

VALIDATION OF STANDARDIZED CREATININE AND CYSTATIN C GFR ESTIMATING EQUATIONS IN A LARGE MULTICENTRE EUROPEAN COHORT OF CHILDREN

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ABSTRACT

Background Most validations of paediatric glomerular filtration rate (GFR) estimating equations using standardized creatinine (CR) and cystatin C (CYS) assays have comprised relatively small cohorts, which makes accuracy across subgroups of GFR, age, body mass index (BMI) and gender uncertain. To overcome this, a large cohort of children referred for GFR determination has been established from several European medical centres.

Methods Three thousand four hundred eight measurements of GFR (mGFR) using plasma clearance of exogenous substances were performed in 2218 children aged 2-17 years. Validated equations included Schwartz-2009_{CR}/2012_{CR/CYS/CR+CYS}, FAS_{CR/CYS/CR+CYS}, LMR_{CR}, Schwartz-Lyon_{CR}, Berg_{CYS}, CAPA_{CYS}, CKD-EPI_{CYS}, Anders_{CR+CYS} and arithmetic means of the best single-marker equations in explorative analysis. Five metrics were used to compare the performance of the GFR equations: bias, precision and three accuracy measures including the percentage of GFR estimates (eGFR) within $\pm 10\%$ (P₁₀) and $\pm 30\%$ (P₃₀) of mGFR.

Results Three of the cystatin C equations, Berg_{CYS}, CAPA_{CYS} and CKD-EPI_{CYS}, exhibited low bias and generally satisfactory accuracy across all levels of mGFR; CKD-EPI_{CYS} had more stable performance across gender than the two other equations. Among creatinine equations, Schwartz-Lyon_{CR} had the best performance but was inaccurate at mGFR < 30 mL/min/1.73 m² and in underweight patients. Arithmetic means of the best creatinine and cystatin C equations above improved bias compared to the existing composite creatinine+cystatin C equations.

Conclusions The present study strongly suggests that cystatin C should be the primary biomarker of choice when estimating GFR in children with decreased GFR. Arithmetic means of well-performing single-marker equations improve accuracy further at most mGFR levels and have practical advantages compared to composite equations.

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Introduction

Accurate assessment of glomerular filtration rate (GFR) in children is essential for correct classification and management of chronic kidney disease (CKD) and for adjusting drug dosage to avoid toxic effects from certain cytostatic drugs, antibiotics and radiographic contrast media. Measuring GFR (mGFR) in terms of clearance of exogenous substances is relatively costly, inconvenient in daily clinical practice and even unavailable in many hospitals, making equations based on plasma/serum concentrations of creatinine or cystatin C to estimate GFR (eGFR) a necessary alternative to evaluate renal function in many situations.

Most validation studies regarding paediatric GFR equations based on standardized creatinine and cystatin C assays have been small, typically with fewer than 400 GFR measurements and with small numbers of patients with decreased GFR [1-13]. Therefore, the accuracy of even commonly used GFR equations in clinical practice is largely unknown across subgroups of GFR, age, body mass index (BMI) and sex.

The aim of the present study was to validate commonly used GFR equations calibrated to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)- standardized creatinine and cystatin C assays in a large cohort of children pooled from several medical centres across Europe.

Material and methods

DATA SOURCES AND PARTICIPANTS

A new European work group named “European Kidney Function Consortium” (EKFC) has recently been set up at two meetings (Malmö on September 25, 2017 and Copenhagen on May 26, 2018). EKFC took the initiative to collect retrospective data on subjects referred for GFR measurement including sCr, sCys, age, sex, height and weight. For this study, we selected a subset of all available data from this database, restricted to children 2 to 17 full years of age. Five different cohorts (see descriptions in Supplementary Material including Table S1) used in ongoing or published cross-sectional studies between 2004 and 2016 were included from the Netherlands (Amsterdam [7, 14-17]), Belgium (Leuven [13]), France (Lyon [12, 13]) and Sweden (Lund [7] and Stockholm). Common reasons for determination of mGFR were congenital malformations, hereditary disorders, glomerulopathies, tubular diseases, vascular disorders, malignancies, urinary tract infections, liver diseases, transplantation follow-up and to dose drugs cleared by the kidneys. A study database, fully anonymous and without personal information, was pooled and centralized for the present study at Lund University, Sweden, comprising 3408 measurements of GFR in 2218 children with complete data on sCr, sCys, age, gender, height and weight. All procedures involving subjects and data agreed with the ethical principles for medical research involving human subjects established in the World Medical Association Declaration of Helsinki of 1975, as revised in 2000. The study was reviewed and approved by the Regional Ethical Board in Lund, Sweden.

LABORATORY ASSAYS

All centres used creatinine assays traceable to isotope dilution mass spectrometry (IDMS). The cystatin C assays were calibrated against the international cystatin C reference material, ERM-DA471/IFCC, except for Lyon before April 2011 where the values were multiplied by 1.11 [13] and for Amsterdam before December 2012 where the values were multiplied by 1.174 [18] for conversion. For the data originating from the Caucasian, Asian, Paediatric and Adult (CAPA) cohort (Lund and part of Amsterdam cohort), extra care was undertaken to assure that the cystatin C assay used was commutable with regard to the reference material [7]. All assays for cystatin C and creatinine for the CAPA cohort were run on the same instrument (Architect ci8200) at one laboratory (Uppsala University Hospital, Sweden). An external quality assessment system (Equalis AB, Sweden) was used to monitor these analyses as well as those from Stockholm.

Details of clearance methods and creatinine and cystatin C assays used in Amsterdam, Leuven, Lund and Lyon have been published earlier (see references in Supplementary Material and Table S1), while unpublished techniques used in Stockholm are described in more detail in Supplementary Material. External quality control was provided by Equalis AB (Uppsala, Sweden, www.equalis.se/en) regarding the iohexol measurements in Lund and Stockholm.

