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GFR Estimation After Cystatin C Reference Material Change

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Introduction: Glomerular filtration rate (GFR) is routinely estimated with cystatin C. In June 2010, the International Federation of Clinical Chemistry (IFCC) released a certified cystatin C reference material (ERM-DA471/IFCC), and new cystatin C glomerular filtration rate estimation (eGFR) equations were developed with the IFCC standard. Early in 2018, Siemens discontinued their nonstandardized cystatin C reagent kits and replaced them with IFCC-calibrated kits in the US market. The aim of the current study was to assess the effect of IFCC calibration on cystatin C values and corresponding GFR estimations.

Methods: Cystatin C concentration was measured in 81 pediatric patients using a plasma sample from their nuclear GFR measurement with 99mTc-diethylenetriaminepentaaccetic acid. Calibration curves were generated using Siemens nonstandardized and IFCC-standardized kits to measure paired cystatin C concentrations in each sample. GFR-estimating equations using pre-IFCC and IFCC cystatin C values were compared using Bland-Altman analyses.

Results: The IFCC-standardized assay resulted in a mean increase in the measured cystatin C value of 24%. Estimating equations consistently overestimated GFR prior to IFCC standardization. Following incorporation of the IFCC standard, the Full Age Spectrum equation demonstrated the best overall performance, whereas the Chronic Kidney Disease in Children (CKiD) equation was more accurate in children with decreased GFR.

Conclusion: Incorporation of the IFCC standard significantly increased cystatin C values and affected the performance of GFR estimating equations. Clinical laboratories and providers may need to update the equation used for cystatin C–based estimation of GFR following adoption of the IFCC reference standard.

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Cystatin C has become widely accepted as an endogenous biomarker of GFR and is routinely used in the evaluation of chronic kidney disease.[1–6] Reagents and clinical assays have had considerable differences over time, which has resulted in numerous cystatin C–based estimated GFR equations (eGFR) with different coefficients to account for the variation in concentrations measured.[7,8] The lack of uniformity has made it difficult to share or reproduce data across institutions.[9] There have also been concerns with calibration changes by individual manufacturers during the past 10–20 years. Specifically, a downward drift in Siemens' particle-enhanced nephelometric immunoassay was observed, resulting in progressively higher estimations of GFR.[10]

Thus, in June of 2010, the IFCC and Laboratory Medicine Working Group on Standardization of Cystatin C released an international certified cystatin C reference material (ERM-DA471/IFCC).[11] Several cystatin C eGFR equations were subsequently developed using the new IFCC standard.[12,13] The reference material was made available to clinical laboratories in late 2010. Early in 2018, Siemens announced it was going to discontinue the nonstandardized cystatin C reagent kits and replace them with IFCC-calibrated cystatin C reagent kits in the US market.[14] They reported that the calibrated values would be 17.4% higher than the pre-IFC values.[14]

In preparation for this reagent change at our pediatric hospital system, we measured cystatin C levels using both the nonstandardized and standardized

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Table 1. GFR estimating equations

Cystatin C formulas	
Precalibration	
Zappitelli	75.94 x (cys C)$^{-1.17}$
Larsson*	77.24 x (cys C)$^{-1.2623}$
Hoek	−4.32 + (80.35/cys C)
Rule	66.8 x (cys C) $^{-1.30}$
Le Bricon	(78/cys C) + 4
CKiD	70.69 x (cys C)$^{-0.931}$
CKD-EPI	≤0.8: 133 × (cys C/0.8) − 0.499 × 0.996 age [× 0.932 if female] >0.8: 133 × (cys C/0.8) − 1.328 × 0.996 age [× 0.932 if female]
Postcalibration	
CAPA	130 x (cys C)$^{-1.069}$ x age$^{-0.117}$ − 7
FAS	107.3 / (cys C/Qcys C) x [0.988 $^{(age - 40)}$] When age > 40 y, Qcys C = 0.82

CAPA, Caucasian, Asian, pediatric and adult; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKiD, Chronic Kidney Disease in Children; Cys C, cystatin C (mg/l); FAS, full age spectrum; GFR, glomerular filtration rate.
GFR in ml/min per 1.73 m^2 unless indicated by *ml/min.

