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Effect of Two Cystatin C Reagents and Four Equations on Glomerular Filtration Rate Estimations After Standardization

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Background: Serum cystatin C (cysC), which is less affected by sex, race, and muscle mass than creatinine, is a useful biomarker of the estimated glomerular filtration rate (eGFR). The standardization of cysC measurements remains controversial, although a certified reference material (ERM-DA471/IFCC) is available. Moreover, the effect of combinations of cysC reagents and equations for eGFR is unclear.

Methods: We conducted a simulation analysis of cysC measured using two reagents standardized against ERM-DA471/IFCC—Gentian cystatin C immunoassay ($Gentian_{cys}$; GentianAS, Moss, Norway) and Roche Tina-quant Cystatin C Gen.2 ($Roche_{cys}$; Roche, Mannheim, Germany)—on a Cobas c702 system (Roche) and eGFR generated by eight combinations of four equations: 2012 cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equation ($CKD\text{-}EPI_{cys}$); the Caucasian, Asian, pediatric, and adult equation ($CAPA_{eq}$); full age spectrum equation (FAS_{eq}); and 2023 cystatin C-based European Kidney Function Consortium equation ($EKFC_{cys}$).

Results: A total of 148 participants (mean age, 60.5±14.5 years; 43% female) were enrolled. The mean cysC was 1.72±1.44 mg/L for $Gentian_{cys}$ and 1.71±1.35 mg/L for $Roche_{cys}$. Regression analysis showed concordance between the reagents within 0.85–4.40 mg/L when using ±7.61% total allowable error. Lin's concordance correlation coefficient of eGFR, by combining the measuring system and equation, varied from 0.73 to 1.00.

Conclusions: The equivalence of cysC values at low concentrations (<0.85 mg/L) between the two reagents was unsatisfactory. Results obtained with different measurement systems could lead to larger differences in eGFR varying with the combination.

Key Words: Cystatin C, Estimated glomerular filtration rate, Chronic Kidney Disease Epidemiology Collaboration, Caucasian, Asian, European Kidney Function Consortium

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INTRODUCTION

Cystatin C (cysC) is a 13-kDa, low-molecular-weight protein with a high isoelectric point [1]. CysC can be freely filtered through the glomerular membrane and synthesized at a relatively constant rate [2]. Owing to its characteristics, cysC is used as an alternative biomarker for predicting the glomerular filtration rate (GFR) [3], a parameter that reflects kidney function. CysC is less affected by age, race, and muscle mass than creatinine [4], which is the most widely applied marker for GFR estimation.

Therefore, the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines suggest measuring cysC in patients with chronic kidney disease (CKD) confirmed solely by a creatinine-based estimated GFR (eGFR) of 45–60 mL/min/1.73 m^2 [5]. Furthermore, cysC can have advantages over creatinine for the rapid detection of an abrupt decrease in eGFR in critically ill adults and children [6, 7].

There are two important issues related to measuring cysC in clinical settings. Systematic measurement errors can result from variability in the measuring systems such as reagents, instruments, calibrators, and control materials [8-12]. More than five manufacturers have produced cysC assay kits using different measurement principles such as immunoassay, nephelometric, turbidimetric, or spectrophotometric methodologies, resulting in various reagents [10, 11]. Uncertainties attributable to imprecision, bias, and drift have been reported [13-16], and the need for cysC measurement standardization has increased. Various equations are also used for eGFR. In 2012, the 2012 cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI$_{cys}$) was developed [8]. Subsequently, the Caucasian, Asian, pediatric, and adult equations (CAPA$_{eq}$) [17] and full age spectrum equation (FAS$_{eq}$) [18] were developed. Recently, the 2023 cystatin C-based European Kidney Function Consortium equation (EKFC$_{cys}$) [19], which had a scaling factor for cysC that did not differ according to race or sex, was reported to have better accuracy than CKD-EPI$_{cys}$, CAPA$_{eq}$, or FAS$_{eq}$.

These two issues for the measurement of cysC have not been resolved, although manufacturers have altered their cysC measurement procedures using the ERM-DA471/IFCC traceable calibrator. Numerous combinations of measuring systems and novel equations have been generated, depending on the laboratory setting and equation selection. Studies on the effect of cysC reagents and equation combinations for eGFR after cysC standardization are limited [12, 15, 20]. We conducted a simulation analysis of cysC measured using two ERM-DA471/IFCC-standardized cysC reagents and analyzed the correlations among eight eGFR combination methods comprising two reagents and four equations as follows: CKD-EPI$_{cys}$, CAPA$_{eq}$, FAS$_{eq}$, and EKFC$_{cys}$. We aimed to elucidate the effect of the cysC reagent and eGFR equation combinations for GFR estimation.

