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Performance evaluation of a particle-enhanced turbidimetric cystatin C assay using the Abbott Aeroset analyser and assessment of cystatin C-based equations for estimating glomerular filtration rate in chronic kidney disease

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Abstract

Introduction. Measurement of glomerular filtration rate (GFR) is critical for the diagnosis and stratification of chronic kidney disease (CKD). Recent studies have shown that cystatin C is superior to creatinine for the detection of impaired GFR, and several cystatin C-based equations for estimating GFR have been developed for this clinical application. We conducted the present study to assess the applicability of cystatin C as a routine clinical laboratory index and to determine the performance of cystatin C-based equations in estimating GFR in CKD patients in China.

Methods. Performance evaluation of particle-enhanced turbidimetric cystatin C assay on the Abbott Aeroset analyser was carried out according to the National Committee for Clinical Laboratory document EP10-A2. Estimated GFR, which was generated from cystatin C-based equations, was compared with measured GFR, which was detected by plasma clearance of 99mTc-DTPA.

Results. Our cystatin C assay showed a very low total imprecision and linearity drift. All eight cystatin C-based GFR estimating equations underestimated or overestimated GFR as compared with GFR determined by 99mTc-DTPA clearance.

Conclusion. Although the cystatin C assay is acceptable for routine clinical laboratory monitoring, none of the existing cystatin C-based equations were ideal for estimating GFR in Chinese CKD patients.

Keywords: chronic kidney disease; cystatin C; glomerular filtration rate

Introduction

Glomerular filtration rate (GFR) is one of the commonly used indexes for early detection of chronic kidney disease (CKD). Estimation of GFR is recommended to diagnose and stratify CKD. An accurate, convenient and reproducible method for estimating GFR is important for clinical practice. The 'gold standard' for determining GFR involves measuring the clearance of exogenous substances such as inulin. However, the measurement of inulin is time-consuming, labor-intensive and expensive, which makes it incompatible for routine monitoring. As a result, in clinical practice, the measurement of endogenous serum substances for estimation of GFR (eGFR) is commonly performed. Because earlier studies have mainly focused on serum creatinine (Scr), creatinine clearance has commonly been used as a marker for GFR. Despite its common use, creatinine has several serious limitations as a marker for renal function. For example, creatinine levels are influenced by factors such as age, gender, muscle mass, physical activity and diet [1]. Also, creatinine clearance usually overestimates the true GFR [2].

The use of cystatin C has attracted recent attention as an endogenous substance for measuring GFR. A meta-analysis suggested that cystatin C is superior to creatinine for detecting impaired GFR in cross-sectional studies [3]. Cystatin C offers advantages over creatinine because there is less interference and because it is more sensitive to the change in GFR in children. In clinical practice, serum cystatin C may optimize the early detection of diabetic or hypertensive nephropathy [4,5].

Recently reported formulas for using serum cystatin C concentrations are based on the particle-enhanced immunonephelometric assay (PENIA) or the immunoturbidimetric assay (PETIA) in order to estimate GFR [6–11]. The diagnostic accuracy of three cystatin C-based formulas (Larson, Hoek and Filler formulae) using an immunonephelometric method has been evaluated in liver transplant recipients [12] and in kidney transplant recipients [13]. In both reports, the Hoek formula showed the best overall performance for GFR estimation with respect to bias, precision and accuracy.

However, cystatin C has not been widely used in routine clinical laboratory testing. One problem is a need for adaptation of cystatin C assays to routine chemistry instru-

Table 1. Equations to predict GFR using cystatin C (cystatin C in mg/L)

Equations	Reference	Formula	Methods	Instruments and reagents	Sample size	Population
1	Grubb *et al*. [11]	eGFR = 99.19 × cystatin $C^{-1.713}$ × 0.823 (if female)	PETIA	Hitachi, DakoCytomation	n = 451	Adults
2	Tan *et al*. [25]	eGFR = 87.1 cystatin C − 6.87	PETIA	Roche Cobas, DakoCytomation	n = 40 n = 29	Adults Diabetics
3	Larsson *et al*. [8]	eGFR = 99.434 × cystatin $C^{-1.5837}$	PETIA	DakoCytomation	n = 100	Adults
4	Sjöström *et al*. [10]	eGFR = 124 cystatin C − 22.3	PETIA		n = 381	Adults
5	Hoek *et al*. [7]	eGFR = 80.35 cystatin C − 4.32	PENIA	BN ProSpec Dade Behring	n = 123	CKD patients
6	Filler *et al*. [26]	log(eGFR) = 1.962 + [1.123 × log(1 cystatin C)]	PENIA	BN ProSpec Dade Behring	n = 536	CKD children
7	Larsson *et al*. [8]	eGFR = 77.239 × cystatin $C^{-1.2623}$	PENIA	Dade Behring	n = 100	Adults
8	Rule *et al*. [27]	eGFR = 66.8 × cystatin $C^{-1.30}$	PENIA	BN II Nephelometer Dade Behring	n = 357	CKD patients

