Diagnostic Accuracy of Cystatin C–Based eGFR Equations at Different GFR Levels in Children

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Summary

Background and objectives The diagnostic accuracy of cystatin C estimated GFR (eGFR) by various cystatin C equations have varied in different studies. We hypothesized that the GFR level of enrolled patients affects the diagnostic accuracy of a cystatin C equation.

Design, setting, participants, & measurements We analyzed 240 consecutively enrolled children at a single Canadian center in a prospective and cross-sectional study. Cystatin C was analyzed with nephelometry, and cystatin C eGFR was estimated by the equations validated in children. GFR was measured by technetium-99m–diethylene-triamine penta-acetic acid (^{99m}Tc DTPA).

Results We compared various cystatin C equations across GFR strata <60, <90, ≥135, and ≥150 ml/min per 1.73 m^2 for an accurate prediction and appropriate classification of the measured GFR. The CKiD, Zappitelli-CysEq, and Zappitelli-CysCrEq equations had a higher accuracy, estimated by eGFR values within 10% and 30% of the respective ^{99m}Tc DTPA, in the GFR categories <60 and <90 ml/min per 1.73 m^2, whereas the Bökenkamp, Bouvet, and Filler equations had a greater accuracy in the GFR categories ≥135 and ≥150 ml/min per 1.73 m^2. The Bouvet, CKiD, Filler, Zappitelli-CysEq, and Zappitelli-CysCrEq equations had a greater sensitivity to classify GFR <60 and <90 ml/min per 1.73 m^2, whereas the Bökenkamp equation had a higher sensitivity for GFR ≥135 and ≥150 ml/min per 1.73 m^2.

Conclusions The diagnostic accuracy of various cystatin C equations varies with GFR. This issue needs consideration while applying these equations in clinical practice and for further research on eGFR equations.

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Introduction

Kidney function typically is measured by GFR. In clinical practice, it is most frequently estimated using endogenous surrogate markers. Serum creatinine remains the most widely used endogenous marker. Serum cystatin C is a relatively new endogenous marker that offers the advantage of a constant production by all nucleated body cells and its almost entire catabolism at the proximal tubule (1). In clinical studies, serum cystatin C has been found to be a good marker for predicting GFR (2–9).

The Kidney Disease Outcomes Quality Initiative (KDOQI) recommends the use of predictive equations based on the serum concentrations of these markers (10). Various predictive equations have been established based on serum cystatin C levels (2–8). In children, the validated equations have used serum cystatin C levels either without serum creatinine (*e.g.*, Bökenkamp, Filler, Grubb, and Zapitelli-CysEq equations) (2–4,6) or with serum creatinine (*e.g.*, Bouvet, chronic kidney disease in children [CKiD], and Zapitelli-CysCrEq equations) (3,8,11). The rationale of combining serum creatinine and plasma cystatin C originated from the fact that the sources of error for either marker differ. Serum creatinine levels are confounded by muscle mass and variable tubular secretion, whereas serum cystatin C has a different volume of distribution and may vary with the volume status (12).

Various cystatin C equations consistently demonstrate a variance between the measured GFR and cystatin C estimated GFR (eGFR) in the magnitude of 20% to 30% (2,3,13). With different equations, the estimates of cystatin C eGFR also vary for the same cystatin C level (3,11,14). The equations performed differently in subsequent studies than in the original studies (11). The factors contributing to the variability in the performance of cystatin C equations are not well understood.

The use of different gold standards for measuring GFR cannot completely account for the difference in the estimated and measured GFR (2,3,13). In children, age and body mass do not significantly affect cystatin C eGFR (13). The known confounders affecting cystatin C levels such as corticosteroids and thyroid status cannot explain the variability in the performance of different cystatin C equations either (3,8,11). Previous studies have demonstrated that the variance between cystatin C eGFR and measured GFR increases in the

high GFR range when compared with that in the low GFR range (3,13). The cystatin C eGFR estimates by different equations also show a higher interequation variability at a higher GFR (14). Any change in the diagnostic accuracy of various equations at different GFR levels has not been systematically investigated.

We hypothesized that the diagnostic accuracy of various cystatin C equations may vary with GFR level. We tested this hypothesis in children across different GFR categories with various validated cystatin C equations.

Materials and Methods

The study was approved by the Institutional Review Board, and written consent was obtained from the parents or legal guardians of the patients. In a prospective manner, 240 stable children consecutively referred to the pediatric nephrology clinic in a single tertiary care Canadian center underwent the estimation of serum cystatin C levels and technetium 99m–diethylene-triamine penta-acetic acid (^{99m}Tc DTPA) GFR. We excluded patients with an acute illness, acute kidney injury, or a thyroid disorder. We included patients on a low-dose steroid, as steroid dose up to 2 mg/kg per day does not affect cystatin C level (15).

Baseline data were collected regarding the date of birth, date of assessment, weight, height, and body surface area (BSA). BMI was calculated by the ratio of weight (kg) and square of height (m). BMI *z*-scores were calculated from the age and gender-specific SD published by the US National Center for Health Statistics (16).

Nuclear GFR Estimation

Nuclear GFR was measured using a ^{99m}Tc DTPA renal scan with three-point sampling approach at 2, 3, and 4 hours after injection (17). As per conventional practice, measured ^{99m}Tc DTPA GFR was normalized to a BSA of 1.73 m^2, calculated using the Haycock formula (18). To ensure reliability of ^{99m}Tc DTPA measurements, standard radiochemical and radiopharmaceutical purity were performed on each preparation of ^{99m}Tc DTPA. The average purity, obtained from our radiopharmacy laboratory, was approximately 99%. ^{99m}Tc DTPA has shown to be in good agreement with inulin and iothalamate clearance (19).