METHODS

Study design and participants

The equation for the Gentian cystatin C immunoassay (Gentian$_{cys}$; GentianAS, Moss, Norway; Getian$_{eq}$) was used to estimate the GFR with a single factor of the Gentian$_{cys}$ value [21]. Using a combination of Gentian$_{cys}$ and Getian$_{eq}$, each eGFR value was categorized as follows: ≥90 mL/min/1.73 m^2 (G1), 60–89 mL/min/1.73 m^2 (G2), 30–59 mL/min/1.73 m^2 (G3), 15–29 mL/min/1.73 m^2 (G4), or <15 mL/min/1.73 m^2 (G5). Gentian$_{cys}$ values of 1.98 and 1.22 mg/L produced eGFR results of approximately 30 mL/min/1.73 m^2 and 60 mL/min/1.73 m^2, respectively.

We reviewed the records of 413 participants who were referred for serum Gentian$_{cys}$ measurements at Samsung Medical Center Seoul, Korea, during a 7-day period in July 2021. Of these, 16 participants younger than 18 years of age were excluded; of the remaining 397 participants, 37 had a recorded Gentian$_{cys}$ of >1.98 mg/L and were enrolled (Q1). We selected 37 participants with recorded Gentian$_{cys}$ values ranging between 1.22 and 1.97 mg/L (Q2). As CKD-EPI$_{cys}$ has an inflection point at 0.8 mg/L of cysC, we additionally selected 37 participants with Gentian$_{cys}$ values ranging between 0.80 and 1.21 mg/L (Q3) and 37 participants with Gentian$_{cys}$ values <0.80 mg/L (Q4). Each selected group was matched by age and sex to the group with recorded Gentian$_{cys}$ values >1.98 mg/L. In total, 148 residual samples were analyzed in this study. The participants were categorized as G1 (N=44), G2 (N=36), G3 (N=31), G4 (N=20), and G5 (N=17), according to the eGFR for combined Gentian$_{cys}$ and Getian$_{eq}$. The study was approved by the Institutional Review Board of Samsung Medical Center Hospital (IRB file no. 2021–06–180). A waiver of consent was obtained given the retrospective nature of the study.

Sample collection and storage

Peripheral venous blood samples were collected via venipuncture into a BD Vacutainer SST tube (BD, Plymouth, UK) and centrifuged for 10 minutes at 2,270×*g*. Separated serum aliquots were stored in an Eppendorf tube (Eppendorf, Hamburg, Germany) at 4°C until analysis.

cysC measurement

The cysC value of each aliquot was simultaneously measured using the Gentian$_{cys}$ and Tina-quant Gen.2 assays (Roche$_{cys}$; Roche, Mannheim, Germany), both on a Cobas c702 automatic analyzer (Roche). Each instrument was set based on the manufacturer's recommendations and application notes. The Gentian$_{cys}$ calibrator (REF 1012, GentianAS) and Roche$_{cys}$ calibrator (C.f.a.s. Cystatin C, Roche) were standardized against the ERM-DA471/IFCC. The details of the cysC assay conditions are presented in Supplemental Data Table S1.

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Table 1. Comparison of cystatin C-based eGFR equations used in the current study

Name	Reference	Year	Age (yr)	Sex	cysC (mg/L)	Equation
$Gentian_{eq}$	[21]	2007	-	-	-	$79.901/ Gentian_{cys}^{1.4389}$
$CKD\text{-}EPI_{cys}$	[8]	2011	-	Male	≤0.8	$133\times(cysC/0.8)^{-0.499}\times0.996^{age}$
					>0.8	$133\times(cysC/0.8)^{-1.328}\times0.996^{age}$
			-	Female	≤0.8	$133\times(cysC/0.8)^{-0.499}\times0.996^{age}\times0.932$
					>0.8	$133\times(cysC/0.8)^{-1.328}\times0.996^{age}\times0.932$
$CAPA_{eq}$	[17]	2014	-	-	-	$130\times cysC^{-1.069}\times age^{-0.117}-7$
FAS_{eq}*	[18]	2017	≤40	-	-	$107.3/(cysC/Q_{cys})$
			40<age<70			$107.3/(cysC/Q_{cys})\times0.988^{(age-40)}$
			≥70			$107.3/(cysC/Q_{cys})\times0.988^{(age-40)}$
$EKFC_{cys}$†	[19]	2023	≤40	-	-	$107.3/(cysC/Q_{cys})$
			40<age<50			$107.3/(cysC/Q_{cys})\times0.990^{(age-40)}$
			≥50			$107.3/(cysC/Q_{cys})\times0.990^{(age-40)}$

*For Q_{cys} of FAS_{eq}, 0.82 mg/L for ages <70 years and 0.95 mg/L for ages ≥70 years are used; †For Q_{cys} of $EKFC_{cys}$, 0.83 mg/L for ages <50 years and 0.83+0.005×(age–50) mg/L for ages ≥50 years are used.
Abbreviations: eGFR, estimated glomerular filtration rate; $CKD\text{-}EPI_{cys}$, CKD-EPI cystatin C equation; $CAPA_{eq}$, Caucasian, Asian, pediatric, and adult participants equation; FAS_{eq}, full age spectrum equation; $Gentian_{eq}$, equation of Gentian manufacturer recommendation; $Gentian_{cys}$, Gentian cystatin C immunoassay; cysC, cystatin C; Q_{cys}, population-normalized cystatin C.