GFR EQUATIONS

The following GFR equations were analyzed (for equation expressions, see Supplementary Material):

1. *Creatinine-based, general-purpose equations*: The Full Age Spectrum based on age (FAS-Age_{CR}) and height (FAS-Ht_{CR}) [12, 13, 19] and the Lund-Malmö revised equation (LMR_{CR}) [20], which was developed for adults but has also been validated in children [21]
2. *Creatinine-based, paediatric equations*: Schwartz-2009_{CR} [1], Schwartz-2012_{CR} [3] and Schwartz-Lyon_{CR} [4]
3. *Cystatin C-based, general-purpose equations*: CAPA_{CYS} [7], FAS_{CYS} [13] and the Chronic Kidney Disease Epidemiology collaboration equation (CKD-EPI_{CYS}) [22], which was developed for adults but has also been validated in children [7]
4. *Cystatin C based, paediatric equations*: Berg_{CYS} [9] and Schwartz-2012_{CYS} [3]. For the latter, the cystatin C values were divided by 1.174 to make the equation suitable for cystatin C values compatible with the international calibrator [18]
5. *Composite creatinine and cystatin C-based, general-purpose equations*: FAS-Age_{CR+CYS} and FAS-Ht_{CR+CYS} [13]
6. *Composite creatinine and cystatin C-based, paediatric equations*: Schwartz-2012_{CR+CYS} [3] and Andersens_{CR+CYS} [5], the latter with absolute values normalized to 1.73-m body surface area

STATISTICAL EVALUATION

All statistical evaluations were conducted using IBM SPSS Statistics (version 25; IBM Corp.), STATA (version 14.2; StataCorp), PROC QUANTREG in SAS 9.4, (SAS Institute Inc., Cary, NC, US) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Five performance metrics were used:

Bias: Median individual differences eGFR - mGFR (mL/min/1.73 m²)

Precision: Interquartile range (IQR) of the differences eGFR - mGFR

Accuracy: Median absolute percentage error (100 |eGFR - mGFR| / mGFR) and the percentage of estimates within ± 10 and ± 30 % of mGFR (labelled P₁₀ and P₃₀). P₃₀ accuracy above 75% is considered "sufficient for good clinical decision-making" but the benchmark is to reach P₃₀ > 90% [23]

Main results are presented stratified for mGFR below and above 75 mL/min/1.73 m², as abnormal GFR in children has been regarded to start below this threshold [24], and by gender. Quantile regression with linear and quadratic fractional polynomials was used to model the relation between age or mGFR and the 50th percentile of eGFR-mGFR (i.e., median bias). Non-parametric and asymptotic 95% confidence intervals (CI) were calculated for the main results as measures of the statistical uncertainty in medians and proportions (P₁₀ and P₃₀). We used McNemar's exact test for pairwise comparisons of P₃₀ across different equations for the main results.

In explorative analysis, we evaluated the performance of the arithmetic means of the most accurate creatinine and cystatin C equations compared to composite equations using a more detailed stratification of mGFR (< 30, 30-44, 45-59, 60-74, 75-89, 90-119 and ≥ 120 mL/min/1.73 m²).

As supplementary results, we restricted the analysis to one randomly chosen measurement per patient in order to obtain statistically independent observations. We also stratified results by age (2-7, 8-12 and 13-17 full years) and by age- and gender-specific BMI percentiles (< 5% underweight, 5 to <85% healthy weight, 85 to <95% overweight and ≥ 95 % obese) [25] representative for Dutch children [26]. LMS values (*L* curve, skewness; *M* curve, median; and *S* curve, coefficient of variation) from reference [26] were converted into BMI percentiles via the *z* score which is calculated from $Z = (y / M)^L - 1 / (S \times L)$ when $L \neq 0$. The corresponding percentile was obtained from the standard normal distribution, e.g., $z = -1.96$ corresponds to the 2.5th percentile, $z = 0$ to the 50th percentile and $z = +1.96$ to the 97.5th percentile. We also present results stratified by organ transplantation and individual cohorts.

Results

Patient characteristics for the entire cohort ($n = 3408$ measurements) are presented in Table 1 and split by participating centres in Supplementary Material (Table S2). The proportion of organ transplant recipients was substantially higher in patients with mGFR below 75 mL/min/1.73 m² (26 versus 4.2%). Patients with mGFR <75 mL/min/1.73m² tended to be older than those with mGFR \geq 75 mL/min/1.73 m² (median age 13.0 versus 11.9 years), while there were no important differences in gender distribution, weight or height. There was no marked difference in mGFR distribution between boys and girls at any age. Boys and girls also had similar sCr and sCys values overall until puberty. In boys aged 13 to 17 years, however, both sCr and sCys concentrations were about 15% higher in boys than in girls of the same age.

Table 1 Patient characteristics of children 2 to < 18 years of age with descriptive measures given as median values (2.5 and 97.5 percentiles) if not stated otherwise. The numbers (n) and percentages (%) refer to GFR measurements/unique children