Calculation of the Estimated GFR

Serum cystatin C was measured using an N Latex cystatin C kit (Siemens Healthcare, Mississauga, Canada) on a Behring BN ProSpec analyzer (Dade Behring, Marburg, Germany). The detailed method is described elsewhere (6). The coefficient of variation of serum cystatin C was 3.1% at 1.06 mg/L, 3.5% at 2.04 mg/L, and 6.7% at 5.26 mg/L.

Cystatin C eGFR was calculated using the previously published Bökenkamp (2), Bouvet (8), CKiD (11), Filler (6), Grubb (4), and Zappitelli (3) equations. The published equations are shown in Table 1. While employing the CKiD equation, we converted enzymatic serum creatinine to isotope dilution mass spectroscopy (IDMS) standardized creatinine as done in the original study (11).

Evaluation of the Estimated GFR

We compared the correlation, bias, precision, and accuracy of serum cystatin C eGFR with respect to ^{99m}Tc DTPA GFR in the GFR groups of <60, <90, ≥135, and ≥150 ml/min per 1.73 m^2. These cutoffs have been used previously to categorize CKD (5,20), and to define hyperfiltration (21).

We calculated the bias, precision, and accuracy, as recommended by the National Kidney Foundation (10):

Table 1. Published cystatin C–based estimated GFR equations

Equation Name	Equation
Equations with serum cystatin C	
Bökenkamp *et al.* (2)	GFR (ml/min per 1.73 m^2) = 137/serum cystatin C − 20.4
Filler and Lepage (6)	GFR (ml/min per 1.73 m^2) = 10^(1.962 + (1.123 × LOG(1/Cys C))
Grubb *et al.* (4)	GFR (ml/min per 1.73 m^2) = 84.69 × serum cystatin $C^{-1.68}$ × 1.384 for age <14 years
Zappitelli *et al.* (CysEq) (3)	GFR (ml/min per 1.73 m^2) = 75.94/[serum cystatin $C^{1.17}$] if renal transplant, ×1.2
Equations with serum cystatin C and serum creatinine	
Bouvet *et al.* (8)	[(SCr (μM)/96)(−0.35 (±0.20))] · [(serum cystatin C (mg/L)/1.2)(−0.56(±0.19))] · [(body weight (kg)/45)(0.30(±0.17))] · [age (years)/14)(0.40 (±0.16))].
CKiD[a] (11)	eGFR= a[height (m)/Scr (mg/dl)]^b[1.8/cystatin C (mg/L)]^c[30/BUN (mg/dl)]^d[e^{male}][height/1.4]^f
Zappitelli *et al.* (CysCrEq) (3)	GFR (ml/min per 1.73 m^2) = (507.76$e^{0.003\times height}$)/($CysC^{0.635}$ × $SCr^{0.547}$ [μmol/L]) If renal transplant, ×1.165 If spina bifida, ×($SCr^{0.925}$ [μmol/L])/40.45

[a]We analyzed the CKiD equation with the model that did not include urea. In the original study, the inclusion of urea level in the equation improved the performance of CKiD equation to some extent as the model without urea had R^2 of 69.4% and percentage of estimated GFR within 30% of measured GFR of 84% which was slightly lower than the respective values of 75% and 87.7% with the equation with urea (11). BUN, blood urea nitrogen.

- Bias = mean difference between ^{99m}Tc DTPA GFR and cystatin C eGFR
- Relative bias = mean % difference = 100 × [(^{99m}Tc DTPA GFR − cystatin C eGFR)/^{99m}Tc DTPA GFR]
- Precision = SD of bias (an increase in the SD means a decrease in the precision)
- Relative precision = SD of relative bias
- Accuracy

= percentage of cystatin C eGFR values within 10 and 30% of the respective ^{99m}Tc DTPA GFR measurements

= area under curve (AUC) for the GFR <60, <90, ≥135, and ≥150 ml/min per 1.73 m^2 (5,20).

Statistical Analyses

Continuous data were tested for normal distribution using the D'Agostini Pearson omnibus test. Normally distributed data were analyzed using parametric methods (mean, SD, *t* test, Pearson correlation). Otherwise, nonparametric methods (median, interquartile range, Wilcoxon matched pairs test, and Spearman rank correlation) were applied. The agreement between ^{99m}Tc DTPA GFR and cystatin C eGFR was analyzed by Bland and Altman analysis (22). The AUC, sensitivity, and specificity of cystatin C eGFR for different GFR cutoffs were analyzed by receiver operating characteristic (ROC) plots using Medcalc software (23). We used GraphPad Prism software, version 4.02 (GraphPad, Inc., San Diego, CA) and SPSS version 17 (SPSS, Inc., Chicago, IL) for statistical analysis.

Results

In the study group of 240 patients, median age was 11.7 years (range 2.0 to 17.9 years) and 107 (45%) were girls. The reasons for GFR measurement included abnormal kidney morphology (19.5%), glomerulopathies (14.4%), obstructive uropathy (13.4%), reflux nephropathy (13.0%), proteinuria (9.9%), oncologic disease-associated nephropathy (6.8%), and others (23%).

The percentage error of the eGFR by all of the equations with respect to the measured GFR is shown in Figure 1. In the whole group, the correlation coefficient between the percentage error and measured GFR was significant for all of the equations.

Patient characteristics in the five GFR groups, GFR <60 (n = 31), <90 (n = 74), 90 to 134 (n = 84), ≥135 (n = 81), and ≥150 (n = 41) ml/min per 1.73 m^2, are shown in Table 2. These groups were similar with respect to the age, gender, and number of adolescent patients. BMI *z*-score was lower in the GFR <60 and <90 ml/min per 1.73 m^2 groups; however, the proportion of obese patients was evenly distributed among the groups.