Equations for cysC-based eGFR

We calculated and compared eGFRs with $Gentian_{cys}$ combined with $Gentian_{eq}$ and each cysC-based eGFR using the two reagents combined with the four equations of $CKD\text{-}EPI_{cys}$, $CAPA_{eq}$, FAS_{eq}, and $EKFC_{cys}$ [16-19, 21] (Table 1). The population-normalized cysC (Q_{cys}) of FAS_{eq} was referenced from the FAS_{eq} study, which analyzed 6,132 participants from 11 cohorts [18], and the Q_{cys} of $EKFC_{cys}$ was referenced from the $EKFC_{cys}$ study, which analyzed 227,643 participants [19]. For the Q_{cys} of FAS_{eq}, values of 0.82 mg/L for ages <70 years and 0.95 mg/L for ages ≥70 years were used, and for the Q_{cys} of $EKFC_{cys}$, 0.83 mg/L for ages <50 years and 0.83+0.005×(age–50) mg/L for ages ≥50 years were used.

Statistical analysis

The Reed–Dixon analysis was performed to check for the absence of outliers. As an acceptable bias limit, ±7.61% of the total allowable error of the serum cysC measurements was used [22]. Bland–Altman plots were constructed to compare the concordance of each cysC-based eGFR equation and the measuring system. Passing–Bablok regression was performed to obtain a regression line between the $Gentian_{cys}$ and $Roche_{cys}$ values. To compare $Getian_{eq}$ with a single $Gentian_{cys}$ value and $CKD\text{-}EPI_{cys}$, scatter plots and regression lines were constructed. Lin's concordance correlation coefficient (ρ_c) [23] was used to assess the concordance between the eGFR calculations. Statistical Package for the Social Sciences version 25.0 (IBM Corporation, Armonk, NY, USA) and MedCalc (version 11.5.1.0; MedCalc Software, Mariakerke, Belgium) were used for statistical analyses and graph construction. Statistically, $P<0.05$ was considered significant.

RESULTS

The characteristics of the 148 enrolled participants are presented in Table 2. The mean age of the participants was 60.5 years (SD±14.5 years), and 43% were female. The mean cysC was 1.72±1.44 mg/L for $Gentian_{cys}$ and 1.71±1.35 mg/L for $Roche_{cys}$. There was no significant difference in the mean cysC between $Gentian_{cys}$ and $Roche_{cys}$.

The arithmetic mean of the percent difference was –2.74%±6.62%, and the lower and upper limits of the 95% confidence interval (CI) were –15.72% and 10.23%, respectively (Fig. 1A). Using a ±7.61% difference as the limit of concordance, the calculated cysC values exceeded the limits. The percent difference plot showed that the bias might have affected the measurements at low concentrations. The percentage difference converged to zero for higher concentrations, although it again widened for extremely high values >5 mg/L.

The obtained regression equation between $Gentian_{cys}$ and $Roche_{cys}$ was $Roche_{cys}=0.13$ (95% CI, 0.12–0.15)+0.91 (0.90 to 0.93)×$Gentian_{cys}$ (Fig. 1B). According to the regression equa-

Table 2. Characteristics of enrolled participants according to categorization of cystatin C-based eGFR with $Gentian_{cys}$ and $Gentian_{eq}$

Parameter	Categorization of cystatin C-based eGFR with $Gentian_{cys}$ and $Gentian_{eq}$*					
	G1 (N=44)	G2 (N=36)	G3 (N=31)	G4 (N=20)	G5 (N=17)	Total (N=148)
Age, yr, mean±SD	58.4±14.9	61.0±11.7	57.8±14.9	62.5±17.7	67.8±12.7	60.5±14.5
Age range, yr	20–85	36–82	20–81	27–91	49–88	20–91
Female, N (%)	22 (50)	12 (33)	13 (42)	8 (40)	8 (47)	63 (43)
ICU admission, N (%)	14 (32)	7 (19)	13 (42)	7 (35)	9 (53)	50 (34)
Acute kidney injury, N (%)	0 (0)	2 (6)	3 (10)	3 (15)	4 (24)	12 (8)
Hemodialysis or CRRT, N (%)	0 (0)	1 (3)	4 (13)	4 (20)	7 (41)	16 (11)
Creatinine, mg/dL, mean±SD	0.63±0.20	0.90±0.29	1.04±0.4	1.43±0.52	3.37±1.68	1.20±1.04
eGFR $CKD\text{-}EPI_{Crea}$, mL/min/1.73 m^2, mean±SD	105.0±19.6	84.3±20.7	74.5±26.0	55.2±26.2	24.0±22.4	77.5±33.7
$Gentian_{cys}$ value, mg/L, mean±SD	0.72±0.11	1.09±0.08	1.51±0.18	2.37±0.36	5.24±1.22	1.72±1.44
$Roche_{cys}$ value, mg/L, mean±SD	0.80±0.10	1.12±0.08	1.50±0.18	2.29±0.36	5.03±1.03	1.71±1.35
eGFR with $Gentian_{cys}$ and $Gentian_{eq}$, mL/min/1.73 m^2, mean±SD	133.9±30.0	70.9±8.1	45.1±7.7	23.9±4.8	8.1±2.6	70.7±48.8