Variables	All ($n = 3408/2218$)	mGFR <75 mL/min/1.73 m ² ($n = 1068/558$)	mGFR \geq 75 mL/min/1.73 m ² ($n = 2340/1806$)	Girls ($n = 1411/924$)	Boys ($n = 1997/1294$)
Girls, number (%)	1411/924 (41/42)	425/232 (40/42)	986/751 (42/42)	NA	NA
Transplants, number (%)	378/140 (11/6.3)	279/99 (26/18)	99/63 (4.2/3.5)	127/49 (9.0/5.3)	251/91 (13/7.0)
Age (years)	12.3 (2.6-17.8)	13.0 (3.1-17.7)	11.9 (2.5-17.8)	12.0 (2.6-17.8)	12.5 (2.6-17.7)
Weight (kg)	41 (13-86)	42 (14-86)	40 (13-86)	40 (13-80)	42 (14-88)
Height (cm)	148 (90-184)	149 (91-183)	148 (90-185)	147 (90-176)	150 (90-186)
Body surface area (m ²)	1.31 (0.57-2.0)	1.33 (0.57-2.02)	1.29 (0.56-2.06)	1.29 (0.56-1.91)	1.33 (0.57-2.10)
Body mass index (kg/m ²)	18 (14-29)	18 (14-31)	18 (14-29)	18 (13-29)	18 (14-30)
Plasma creatinine μ mol/L)	57 (21-189)	90 (39-328)	48 (19-91)	54 (21-179)	60 (21-201)
Plasma cystatin C (mg/L)	1.00 (0.62-3.00)	1.55 (0.94-4.00)	0.90 (0.60-1.39)	0.95 (0.60-2.89)	1.05 (0.64-3.16)
Measured GFR(mL/min/1.73 m ²)	90 (23-165)	56 (15-74)	103 (77-172)	90 (24-165)	91 (23-164)
Measured GFR, number (%)					
\geq 120 mL/min/1.73 m ²	607 (18)	NA	607 (26)	244 (17)	363 (18)
90-119 mL/min/1.73 m ²	1149 (34)	NA	1149 (49)	485 (34)	664 (33)
75-89 mL/min/1.73 m ²	584 (17)	NA	584 (25)	257 (18)	327 (16)
60-74 mL/min/1.73 m ²	449 (13)	449 (42)	NA	176 (12)	273 (14)
45-59 mL/min/1.73 m ²	285 (8.4)	285 (27)	NA	121 (8.6)	164 (8.2)
30-44 mL/min/1.73 m ²	185 (5.4)	185 (17)	NA	75 (5.3)	110(5.5)
<30 mL/min/1.73 m ²	149 (4.4)	149 (14)	NA	53 (3.8)	96 (4.8)

mGFR measured glomerular filtration rate, NA not applicable

MAIN RESULTS

CREATININE EQUATIONS

The paediatric Schwartz-Lyon_{CR} equation exhibited the best or second-best results among the creatinine equations in terms of bias, precision and all three accuracy measures both below and above an mGFR of 75 mL/min/1.73 m² (Table 2). The P₃₀ accuracy at mGFR <75 mL/min/1.73 m² was significantly higher than for all other creatinine equations ($p < 0.001$) except for Schwartz-2012_{CR} ($p = 0.35$). Schwartz-Lyon_{CR} also exhibited the most stable performance across the whole spectrum of mGFR, especially at the lowest and highest mGFR intervals (Supplementary Material and Table S3). Underestimations exceeding 20 mL/min/1.73 m were noted for Schwartz-2012_{CR} at mGFR >120 mL/min/1.73 m² and for LMR_{CR} at mGFR >130 mL/min/1.73 m² (Fig. 1a). All creatinine

equations, however, had poor performance at $mGFR < 30 \text{ mL/min/1.73 m}^2$ with P_{30} values $\leq 60\%$ (Supplementary Material and Table S3).

CYSTATIN C EQUATIONS

Both the general-purpose (CAPA_{CYS}, CKD-EPI_{CYS} and FAS_{CYS}) and the paediatric (Berg_{CYS} and Schwartz-2012_{CYS}) cystatin C equations had P_{30} accuracy close to 80% at $mGFR < 75 \text{ mL/min/1.73 m}^2$ and between 84 and 89% $\geq 75 \text{ mL/min/1.73 m}^2$ (Table 2). However, Schwartz-2012_{CYS} markedly underestimated $mGFR \geq 75 \text{ mL/min/1.73 m}^2$, resulting in higher absolute percentage error and lower P_{10} compared to the other cystatin C equations. Underestimation exceeding 20 mL/min/1.73 m was noted for Schwartz-2012_{CYS} at $mGFR > 120 \text{ mL/min/1.73 m}^2$ but also for FAS_{CYS} at $mGFR > 135 \text{ mL/min/1.73 m}^2$ (Fig. 1b). Berg_{CYS}, CAPA_{CYS} and CKD-EPI_{CYS} exhibited low bias and generally satisfactory accuracy across all levels of $mGFR$, while Schwartz-2012_{CYS} and FAS_{CYS} had noticeably poor performance at $mGFR < 30 \text{ mL/min/1.73 m}^2$ with $P_{30} < 40\%$ (Supplementary Material and Table S3).

COMPOSITE CREATININE AND CYSTATIN C EQUATIONS

The composite creatinine and cystatin C equations were consistently more precise than the single-marker equations at all $mGFR$ levels. However, at $mGFR < 75 \text{ mL/min/1.73 m}^2$, all composite equations had a positive bias (Fig. 1c). Therefore, accuracy was not superior to the most accurate single-marker equations at $mGFR < 75 \text{ mL/min/1.73 m}^2$ (Table 2). All composite equations had poor P_{30} accuracy at $mGFR < 30 \text{ mL/min/1.73 m}^2$ (Supplementary Material and Table S3). P_{30} accuracy of all composite equations exceeded 90% at $mGFR \geq 75 \text{ mL/min/1.73 m}^2$, which was higher than for any of the single-marker equations (Table 2). Underestimations exceeding 20 mL/min/1.73 m were noted for Schwartz-2012_{CR+CYS} at $mGFR > 140 \text{ mL/min/1.73 m}^2$ (Fig. 1c).

