Improved GFR estimation by combined creatinine and cystatin C measurements

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Plasma creatinine may not reflect glomerular filtration rate (GFR) especially in the early stages of chronic kidney disease (CKD). Plasma cystatin C (cysC), however, has the potential to more accurately determine early GFR reduction. We sought to improve the creatinine-based GFR estimation by including cysC measurements. We derived a reference GFR from standard dual plasma sampling ^{99m}Tc-DTPA clearance in a training cohort of 376 randomly selected adult Chinese patients with CKD. We compared reference values to estimated GFR and applied multiple regression models to one equation based solely on cysC, and to another combining plasma creatinine (Pcr) and cysC measurements of the training cohort. The results were validated by testing an additional 191 patients. The difference, precision, and accuracy of the two estimates were compared with the modified Modification of Diet in Renal Disease (MDRD) equation for Chinese patients, and another estimate combining cysC and modified MDRD calculations. The estimated GFR combining Pcr and cysC measurements more accurately matched the reference GFR at all stages of CKD than the other equations, particularly in patients with near-normal kidney function.

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The number of patients with chronic kidney disease (CKD) is growing worldwide;[1,2] CKD has been recognized as one of the independent risk factor of cardiovascular disease (CVD), even in its early stages.[3,4] Detection of decreased kidney function and identification of CKD in its early stage are thus very important, but because overestimation of true glomerular filtration rate (GFR) will lead to insufficient treatment and underestimation of true GFR will lead to an unnecessary intervention and waste of medical resources,[5] a simple and accurate GFR estimating method is of great clinical importance.

The Modification of Diet in Renal Disease (MDRD) equations provided reasonable accuracy in Whites and African Americans in GFR estimation,[6] and were recommended by the Kidney Disease Outcome Quality Initiatives clinical practice guidelines.[7] Recently, Levey *et al.* re-expressed MDRD equations using the standardized plasma creatinine (Pcr) assay,[8] and claimed that clinical laboratories can report more accurate GFR estimates in patients with true GFR of less than $90\,ml\,min^{-1}\,1.73\,m^{-2}$. Also, modified MDRD equations based on Chinese CKD patients showed significant improvement compared with the original ones.[9]

Both original MDRD equations and the modified MDRD equations underestimated GFR in CKD stage 1 and 2,[9,10] and re-expressed MDRD equations, however, do not perform well in patients with higher GFR levels.[8] This is partly because the main GFR predictor in these equations is the Pcr, and Pcr's relationship with GFR is different among stages of CKD,[9,11] and different levels of Pcr do not necessarily reflect the true variation of GFR.[12] For example, there was no significant elevation of Pcr levels with the decrease of GFR at the very early stages of CKD because of tubular secretion. These phenomena contribute to the inaccuracy of Pcr-based equations in early stages of CKD, and may be an unavoidable pitfall of Pcr-based GFR estimation equations.

Plasma cystatin C level (cysC) increases earlier than Pcr as GFR decreases, and may be a valuable marker in detecting

early renal function impairment.[13,14] It fulfills a number of criteria for endogenous marker of GFR: It is freely filtered and catabolized in the proximal tubule, without being secreted.[15] Unlike Pcr, cysC does not depend on sex and muscle mass, and does not change with age between 1 and 50 years.[15] In several studies[14,16,17] and in one meta-analysis,[18] cysC has been reported to be superior to Pcr in GFR estimation, particularly in patients with near-normal kidney function.

Researchers have developed cysC-based GFR-estimating equations, and have compared their performance with original MDRD equations. White *et al.*[19] demonstrated that cysC-based equations are more accurate in GFR prediction in renal transplant recipients, than abbreviated MDRD equations. Poge *et al.*[20] found that cysC-based equations might offer advantages compared with abbreviated MDRD equations, in cirrhotic patients. Grubb *et al.*[21] reached the similar conclusions in patients with various renal diseases. More recently, Rule *et al.*[22] claimed that estimating the geometric mean of a cysC-based equation and a Pcr-based equation improved GFR estimation.

In this study, an GFR equation was developed by a log transformed regression model, which simultaneously used Pcr and cysC as independent variables, and compared with the modified MDRD equation for Chinese and a composite equation constituted from the modified MDRD equation and a solely cysC equation.