*According to the 2012 KDIGO classification, each eGFR value was categorized as eGFR ≥90 mL/min/1.73 m^2 (G1), 60–89 mL/min/1.73 m^2 (G2), 30–59 mL/min/1.73 m^2 (G3), 15–29 mL/min/1.73 m^2 (G4), or <15 mL/min/1.73 m^2 (G5).
Abbreviations: eGFR, estimated glomerular filtration rate; $Gentian_{eq}$, the equation of Gentian manufacturer; $Gentian_{cys}$, Gentian cystatin C immunoassay; ICU, intensive care unit; CRRT, continuous renal replacement therapy; $CKD\text{-}EPI_{cys}$, CKD-EPI cystatin C equation; $CKD\text{-}EPI_{crea}$, CKD-EPI creatinine equation; $Roche_{cys}$, Tina-quant Gen.2 assay.

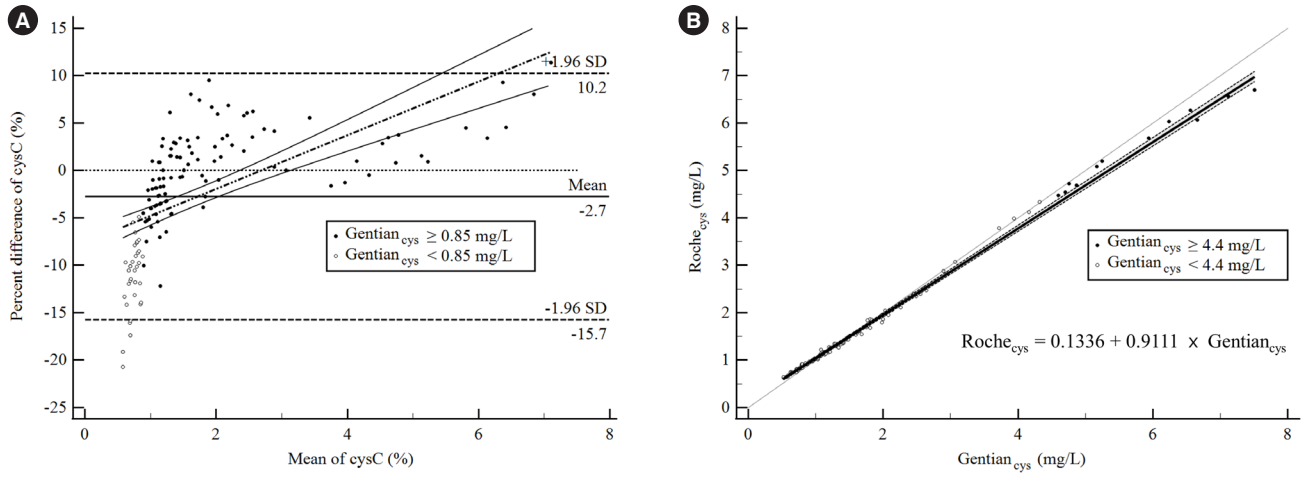

Fig. 1. Bland–Altman plots of cysC values between $Gentian_{cys}$ and $Roche_{cys}$ (A) and the regression line between $Gentian_{cys}$ and $Roche_{cys}$ (B). The Y-axis of (A) is the percentage difference of cysC values between $Gentian_{cys}$ and $Roche_{cys}$.
Abbreviations: cysC, cystatin C; $Gentian_{cys}$, Gentian cystatin C immunoassay; $Roche_{cys}$, Tina-quant Gen.2 analysis.

tion, the limit of the maximum acceptable difference, which satisfied a ±7.61% difference, was 0.85–4.40 mg/L based on $Gentian_{cys}$. Of the 148 participants, 38 (25.7%) had values <0.85 mg/L and 12 (8.1%) had values ≥4.40 mg/L.

The end point for the linear portion of the regression line was approximately 60 mL/min/1.73 m^2 based on a visual assessment (Fig. 2A). Comparing the eGFR of $Gentian_{cys}$ combined with $Getian_{eq}$ and that of $Roche_{cys}$ combined with $CKD\text{-}EPI_{cys}$, the end point for the linear portion of the regression line was 60 mL/min/1.73 m^2, after which the distance to the ideal line was greater than that of the combination of $Gentian_{cys}$ and $CKD\text{-}EPI_{cys}$ (Fig. 2B). Bland–Altman analysis results showed that differences between $Getian_{eq}$ and $CKD\text{-}EPI_{cys}$ increased by approximately 60 mL/min/1.73 m^2 (Fig. 2C and D).