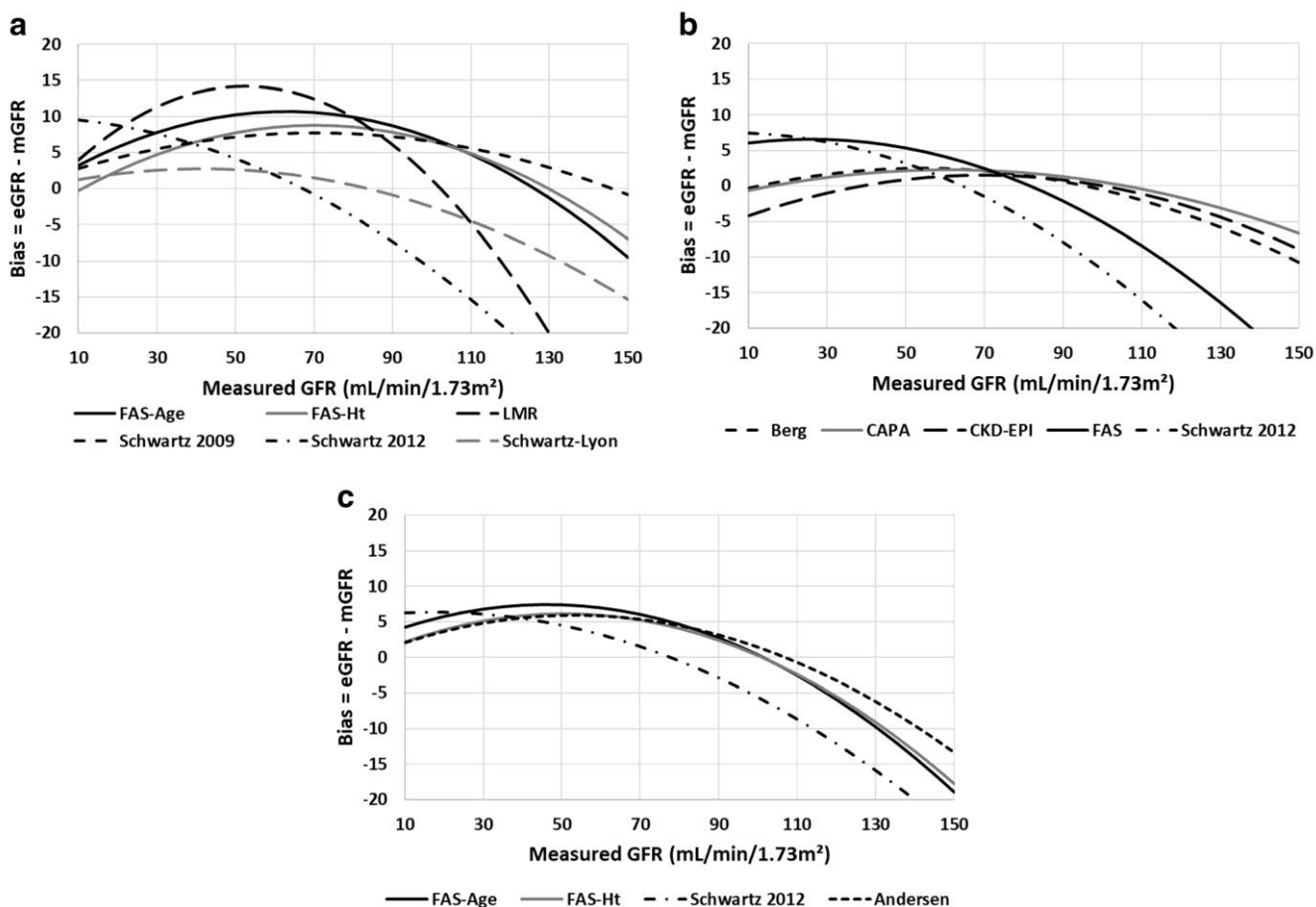


Fig. 1 Quantile regression for the relation between $mGFR$ and median bias (50th percentile of $eGFR - mGFR$) in mL/min/1.73 m^2 of **a** creatinine equations, **b** cystatin C equations and **c** composite creatinine and cystatin C equations. $eGFR$ estimated glomerular filtration rate, $mGFR$ measured glomerular filtration rate, CAPA Caucasian, Asian, Paediatric and Adult equation, CKD-EPI Chronic Kidney Disease Epidemiology collaboration equation, FAS

Full Age Spectrum equation, Ht height, LMR Lund-Malmo revised equation

Table 2 Bias, precision and accuracy (95% confidence intervals) of GFR estimating equations based on creatinine, cystatin C and their combinations in children 2 to < 18 years (n = 3408 measurements) and stratified for measured GFR < 75 (n = 1068) and ≥ 75 mL/min/1.73 m² (n = 2340)