ments to minimize turnaround times and to allow 24 h/day availability. In addition, despite the demonstrated advantages of the above equations, further verification is needed in larger and more diverse populations. We therefore conducted the present study to evaluate the applicability of cystatin C as a routine clinical laboratory index using the Abbott Aeroset analyser with a particle-enhanced turbidimetric cystatin C kit and to evaluate the diagnostic performance of cystatin C-based formulas in CKD patients in China.

Materials and methods

Cystatin C measurement and performance evaluation of a particle-enhanced turbidimetric cystatin C assay on the Abbott Aeroset analyser

Serum cystatin C was measured using a particle-enhanced turbidimetric cystatin C kit (Jingyuan, Medical Appliance Ltd, Shanghai, China) on Architect Aeroset analyzer (Abbott Laboratories, Abbott Park, IL, USA). The performance of the particle-enhanced turbidimetric cystatin C method was evaluated according to National Committee for Clinical Laboratory Standards (NCCLS) document EP10-A2 [14]. To establish the accuracy of this method, plasma cystatin C measurements were also performed by latex-enhanced reagent (N Latex Cystatin C, Dade Behring, Deerfield, IL, USA) using a BN ProSpec analyser (Dade Behring) and calibrators from Dade Behring. The test was performed according to the recommendation of the manufacturer. Using a statistical technique, the linearity, proportional and constant bias, linear drift and precision of each clinical laboratory method were given preliminary evaluation. Correlations between the two methods were also evaluated.

Patient population and samples

Plasma cystatin C was measured in 95 CKD patients (age range, 15.6–74.0 years; 41 females and 54 males) that were hospitalized in The First Affiliated Hospital of Sun Yat-sen University and that had been consecutively referred for determination of GFR by 99mTc-DTPA clearance measurements during a period of 12 months (January to December 2005). Common causes for referral of patients were primary or secondary glomerular disease, hypertension, obstructive kidney disease, renal-vascular disease, chronic tubulointerstitial disease, diabetic nephropathy, polycystic kidney disease, other causes and causes unknown. CKD was diagnosed and classified according to the Kidney Disease Outcome Quality Initiative clinical practice guideline [15]. Patients with acute kidney function deterioration, edema, skeletal muscle atrophy, pleural effusion or ascites, malnutrition, amputation, heart failure, ketoacidosis, hypothyroidism, hyperthyroidism [16] or high-dose steroid use [17], which have significant influences on cystatin C, were excluded. All subjects gave their written informed consent, and the Ethics Committee of Sun Yat-sen University approved the study.

GFR measurement

GFR was measured by the plasma clearance of 99mTc-DTPA [18,19]. Ten millicuries (370 Mbq) of 99mTc-DTPA was given as a single injection with plasma samples drawn at 120, 180 and 240 min after injection [20,21]. GFR was assessed using a dual plasma sampling method [22,23], standardized for body surface area [24], and was calculated using the measured GFR (mGFR) equation:

$$\text{rGFR (ml min}^{-1}/1.73\ \text{m}^{-2}) = \{D\ln(\text{P1/P2})/(\text{T2} - \text{T1})\}\exp\{[(\text{T1ln P2}) - (\text{T2ln P1})]/(\text{T2} - \text{T1})\} \times 0.93 \times 1.73/\text{BSA}$$

where D is dosage of drug injected, T1 is time of first blood sampling (~2 h), P1 is plasma activity at T1, T2 is time of second blood sampling (~4 h) and P2 is plasma activity at T2. The units of measurement were counts per minute, per millilitre for D, P1 and P2 and minutes for T1 and T2. Body surface area is abbreviated as BSA.

To ensure reliability of the 99mTc-DTPA measurements, the 99mTc-DTPA drug was strictly selected (radiochemical purity greater than 95% and percentage of 99mTc-DTPA bound to plasma protein <5%). In addition, the well counter was verified weekly for counting reproducibility. GFR was corrected for standard body surface area by multiplying the measured value by 1.73 and dividing by the patient's body surface area, as estimated by the DuBois formula [24]. On the day of the 99mTc-DTPA GFR measurement, patients were weighed; their height was measured, and a blood sample was taken for serum cystatin C. Gender, race and age of the patients were recorded.