RESULTS

Patients' characteristics

Five hundred and sixty-seven patients with CKD (average age was 49.8 ± 16.3 years), including 298 males and 269 females participated in the study. The average Pcr and cysC levels of enrolled patients were $1.9 \pm 1.7\,\mathrm{mg\,dl^{-1}}$ (range 0.59–18.6) and $2.1 \pm 1.5\,\mathrm{mg\,l^{-1}}$ (range 0.43–8.3), respectively. The average of reference GFR (rGFR) measured by ^{99m}Tc-DTPA plasma clearance was $57.9 \pm 36.4\,\mathrm{ml\,min^{-1}\,1.73\,m^{-2}}$ (range 4.7–167.4). Causes of CKD and CKD stage classification are shown in Table 1.

Table 1 | Patients' characteristics

Causes of CKD	
Primary or secondary glomerular disease, *N* (%)	231 (40.7%)
Hypertension, *N* (%)	76 (13.4%)
Obstructive kidney disease, *N* (%)	78 (13.7%)
Renovascular disease, *N* (%)	69 (12.3%)
Chronic tubulointerstitial disease, *N* (%)	36 (6.3%)
Diabetic nephropathy, *N* (%)	27 (4.7%)
Polycystic kidney disease, *N* (%)	14 (2.6%)
Other causes or causes unknown, *N* (%)	36 (6.3%)
CKD stages	
Stage 1, *N* (%)	121 (21.3%)
Stage 2, *N* (%)	125 (22.1%)
Stage 3, *N* (%)	166 (29.3%)
Stage 4, *N* (%)	87 (15.4%)
Stage 5, *N* (%)	68 (11.9%)

BSA, body surface area (m^2); CKD, chronic kidney disease.

Development of cysC-based equations

Two cysC-based GFR-predicting models were constructed from a random sample of 376 of the 567 patients. In model 1, inclusion of age and gender only increased the R^2 by 0.01, and did not improve model fit significantly, and so they were not included in the model ($R^2 = 0.85$, standard deviation of residual = 0.13, $P < 0.001$ for model fitting):

$$\mathrm{eGFR}_1 = 86 \times \mathrm{cysC}^{-1.132} \quad (1)$$

where GFR is in units of $\mathrm{ml\,min^{-1}\,1.73\,m^{-2}}$ and cysC is in units of $\mathrm{mg\,l^{-1}}$.

Model 2, which included Pcr, age, and gender in addition to cysC, gave better model fit ($R^2 = 0.91$, standard deviation of residual = 0.103, $P < 0.001$ for model fitting) compared with model 1:

$$\mathrm{eGFR}_2 = 176 \times \mathrm{Pcr}^{-0.607} \times \mathrm{cysC}^{-0.638} \times \mathrm{Age}^{-0.171}\,(\mathrm{Female} \times 0.85) \quad (2)$$

where Pcr is in units of $\mathrm{mg\,dl^{-1}}$ and age is in units of years.

The residual plot for model 2 is shown in Figure 1. The residuals distributed around zero.

The diagnostic performances of the equations

First, the overall diagnostic performances were compared among eGFR_1, eGFR_2, $\mathrm{eGFR}_3 = 175 \times \mathrm{Pcr}^{-1.234} \times \mathrm{age}^{-0.179}$ (female $\times$ 0.79) (the modified MDRD equation for Chinese), and

$$\mathrm{eGFR}_4 = \sqrt{(86 \times \mathrm{cysC}^{-1.132}) \times [175 \times \mathrm{Pcr}^{-1.234} \times \mathrm{Age}^{-0.179} \times (\mathrm{Female} \times 0.79)]}$$

(the composite equation constituted from eGFR_1 and eGFR_3). All of the eGFRs correlated well with rGFR. Linear regressions were made using estimated GFR (eGFRs) against rGFR. eGFR_1 and eGFR_2 showed less intercepts on *y*-axis compared with those of GFR_3 and eGFR_4, and they also had higher slope that were significantly closer to the identical line compared with eGFR_3 and eGFR_4.

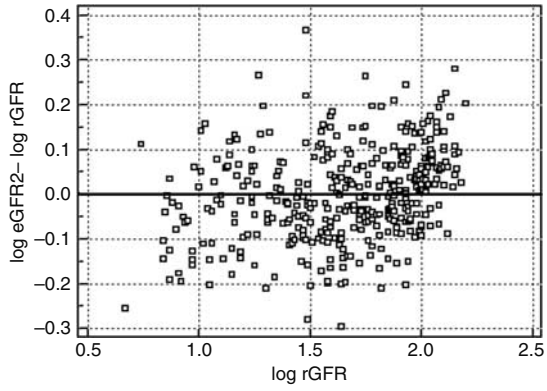

Figure 1 | Zero residual scatterplot for model 2. Abbreviations are as follows: rGFR, reference GFR; eGFR: estimated GFR; model 2: model constructed from a combination of cystatin C, Pcr, age, and gender, using the training sample data.