Table 2. Baseline characteristics

Characteristics	Values
Subjects, *n*	81
Measurements, *n*	105
Age, yr	9 (4, 16)
Sex, male, *n* (%)	45 (56)
Race, *n* (%)	
Caucasian	66 (81)
African American	9 (11)
Other	6 (7)
Primary diagnosis, *n* (%)	
Malignancy	66 (81)
Solid organ transplant	6 (7)
CKD	3 (4)
Other	6 (7)
Height, cm	133.5 (105.6, 167)
Weight, kg	31.3 (17.9, 65.2)
Cystatin C, mg/l	
Non-IFCC assay	0.64 (0.56, 0.72)
IFCC assay	0.78 (0.67, 0.94)

CKD, chronic kidney disease; HSCT, hematopoietic stem cell transplant; IFCC, International Federation of Clinical Chemistry.
Continuous variables are presented as median (interquartile range).

cystatin C reagent kits in a cohort of 81 pediatric patients who receive a nuclear medicine GFR (NMGFR). We aimed to assess the mean change in cystatin C value between the 2 reagent kits and to determine which equation would produce the most accurate cystatin C eGFR. To accomplish this, we compared eGFR values from each equation to the measured NMGFR, using both pre-IFCC and IFCC-calibrated cystatin C values.

MATERIALS AND METHODS

We conducted this clinical laboratory quality improvement project using a prospective cohort of 81 consecutive patients who obtained NMGFR at Cincinnati Children's Hospital Medical Center between February and August 2019. Patients less than 2 years of age were excluded. Briefly, GFR was computed using plasma 99mTc-diethylenetriaminepentaaccetic acid (DTPA) disappearance curves obtained from 4 time points (120, 150, 180, 210 minutes) after a single injection of a known amount of compound. ^{99m}Tc-DTPA has previously demonstrated good agreement with inulin clearance.[15] A single dose of ^{99m}Tc-DTPA was administered with direct visualization and intermittent aspiration of blood while injecting to ensure delivery of the isotope intravenously. Each plasma disappearance curve had a correlation coefficient of greater than 0.98, indicating that intercompartmental equilibration had occurred. A quadratic correction factor was then used to adjust the slope-intercept GFR to a 2-compartment model according to the methods of Brochner-Mortensen.[16] After GFR was measured, the 4 time-point samples from each subject were pooled and stored at −80°C until radioisotope decay occurred before the measurement of cystatin C for each subject. All samples were frozen and thawed once. Cystatin C was measured using the NMGFR samples using particle-enhanced immunonephelometry (Siemens BN-II, Siemens AG) in the Cincinnati Children's Hospital Division of Nephrology Clinical Laboratory. For each sample, cystatin C values were obtained using both nonstandard and IFCC-standardized reagents. Cystatin C eGFR was calculated using equations developed both pre- and poststandardization,[12,13,17–23] as shown in Table 1.

Descriptive statistics for continuous variables were reported as mean and standard deviation (SD), or median and interquartile ranges (IQRs) when appropriate. Lin's concordance correlation coefficient and linear regression analysis were used to investigate the relationship between IFCC-calibrated cystatin C values to those obtained pre–IFCC calibration. Bland-Altman analyses were conducted to compare the precision and accuracy of each cystatin C–based equation, using the results from the standardized and nonstandardized reagent kits. The mean bias and 95% limits of agreement were reported.[24] The proportion of the estimated GFR for each formula within 10% and 30% of the NMGFR were also calculated. All analyses were conducted using SAS statistical software (version 9; SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

The demographics and clinical characteristics of the cohort are shown in Table 2. Mean NMGFR was 103.3 ml/min per 1.73 m^2 (SD 32.6, min 17, max 187 ml/min per 1.73 m^2). Twenty-seven patients had NMGFR <90 ml/min per 1.73 m^2, 40 patients had an NMGFR