According to the groups with $Gentian_{cys}$ values, the eGFR results calculated for each combination are shown in Table 3. The mean eGFR range of all combinations was 63.4±38.0–58.8±28.6 mL/min/1.73 m^2. Differences in eGFRs between $Gentian_{cys}$

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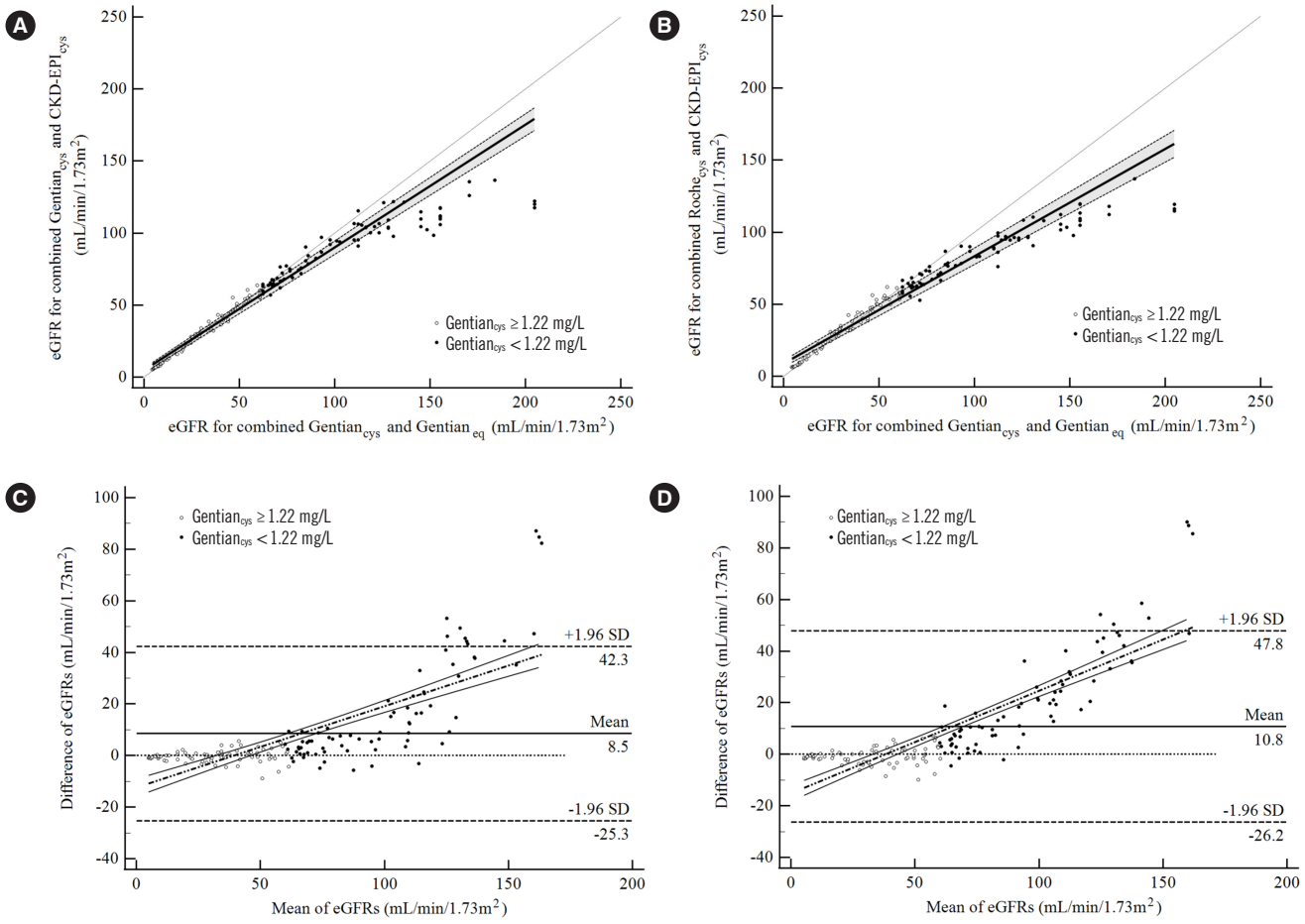

Fig. 2. Regression lines (A and B) and Bland–Altman plots (C and D) of eGFR values between Gentian$_{eq}$ and CKD-EPI$_{cys}$ using cysC values with Gentian$_{cys}$ and Roche$_{cys}$. The Y-axes of (C) and (D) are the differences in eGFR between Gentian$_{eq}$ and CKD-EPI$_{cys}$.
Abbreviations: eGFR, estimated glomerular filtration rate; cysC, cystatin C; Gentian$_{cys}$, Gentian cystatin C immunoassay; Roche$_{cys}$, Tina-quant Gen.2 analysis; Gentian$_{eq}$, equation of Gentian manufacturer; CKD-EPI$_{cys}$, CKD-EPI cystatin C equation.

and Roche$_{cys}$ were found mainly in the Q4 (6.8–11.7 mL/min/1.73 m^2) and Q3 (2.4–3.4 mL/min/1.73 m^2) groups rather than in the Q1 (–0.3 to –0.4 mL/min/1.73 m^2) or Q2 (–0.8 to –1.0 mL/min/1.73 m^2) groups.