GFR estimation

Estimated GFR (eGFR) was assessed by eight equations that used serum cystatin C [7,8,10,11,25–27] (Table 1).

Statistical analyses

Statistical analysis was performed, and figures were made using Excel 2007 (Microsoft Corporation, Seattle, WA, USA) and SPSS for Windows, release 13.0.1(SPSS Inc., Chicago, IL, USA). Multiple regressions and Student's t statistics were used for evaluation of cystatin C methods. Decisions on the significance of the various components (slope, carry-over, nonlinearity or drift) were made by comparing t-values compared to the significance levels. Evaluation of the prediction equations was performed by calculating the bias, precision and accuracy as recommended in the National Kidney Foundation guidelines on chronic kidney disease [28]. Bias was defined as the mean difference between the mGFR (using 99mTc-DTPA) and eGFR [28]. Precision was defined as the standard deviation (SD) of the difference between the mGFR and eGFR [28]. Accuracy was defined as the percentage of GFR estimates lying within 15%, 30% and 50% of the mGFR (using 99m Tc-DTPA) [28]. The analysis was repeated after stratifying patients by eGFR (<60 and ≥60 ml/min per 1.73 m^2). Pearson's correlations were used for comparisons between groups. Significance of differences between means was calculated using t-tests. Accuracy calculations were performed using an Excel spreadsheet. Medcalc software version 8.0 (Medcalc Software, Mariekerke, Belgium) was used for a Bland and Altman analysis [29] to compare GFR estimates with mGFR. Systematic

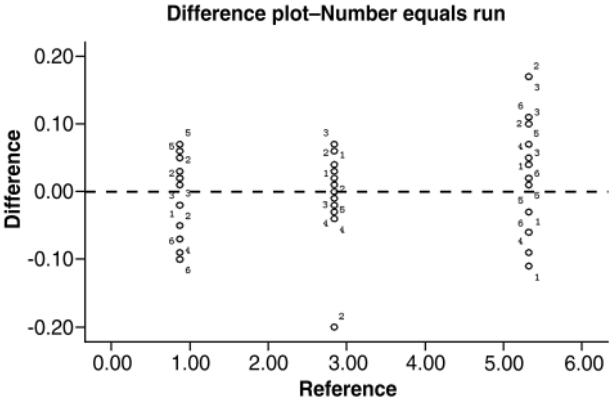

Fig. 1. Raw data difference plot of cystatin C data. One run on each of 6 days; (Day 2 declared an outlier day).

increases or decrease in the difference, assessed by increasing GFR, was checked by inspection of the graph. The limits of agreement are given by the mean ± 1.96 SD, containing 95% of the values. For all analyses, $P < 0.05$ was considered statistically significant.

Results

Performance evaluation

Imprecision data. For total imprecision estimates, bias can be estimated by the difference between the observed mean values and the assigned values at each concentration. The bias in the low, mid, and high levels were 0.00, 0.01 and 0.03 mg/L, respectively, which was far lower than the allowable goal for bias.

Linearity. The *x*-axis is the expected (labeled) concentration. The *y*-axis represents the observed difference between the assay and reference. A zero difference line on the plot was drawn for reference. Each individual point (15 at each level) was plotted. The linearity of these data is shown in Figure 1.

Analysis of the data for imprecision. According to the standard based on biological variation, the total allowable imprecision should be less than half of intra-individual variation. Because intra-individual variation of cystatin C is 13.3%, the allowable imprecision for cystatin C is 6.6% [30]. The results in Table 2 show that the total coefficients of variation were 5.63%, 2.21% and 1.66% in low, mid and high levels, respectively, which were lower than the 6.6% allowable range.

Correlation between cystatin C analysed on ProSpec and Architect Aeroset. There was a strong correlation between cystatin C values analysed with Dade Behring and Jingyuan reagents ($R^2 = 0.997$, Figure 2). Linear regression analysis showed a slope very close to 1.00 and an intercept close to 0 [$y = 0.957 \times (\text{error} = 0.007) - 0.023(\text{error} = 0.022)$]. The bias plot (Figure 3) displayed a good agreement between the two methods within the studied range but slightly lower values for the Jingyuan reagent.