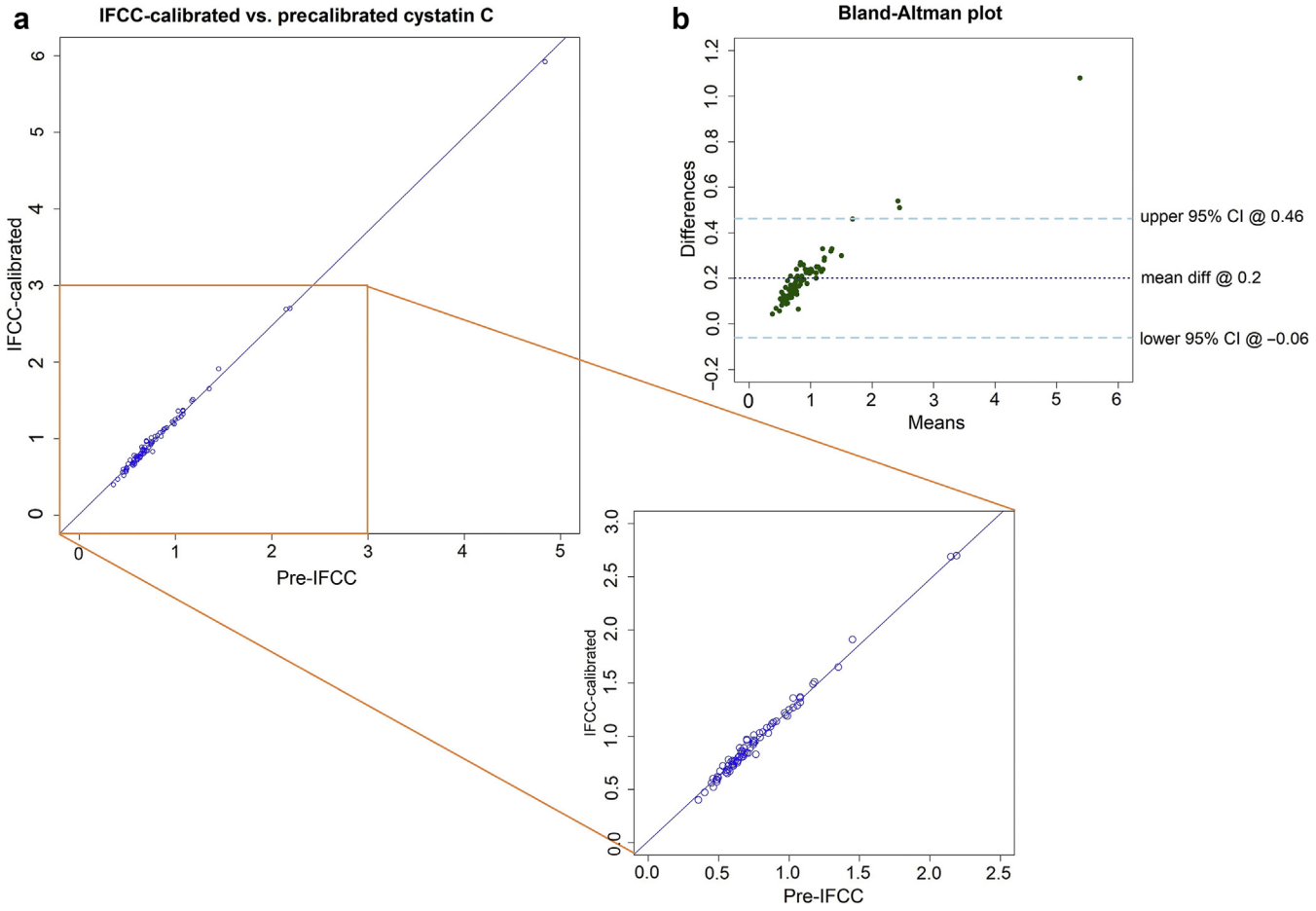

Figure 1. Pre- and post–International Federation of Clinical Chemistry (IFCC) calibration cystatin C values. (a) Linear regression analysis comparing cystatin C values obtained using the IFCC-calibrated and pre–IFCC calibration reagents ($r = 0.998$). (b) Bland-Altman plot of IFCC-calibrated cystatin C. Pre–IFCC calibration cystatin C shows a larger difference between pre- and postcalibrated values as the cystatin C value increased. Cystatin C is measured in milligrams per liter. CI, confidence interval.

between 90 and 135 ml/min per 1.73 m^2, and 14 patients had NMGFR >135 ml/min per 1.73 m^2.