Equations	Bias (mL/min/1.73 m ²)	Precision (mL/min/1.73 m ²)	Absolute percentage error	P ₁₀ (%)	P ₃₀ (%)
<i>mGFR < 75 (median 56) mL/min/1.73 m² Creatinine</i>					
FAS-Age	9.5 (8.5 to 10.2)	15.9	20.3 (18.8 to 22.0)	27.0 (24.3 to 29.6)	66.8 (63.9 to 69.6)
FAS-Height	6.6 (5. To 7.2)	15.8	16.4 (15.5 to 18.0)	33.1 (30.2 to 35.9)	72.3 (69.6 to 75.0)
LMR	13.1 (11.8 to 14.0)	19.1	25.7 (23.9 to 27.5)	22.8 (20.2 to 25.3)	56.7 (53.8 to 59.7)
Schwartz-2009	6.9 (5.9 to 7.6)	15.1	16.7 (15.3 to 17.8)	31.0 (28.2 to 33.8)	73.0 (70.4 to 75.7)
Schwartz-2012	4.1 (3.3 to 4.7)	11.5	13.1 (12.4 to 14.0)	41.5 (38.5 to 44.4)	81.1 (78.7 to 83.4)
Schwartz-Lyon	2.3 (1.5 to 3.1)	13.0	12.7 (12.0 to 13.7)	39.4 (36.5 to 42.4)	82.0 (79.7 to 83.4)
<i>Cystatin C</i>					
Berg	1.1 (0.2 to 1.7)	13.1	13.7 (12.7 to 14.8)	37.2 (34.3 to 40.1)	81.1 (78.7 to 84.4)
CAPA	1.1 (0.4 to 1.9)	15.2	15.4 (14.3 to 16.6)	33.7 (30.9 to 36.5)	79.0 (76.6 to 81.5)
CKD-EPI	-1.1 (-1.7 to -0.5)	13.4	14.9 (13.8 to 15.9)	36.6 (33.7 to 39.5)	80.3 (78.0 to 82.7)
FAS	4.5 (3.6 to 5.0)	12.2	14.5 (13.4 to 15.6)	36.6 (33.7 to 39.5)	77.6 (75.1 to 80.1)
Schwartz-2012	2.4 (1.7 to 3.1)	12.4	13.3 (12.1 to 14.7)	39.7 (36.8 to 42.6)	81.6 (79.2 to 83.9)
<i>Creatinine+cystatin C</i>					
FAS-AGE	6.1 (5.4 to 6.9)	10.9	15.0 (13.6 to 16.3)	37.8 (34.9 to 40.7)	77.4 (74.9 to 79.9)
FAS-Height	4.9 (4.4 to 5.4)	11.4	13.0 (12.0 to 14.0)	39.8 (36.9 to 42.7)	81.5 (79.1 to 83.8)
Schwartz-2012	4.4 (3.8 to 4.9)	10.6	12.9 (12.0 to 13.7)	40.9 (38.0 to 43.9)	81.9 (79.6 to 84.2)
Andersen	4.0 (3.3 to 4.6)	11.6	12.1 (11.1 to 13.1)	43.0 (40.0 to 45.9)	82.4 (80.1 to 84.7)
<i>mGFR ≥ 75 (median 103) mL/min/1.73 m²</i>					
<i>Creatinine</i>					
FAS-Age	5.8 (4.6 to 6.7)	27.9	13.5 (12.9 to 14.1)	38.6 (36.7 to 40.6)	82.4 (80.8 to 83.9)
FAS-Height	5.4 (4.3 to 6.4)	29.1	13.6 (12.7 to 14.4)	38.2 (36.2 to 40.2)	82.0 (80.4 to 83.5)
LMR	-4.1 (-5.0 to -3.1)	26.2	12.5 (11.8 to 13.3)	41.1 (39.1 to 43.1)	88.2 (86.9 to 89.5)
Schwartz-2009	5.2 (4.1 to 6.6)	29.8	14.7 (14.1 to 15.5)	35.2 (33.2 to 37.1)	81.5 (79.9 to 83.1)
Schwartz-2012	-13.2 (-14.0 to -12.3)	22.7	15.2 (14.6 to 16.8)	32.5 (30.6 to 34.4)	85.9 (84.4 to 87.3)
Schwartz-Lyon	-3.6 (-4.7 to -2.3)	26.0	13.5 (12.7 to 14.1)	38.8 (36.8 to 40.8)	87.1 (85.7 to 88.4)
<i>Cystatin C</i>					
Berg	-1.1 (-2.2 to -0.2)	28.3	13.8 (13.0 to 14.3)	38.5 (36.5 to 40.4)	83.8 (82.3 to 85.3)
CAPA	-0.4 (-1.5 to -0.6)	30.1	14.4 (13.6 to 15.0)	36.7 (34.7 to 38.6)	84.6 (83.2 to 86.1)
CKD-EPI	-1.6 (-2.6 to -0.7)	27.2	13.1 (12.5 to 13.7)	39.6 (37.6 to 41.6)	87.5 (86.1 to 88.8)
FAS	-6.2 (-7.0 to -5.4)	24.3	12.9 (12.2 to 13.7)	40.1 (38.1 to 42.1)	88.9 (87.7 to 90.2)
Schwartz 2012	-13.3 (-14.1 to -12.3)	22.7	15.0 (14.2 to 15.6)	31.9 (30.0 to 33.8)	85.9 (84.4 to 87.3)
<i>Creatinine+cystatin C</i>					
FAS-AGE	-0.9 (-1.7 to -0.2)	21.2	10.2 (9.7 to 10.8)	49.2 (47.2 to 51.2)	93.4 (92.4 to 94.4)
FAS-Height	-1.2 (-2.0 to -0.5)	20.8	10.1 (9.7 to 10.6)	49.2 (47.2 to 51.3)	93.2 (92.1 to 94.2)
Schwartz-2012	-7.0 (-7.9 to -6.3)	19.7	11.0 (10.5 to 11.6)	46.1 (44.0 to 48.1)	93.1 (92.0 to 94.1)
Andersen	-1.3 (-2.1 to -0.5)	21.7	10.8 (10.3 to 11.4)	46.8 (44.8 to 48.9)	91.4 (90.2 to 92.5)

Median bias (eGFR - mGFR) and precision (interquartile range) expressed in mL/min/1.73 m² and accuracy expressed as median absolute percentage error (100 · |eGFR - mGFR| / mGFR) and P₁₀ and P₃₀ accuracy (percentage of GFR estimates within ± 10 and ± 30% of mGFR). □P₃₀ < 75%, ▣P₃₀ 75 to < 90%, ■P₃₀ ≥ 90%

CAPA Caucasian, Asian, Paediatric and Adult, CKD-EPI Chronic Kidney Disease Epidemiology collaboration, FAS Full Age Spectrum, LMR Lund- Malmo revised, eGFR estimated glomerular filtration rate, mGFR measured glomerular filtration rate

STRATIFICATION FOR GENDER

Most creatinine equations had equal performance in girls and boys both below and above an mGFR of 75 mL/min/1.73 m (Table 3). Notably, Schwartz-Lyon_{CR}, a modification of Schwartz-2009_{CR} with a lower *k* factor (32.5 instead of 36.5) in all girls and in boys < 13 years, performed better than Schwartz-2009_{CR} both below and above an mGFR of 75 mL/min/1.73 m². CKD-EPI_{CYS}, the only cystatin C equation with a gender factor, exhibited equal performance among girls and boys both below and above mGFR 75 mL/min/1.73 m², while the other cystatin C-based equations had lower P₃₀ accuracy in girls compared to boys at mGFR <75 mL/min/1.73 m (Table 3). The improvement in performance of the composite equations at mGFR ≥ 75 mL/min/1.73 m was noted in both boys and girls.