Table 2. Calculation of Imprecision

Projects	Low	Mid	High
(*R*) Pooled within-run variance	0.002	0.004	0.006
(*S*) Variance of daily means	0.001	0.001	0.004
(*T*) Adjusted between-day variance, (*S*) − (*R*)/3	0.000	0.000	0.002
(*U*) Total variance, (*R*) + (*T*)	0.002	0.004	0.008
(*V*) Total standard deviation = $\sqrt{U}$	0.049	0.063	0.089
Grand mean value	0.87	2.85	5.35
(*W*) Total CV% = (*V*)/Grand mean value 100%	5.63	2.21	1.66

Because all days have the same number of data points, it is permissible to simply take the mean within-run variance for all accepted runs at each level. The data were calculated as the variance of daily means from data for each level. Values <0 were set equal to zero.

CKD patient characteristics

Ninety-five patients with CKD were used in the final analysis, which included 54 males and 41 females. The average age was 43.5 ± 13.7 year. The average mGFR using 99mTc-DTPA was 43 ± 27 ml/min per 1.73 m^2 with a range of 6 to 112 ml/min per 1.73 m^2. The mean, median and range of the eGFRs with the different prediction quotations are shown in Table 3.

Diagnostic performance of the equations for estimated GFR

First, the overall diagnostic performance was compared among equations 1 to 8. Linear regressions were made using eGFR against mGFR. The performance of the various estimates of GFR is shown in Table 4 and Figure 4. The Sjöström (PETIA), Larsson (PETIA) and Filler (PENIA) equations had the least bias (0.672, 1.483 and −2.284 ml/min per 1.73 m^2, respectively). Equations 2 and 5 to 7 had similar accuracy and nearly 80% of the GFR estimates were within 50% of the measured 99Tc-DTPA GFR. Among the eight equations, the Filler (PENIA) equation had the best precision (19.691), the highest percentage of values that fell within 30% of the true GFR (55.8%) and the least bias.

Performances of the GFR equations when the estimated GFR was <60 or ≥60 ml/min per 1.73 m^2 are shown in Table 5. None of the eight equations were accurate when the eGFR was either above or below 60 ml/min per 1.73 m^2. Table 6 and Figure 5 show the dependent relations between the eGFR and mGFR by comparing the *D*-value and average of eGFR and mGFR. *F* tests were performed when there was a demonstrated relationship. When there was none, the Bland–Altman plot was drawn to obtain the limits of agreement. Equations 1, 3, 4 and 6 had significant correlations between the eGFR and mGFR, and *F* tests revealed statistical significance for equations 1, 3 and 4, which suggested that eGFRs from equations 1, 3 and 4 were inconsistent with mGFR. The *F* test for estimation of equation 6 was not statistically significant, suggesting that estimates from the Filler equation were consistent with the mGFR. The Bland–Altman plots for equations 2, 5, 7 and 8 showed that the estimates from equations 2, 5, 7 and 8 were not consistent with mGFR because the limits of agreement obtained

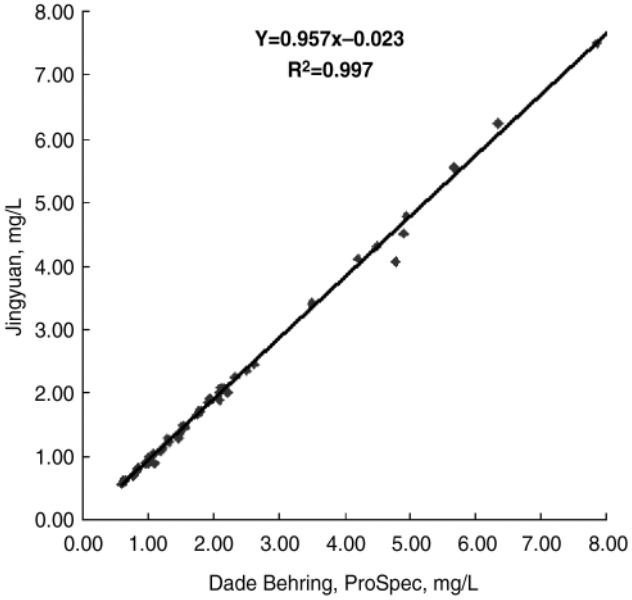

Fig. 2. Correlation between patient samples analysed with Dade Behring and Jingyuan reagents.

from the four equations were larger than the professional boundary (60 mL/min/1.73 m^2) of 30% [31].