Comparison of Cystatin C Values Using Nonstandard and IFCC-Standardized Reagents

The median cystatin C value using the nonstandard calibrating reagent was 0.64 mg/l (interquartile range 0.56–0.72). In comparison, the median cystatin C values using the new assay was 0.78 mg/l (interquartile range 0.67–0.94). There was moderate concordance between the nonstandard and IFCC-standardized reagents with a concordance correlation coefficient of 0.927 (95% confidence interval = 0.903–0.945). On average, values from the new standardized assay were 24.5% higher compared to the nonstandardized assay, with a 95% confidence interval of 23.3% to 25.8%. Linear regression analysis comparing the 2 cystatin C assays yielded the following equation (Figure 1): cystatin C (standardized) = 1.232 × cystatin C (nonstandardized) + 0.008. The intercept was small and not significantly different from 0 ($P = 0.14$), and the correlation coefficient using this model was very high ($r = 0.998$). Using a model without an intercept yielded a factor of 1.242, or 24.2% increase, which was nearly identical to the crude analysis value of 24.5%. These results indicate that a 24% increase in cystatin C values obtained using the IFCC-standardized reagents could be reliably predicted from nonstandardized values with minimal residual error.

Comparison of Cystatin C–Based Equations Using Nonstandard and IFCC-Standardized Reagents

The performance of each cystatin C–based formula to predict GFR using the nonstandardized and IFCC-standardized reagents are shown in Tables 3 and 4, and Bland-Altman plots of pre– and post–IFCC reagent eGFRs are shown in Figure 2. Bland-Altman analyses demonstrated that the difference in eGFR (post- to pre-IFCC) became more pronounced with increasing cystatin C values. Stated similarly, compared to GFR estimations using uncalibrated cystatin, the eGFR using

Table 3. Overall performance of GFR-estimating equations using uncalibrated cystatin C

	mGFR total group (n = 81)					mGFR < 90 ml/min per 1.73 m^2 (n = 27)				
	Mean (SD)	Mean bias (95% CI)	95% limits of agreement	P30 (%)	P10 (%)	Mean (SD)	Mean bias (95% CI)	95% limits of agreement	P30 (%)	P10 (%)
NMGFR	103.3 (32.6)	N/A	N/A	N/A	N/A	67.0 (18.9)	N/A	N/A	N/A	N/A
Cystatin C formulas (prestandardization)										
Zappitelli	117.9 (42.5)	14.6 (7.8, 21.5)	−46.0, 75.2	66.7	24.7	86.5 (33.0)	19.5 (11.5, 27.6)	−29.0, 27.8	48.2	22.2
Larsson	124.9 (48.2)	21.6 (13.8, 29.4)	−47.2, 90.4	59.3	21.0	89.5 (36.1)	22.5 (13.3, 31.7)	−31.3, 31.9	48.2	22.2
Hoek	111.7 (36.4)	8.4 (2.4, 14.4)	−50.3, 67.2	71.6	32.1	84.6 (30.0)	17.6 (10.6, 24.5)	−26.1, 25.3	55.6	29.6
Rule	109.8 (43.5)	6.5 (−0.5, 13.6)	−56.0, 69.1	65.4	28.4	78.0 (32.2)	10.9 (3.1, 18.8)	−36.2, 18.5	55.6	22.2
Le Bricon	116.6 (35.3)	13.4 (7.5, 19.2)	−38.5, 65.2	67.9	24.7	90.3 (29.1)	23.3 (16.6, 29.9)	−19.0, 30.7	40.7	14.8
CKiD	99.2 (29.2)	−4.1 (−9.4, 1.2)	−51.2, 43.0	80.3	34.6	77.4 (24.7)	10.3 (5.0, 15.6)	−25.6, 16.9	77.8	29.6
CKD-EPI	124.7 (39.8)	21.5 (15.9, 27.1)	−28.0, 71.0	56.8	22.2	99.1 (36.4)	32.1 (22.8, 41.3)	−13.8, 77.9	33.3	3.7
Cystatin C formulas (poststandardization)										
CAPA	145.2 (56.7)	42.0 (32.8, 51.1)	−39.2, 123.1	37.0	11.1	106.3 (45.3)	39.3 (26.2, 52.4)	−32.8, 62.7	33.3	14.8
FAS	127.0 (39.8)	23.8 (17.4, 30.2)	−32.8, 80.4	59.3	16.1	97.4 (32.9)	30.3 (22.5, 38.2)	−18.1, 39.4	33.3	7.4

CAPA, Caucasian, Asian, pediatric and adult; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKiD, Chronic Kidney Disease in Children; eGFR, estimated glomerular filtration rate; FAS, full age spectrum; GFR, glomerular filtration rate; mGFR, measured GFR; N/A, not applicable; NMGFR, nuclear medicine glomerular filtration rate.

IFCC-calibrated cystatin C values was progressively lower. The CKD-EPI equation is the only one that did not follow this trend, owing to the spline term at the cystatin C value of 0.8 mg/l.