According to the groups with Gentian$_{cys}$ values, comparisons of the ρ_c between the two reagents are described in Supplemental Data Table S2. In the Q1 and Q2 groups, there was a negligible difference between Gentian$_{cys}$ and Roche$_{cys}$, whereas the difference between Gentian$_{cys}$ and Roche$_{cys}$ was significant in the Q3 and Q4 groups. Comparisons of the ρ_c among the four equations with the same reagent are described in Supplemental Data Table S3. In the Q1 and Q2 groups, FAS$_{eq}$ and EKFC$_{cys}$ resulted in higher eGFR values than CKD-EPI$_{cys}$ and CAPA$_{eq}$. In contrast, FAS$_{eq}$, but not EKFC$_{cys}$, resulted in lower eGFR values in the Q3 and Q4 groups. The largest difference in eGFR was found between Roche$_{cys}$ combined with FAS$_{eq}$ and Gentian$_{cys}$ combined with CAPA$_{eq}$, but this was not the case for Gentian$_{cys}$ combined with FAS$_{eq}$, Roche$_{cys}$ combined with CAPA$_{eq}$, and Roche$_{cys}$ combined with EKFC$_{cys}$ (Supplemental Data Table S4).

DISCUSSION

Initially, simple equations such as "eGFR = A × cysC^{-B}" were developed for cysC-based GFR calculation [21, 24-27]. Potentially different results obtained with different measurement systems could potentially lead to larger differences in eGFR [8]. Each equation is recommended for use with a specific associated measuring system because of the discrepancy in eGFR among equations despite a fixed cysC value [14, 21, 24, 26, 28]. For the standardization of cysC, a certified reference material, ERM-DA471/

Table 3. Mean eGFR values calculated using each combination of reagents and equations according to groups based on $Gentian_{cys}$ values

Reagent	Equation	Mean eGFR values (mL/min/1.73 m^2) according to groups based on $Gentian_{cys}$ values*				Total
		Q1 (N=37)	Q2 (N=37)	Q3 (N=37)	Q4 (N=37)	
$Gentian_{cys}$	$Gentian_{cys}$, mean±SD (range), mg/L	3.69±1.68 (1.98–7.51)	1.46±0.20 (1.23–1.83)	1.04±0.10 (0.80–1.19)	0.69±0.09 (0.52–0.80)	1.72±1.44 (0.52–7.51)
	$CKD\text{-}EPI_{cys}$	17.4±9.2	47.3±9.5	73.3±11.1	109.7±10.9	62.2±35.7
	$Gentian_{eq}$ (%diff, *P*)	16.6±8.9	47.7±9.2	77.3±11.6	141.0±27.3	70.7±48.8
		(–5.2, *P*<0.001)	(1.1, *P*<0.001)	(5.6, *P*<0.001)	(27.8, *P*<0.001)	(7.2, *P*<0.001)
	$CAPA_{eq}$ (%diff, *P*)	17.3±10.3	48.0±8.2	71.3±9.0	115.9±19.4	63.4±38.4
		(–6.9, *P*<0.001)	(2.2, *P*<0.001)	(–2.4, *P*<0.001)	(5.6, *P*<0.001)	(–0.4, *P*<0.001)
	FAS_{eq} (%diff, *P*)	22.6±10.2	50.3±8.8	68.9±10.6	105.8±20.8	62.1±33.4
		(38.9, *P*<0.001)	(7.5, *P*<0.001)	(–5.6, *P*<0.001)	(–3.8, *P*<0.001)	(9.3, *P*<0.001)
	$EKFC_{cys}$ (%diff, *P*)	24.9±10.6	54.4±8.5	75.8±9.3	116.3±18.6	67.8±35.6
		(53.8, *P*<0.001)	(16.6, *P*<0.001)	(–3.3, *P*<0.001)	(5.2, *P*<0.001)	(19.6, *P*<0.001)
$Roche_{cys}$	$Roche_{cys}$, mean±SD (range), mg/L	3.55±1.57 (1.96–6.70)	1.45±0.20 (1.20–1.85)	1.08±0.09 (0.92–1.20)	0.77±0.08 (0.63–0.92)	1.71±1.35 (0.63–6.70)
	$CKD\text{-}EPI_{cys}$ (%diff, *P*)	18.2±9.8	47.7±9.2	69.9±9.7	102.9±12.2	59.9±32.9
		(5.0, *P*<0.001)	(1.0, *P*<0.001)	(–4.7, *P*<0.001)	(–6.1, *P*<0.001)	(–1.2, *P*<0.001)
	$CAPA_{eq}$ (%diff, *P*)	18.3±10.9	48.4±8.0	68.3±7.8	101.2±14.4	59.3±32.3
		(0.1, *P*<0.001)	(3.1, *P*<0.001)	(–6.5, *P*<0.001)	(–7.7, *P*<0.001)	(–2.8, *P*<0.001)
	FAS_{eq} (%diff, *P*)	23.4±10.6	50.7±8.9	66.5±9.8	94.1±17.6	58.8±28.6
		(44.1, *P*<0.001)	(8.4, *P*<0.001)	(–8.9, *P*<0.001)	(–14.4, *P*<0.001)	(7.3, *P*<0.001)
	$EKFC_{cys}$ (%diff, *P*)	25.8±11.1	54.7±8.4	73.0±8.3	103.4±14.9	64.2±30.3
		(59.6, *P*<0.001)	(16.9, *P*<0.001)	(–0.4, *P*<0.001)	(–6.4, *P*<0.001)	(17.4, *P*<0.001)