Table 3 shows the mean, median, and correlation coefficients of the measured and estimated GFR. The correlation coefficient decreased with all of the equations with the increase in GFR, except no interval change with the Bouvet equation across the GFR.

Table 4 shows the Bland and Altman analysis for the agreement of eGFR by different equations and measured GFR. As the bias and SD of bias of the equations increased with GFR, we analyzed the relative (%) bias and relative SD of bias as recommended (22,24,25). Unlike the bias and SD of bias, the relative bias and relative SD of bias of the equations changed variably with GFR. The diagnostic accuracy of the equations estimated by eGFR values within 10% and 30% of the respective ^{99m}Tc DTPA also varied with GFR. The CKiD, Zappitelli-CysEq, and Zappitelli-CysCrEq equations had a higher accuracy in the GFR categories <60 and <90 ml/min per 1.73 m^2 than in the GFR ≥135 and ≥150 ml/min per 1.73 m^2. The Bökenkamp, Bouvet, and Filler equations had a greater accuracy in the GFR categories ≥135 and ≥150 ml/min per 1.73 m^2. The Grub equation did not have much change in the accuracy across the GFR categories.

Table 5 shows the area under the ROC curves (AUC), sensitivity, and specificity of various equations to appropriately categorize the measured GFR. The Bouvet equation had the AUC of 1.0 over all GFR categories, whereas all other equations had the AUC of 0.97 to 0.99 for GFR <60 and <90 ml/min per 1.73 m^2, which decreased to 0.83 to 0.85 for GFR ≥135 and ≥150 ml/min per 1.73 m^2. For GFR <60 and <90 ml/min per 1.73 m^2, the CKiD, Zappitelli-CysEq, and Zappitelli-CysCrEq equations had >90% sensitivity for categorizing the GFR. For GFR ≥135 and ≥150 ml/min per 1.73 m^2, the Bökenkamp equation had >90% sensitivity. All of the equations had >90% specificity for GFR <60 ml/min per 1.73 m^2. The specificity was >90% for the Bökenkamp, Bouvet, Filler, and Grubb equations at the GFR <90 ml/min per 1.73 m^2, for the Bouvet, CKiD, Zappitelli-CysEq, and Zappitelli-CysCrEq equations at the GFR ≥135 ml/min per 1.73 m^2, and for the Bouvet, CKiD, Filler, Zappitelli-CysEq, and Zappitelli-CysCrEq equations at the GFR ≥150 ml/min per 1.73 m^2.

Discussion

The main finding of the study was that the diagnostic accuracy of various cystatin C equations changed with GFR. This change in the diagnostic accuracy occurred in both classifying and predicting the measured GFR. Notably, the pattern of change in the diagnostic accuracy with GFR varied among the equations. Some equations performed better at a low GFR and others at a high GFR. To the best of our knowledge, this is the first study that demonstrated the variation in the diagnostic accuracies of different cystatin C eGFR equations with GFR. This observation becomes clinically relevant as it can provide insight into the clinical applicability of the equations at different GFR levels. It can also explain the variability in the performance of various equations in different studies (3,11,26).

As per standard methodology, we analyzed the diagnostic accuracy of various equations by two methods: first, by the ability of the equations to classify the measured GFR appropriately, as tested by the AUC, sensitivity, and specificity; and second, by the accuracy of the equations in predicting the measured GFR, as tested by the relative bias, relative SD of bias, and eGFR values within 10% and 30% of the respective ^{99m}Tc DTPA. The equations were compared over the GFR categories <60, <90, ≥135, and ≥150 ml/min per 1.73 m^2, which were consistent with the KDOQI recommendations on GFR categorization (10), and also with previous studies testing eGFR equations in decreased GFR and hyperfiltration (5,20,21).

The AUC of all cystatin C equations (with the exception of the Bouvet equation, which did not change with GFR)