When using pre–IFCC calibrated cystatin C values, most of the formulas that were derived prior to standardization of reagent kits overestimated NMGFR, both overall and in those patients with measured GFR (mGFR) <90 ml/min per 1.73 m^2 (Table 3). This overestimation of NMGFR was even more pronounced when using formulas derived following implementation of the IFCC-standardized reagents (CAPA and FAS equations). In contrast, in general there were improvements in GFR estimation when the IFCC-standardized reagents were used (Table 4). Specifically, the FAS equation performed best overall with a mean bias that was not significantly different from 0. However, this formula overestimated GFR in those with an NMGFR < 90 ml/min/1.73m^2. In this group, the CKID, Hoek, Larsson, and Zappitelli equations performed the best.

DISCUSSION

This analysis comparing cystatin C levels and the corresponding GFR estimations generated before and after incorporation of standardized IFCC reference material revealed 2 main findings. First, post–IFCC calibration levels were reliably 24% higher compared to pre-IFCC levels. Second, noticeable improvement occurred in GFR estimations following adoption of IFCC-calibrated cystatin C values. Specifically, the FAS equation provided the most overall accurate estimation of GFR in our cohort. However, in those with decreased GFR, pediatric equations derived in children with CKD (Zappitelli and CKiD equation) performed better.

Cystatin C–based estimations of kidney function are now commonplace in the care of children, both with and without kidney disease.[25,26] The accuracy and reliability of cystatin C lab values are directly affected by variations

Table 4. Overall performance of GFR-estimating equations using IFCC-calibrated cystatin

	mGFR total group (n = 81)					mGFR < 90 ml/min per 1.73 m^2 (n = 27)				
	Mean (SD)	Mean bias (95% CI)	95% limits of agreement	P30 (%)	P10 (%)	Mean (SD)	Mean bias (95% CI)	95% limits of agreement	P30 (%)	P10 (%)
NMGFR	103.3 (32.6)	N/A	N/A	N/A	N/A	67.0 (18.9)	N/A	N/A	N/A	N/A
Cystatin C formulas, prestandardization										
Zappitelli	91.9 (35.4)	−11.4 (−17.5, −5.3)	−65.3, 42.5	74.1	23.5	66.5 (25.7)	−0.6 (−6.3, 5.2)	−29.0, 27.8	85.2	22.2
Larsson	95.5 (39.6)	−7.8 (−14.4, −1.2)	−66.7, 51.1	74.1	24.7	67.4 (27.7)	0.3 (−6.0, 6.7)	−31.3, 31.9	81.5	22.2
Hoek	89.3 (31.2)	−13.9 (−19.5, −8.3)	−63.4, 35.6	80.3	27.2	66.6 (24.3)	−0.4 (−5.6, 4.8)	−26.1, 25.3	96.3	33.3
Rule	83.3 (35.5)	−20.0 (−26.1, −13.8)	−74.7, 34.8	63.0	16.1	58.2 (24.4)	−8.8 (−14.4, −3.3)	−36.2, 18.5	74.1	14.8
Le Bricon	94.9 (30.3)	−8.3 (−13.9, −2.8)	−57.3, 40.6	77.8	32.1	72.9 (23.6)	5.8 (0.8, 10.9)	−19.0, 30.7	85.2	40.7
CKiD	81.2 (25.3)	−22.0 (−27.3, −16.8)	−68.5, 24.4	72.8	23.5	62.7 (20.3)	−4.3 (−8.6, −0.0)	−25.6, 16.9	100	37.0
CKD-EPI	105.2 (35.5)	2.0 (−3.8, 7.7)	−49.0, 52.9	70.4	29.6	78.2 (32.6)	11.2 (3.0, 19.3)	−29.2, 51.5	59.3	18.5
Cystatin C formulas, poststandardization										
CAPA	114.2 (48.0)	11.0 (3.2, 18.7)	−58.0, 79.9	63.0	28.4	82.0 (35.8)	14.9 (5.3, 24.6)	−32.8, 62.7	59.3	18.5
FAS	102.6 (34.2)	−0.7 (−6.6, 5.1)	−52.5, 51.1	74.1	32.1	77.7 (26.6)	10.7 (4.9, 16.5)	−18.1, 39.4	70.4	29.6

CAPA, Caucasian, Asian, pediatric and adult; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKiD, Chronic Kidney Disease in Children; GFR, glomerular filtration rate; FAS, full age spectrum; GFR, glomerular filtration rate; IFCC, International Federation of Clinical Chemistry; mGFR, measured GFR; N/A, not applicable; NMGFR, nuclear medicine glomerular filtration rate.