*According to previously recorded $Gentian_{cys}$ values, participants were divided into four groups: Q1 (>1.98 mg/L), Q2 (1.22–1.98 mg/L), Q3 (1.21–0.80 mg/L), and Q4 (<0.80 mg/L). The %diff was calculated as (A–B)/B×100, where B is the eGFR value of $CKD\text{-}EPI_{cys}$ with $Gentian_{cys}$. All groups showed significant differences (*P*<0.001) when using the Wilcoxon signed-rank analysis to compare the difference between each eGFR combination and $CKD\text{-}EPI_{cys}$ with $Gentian_{cys}$.

Abbreviations: eGFR, estimated glomerular filtration rate; $Gentian_{cys}$, Gentian cystatin C immunoassay; $Roche_{cys}$, Tina-quant Gen.2 analysis; $Gentian_{eq}$, equation of Gentian manufacturer; $CKD\text{-}EPI_{cys}$, CKD-EPI cystatin C equation; $CAPA_{eq}$, equation of Caucasian, Asian, pediatric, and adult participants; FAS_{eq}, equation of full age spectrum; $EKFC_{cys}$, EKFC cystatin C equation; %diff, percent difference.

IFCC, was produced by the Working Group for the Production of an International Cystatin C Calibrator (WG-SCC) in 2010 [29]. The 2012 cysC-based $CKD\text{-}EPI_{cys}$ was developed to include age and sex factors as well as the standardized cysC value [8], and the KDIGO guidelines recommend use of $CKD\text{-}EPI_{cys}$ for reporting cysC-based eGFR or the alternative equations shown to improve the accuracy of GFR estimation compared with $CKD\text{-}EPI_{cys}$ [5]. Therefore, $CKD\text{-}EPI_{cys}$ appears to be the most broadly applicable equation [30], although $CAPA_{eq}$ [17], FAS_{eq} [18], and $EKFC_{cys}$ [19] were suggested as their GFR estimation showed improved accuracy compared with that of $CKD\text{-}EPI_{cys}$.

In 2010, ERM-DA471/IFCC was approved for cysC measurements in specific systems with successful calibration and negligible method bias [20, 31, 32]. Using ERM-DA471/IFCC as a calibrator or measurement trueness control material, each manufacturer generated adjusted calibration curves with improved measurement uncertainty [20, 31, 33, 34]. However, the outcome of harmonization and standardization for cysC measurement remains controversial [10, 11], although a few studies have reported improvements in measurement uncertainty for specific measuring systems [20, 31, 34]. Both $Gentian_{cys}$ and $Roche_{cys}$, which use the particle-enhanced immunoturbidimetric (PETIA) method, have low analytical variability and bias [11, 32]. ERM-DA471/IFCC requires dilution because it is a single-level calibrator with a high cysC concentration of 5.48 mg/L. The results of the College of American Pathologists 2014 CYS Survey, international proficiency testing programs [11], and the 2015 survey involving seven clinical laboratories located in France and Belgium [10] showed limitations in the metrological traceability of commercial measuring systems for cysC. We found significant

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differences between $Gentian_{cys}$ and $Roche_{cys}$ at concentrations <0.85 mg/L and cysC concentrations ≥4.40 mg/L calculated using $Gentian_{cys}$. $Gentian_{cys}$ resulted in higher values than $Roche_{cys}$ at very high cysC concentrations, similar to the results of a previous report [10]. In contrast, the Bland–Altman analysis suggested that $Roche_{cys}$ shows higher values than $Gentian_{cys}$ at low concentrations (<0.85 mg/L). These results are in line with a 2015 survey [10], which reported that clinically significant positive bias decreased with higher concentrations of $Roche_{cys}$ on the Cobas c502 system. Based on our results, the equivalence of cysC values at low concentrations (<0.85 mg/L) between the two PETIA-based measuring systems was unsatisfactory.