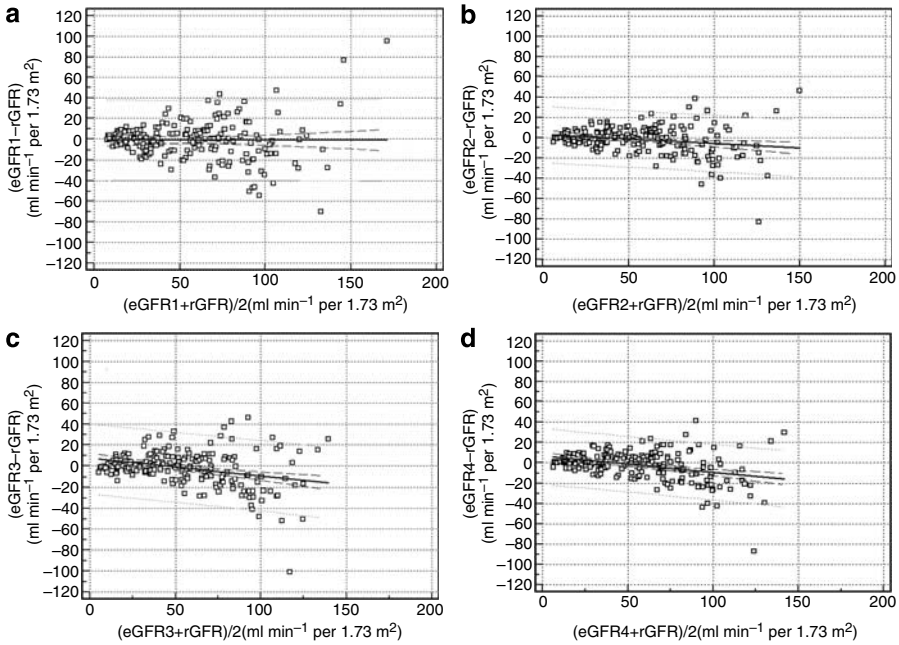

Figure 2 | Bland–Altman plots showing the disagreement between eGFR and rGFR. The solid line represents the regression line of difference between eGFR and rGFR against average of two methods, dashed lines represent 95% confidence intervals for the regression line, and dotted lines represent 95% limits of agreement. Abbreviations are as follows: rGFR, reference GFR; $eGFR_1$, GFR estimated by equation based solely on cysC; $eGFR_2$, GFR estimated by equation based on a combination of cysC and Pcr; $eGFR_3$, GFR estimated by modified MDRD equation; $eGFR_4$; GFR estimated by the composite equation constituted from modified MDRD equation and the solely cysC-based equation; units for GFR were all ml min^{-1} 1.73 m^{-2}. **a**, **b**, **c** and **d** represent for the results of $eGFR_1$, $eGFR_2$, $eGFR_3$ and $eGFR_4$ respectively.

Table 2 | Overall performances of eGFR compared with rGFR: difference, absolute difference, precision, and accuracy

	$eGFR_1$ (ml min^{-1} 1.73 m^{-2})	$eGFR_2$ (ml min^{-1} 1.73 m^{-2})	$eGFR_3$ (ml min^{-1} 1.73 m^{-2})	$eGFR_4$ (ml min^{-1} 1.73 m^{-2})
b (95% CI)	8.17 (2.93, 13.41)	7.64 (3.99, 11.31)	12.81 (8.62, 17.01)	10.12 (6.67, 13.58)
m (95% CI)	0.84 (0.77, 0.92)	0.84 (0.78, 0.89)	0.74 (0.68, 0.80)	0.78 (0.73, 0.84)
R	0.84	0.91	0.86	0.91
r^2	0.71	0.83	0.75	0.83
Median of difference (25%, 75% percentile) (ml min^{-1} 1.73 m^{-2})	0.08 (−10.53, 7.88)	−0.51 (−7.6,6.65)	−1.16 (−10.83,7.9)	−0.44 (−9.46, 5. 68)
Median of absolute difference (25, 75% percentile) (ml min^{-1} 1.73 m^{-2})	8.8 (3.7, 19.4)	6.89* (3.28,13.82)	8.51 (3.99,16.71)	6.53* (3.31, 14.66)
Bias (arbitrary units)	495	608	875	885
Precision (ml min^{-1} 1.73 m^{-2})	76.6	56	68.6	56.9
15% accuracy (%)	41.8	50.3	42.9	50.8
30% accuracy (%)	64.4	80.6	76.4	83.2
50% accuracy (%)	89	95.3	91.1	94.2