ARITHMETIC MEANS OF CREATININE AND CYSTATIN C EQUATIONS

In the explorative analysis, the performance of the arithmetic means of the most accurate creatinine and cystatin C equations was evaluated; Schwartz-Lyon_{CR} combined with Berg_{CYS}, CAPA_{CYS} or CKD-EPI_{CYS} (Supplementary Material, Table S3 and Fig. S1). All three performed similarly, generally with decreased bias irrespective of mGFR level and improved P₃₀ accuracy below 45 mL/min/1.73 m compared to the composite equations. The P₃₀ accuracy was equal or higher than for any of the three corresponding cystatin C equations at all mGFR levels ≥ 30 mL/min/1.73 m².

SUPPLEMENTARY RESULTS

Restricting the analysis to one randomly chosen measurement per patient did not change overall results markedly but reduced statistical precision. As an example, P₃₀ for Schwartz-Lyon_{CR} was 79.0% (95% CI 75.3 to 82.6%) among 480 unique patients with mGFR < 75 mL/min/1.73 m² compared with 82.0% (95% CI 79.7 to 83.4%) among all measurements (Table 2). Corresponding figures for 1738 unique patients with mGFR > 75 mL/min/1.73 m² were 85.7% (95% CI 84.0 to 87.3%) compared with 87.1% (95% CI 85.7 to 88.4%) among all measurements.

STRATIFICATION FOR AGE

Schwartz-Lyon_{CR}, Berg_{CYS} and CKD-EPI_{CYS} had relatively stable and adequate performance in all age groups both below and above mGFR of 75 mL/min/1.73 m² (Fig. 2a-c, Supplementary Material and Table S4). The improvement in performance for the composite equations at an mGFR ≥ 75 mL/min/1.73m² was seen across all age groups. The arithmetic means of Schwartz-Lyon_{CR} combined with Berg_{CYS}, CAPA_{CYS} or CKD-EPI_{CYS} had a stable performance across all age groups except for the combination with CAPA below the age of 6 years (Supplementary Material and Fig. S2).

STRATIFICATION FOR BMI

In general, all creatinine equations had a marked positive bias in underweight patients both below and above mGFR of 75 mL/min/1.73 m², while bias of the cystatin C equations was lower (Supplementary Material and Table S5A and B). Despite this, none of the cystatin C equations had P₃₀ accuracy above 75% in underweight patients at mGFR <75 mL/min/1.73 m², while this was the case at mGFR > 75 mL/min/1.73 m² and in obese patients irrespective of mGFR level.

PATIENTS WITH ORGAN TRANSPLANTS

All equations performed at least as well in transplanted as in the other patients, both at mGFR below and above 75 mL/min/1.73 m² (Supplementary Material and Table S6).

RESULTS IN INDIVIDUAL COHORTS

Bias and P₃₀ accuracy of the various GFR equations in the individual cohorts are presented in Supplementary Material (Table S7A and B). In general, the bias of the cystatin C equations varied more across the cohorts compared with the creatinine equations. Of note, in the Lund Cohort, all cystatin C equations had a negative bias at mGFR ≥ 75 mL/min/1.73 m². The cohort from Leuven had only 16 measurements below 75 mL/min/1.73 m² and these results should therefore be interpreted with caution.

Table 3 Bias and accuracy (P_{30}) of GFR estimating equations based on creatinine, cystatin C and their combinations in children 2 to < 18 years old ($n = 3408$ measurements) and stratified by gender and measured GFR < 75 and ($n = 1068$) ≥ 75 mL/min/1.73 m² ($n = 2340$)

Equations	n (%)	Median mGFR (mL/min / 1.73 m ²)	Creatinine						Cystatin C					Creatinine and cystatin C			
			FAS Age	FAS Height	LMR	Schwartz 2009	Schwartz 2012	Schwartz Lyon	Berg	CAPA	CKD-EPI	FAS	Schwartz 2012	FAS Age	FAS Height	Schwartz 2012	Andersen
mGFR < 75 mL/min/1.73 m ²																	
Bias																	
Girls	425 (40)	55	10.0	7.5	9.3	8.9	5.6	2.5	4.0	4.1	0.1	7.4	5.4	8.4	6.7	6.5	5.8
Boys	643 (60)	56	8.9	5.5	16.0	5.1	2.3	2.1	-0.9	-0.7	-1.8	2.3	0.6	4.8	3.6	2.8	2.5
P_{30} (%)																	
Girls			65	73	70	71	78	83	77	74	80	72	79	72	78	78	80
Boys			68	72	48	75	83	81	84	82	81	81	84	81	84	85	84
mGFR ≥ 75 mL/min/1.73 m ²																	
Bias																	
Girls	986 (42)	102	7.1	7.0	-6.4	9.3	10.6	-2.5	4.7	5.7	-0.3	-2.0	-9.1	2.2	1.6	-3.5	3.1
Boys	1,354 (58)	103	4.6	4.3	-1.7	2.4	-15.1	-4.2	-5.2	-4.9	-2.6	-9.4	-16.2	-3.6	-3.4	-10.0	-4.0
P_{30} (%)																	
Girls			82	83	89	81	90	87	81	84	87	89	89	92	92	94	89
Boys			83	81	88	82	83	87	86	85	88	89	84	94	94	92	93

Median bias (eGFR - mGFR) expressed in mL/min/1.73 m and P_{30} accuracy as percentage of GFR estimates within $\pm 30\%$ of mGFR. $\square P_{30} < 75\%$, $\square P_{30} 75$ to < 90%, $\square P_{30} \geq 90\%$

CAPA Caucasian, Asian, Paediatric and Adult, CKD-EPI Chronic Kidney Disease Epidemiology collaboration, FAS Full Age Spectrum, LMR Lund-Malmö revised, eGFR estimated glomerular filtration rate, mGFR measured glomerular filtration rate

