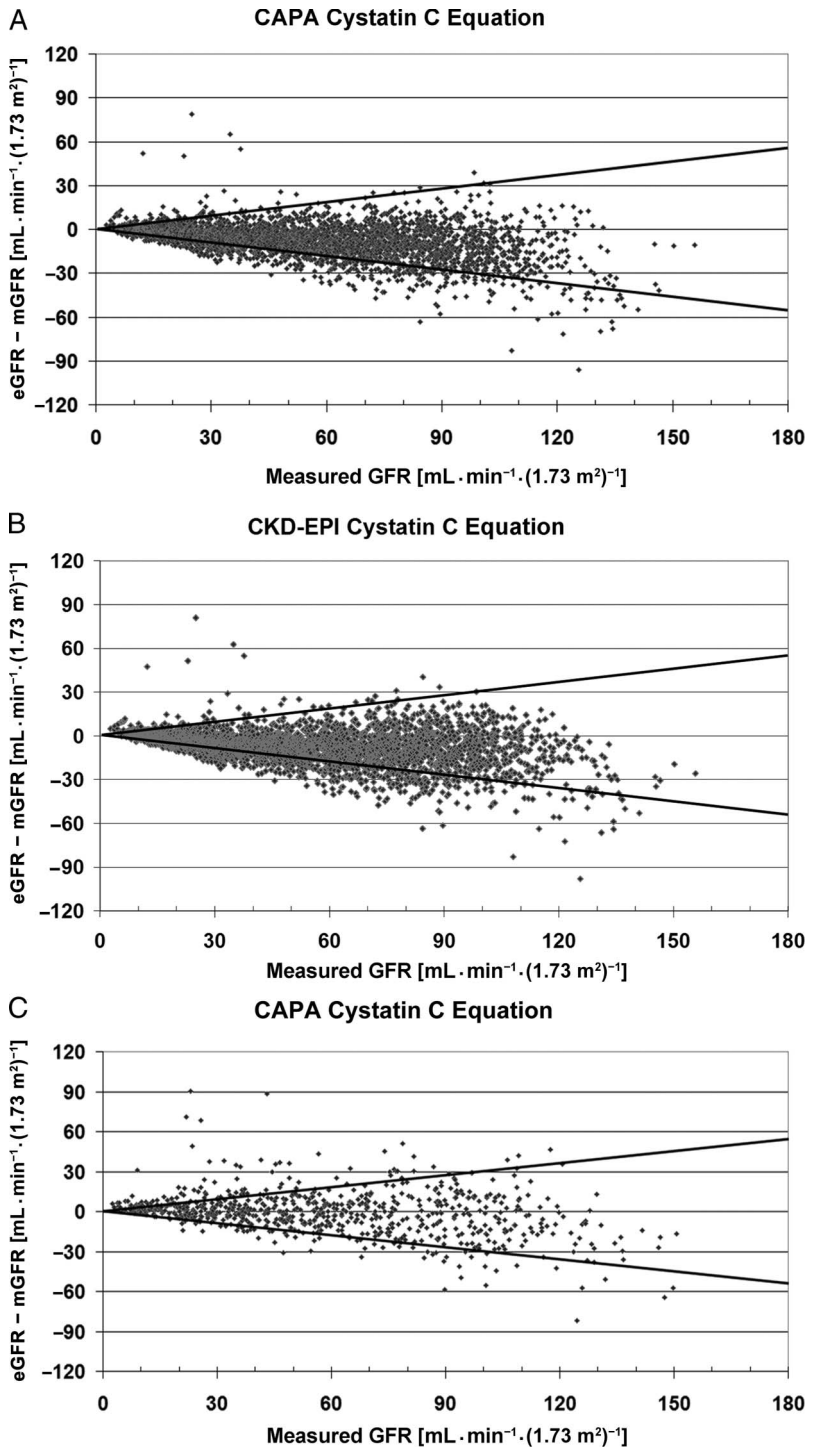

Fig. 1. Individual errors (eGFR − mGFR) of the CAPA and CKD-EPI cystatin C equations at different levels of mGFR in the combined data sets (development + validation).

(A and B), Swedish adults, n = 3495; (C and D), Japanese adults, n = 763; (E and F), children, n = 702. The lines represent 30% limits in relation to mGFR. The CKD-EPI equation is not intended for use among children; results presented only for comparison.