Discussion

Before using a new method, kit or instrument for *in vitro* diagnostic use, it is first necessary to make a test for acceptability. This initial performance check is neither a rigorous investigation into the long-term performance of the method nor an evaluation of the many factors that can affect results produced by the device. Here, we referred to the NCCLS EP10-A2 document for evaluating serum cystatin C measurements using a particle-enhanced turbidimetric cystatin C kit by the Abbott Aeroset analyser. The NCCLS EP10-A2 document describes a procedure for the evaluation of linearity, proportional and constant bias, linear drift, sample carry-over and precision of these clinical laboratory methods.

The validation report of the NCCLS EP10-A2 protocol revealed that the total imprecision and bias is less than the allowance [30]. Linear drift or cross-contamination has little influence on the results. Recently, particle-enhanced turbidimetric cystatin C assays on other systems, such as the Hitachi 917 analyser [32] and the Abbott ci8200 analyzer [33], have reported good performance and close correlations with standard cystatin C assays. These findings show that this method is suitable for *in vitro* measurement of cystatin C in routine clinical laboratory testing.

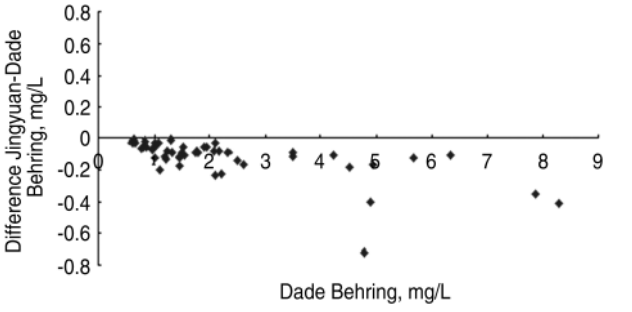

Fig. 3. Bland–Altman bias plot for patient samples analyzed with Dade Behring and Jingyuan reagents. The results are presented as the Dade Behring results (*x*-axis) plotted against the difference between the two methods (*y*-axis).

Table 3. Measured and estimated GFR

	Mean	Median	Range
Measured values			
^{99m}Tc-DTPA GFR	43 ± 27	33	6 to 112
Estimates using cystatin C			
Grubb	36 ± 34	20	2 to 125
Tan	38 ± 28	30	3 to 105
Larsson(PETIA)	41 ± 38	26	3 to 147
Sjöström	42 ± 40	30	−8 to 137
Hoek	37 ± 26	30	5 to 99
Filler	45 ± 31	35	8 to 121
Larsson(PENIA)	36 ± 27	26	5 to 106
Rule	30 ± 24	22	4 to 92

To evaluate the diagnostic performance of the equations for eGFR, 95 Chinese adult patients were consecutively referred to our hospital for determination of GFR by 99mTc-DTPA clearance measurements during a 12-month period. In this patient cohort with CKD, it is necessary to determine GFR using gold standard methods. We compared GFR estimates of all patients using eight formulae based only upon a single parameter, the cystatin C level in milligrams per liter, with the 'true' GFR obtained by using invasive 99mTc-DTPA clearance determinations.

Analysis of the data revealed that the eGFRs from the eight cystatin C-based formulae were significantly correlated with the 'true' mGFR. All eight cystatin C-based GFR estimates showed underestimation or overestimation of GFR when compared with GFR determined by 99mTc-DTPA clearance. Accuracy was measured as the proportion of patients with GFR estimates within the 15%, 30% and 50% intervals of the 99mTc-DTPA-based GFR. Prediction accuracy of the five cystatin C-based formulas did differ significantly. We found that the cystatin C-based prediction equations of the Larsson (PETIA), Sjöström (PETIA) and Filler equations were more accurate at estimating GFR than the other cystatin C-based equations in CKD patients. Prediction accuracy was higher for the cystatin C-based estimates from the Larsson (PETIA), Sjöström (PETIA) and Filler (PENIA) equations. Importantly, among the eight equations, the Filler (PENIA) equation has the best precision, the highest percentage of values that fell within 30% of the true GFR and the least bias. However, none of the eight equations performed well at GFR values above or below 60 ml/min per 1.73 m^2. The Hoek and Larsson equations were the most accurate and were not significantly different from the mGFR when the eGFR was ≥ 60 ml/min per 1.73 m^2. This represented a significant advantage over the other cystatin C-based equations where the accuracy was considerably lower at GFR values above or below 60 ml/min per 1.73 m^2.