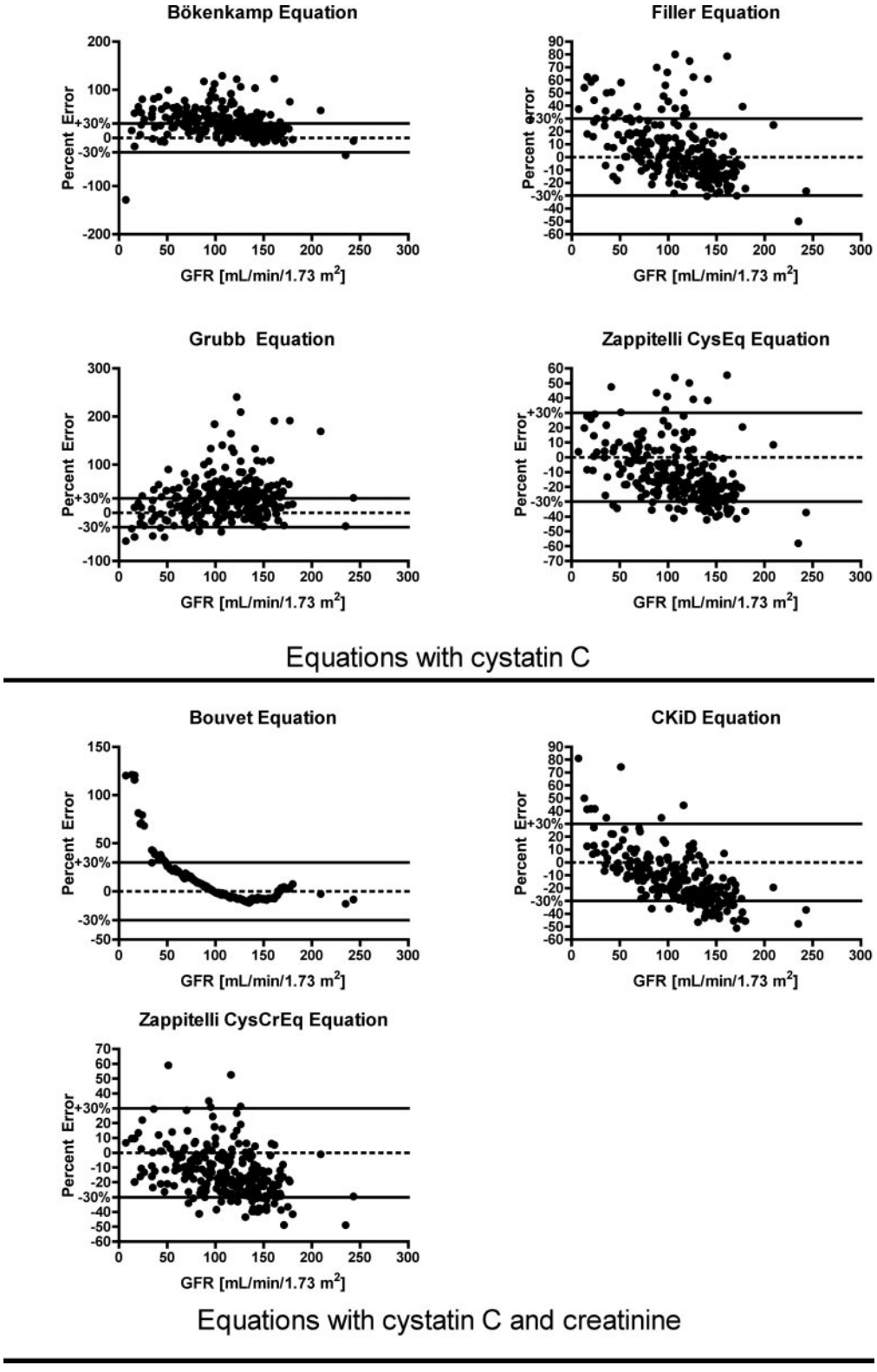

Figure 1. | **Relationship between the percentage errors of different eGFR equations with measured GFR.** Percentage error = (measured GFR − eGFR/measured GFR) × 100. Correlation coefficient between the percent error and measured GFR for equations with only cystatin C: Bökenkamp: $r = -0.22$ (95% confidence interval [CI], −0.34 to −0.10), Filler: $r = -0.48$ (95% CI, −0.57 to −0.37), Grubb: $r = 0.23$ (95% CI, 0.11 to 0.35), Zappitelli-CysEq: $r = -0.43$ (95% CI, −0.53 to −0.32). Correlation coefficient between the percent error and measured GFR for equations with cystatin C and creatinine: Bouvet: $r = -0.76$ (95% CI, −0.81 to −0.70), CKiD: $r = -0.69$ (95% CI, −0.75 to −0.62), Zappitelli-CysCrEq: $r = -0.42$ (95% CI, −0.51 to −0.31). $P < 0.05$ for correlation coefficients with all of the equations.

Table 2. Patient characteristics in different GFR categories (ml/min per 1.73 m^2)

	Whole Group (n = 240)	GFR <60 (n = 31)	GFR <90 (n = 74)	GFR 90 to 134 (n = 84)	GFR ≥ 135 (n = 81)	GFR ≥ 150 (n = 41)
Age, years	11.65[a] (6.92 to 15.35)	11.51 (4.42)	11.70[a] (7.87 to 16.0)	11.70[a] (6.86 to 15.0)	11.40[a] (6.55 to 14.70)	10.61 (4.72)
Age >10 years, %	114 (47.5%)	16 (51.61%)	36 (49%)	5 (6%)	38 (46.91%)	19 (46.34%)
Girls, %	107 (44.58%)	12 (38.70)	30 (41%)	11 (13%)	35 (43.20%)	19 (46.34%)
Renal transplant	20 (8.33%)	3 (9.67%)	13 (17.56%)	1 (1.19%)	0	0
Height, cm	144.0[a] (119.0 to 162.0)	137.4 (24.43)	139.0[a] (121.5 to 162.0)	139.8 (26.54)	145.0[a] (117.0 to 162.0)	139.2 (27.35)
Weight, kg	41.25[a] (22.60 to 54.18)	30.80[a] (22.00 to 53.00)	35.20[a] (22.98 to 52.03)	41.97 (20.37)	41.60[a] (21.60 to 56.35)	40.30 (19.61)
BMI z-score	0.3472 (1.22)	−0.10 (1.65)	0.05[a] (−0.78 to 0.97)	0.49 (1.19)	0.49 (1.07)	0.44 (1.06)
BMI z-score ≥2.0	18 (7.5%)	2 (6.4%)	5 (6.7%)	6 (7.14%)	3 (3.7%)	2 (4.8%)

Data are presented as mean (SD). GFR was measured by technetium-99m–diethylene-triamine penta-acetic acid (^{99m}Tc DTPA) and presented in ml/min per 1.73 m^2. Age >10 years and BMI z-score ≥2.0 are reported to identify adolescents and obese patients, respectively. BMI, body mass index.
[a]Data are presented as median (interquartile range).

decreased from 0.98 to 0.99 for GFR <60 and <90 ml/min per 1.73 m^2 to 0.83 to 0.85 for GFR ≥135 and ≥150 ml/min per 1.73 m^2. In clinical context, the AUC of 0.90 to 0.99 is deemed excellent and that of 0.80 to 0.89 indicates good performance (23). The AUC of an equation combines its sensitivity and specificity. There was not only a change in the sensitivities and specificities of the equations with GFR but also the extent of change varied among the equations. The CKiD, Zappitelli-CysEq, and Zappitelli-CysCrEq equations had >90% sensitivity for classifying GFR <60 and <90 ml/min per 1.73 m^2, whereas the Bökenkamp equation had a similar sensitivity for GFR ≥135 and ≥150 ml/min per 1.73 m^2. The specificities of the equations also varied with GFR.