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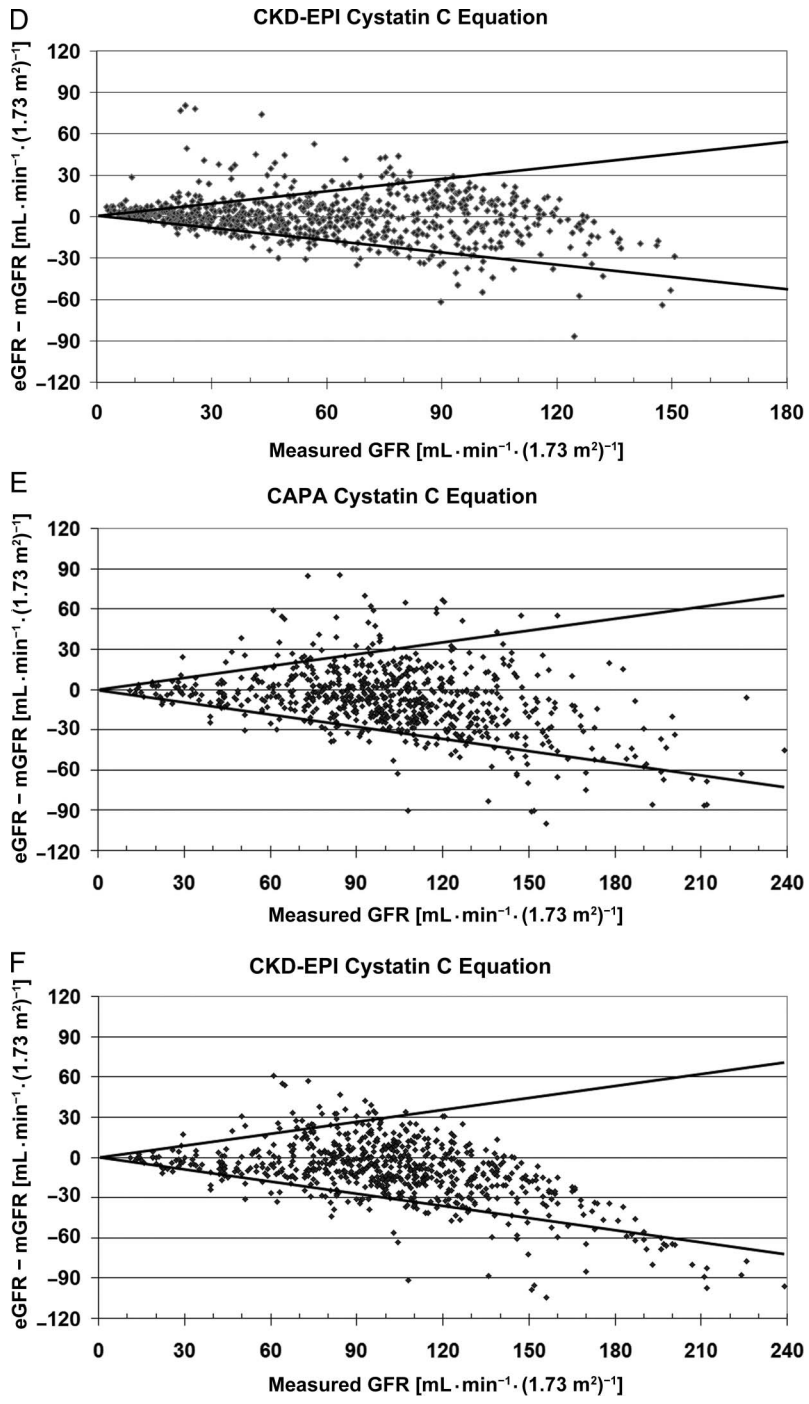

Fig. 1. Continued.

The P30 of the final CAPA equation in the complete data set was 80.6% among Swedish adults, 77.5% among Japanese adults, and 82.2% among children. The difference between eGFR and mGFR at different levels of mGFR by use of this final CAPA equation and the CKD-EPI equation on the entire data set of Swedish and Japanese adult cohorts as well as among children is presented together with limits for P30 accuracy in Fig. 1.