Table 4. Overall performance of eGFR equations compared with rGFR: difference, bias, precision, and accuracy[a]

	PETIA				PENIA			
Parameter	Grubb	Tan	Larsson	Sjöström	Hoek	Filler	Larsson	Rule
Intercept (95% CI)	−4.58 (−13.41,4.23)	3.73 (−3.00,10.48)	−4.61 (−14.08,4.85)	−7.19 (−16.79,2.40)	5.46 (−0.75,11.68)	6.906 (−0.57,14.39)	2.390 (−4.26,9.04)	1.349(−4.47,7.17)
Slope (95% CI)	0.94 (0.77,1.12)	0.81 (0.67,0.94)	1.07 (0.88,1.26)	1.15 (0.96,1.34)	0.74 (0.62,0.87)	0.89 (0.74,1.04)	0.78 (0.65,0.91)	0.68 (0.56,0.79)
R^2	0.55	0.60	0.58	0.608	0.60	0.60	0.59	0.59
Bias:mean of difference (95% CI of lower, upper)	6.84 (2.16,11.53)	4.35 (0.63,8.08)	1.48 (−3.55,6.51)	0.67 (−4.48,5.82)	5.30 (1.72,8.88)	−2.28 (−6.29,1.72)	6.87 (3.15,10.59)	12.15 (8.60,15.69)
Precision	23.00	18.27	24.71	25.31	17.60	19.69	18.27	17.40
t-value	2.90	2.32	0.58	0.25	2.94	−1.13	3.66	6.80
P-value	0.005*	0.022*	0.560	0.796	0.004*	0.261	0.000*	0.000*
Accuracy within (%)								
15%	12.6	30.5	15.8	20.0	30.5	27.4	27.4	14.7
30%	25.3	48.4	38.9	31.6	48.4	55.8	44.2	38.9
50%	57.9	78.9	62.1	54.7	77.9	78.9	76.8	66.3

[a]The estimated GFRs (eGFRs) that resulted from these eight equations were all significantly correlated with mGFR. Linear regressions were made using eGFR against mGFR. The eight intercepts and slopes were different. CI, confidence interval.
*$P < 0.05$ compared with mGFR.

Fig. 4. Accuracy of GFR prediction equations. Accuracy was defined as the proportion of values that were within 15%, 30% or 50% of the measured (radiolabeled diethylenetriaminepentaacetic acid) GFR.

In a previous study by Risch and Huber [34], GFR was estimated using a single cystatin C-based equation. They found that the eGFR from the cystatin C-based Larsson equation yielded a bias of −4.7 ml/min per 1.73 m^2 and that 69% of estimates were within 30% of the mGFR, which represented findings that were superior to ours using CKD patients. These discrepant results are likely due to different methods used to measure cystatin C and to estimate GFR. Larsson *et al*. [8] published two separate equations that estimated GFR according to the cystatin C assay that was used. In the paper by Risch and Huber [34], cystatin C was measured using the Dako turbidimetric immunoassay, whereas we used a particle-enhanced turbidimetric cystatin C kit on the Architect Aeroset System. Although Risch and Huber [34] found that the Dako-based Larsson equation was more accurate than the Larsson equation used in the present study, it was far less accurate at estimating GFR than either the Filler or Le Bricon equations.

More recently, a high-profile group reported their findings from 3418 CKD patients pooled from a collection of research studies [35]. Using newly developed equations, percentages of eGFR within 30% of mGFR for equations using serum cystatin C alone, serum creatinine alone or both measurements, adjusted for age, sex and race, were 81%, 83% and 89%, respectively. These findings indicated that an equation using serum cystatin C levels in combination with serum creatinine levels and that included age, sex, and race provides the most accurate estimates of GFR.

Although many have reported that cystatin C is independent of factors such as age and weight, other groups have reported contradictory findings [36]. In a study involving 8058 patients from the Netherlands, Knight *et al*. [36] showed that increasing age, male gender, increasing weight, current smoking and higher C-reactive protein levels were independently associated with a higher cystatin C concentration. These associations were independent of renal function as measured by clearance of urinary creatinine [36]. These findings suggest that cystatin C concen-

Table 5. Bias, precision and accuracy of cystatin C estimates for patients with estimated GFR < 60 and ≥ 60 ml/min per 1.73 m^{2a}