The diagnostic accuracy of various equations assessed for an accurate prediction of the measured GFR again changed variably with GFR, except no interval change for Grubb's equation with GFR (10). As the bias of various equations increased with the GFR, it was important to understand the implication of this change. A bias of 15 ml/min per 1.73 m^2 at a GFR of 30 ml/min per 1.73 m^2 would mean a relative (%) bias of 50%, whereas the same bias at a GFR of 120 ml/min per 1.73 m^2 signifies a relative bias of 12.5%. Therefore, we estimated the relative bias and SD of bias for all of the equations (22,24). Unlike the bias and SD of bias, the relative bias and SD of bias of the equations changed variably across the GFR categories. Furthermore, the diagnostic accuracy of the equations estimated by eGFR values within 10% and 30% of the respective ^{99m}Tc DTPA GFR also varied with GFR. The CKiD, Zappitelli-CysEq, and Zappitelli-CysCrEq equations had a higher accuracy in the GFR ranges <60 and <90 ml/min per 1.73 m^2, whereas the Bökenkamp, Bouvet, and Filler equations had greater accuracy in the GFR categories ≥135 and ≥150 ml/min per 1.73 m^2. As an individual's day-to-day GFR varies by 17% (27–29), we looked at the equations with >80% cystatin C eGFR values within 30% of the measured GFR across different GFR ranges. This cutoff of accuracy was met by the Zappitelli-CysEq and Zappitelli-CysCrEq equations for GFR <60 ml/min per 1.73 m^2, by the CKiD, Zappitelli-CysEq, and Zappitelli-CysCrEq equations for GFR <90 ml/min per 1.73 m^2, and by the Bökenkamp, Bouvet, and Filler equations for the GFR ≥135 and ≥150 ml/min per 1.73 m^2.

There was an apparent discrepancy in the diagnostic accuracy of eGFR equations in GFR categorization and GFR prediction. For example, the Bouvet equation had an excellent AUC and correlation coefficient overall; however, it had a relatively large relative bias and lower predictive accuracy in the low GFR range. It is important to consider that the AUC and sensitivity of an equation depends on the cutoff points selected for GFR categorization and the equation's tendency to underestimate or overestimate the GFR. On the other hand, the accuracy of an equation for GFR prediction varies by the closeness of an eGFR to the measured GFR, regardless of the equation's tendency to underestimate or overestimate the measured GFR. This point was further evident from a higher percentage error of the Bouvet equation at the low GFR. Unlike other equations, the Bouvet equation employed a Baysian approach for GFR

Table 3. GFR levels estimated by various cystatin C equations and correlation coefficients between the estimated GFR and measured GFR across different GFR categories (ml/min per 1.73 m^2)

	Whole Group (n = 240)	GFR <60 (n = 31)	GFR <90 (n = 74)	GFR 90 to 134 (n = 84)	GFR ≥ 135 (n = 81)	GFR ≥ 150 (n = 41)
Mean; median						
measured GFR	109.7; 112	37.26; 37	59.14; 66	111.4; 110.5	153.9; 150	166.6; 162
equations with serum cystatin C						
Bökenkamp *et al.*	140.5; 143.1	52.05; 51	81.49; 87.9	153.6; 145.6	180.6; 169.9	191; 179.3
Filler and Lepage	110.6 to 111.8	45.18; 44	66.23; 70.4	120.1; 113.7	141.2; 132.5	149.5; 139.9
Grubb *et al.*	148.4; 141.4	40.56; 36.9	68.09; 70.5	163.2; 150.9	206.6; 189.2	227.9; 202.1
Zapitelli-CysEq	93.92; 93.75	37.29; 35.4	56.35; 58.5	102.4; 96.0	119.3; 111.5	126.7; 118
equations with serum cystatin C and serum creatinine						
Bouvet *et al.*	108.5; 107.1	51.85; 51.3	70.72; 76.45	106.6; 106.5	144.9; 137.7	160.2; 152.9
CKiD	89.58; 91.1	41.83; 41.3	57.82; 61.4	97.03; 94.19	110.9; 109.6	116.8; 112.3
Zapitelli-CysCrEq	92.25; 94.65	36.43; 34.6	54.19; 58	100.8; 96.0	118.2; 115.6	125.6; 120
Correlation coefficient (95% CI)						
equations with serum cystatin C						
Bökenkamp *et al.*	0.82 (0.78 to 0.86)	0.88 (0.76 to 0.94)	0.84 (0.76 to 0.90)	0.36 (0.16 to 0.54)	0.42 (0.22 to 0.59)	0.43 (0.14 to 0.66)
Filler and Lepage	0.82 (0.78 to 0.86)	0.88 (0.76 to 0.94)	0.84 (0.76 to 0.90)	0.45 (0.25 to 0.61)	0.42 (0.22 to 0.59)	0.43 (0.14 to 0.66)
Grubb *et al.*	0.80 (0.74 to 0.84)	0.84 (0.69 to 0.92)	0.77 (0.66 to 0.85)	0.31 (0.11 to 0.49)	0.36 (0.15 to 0.54)	0.34 (0.02 to 0.59)
Zapitelli-CysEq	0.81 (0.76 to 0.85)	0.87 (0.75 to 0.94)	0.83 (0.74 to 0.89)	0.40 (0.20 to 0.56)	0.42 (0.22 to 0.59)	0.43 (0.14 to 0.66)
equations with serum cystatin C and serum creatinine						
Bouvet *et al.*	0.99 (0.99 to 0.99)	0.99 (0.99 to 0.99)	0.99 (0.99 to 0.99)	0.99 (0.99 to 0.99)	0.99 (0.99 to 0.99)	0.99 (0.99 to 0.99)
CKiD	0.81 (0.76 to 0.85)	0.92 (0.84 to 0.96)	0.82 (0.72 to 0.88)	0.40 (0.21 to 0.57)	0.29 (0.07 to 0.48)	0.30 (−0.01 to 0.56)
Zapitelli-CysCrEq	0.82 (0.78 to 0.86)	0.91 (0.82 to 0.95)	0.84 (0.75 to 0.89)	0.39 (0.19 to 0.56)	0.36 (0.15 to 0.54)	0.39 (0.09 to 0.63)