Discussion

The present study concerns whether use of the certified reference material and minor further adjustments of 6 commercially available cystatin C assays can increase agreement among the assays so that a common, virtually assay-independent, cystatin C–based equation for estimation of GFR can be established by use of a large population of about 5000 individuals with known GFR. The interassay CV of 9.7% for 4 of the original assays, run for several years in a quality assessment system, was reduced to 2.8% by the use of certified reference material and by further optimizations of the assays. The eGFR equation generated by use of the mean cystatin C values of 2 of the adjusted assays applied in the patient population could be used by all 6 assays, as well as the Alfresa assay, with only minor differences in the resulting GFR estimations (online Supplemental Fig. 4B shows the results for 4 of the assays). The remaining differences in the GFR estimations by all 7 assays are generally too small to influence clinical decision making in most cases. For example, at the clinically relevant cystatin C concentrations of 1.22, 2.1, and 3.5 mg/L, corresponding to eGFRs of approximately 15, 30, and 60 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$, the total eGFR ranges determined by the 7 assays in this study were 14–16, 29–32, and 59–63 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$, respectively.

Some of the assay adjustments performed in the present study can easily be applied to decrease the interassay variability of assays other than those included in this study. This is particularly evident for the position of the calibrator points (see online Supplemental Fig. 2). The most frequent decision making concerns whether an individual has normal or decreased GFR, and it would therefore seem appropriate to have the majority of the calibrator points in the corresponding cystatin C concentration interval of about 0.8–2.0 mg/L, which corresponds to GFR estimations from about 110 and down to 30 $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$ by use of the CAPA GFR-estimating equation of this work.

The performance of the CAPA equation was compared to that of another recent cystatin C–based equation, CKD-EPI, by use of a cystatin C assay providing results traceable to the certified reference material, ERM-DA471/IFCC *(36)*. The populations used to generate the 2 equations differed considerably. Whereas the population used to generate the CKD-EPI equation comprised an adult population of Caucasians and African Americans, the population used to develop the CAPA equation comprised large subgroups of adults and children as well as of Asians and Caucasians. There is also another difference in the generation of the 2 equations. The CKD-EPI equation is based on finding a mathematical-statistical connection between mGFR and cystatin C, sex, and age of the individuals of the population studied without attempting to express the equation so that it might be given a biological interpretation. When we developed the CAPA equation, we tried to use not only a mathematical-statistical procedure, as described above, but also a procedure subject to conditions that might generate an equation with a possible biological interpretation, i.e., in which all, or most of, the terms correspond to a physiological process *(35, 40)*. These conditions were that the equation should comprise a term close to cystatin C to the power of −1, which corresponds to the renal excretion of a freely filtered substance, and that there should be a term for nonrenal clearance of cystatin C, as we know that such a clearance exists. We generated both types of equations and compared their diagnostic performances. Because these did not differ, we chose to use the equation with a possible biological interpretation. An interesting difference between the CKD-EPI and CAPA equations is that the CAPA equation does not require a sex factor. This might be of practical value, since sex, like race, is not always clearly defined. For example, several countries today recognize >2 sexes *(41)*.

Although all-purpose equations have many practical advantages, lower performance in certain subgroups defined by, e.g., GFR level, BMI, or age is inevitable. In the present study, underestimations were noted in patients with a GFR within reference intervals, most noticeably among children. However, the P30 accuracy was still acceptable, but it cannot be ruled out that population-specific equations would increase performance further. Although the CAPA and CKD-EPI cystatin C–based equations were produced in different ways, they showed similar and sufficient accuracy overall and across subgroups. The CAPA equation has the advantage of being developed on the basis of more diverse populations. Another advantage is that the CAPA equation has a less complex formula expression that does not include a sex factor. It should be noted that the sex factor of CKD-EPI did not contribute to decreased differences in performance between males and females.

Although the procedures to generate the CAPA and the CKD-EPI equations differed significantly in several respects, they had one common element, the use of cystatin C assays adjusted to the certified reference material ERM-DA471/IFCC. We believe that this is the critical step in reducing the number of cystatin C–based GFR estimating equations required for clinical use in the future and in reaching the full potential of cystatin C in classifying kidney disease *(42)*.

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