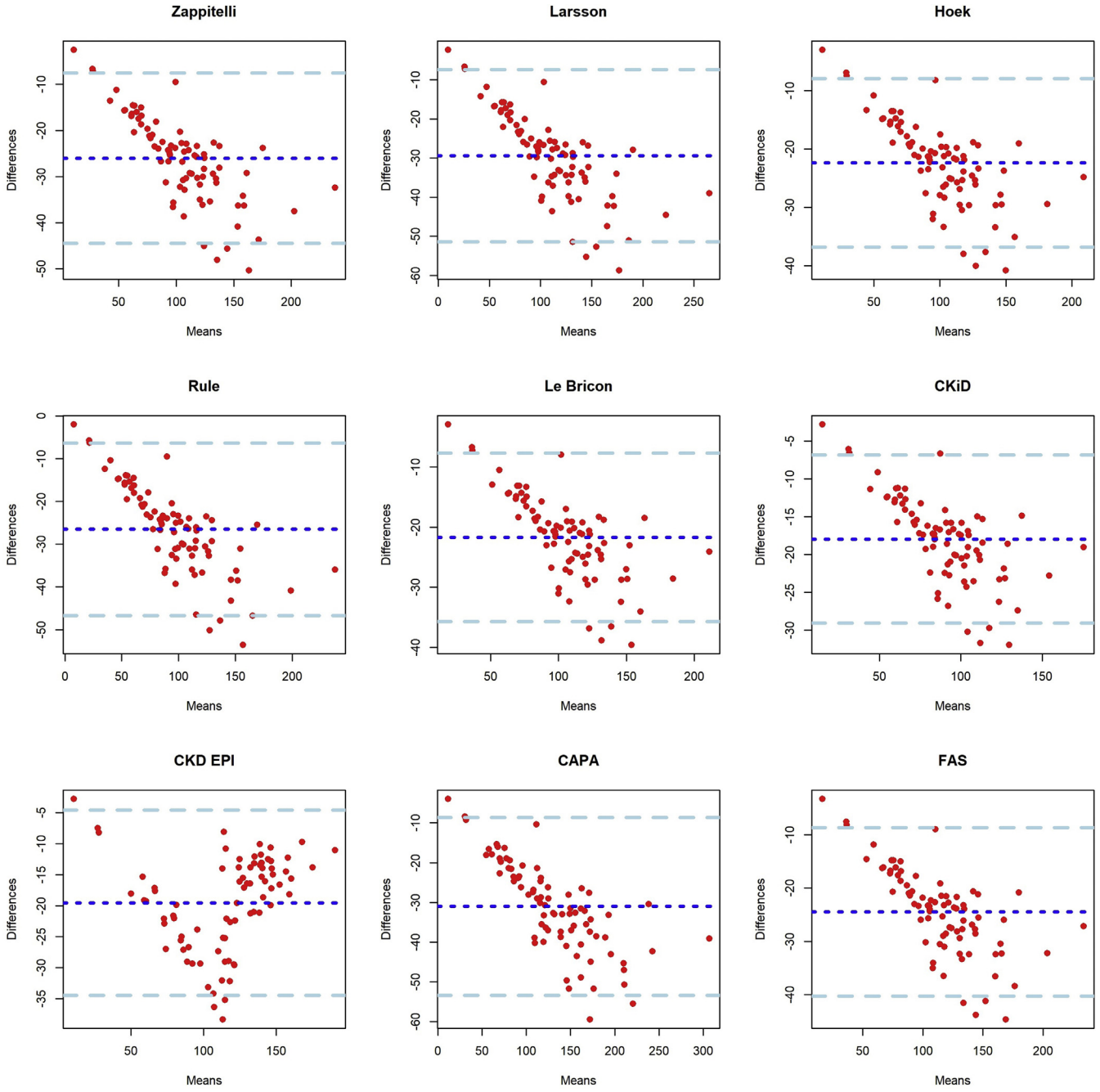

Figure 2. Bland-Altman plots of eGFRs using pre- and post–International Federation of Clinical Chemistry (IFCC) cystatin C values. Bland-Altman plots of estimated glomerular filtration rate (eGFR) using the IFCC-calibrated cystatin C value: the eGFR using the pre-IFCC calibration cystatin C value for each evaluated equation. Dark blue dashed line is the mean difference. Light blue dashed lines are upper and lower 95% confidence intervals. eGFR is calculated in milliliters per minute per 1.73 m^2. CAPA, Caucasian, Asian, pediatric and adult; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKiD, Chronic Kidney Disease in Children; FAS, full age spectrum.