CI, confidence interval.

Table 4. Bland and Altman analysis for the agreement of technetium-99m–diethylene-triamine penta-acetic acid (^{99m}Tc DTPA) GFR and cystatin C estimated GFR (estimated by various cystatin C equations) across different GFR categories (ml/min per 1.73 m^2)

	Bias	SD of Bias	Relative Bias (%)	Relative SD of Bias (%)	95% Limit of Agreement for Relative SD of Bias	Accuracy (%) Within	
						10%	30%
Whole group							
equations with serum cystatin C							
Bökenkamp *et al.*	−30.76	31.45	−23.18	31.51	−84.95, 38.58	19.17%	55.83%
Filler and Lepage	−2.70	20.52	2.71	20.53	−42.94, 37.52	37.50%	85.42%
Grubb *et al.*	−38.70	59.59	−21.07	31.48	−82.79, 40.64	17.92%	49.58%
Zapitelli-CysEq	15.78	25.67	13.90	20.88	−27.01, 54.83	28.75%	80.83%
equations with serum cystatin C and serum creatinine							
Bouvet *et al.*	1.17	10.28	−3.53	16.81	−36.48, 29.41	27.08%	79.58%
CKiD	20.12	24.07	16.08	21.72	−26.49, 58.66	26.67%	78.33%
Zapitelli-CysCrEq	17.45	22.49	15.77	18.78	−21.03, 52.58	29.58%	85.42%
GFR < 60 ml/min per 1.73 m^2							
equations with serum cystatin C							
Bökenkamp *et al.*	−14.79	13.81	−17.79	73.35	−161.56, 125.98	16.13%	38.71%
Filler and Lepage	−7.91	8.18	−20.76	18.68	−57.39, 15.86	19.35%	58.06%
Grubb *et al.*	−3.3	14.86	2.92	36.94	−69.48, 75.33	16.13%	51.61%
Zapitelli-CysEq	−0.02	7.52	−0.74	18.85	−37.69, 36.22	48.39%	87.10%
equations with serum cystatin C and serum creatinine							
Bouvet *et al.*	−14.59	2.39	−38.20	18.43	−74.33, −2.07	0.00%	32.26%
CKiD	−4.57	8.08	−13.67	18.25	−49.44, 22.09	45.16%	77.42%
Zapitelli-CysCrEq	0.83	7.97	3.28	17.26	−30.55, 37.12	38.71%	96.77%
GFR < 90 ml/min per 1.73 m^2							
equations with serum cystatin C							
Bökenkamp *et al.*	−27.80	17.85	−30.28	15.31	−60.30, −0.26	10.81%	39.19%
Filler and Lepage	−6.49	13.67	−7.54	15.08	−37.10, 22.01	29.73%	78.38%
Grubb *et al.*	−13.03	24.10	−12.63	25.57	−62.75, 37.47	24.32%	59.46%
Zapitelli-CysEq	4.82	12.40	7.44	16.13	−24.19, 39.07	50.00%	90.54%
equations with serum cystatin C and serum creatinine							
Bouvet *et al.*	−11.58	3.59	−23.09	17.80	−57.98, 11.80	18.92%	71.62%
CKiD	5.56	9.77	7.90	13.53	−18.62, 34.43	45.95%	89.19%
Zapitelli-CysCrEq	7.91	10.29	11.68	14.70	−17.14, 40.50	44.59%	94.59%
GFR 90 to 134 ml/min per 1.73 m^2							
equations with serum cystatin C							
Bökenkamp *et al.*	−41.97	31.25	−29.87	18.31	−65.76, 6.01	11.76%	47.06%
Filler and Lepage	−8.50	24.68	−5.75	18.77	−42.53, 31.03	51.76%	84.71%
Grubb *et al.*	−51.19	58.57	−31.42	30.01	−90.24, 27.39	15.29%	40.00%
Zapitelli-CysEq	9.22	21.93	10.0.32	19.54	−27.97, 48.61	27.06%	87.06%
equations with serum cystatin C and serum creatinine							
Bouvet *et al.*	4.91	5.24	4.10	4.48	−4.68, 12.89	90.59%	100.00%
CKiD	14.69	17.95	15.02	16.80	−17.90, 47.94	29.41%	90.59%
Zapitelli-CysCrEq	10.97	20.56	11.73	18.76	−25.03, 48.50	30.59%	89.41%
GFR ≥135 ml/min per 1.73 m^2							
equations with serum cystatin C							
Bökenkamp *et al.*	−26.69	37.93	−14.46	17.63	−49.02, 20.10	34.57%	80.25%
Filler and Lepage	12.61	31.20	9.96	18.10	−25.52, 45.44	29.63%	92.59%
Grubb *et al.*	−52.77	73.57	−23.89	27.90	−78.58, 30.79	14.81%	50.62%
Zapitelli-CysEq	34.53	28.25	26.71	18.61	−9.76, 63.19	11.11%	65.43%
equations with serum cystatin C and serum creatinine							
Bouvet *et al.*	8.92	7.00	6.31	4.48	−2.47, 15.09	93.83%	100.00%
CKiD	43.01	20.16	32.93	14.99	3.53, 62.33	6.17%	55.56%
Zapitelli-CysCrEq	35.69	21.24	26.98	15.65	−3.69, 57.66	14.81%	72.84%