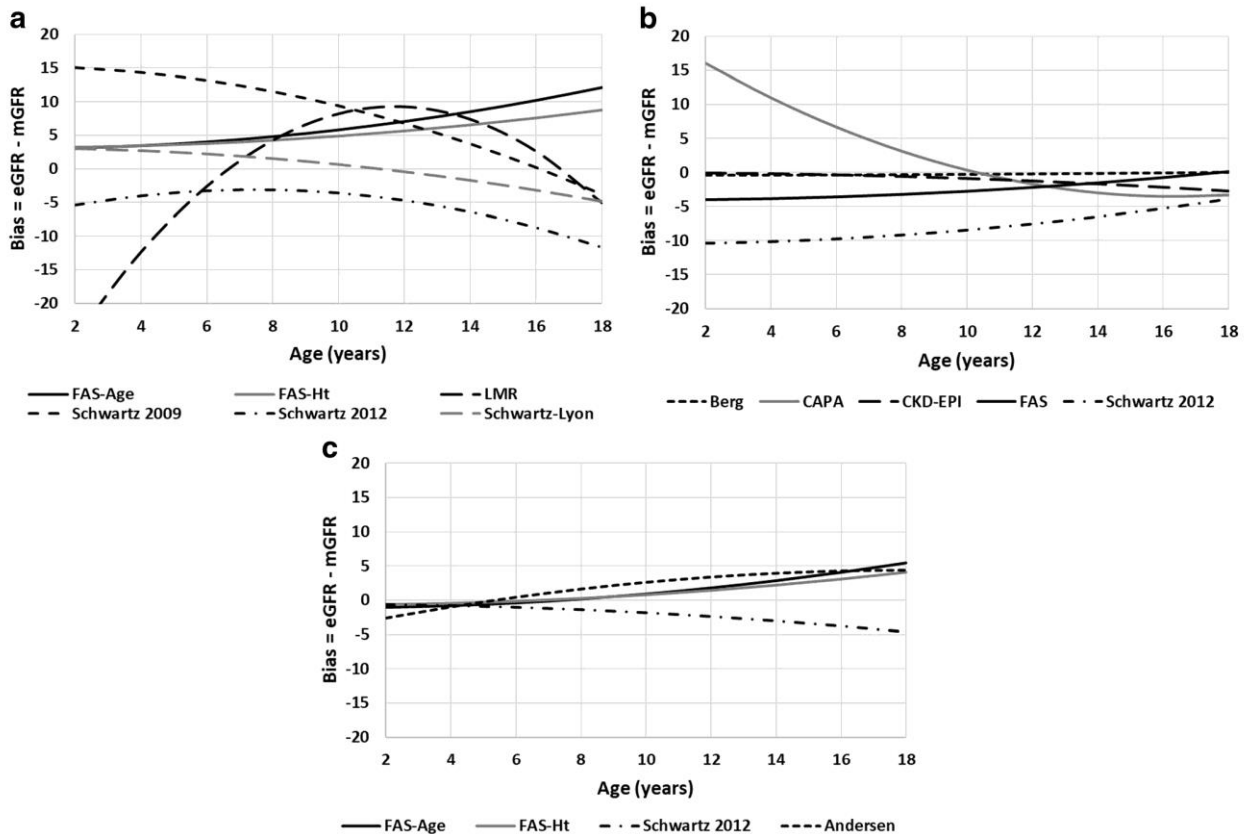


Fig. 2 Quantile regression for the relation between age and median bias (50th percentile of eGFR-mGFR) in mL/min/1.73 m² of **a** creatinine equations, **b** cystatin C equations and **c** composite creatinine and cystatin C equations. eGFR estimated glomerular filtration rate, mGFR measured glomerular filtration rate, CAPA Caucasian, Asian, Paediatric and Adult equation, CKD-EPI Chronic Kidney Disease Epidemiology collaboration equation, FAS

Full Age Spectrum equation, Ht height, LMR Lund-Malmo revised equation

Discussion

Three cystatin C equations, i.e. Berg_{CYS}, CAPA_{CYS} and CKD-EPI_{CYS}, had low bias and generally satisfactory accuracy across all levels of mGFR. By contrast, the creatinine equations were generally hampered by substantial overestimation and lack of accuracy in children with decreased renal function. Only the paediatric Schwartz-Lyon_{CR} equation had satisfactory performance at all mGFR levels ≥ 30 mL/min/1.73 m². Arithmetic means of these best-performing creatinine and cystatin C equations generally lead to further improvement in accuracy at all mGFR levels ≥ 30 mL/min/1.73 m².

The present, multi-centre study is the largest validation of GFR equations in children conducted to date. The cohort represents children, mainly of Caucasian descent, from several European countries and covers the age span between 2 and 17 full years of age, both girls and boys and with a broad spectrum of diagnoses. Therefore, the findings are applicable in daily clinical practice and extend beyond the populations where the various GFR equations were developed. Importantly, both creatinine and cystatin C were analyzed using IFCC standards at all participating centres.

The varying results for the cystatin C equations across the different cohorts suggest that residual differences remain in calibration despite the international standard [27-29]. In the Lund cohort, all cystatin C equations had a marked negative bias at mGFR ≥ 75 mL/min/1.73 m², although a meticulous technique was used to make the cystatin C assay traceable to the international calibrator in this cohort [7]. This may indicate that the cystatin C assays used when developing Berg_{CYS}, CKD-EPI_{CYS}, FAS_{CYS} and Schwartz-2012_{CYS} were suboptimized in relation to the international calibrator. Although the Lund cohort was used for the development CAPA_{CYS}, it also had a marked negative bias. This may be explained by the fact that the Lund paediatric cohort formed only a minor part of the entire development cohort, which was dominated by Swedish and Japanese adults. This may have affected the coefficients of CAPA_{CYS} and resulted in the negative bias in children observed in our study. It should also be noted that roughly 20% of the included measurements were used previously in the development or internal validation of CAPA_{CYS}, Schwartz-Lyon_{CR} and the FAS equations, which may have favored the performance of these equations. Blood urea nitrogen was not available in all cohorts so that the most elaborate CKiD-3 equation [3] could not be tested. The choice of the arithmetic mean equations was driven by the results for the single-marker equations. This may have favored the performance of arithmetic means when compared to the composite creatinine-cystatin C equations.