GFR estimates	*N*	Bias:mean of difference (95% CI of lower, upper)	Precision	*T*-value	Accuracy 15%	30%	50%
PETIA method							
Grubb							
<60	72	14.34(11.23,17.46)	13.25	9.18**	9.7	20.8	51.4
≥60	23	−16.62(−29.84,−3.40)	30.57	−2.60*	26.1	39.1	78.3
Tan							
<60	69	7.51(4.39,10.63)	12.98	4.80**	31.9	43.5	78.3
≥60	26	−4.02(−14.68,6.63)	26.39	−0.77	26.9	61.5	80.8
Larsson							
<60	66	11.540(8.46,14.61)	12.51	7.49**	12.1	34.8	62.1
≥60	29	−21.40(−32.85,−9.95)	30.10	−3.82**	24.1	42.4	62.1
Sjöström							
<60	62	12.77(9.37,16.18)	13.42	7.49**	11.3	22.6	51.6
≥60	33	−22.07(−31.59,−12.55)	26.84	−4.72**	36.4	48.5	60.6
PENIA method							
Hoek							
<60	71	7.37(4.32,10.43)	12.91	4.81**	26.8	45.1	77.5
≥60	24	−0.82(−11.99,10.34)	26.45	−0.15	41.7	58.3	79.2
Filler							
<60	65	3.51(0.41,6.61)	12.51	2.26*	26.2	55.4	81.5
≥60	30	−14.85(−24.53,−5.16)	25.93	−3.13**	30.0	56.7	73.3
Larsson							
<60	75	9.74(6.71,12.77)	13.16	6.41**	24.0	40.0	76.0
≥60	20	−3.89(−17.30,9.52)	28.67	−0.60	40.0	60.0	80.0
Rule							
<60	82	14.32(11.09,17.55)	14.71	8.81**	11.0	32.9	63.4
≥60	13	−1.54(−17.25,14.16)	25.99	−0.21	38.5	76.9	84.6

[a]Bias was defined as the mean difference between measured (99Tc-DTPA) and estimated GFR; precision was defined as the SD of the difference between measured (99Tc-DTPA) and estimated GFR. Both precision and bias were expressed as millilitre per minute per 1.73 m^2; accuracy was defined as the proportion of values that were within 15%, 30% or 50% of the measured (99Tc-DTPA) GFR.
*$P < 0.05$; **$P < 0.01$.

trations may be influenced by factors other than renal function. Although certain factors do influence cystatin C concentrations, Stevens *et al*. [35] developed an estimating equation based on cystatin C using a pooled data set of four studies comprising 3134 individuals with CKD and found that age, sex and race coefficients were significant but were substantially smaller than in the Modification of Diet in Renal Disease Study equation. These factors should be taken into consideration when interpreting the present results.

Table 6. The relationship between bias GFR (average of estimated and measured)

Means	Bias(mGFR-eGFRi) *R*	Intercept	Slope	*F* test
Grubb	−0.339 **	17.135	−0.285	8.151**
Tan	−0.06	–	–	–
Larsson(PETIA)	−0.475**	17.575	−0.384	13.785**
Sjöström	−0.540**	18.934	−0.432	19.146**
Hoek	0.068	–	–	–
Filler	−0.214*	4.458	−0.154	2.889
Larsson(PENIA)	−0.019	–	–	–
Rule	0.190	–	–	–

Means = eGFR + mGFR/2*$P < 0.05$; **$P < 0.01$.

In general, we found that none of the eight equations were ideal for estimating GFR in our population of CKD patients. Many factors may have contributed to this result, including the methods for measuring cystatin C, race and sex. However, our population was not large enough to establish a new cystatin C-based equation that is appropriate for GFR estimations in Chinese CKD patients. Massive multiple-centre studies will be necessary to develop convenient, precise and unified methods for measuring cystatin C using cystatin C-based GFR estimation equations. Findings from these studies may help in developing a more accurate equation that includes race, sex, age, and other factors.

Conclusions

In conclusion, measurement of cystatin C using a particle-enhanced turbidimetric cystatin C kit on the Abbott Aeroset analyser was acceptable for routine clinical laboratory monitoring. These findings will help to evaluate the diagnostic performance of cystatin C in a wide range and lower cost. We recommended this method for measuring cystatin C in future studies. However, we found that GFR was not accurately estimated in Chinese CKD patients using the existing cystatin C-based prediction equations. For this patient population, further studies will be needed to establish a more accurate cystatin C-based GFR estimation equation.