in each component of the measuring system, including the platform, reagents, control materials, and specific to this analysis, the reference materials used to generate the calibration curve.[27,28] Development of high-quality calibration curves, traceable to a reference standard, was essential to ensure the accuracy of GFR estimation.[28,29] It also allowed for creation of universal GFR estimation equations that should no longer be dependent on laboratory methodologies.

During the past decade, however, notable limitations of cystatin C–based GFR estimations have been recognized owing to the lack of widespread incorporation of the ERM-DA471/IFCC standard introduced in 2010. The 2014 College of American Pathologists surveillance survey of 141 laboratories showed persistent and substantial method-based bias between different manufacturers that was comparable to the bias observed in a French Society of Clinical Biology survey that took

place in 2008, prior to IFCC standardization.[30,31] A 2015 study across 7 clinical laboratories located in France and Belgium also showed a similar persistence of unacceptably high biases in 7 of the 8 commercial assays tested.[27] Only the Siemens reagent on the Siemens systems, which we use in our laboratory, met the desired performance criterion. However, because of delayed transition to the IFCC-standardized assay in the United States, and a "downward drift" in cystatin C values during the past 10–20 years resulting in biases ranging from 12% to 20%, it was necessary to assess the institutional impact of the reagent change.[10,32–34]

In our cohort, we found that post–IFCC calbrated values were reliably 24% higher than precalibrated values. This is a clinically significant difference in terms of eGFR calculation for innumerable scenarios, including chronic kidney disease staging, oncology therapy planning, and medication dosing. Cystatin C has also been well established as an independent predictor of morbidity and mortality in a variety of populations for which falsely elevated values could lead to unnecessary testing or treatments.[26] Our difference was larger than was recently published by Schwartz *et al.*, who compared pre– and post–IFCC calibrated results in 113 samples from the CKiD study. They reported a 17% increase in cystatin C values post-calibration.[14] Assuming commutability of the IFCC reference material between our respective anayses, this indicated that our precalibrated values were 5.6% lower compared with those in the CKiD study. This difference, although significant, is not outside the expected variation in cystatin C measurments between different laboratories prior to calibration with the IFCC reference material. Reported interlaboratory coefficients of varation have ranged from 7.3% to 10.7% for Seimens-based platforms,[30] indicating our results are within 1 standard deviaton of the expected variability.

The higher post–IFCC calibrated values in our study could also have been explained by further downward drift of the Seimen-Dade-Behring assay that occurred since the publication of preivous reports. To assess this possibility, we compared the cystatin C values between our curent cohort and a previously reported cohort of patients from our institution in 2010.[35] The mean cystatin C value among those with a normal NMGFR ($\geq$90 ml/min per 1.73 m^2) decreased from 0.74 mg/l to 0.66 mg/l between 2010 and 2019. This "normal value" of 0.66 is less than the 10th percentile of reported values in healthy subjects of the National Health and Nutrition Examination Survey (NHANES) cohort.[36] Therefore, we suspect pre–IFCC calibrated values in our current cohort were at the lower end of the distribution of reported cystatin C values prior to adoption of the IFCC standard.

The FAS equation provided the most accurate estimation of GFR for our cohort as a whole using IFCC-calibrated cystatin C values. This equation was developed in 2017, and all cystatin C values were either directly traceable to the IFCC standard or recalculated to the reference standard based on known multiplication factors.[12] A fundamental assumption of this equation is that GFR is inversely related to the population normalized cystatin C value (CysC/Q, where Q represents the population mean). Therefore, Q must be accurate to ensure the validity of the equation. We used a value of 0.82 mg/l, as was used in the derivation of the equation. The mean IFCC-calibrated cystatin C value of those in our cohort with a normal NGFR was 0.82 mg/l, providing internal validation of this value