A low cysC concentration might be the baseline for evaluating abrupt cysC elevations in critically ill adults and children [35, 36], and the bias at low concentrations might dramatically affect GFR estimations [14, 15]. Recently, a study showed that the predicted concentrations from recalibration against ERM-DA471/IFCC were consistently 17% higher than the previously measured values using calibration with the Siemens nephelometer [31]. These values were 5–6% higher than the previous prediction used for the development of $CKD\text{-}EPI_{cys}$.

In the current study, $CKD\text{-}EPI_{cys}$, with an inflection point at 0.8 mg/L of cysC, resulted in the highest ρ_c value between the two reagents in the Q3 and Q4 groups (Supplemental Data Table S2). According to $CKD\text{-}EPI_{cys}$, the exponent number of cysC is altered from –1.33 to –0.50 at 0.8 mg/L of cysC, which leads to a reduction in effectiveness for bias at low cysC concentrations. Conversely, $CAPA_{eq}$ and FAS_{eq}, without a compensation function at low cysC concentrations, resulted in dynamic ρ_c values ranging from 0.73 to 0.93 in the Q3 and Q4 groups, depending on the combination of reagents (Supplemental Data Table S4).

Our study showed that differences in eGFR values between $Getian_{eq}$ and $CKD\text{-}EPI_{cys}$ within $Gentian_{cys}$ were increased by approximately 60 mL/min/1.73 m^2. Although the manufacturer continues to recommend the use of $Getian_{eq}$ for eGFR using $Gentian_{cys}$ values after standardization, $CKD\text{-}EPI_{cys}$ and two equations developed after standardization revealed different trends for eGFRs from approximately 60 mL/min/1.73 m^2 compared with those of $Getian_{eq}$. Thus, the initial equation cannot be confidently used in the absence of evidence indicating the superior accuracy of GFR estimations compared with that of $CKD\text{-}EPI_{cys}$ [5].

FAS_{eq} and $EKFC_{cys}$ tended to represent higher eGFR values for the Q1 group, which mainly consists of G4 and G5 eGFR categorization, compared with those obtained using $CKD\text{-}EPI_{cys}$ or $CAPA_{eq}$. These results might reflect a relatively higher proportion of healthy participants in the FAS_{eq} development population [18, 37]. In addition, FAS_{eq} resulted in a less sensitive reflection of the eGFR in the Q3 and Q4 groups with low cysC concentrations compared with that obtained using $CKD\text{-}EPI_{cys}$, $CAPA_{eq}$, or $EKFC_{cys}$ (Table 3 and Supplemental Data Fig. S1). Because Q_{cys} rescaling would require only a single rescaling factor, specifically the spectrum of age [19], $EKFC_{cys}$ would have the advantage of easily reducing bias based on the measured GFR. Compared with FAS_{eq}, $EKFC_{cys}$ used a modified Q_{cys}, and each Q_{cys} induced a marked difference in eGFR in the Q3 and Q4 groups.

Furthermore, the cumulative frequency distribution of eGFRs from each combination showed the effect of both reagents and equations on the eGFR results (Supplemental Data Fig. S1). The frequency distribution indicated that the equation prominently affects the eGFR at levels less than approximately 60 mL/min/1.73 m^2, whereas the measuring system and equation have either an additive effect or a decreased effect according to each combination at levels greater than approximately 60 mL/min/1.73 m^2. In contrast to a previous study [20], our data indicate that the diversity of cysC reagents is a cause of discordance in accuracy in comparison studies of equations.

To overcome the current limitations of the cysC measurement system, methodological improvements are required. First, the most promising candidate, isotope dilution mass spectrometry (IDMS), will be a reference measurement method for cysC [38, 39], and current commercial methods should be calibrated to be traceable with respect to the IDMS method. Second, it is essential to develop novel reference materials for cysC that are assigned clinically useful values and do not require dilution.

Our study has several limitations. First, we could not measure the GFR, which serves as the reference method for the eGFR. Second, we only performed one-point calibration, whereas multipoint calibration based on serial dilutions of a certified reference material or commercial calibrator would help to adjust the calibration factor. Finally, owing to limited resources, we evaluated only a limited number of participants. Due to these limitations, we could not show the accuracy and bias of each cysC value and eGFR equation.

Taken together, our data show that the equivalence of cysC values at low concentrations (<0.85 mg/L) between the two PETIA-based measuring systems is not satisfied after standardization. Results obtained with different measurement systems could lead to larger differences in the eGFR, which vary based on the combination used. Bias and accuracy analyses for cysC-based eGFR equations should be conducted using a standardized measurement system for cysC, as well as measured GFR.

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AUTHOR CONTRIBUTIONS

Lee HS and Park HD contributed to the conception and design of the study. Lee HS drafted the manuscript. Lee HS, Bae GE, Lee JE, and Park HD were involved in data investigation, review, and editing of the manuscript. All the authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

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