Table 4. (Continued)

	Bias	SD of Bias	Relative Bias (%)	Relative SD of Bias (%)	95% Limit of Agreement for Relative SD of Bias	Accuracy (%) Within	
						10%	30%
GFR ≥150 ml/min per 1.73 m^2							
equations with serum cystatin C							
Bökenkamp *et al.*	−24.4	44.78	−11.77	19.27	−49.55, 26.01	36.59%	85.37%
Filler and Lepage	17.02	37.02	12.56	19.86	−26.38, 51.50	29.27%	90.24%
Grubb *et al.*	−61.31	89.86	−24.68	30.09	−83.67, 34.30	12.20%	51.22%
Zapitelli-CysEq	39.89	33.50	29.01	20.43	−11.02, 69.06	4.88%	60.98%
equations with serum cystatin C and serum creatinine							
Bouvet *et al.*	6.33	9.02	4.07	5.26	−6.24, 14.40	97.56%	100.00%
CKiD	49.75	21.81	35.66	15.26	5.74, 65.57	2.44%	46.34%
Zapitelli-CysCrEq	41.00	23.81	28.99	16.39	−3.13, 61.13	12.20%	70.73%

Bias = mean difference between ^{99m}Tc DTPA GFR and cystatin C eGFR; relative bias = mean % difference = [100 × (^{99m}Tc DTPA GFR − cystatin C eGFR)/^{99m}Tc DTPA GFR]; SD of the bias = precision (an increase in the SD of bias means a decrease in the precision); SD of the relative bias = relative precision; accuracy = percentage of cystatin C eGFR values within 10% and 30% of the respective ^{99m}Tc DTPA GFR measurements. eGFR, estimated GFR; ^{99m}Tc DTPA, technetium-99m–diethylene-triamine penta-acetic acid.

calculations that improved the equation's AUC and correlation coefficient.

The reasons for variation in the accuracy with GFR remain poorly understood. The GFR categories were similar in regard to the distribution of age, gender, obese, and adolescent patients. The size and charge on cystatin C molecule cannot explain the pattern. We noticed that the equations derived from the patients with low GFR levels (CKiD equation, mean GFR 44 ± 15 ml/min per 1.73 m^2; Zappitelli equation, mean GFR 74 ± 36 ml/min per 1.73 m^2) performed better at a low GFR, whereas the equations derived from normal or high GFR levels (Bouvet equation, mean GFR 95 ml/min per 1.73 m^2; Filler equation, mean GFR 103 ± 41 ml/min per 1.73 m^2; Grubb equation, median GFR 113 ml/min per 1.73 m^2 for age <14 years and 99 for 14 to <18 years) performed better at corresponding high GFR. On the basis of this observation, we speculate that an equation has a better diagnostic accuracy at the GFR that is close to that of the study sample used for deriving the equations.

The findings from this study should be interpreted in the light of its limitations. It is important to note that the Bökenkamp, CKiD, and Grubb equations measured cystatin C with turbidometry (PETIA), whereas the Bouvet, Filler, and Zappitelli used a nephelometric immunoassay (PENIA). Different assays may explain some of the variability but cannot fully explain the trend of diagnostic accuracy within a particular equation. We analyzed the CKiD equation with the model that did not include urea because of the unavailability of urea levels for all patients. In the original study, the R^2 of 69.4% and % eGFR of 84% within 30% of the measured GFR was a bit lower than corresponding values of 75.2% and 87.7% with urea (11). The inclusion of urea would have improved the performance of the equation to some extent; however, it cannot explain the change in the diagnostic accuracy of the equation at different GFR levels. Because of a small number, we could not separately analyze for GFR <30 ml/min per 1.73 m^2. None of the included patients had significant edema to induce GFR overestimation from a tracer dilution (30).

With different accuracy tests, the choice of an accuracy test should depend upon the intended objective. If the purpose is to categorize a measured GFR into a CKD category, the sensitivity and specificity of an equation can provide the required information. However, an accurate prediction of the measured GFR becomes clinically relevant if the goal is to monitor the trend of GFR longitudinally. Given the variability in the performance of various equations with GFR, an ideal equation that can be applied to all remains a challenge. Short of an individualized approach based on GFR levels, further research on refining the equations should focus on data pooling, ensuring the quality of the gold-standard method, and choosing a mathematical model that best resembles the naturally occurring decline of isotope measurements in the time concentration curve. Ideally, nonlinear mixed pharmacokinetic models that adjust for extracorporeal volume, gender, ethnicity, and age as well as selection of an appropriate model with the number of compartments should be utilized. Perhaps the Baysian approach for the WinNonLin derived GFR calculations employed by Bouvet *et al.* can maximize the quality of the gold-standard method of measuring GFR (8). Standardized calibration and uniformity in using cystatin C assays can improve the prediction by cystatin C equations (31).