When specifically looking at patients with mGFR $<$ 90 ml/min per 1.73 m^2, the FAS equation actually overestimated the eGFR, and most pre-standardization equations outperformed it. Specifically, the CKiD equation, which was developed in a population of children with a median mGFR of 43.3 (interquartile range 32.6, 55.6), was less biased than the FAS at −4.3 (confidence interval −8.6, −0.0) ml/min per 1.73 m^2 and was the most accurate with a P30 of 100%.[18] Our findings are consistent with those published in the FAS development paper, in which the bias for the FAS equation was more favorable in children overall (CKiD −21.6 [−23.7, −19.6]; FAS −5.1 [−7.2, −3.1]), while the CKiD equation was more accurate in children with an mGFR $<$ 60 ml/min per 1.73 m^2 (CKiD 2.4 [−5.0, 0.2] and FAS at 6.2 [3.1, 9.3]).[12] Variation in the performance of GFR estimating equations at different levels of kidney function has been reported, and this should be considered when applying these formulas in clinical practice.[37]

The results from our study help to inform future estimations of GFR using cystatin C following ongoing incorporation of IFCC-calibrated cystatin C values. As was demonstrated in our study, use of formulas that were developed pre-IFCC may cause a systematic underestimation of GFR when used post–IFCC calibration, in particular among those with normal kidney function. To avoid this resulting inaccuracy of the cystatin C–based CKiD equation, Schwartz *et al.* recommended applying a correction factor to IFCC-standardized cystatin C values. As cystatin C values were consistently 17% higher post-IFCC in the CKiD cohort, one would divide post-IFCC values by 1.17 prior to calculating the eGFR using the CKiD equation.[14] A second option is to use a formula derived from the cystatin C values referenced to the IFCC standard, such as the FAS equation, which is what we have opted for in our institution. Using these equations has the benefit of avoiding possible confusion for clinicians who

manually calculate the eGFR and may not be as familiar with the more esoteric nuances of cystatin C assay measurement and standardization. Regardless of the method chosen to estimate GFR from IFCC-standardized cystatin C levels, intermittent quality checks should be performed by comparing the cystatin C eGFR to true GFR measurements, which are often performed using nuclear medicine–based techniques. As was evident in our study, even formulas derived using IFCC-calibrated cystatin C levels may perform suboptimally.

Strengths of our analysis include that we performed cystatin C measurements on serum samples drawn as part of the NMGFR testing process, eliminating any intrapatient biological variability in GFR that can occur.[38] Furthermore, pre- and post-IFCC cystatin C measurements were performed simultaneously in real time, avoiding any possible measurement variability that may occur in the process of repeat assessments following frozen storage for prolonged periods. A few limitations deserve mention. First, as this was part of a quality improvement effort, we used ^{99m}Tc-DTPA as the gold standard of GFR measurement, which is routinely performed as part of clinical care at our institution. GFR measurement in studies deriving estimation formulas in children have typically used iohexol or iothalamate.[17,18] A particular limitation of DTPA may include binding to plasma proteins and underestimation of GFR. However, ^{99m}Tc-DTPA clearance has been shown to perform similarly to these markers as an assessment of GFR with minimal differences in protein binding.[15,39–41] Second, our cohort included consecutive patients who were referred for GFR measurement for clinical indications, and only 35% had decreased renal function (NMGFR $<$ 90 ml/min per 1.73 m^2). Notably, 14 of the 81 subjects had NMGFR exceeding 135 ml/min per 1.73 m^2, which is considered hyperfiltration by some definitions.[42] This is likely due to the fact that 66 of our 81 patients had malignancies, and hyperfiltration in pediatric oncology patients is a known phenomenon.[43] Thus, although the FAS equation performed best in our cohort, other equations may in fact be more appropriate for use in other populations. For example, the CKiD and Zappitelli formulas were derived in children with known CKD and likely perform better in this patient population.

The purpose of our endeavor was to institutionally assess the effect of transiting to IFCC-standardized cystatin C measurement on the performance of GFR-estimating equations. Based on these results, we now report the eGFR using the FAS equation along with the raw cystatin C value in the electronic medical record. However, our results are not intended to imply the FAS formula is superior to other formulas, as assay methodologies and populations may vary between institutions. Rather, our results should inform the nephrology community of changes in cystatin C estimation of GFR following adoption of the IFCC standard. Further, we encourage similar quality improvement endeavors within institutions to ensure accurate estimation of GFR using cystatin C.

DISCLOSURE

All the authors declared no competing interests.

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