The present study adds substantial evidence suggesting that cystatin C is a more reliable filtration marker than creatinine for GFR estimations in children with decreased GFR. CKD-EPI_{CYS} was originally developed for adults [22] but nevertheless exhibited surprisingly stable performance across all levels of GFR in the present cohort of children. Although the CKD-EPI_{CYS} did not outperform some of the paediatric cystatin C equations with simpler formula expressions, it has the advantage of covering the whole age spectrum from childhood to adulthood, which may prove attractive for direct eGFR reporting by clinical laboratories. This also applies to CAPA_{CYS} and FAS_{CYS}, although overestimation was an issue with FAS_{CYS} in patients with mGFR below 45 mL/min/1.73 m².

The CKD-EPI_{CYS} equation has a factor of 0.932 for females, which to some extent accounts for the lower cystatin C values among girls than boys during puberty at the same GFR in the present study. Gender-specific differences in cystatin C concentrations during puberty have also been observed by others [30, 31]. The other cystatin C equations do not include separate gender factors, which may explain why they generally perform less well in girls with decreased GFR. Based on our findings, inclusion of a—possibly age-dependent—gender factor appears to be a straightforward modification for some of the cystatin C-based equations, which would most likely improve their performance in the future.

The best-performing creatinine equation Schwartz-Lyon_{CR} is a paediatric equation with a simplistic formulation based on creatinine and height only. It confirms the concept that the age dependence of creatinine can be accounted for in most cases by inclusion of height in the estimation, at least when mGFR is above 30 mL/min/1.73 m². Schwartz-Lyon_{CR} equation uses a lower *k* value for boys under the age of 13 and for all girls and clearly outperforms Schwartz-2009_{CR}. As the Schwartz-2009_{CR} equation—in contrast to the original formulation of the Schwartz equation [32]—does not account for gender-specific differences in body composition, this suggests that gender-specific differences in creatinine production need to be accounted for in order to assure optimal performance.

LMR_{CR} was originally developed for adults [20] and has been shown to outperform the CKD-EPI creatinine equation (CKD-EPI_{CR}) in several adult cohorts, both overall and across important subgroups [33-38]. While CKD-EPI_{CR} is unsuitable in children [21], the present study demonstrates that LMR_{CR} performs quite well in children with mGFR 75-119 mL/min/1.73 m² but is unsuitable outside this range.

The FAS-AGE_{CR} equation was developed with the intention of covering the full age spectrum in children and adults of all ages irrespective of GFR level [13, 12]. This approach eliminates the discontinuous course of eGFR at the transition from adolescence to adulthood due to the change of equation, e.g. from a Schwartz- equation to CKD-EPI [39, 40]. As FAS-AGE_{CR}, like LMR_{CR}, does not require height data, eGFR can be reported automatically by the laboratories. Consistent with a previous study [13], we find satisfactory bias and accuracy for all FAS equations in children with normal GFR but overestimation in children with decreased GFR. Validation studies in adults with CKD have shown substandard accuracy of the FAS equations [12, 38], however. The FAS_{CR} equation corrects for differences in sCr levels with respect to gender (starting at adolescence), age and height. The formula assumes a simplistic inverse relationship between GFR and sCr/Q. While this seems to work well within a certain range of normal sCr values, the relation is probably more complex when sCr becomes too high. This is not surprising when looking at most modern GFR equations, which were built using linear regression analysis following logarithmic transformation [41]. In a similar way, the concept of FAS_{CYS} is based on average GFR and cystatin C for a healthy population. It thus seems that the FAS equations in their present form can only be recommended in patients with normal or mildly impaired renal function and need to be reformulated in patients with low GFR.

Previous studies have shown improved GFR estimation performance when creatinine and cystatin C are combined in children [1, 3, 5, 42]. The present study extends on these results by showing that this improvement is not evident in children with decreased GFR when existing composite equations are applied. However, our explorative results suggest that improvement in performance can be obtained at most levels of mGFR if an arithmetic mean of the best-performing creatinine and cystatin C equations is used. This is in line with previous studies showing that the simple arithmetic means of existing single-marker equations tend to perform as well as more complex formulations that include both markers [21, 43, 44]. This approach also alerts the clinician to situations where one of the two GFR markers fails such as creatinine in spina bifida patients or cystatin C during high-dose glucocorticoid treatment [43, 45, 46].

Conclusion

The present study strongly suggests that cystatin C should replace creatinine as the primary biomarker of choice when estimating GFR in children with moderately to severely decreased GFR. Arithmetic means of well-performing creatinine and cystatin C equations can be expected to improve accuracy at most mGFR levels and have practical advantages compared to composite equations. However, if high precision is required, e.g. in patients with severely decreased GFR, GFR measurement by exogenous clearance markers is recommended. EKFC and its data resources offer an excellent opportunity for refining GFR equations further, both among children and adults. Development of full-age-spectrum equations and estimation strategies that utilize both creatinine and cystatin C applicable for both children and adults irrespective of their GFR level would greatly facilitate direct eGFR reporting by the clinical laboratory and solve the problem of abrupt jumps in eGFR during the transition from child- to adulthood due to the changes from a paediatric to an adult GFR equation.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the World Medical Association Declaration of Helsinki of 1975, as revised in 2000.

Informed consent For this type of retrospective study, all extracted data were fully anonymous without any personal information, why informed consent was not required according to the regional ethical board approval in, Lund, Sweden, which approved the study.

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