Conclusions

We conclude that the diagnostic accuracy of various cystatin C equations varies at different GFR levels. Further studies should focus on refining the equations to improve their consistency across all GFR ranges.

Disclosures

None.

Table 5. Diagnostic accuracy (area under the ROC curves, sensitivity, and specificity) of cystatin C estimated GFR estimated by various cystatin C equations across different GFR categories (ml/min per 1.73 m^2)

	Area under the ROC Curve (AUC) (95% CI)	SEM	Sensitivity (%)	Specificity (%)
GFR<60 ml/min per 1.73 m^2				
equations with serum cystatin C				
Bökenkamp *et al.*	0.99 (0.96 to 0.99)	0.005	83.87 (66.3 to 94.5)	100.00 (98.3 to 100.0)
Filler and Lepage	0.99 (0.96 to 0.99)	0.005	83.87 (66.3 to 94.5)	100.00 (98.3 to 100.0)
Grubb *et al.*	0.98 (0.95 to 0.99)	0.008	80.65 (62.5 to 92.5)	98.09 (95.2 to 99.5)
Zapitelli-CysEq	0.99 (0.97 to 0.99)	0.004	96.77 (83.3 to 99.9)	94.74 (90.8 to 97.3)
equations with serum cystatin C and serum creatinine				
Bouvet *et al.*	1.00 (0.98 to 1.00)	0.000	64.52 (45.4 to 80.8)	100.00 (98.3 to 100.0)
CKiD	0.98 (0.95 to 0.99)	0.013	90.32 (74.2 to 98.0)	97.13 (93.9 to 98.9)
Zapitelli-CysCrEq	0.98 (0.96 to 0.99)	0.008	93.55 (78.6 to 99.2)	90.43 (85.6 to 94.1)
GFR<90 ml/min per 1.73 m^2				
equations with serum cystatin C				
Bökenkamp *et al.*	0.97 (0.94 to 0.99)	0.011	60.81 (48.8 to 72.0)	100.00 (97.8 to 100.0)
Filler and Lepage	0.97 (0.94 to 0.99)	0.011	89.19 (79.8 to 95.2)	95.78 (91.5 to 98.3)
Grubb *et al.*	0.97 (0.93 to 0.98)	0.010	78.38 (67.3 to 87.1)	98.19 (94.8 to 99.6)
Zapitelli-CysEq	0.97 (0.94 to 0.98)	0.012	98.65 (92.7 to 100.0)	81.33 (74.6 to 86.9)
equations with serum cystatin C and serum creatinine				
Bouvet *et al.*	1.00 (0.98 to 1.00)	0.000	86.49 (76.5 to 93.3)	100.00 (97.8 to 100.0)
CKiD	0.97 (0.94 to 0.99)	0.007	100.00 (95.1 to 100)	77.11 (70.0 to 83.3)
Zapitelli-CysCrEq	0.98 (0.95 to 0.99)	0.006	98.65 (92.7 to 100.0)	79.52 (72.6 to 85.4)
GFR ≥ 135 ml/min per 1.73 m^2				
equations with serum cystatin C				
Bökenkamp *et al.*	0.85 (0.80 to 0.89)	0.023	97.47 (91.2 to 99.7)	60.87 (52.9 to 68.5)
Filler and Lepage	0.85 (0.80 to 0.89)	0.023	48.10 (36.7 to 59.6)	86.34 (80.0 to 91.2)
Grubb *et al.*	0.83 (0.78 to 0.88)	0.025	88.61 (79.5 to 94.7)	63.35 (55.4 to 70.8)
Zapitelli-CysEq	0.83 (0.78 to 0.88)	0.025	17.72 (10.0 to 27.9)	95.65 (91.2 to 98.2)
equations with serum cystatin C and serum creatinine				
Bouvet *et al.*	1.00 (0.98 to 1.00)	0.000	58.02 (46.5 to 68.9)	100.00 (97.7 to 100.0)
CKiD	0.84 (0.79 to 0.89)	0.024	12.66 (6.2 to 22.0)	96.89 (92.9 to 99.0)
Zapitelli-CysCrEq	0.85 (0.80 to 0.89)	0.023	20.25 (12.0 to 30.8)	96.27 (92.1 to 98.6)
GFR ≥ 150 ml/min per 1.73 m^2				
equations with serum cystatin C				
Bökenkamp *et al.*	0.84 (0.79 to 0.89)	0.025	97.37 (86.2 to 99.9)	65.84 (58.9 to 72.4)
Filler and Lepage	0.84 (0.79 to 0.89)	0.025	39.47 (24.0 to 56.6)	91.58 (86.9 to 95.0)
Grubb *et al.*	0.84 (0.78 to 0.88)	0.026	89.47 (75.2 to 97.1)	59.90 (52.8 to 66.7)
Zapitelli-CysEq	0.83(0.78 to 0.88)	0.026	13.16 (4.4 to 28.1)	98.02 (95.0 to 99.5)
equations with serum cystatin C and serum creatinine				
Bouvet *et al.*	1.00 (0.98 to 1.00)	0.000	51.22 (35.1 to 67.1)	100.00 (98.2 to 100.0)
CKiD	0.84 (0.79 to 0.88)	0.028	7.89 (1.7 to 21.4)	99.50 (97.3 to 100.0)
Zapitelli-CysCrEq	0.85(0.80 to 0.89)	0.026	15.79 (6.0 to 31.3)	98.51 (95.7 to 99.7)

ROC, receiver operating characteristic; AUC, area under curve; CI, confidence interval; CKiD, chronic kidney disease in children. $P < 0.001$ for all AUC estimations.

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