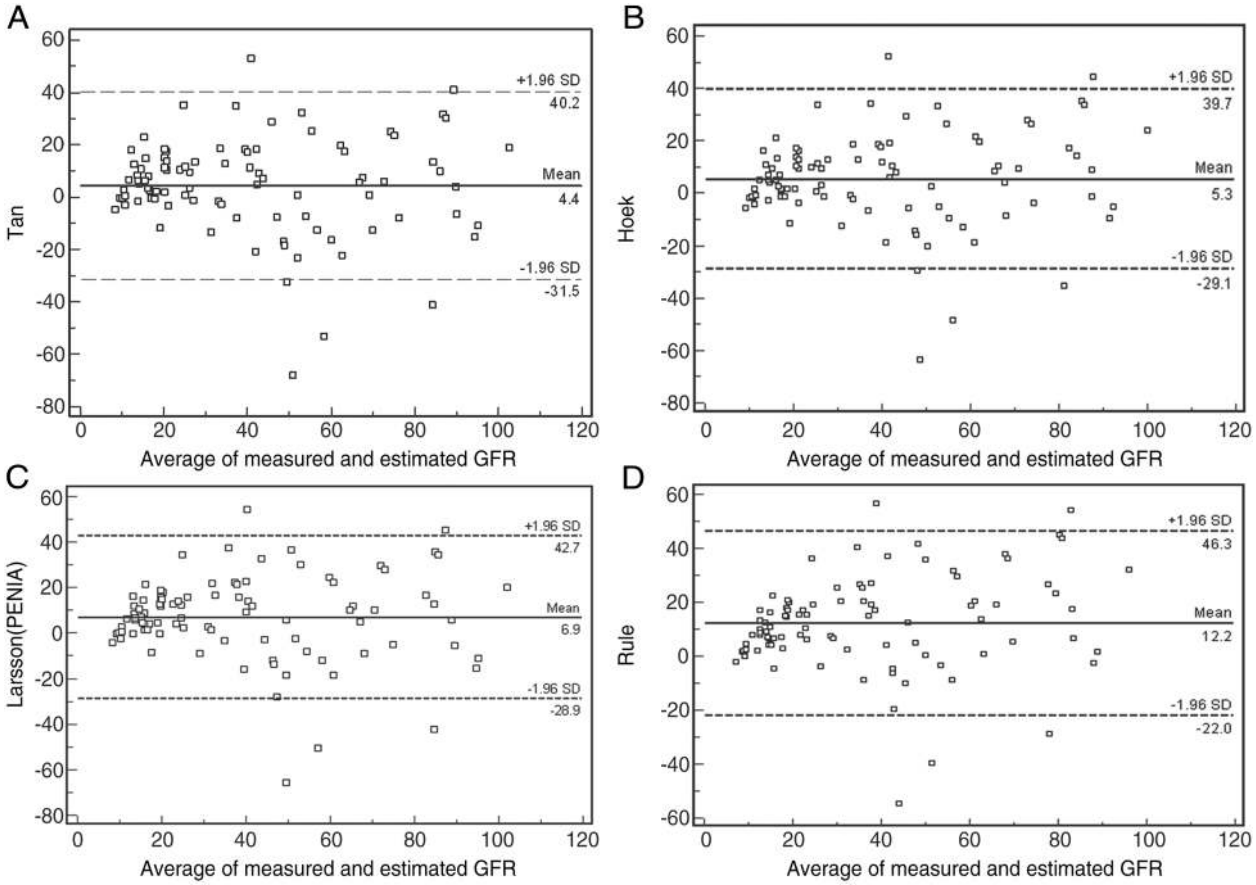

Fig. 5. The disagreement between estimated GFR and mGFR. Bland and Altman plot for differences between estimated GFR and measured GFR. The average GFR is given on the *x*-axis, and the difference in milliliter per minute between the estimated GFR, derived from the cystatin C formula, is on the *y*-axis (**A**. Tan; **B**. Hoek; **C**. Larsson using PENIA method; **D**. Rule). The mean difference (solid lines) and the 1.96 SD limits (dotted lines) are also plotted.

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Conflict of interest statement. None declared.

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Mapping of a new locus for congenital anomalies of the kidney and urinary tract on chromosome 8q24

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Abstract

Background. Congenital anomalies of the kidney and urinary tract (CAKUT) account for the majority of end-stage renal disease in children (50%). Previous studies have mapped autosomal dominant loci for CAKUT. We here report a genome-wide search for linkage in a large pedigree of Somalian descent containing eight affected individuals with a non-syndromic form of CAKUT.

Methods. Clinical data and blood samples were obtained from a Somalian family with eight individuals with CAKUT including high-grade vesicoureteral reflux and unilateral renal agenesis. Total genome search for linkage was performed using a 50K SNP Affymetric DNA microarray. As neither parent is affected, the results of the SNP array were analysed under recessive models of inheritance, with and without the assumption of consanguinity.