CI, confidence interval; $eGFR_1$, GFR estimated by equation based solely on cysC; $eGFR_2$, GFR estimated by equation based on a combination of cysC and Pcr; $eGFR_3$, GFR estimated by modified MDRD equation; $eGFR_4$, GFR estimated by the composite equation constituted from modified MDRD equation and the solely cysC-based equation; rGFR: reference GFR (ml min^{-1} 1.73 m^{-2}).
*$P < 0.05$ comparing with $eGFR_3$.
Units for GFR were all ml min^{-1} 1.73 m^{-2}. Linear regressions of eGFRs against rGFR were made; b represents the intersection with the *y*-axis, m is the slope with the *x*-axis, *r* is the correlated coefficient, and r^2 is the coefficient of determination.

On the Bland–Altman plot (see Figure 2), the bias of $eGFR_1$ and $eGFR_2$ were much less than those of $eGFR_3$ and $eGFR_4$ (495, 608, 875, and 885 for $eGFR_1$, $eGFR_2$, $eGFR_3$, and $eGFR_4$, respectively). Precisions of $eGFR_2$ and $eGFR_4$ were significantly higher than those of $eGFR_1$ and $eGFR_3$ (76.6, 56, 68.6, and 56.9 ml min^{-1} 1.73 m^{-2} for $eGFR_1$, $eGFR_2$, $eGFR_3$, and $eGFR_4$, respectively; Table 2).

The medians of difference between $eGFR_1$, $eGFR_2$, $eGFR_4$, and rGFR were smaller than those of $eGFR_3$ (0.08, −0.51, −1.16, and −0.44 ml min^{-1} 1.73 m^{-2} for $eGFR_1$, $eGFR_2$, $eGFR_3$, and $eGFR_4$, respectively). Also, $eGFR_2$ and $eGFR_4$ showed significantly smaller medians of absolute difference (8.8, 6.89, 8.51, and 6.53 ml min^{-1} 1.73 m^{-2} for $eGFR_1$, $eGFR_2$, $eGFR_3$, and $eGFR_4$, respectively). The 15 and 30%

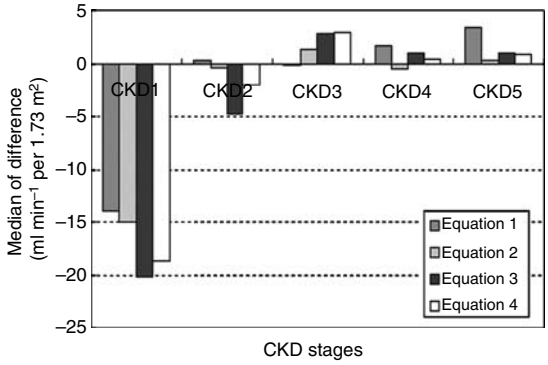

Figure 3 | Comparison of equations: difference between eGFR and rGFR. Abbreviations are as follows: CKD1 to CKD5 stood for chronic kidney disease stage 1 to stage 5, respectively; rGFR, reference GFR; $eGFR_1$, GFR estimated by equation based solely on cysC; $eGFR_2$, GFR estimated by equation based on a combination of cysC and Pcr; $eGFR_3$, GFR estimated by modified MDRD equation; $eGFR_4$; GFR estimated by the composite equation constituted from modified MDRD equation and the solely cysC-based equation; units for GFR were all $ml\,min^{-1}\,1.73\,m^{-2}$.

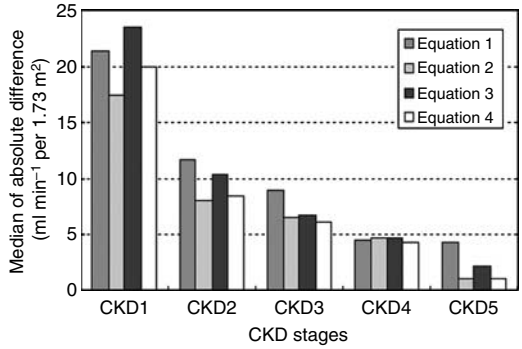

Figure 4 | Comparison of equations: absolute difference between eGFR and rGFR. Abbreviations are as follows: CKD1 to CKD5 stood for chronic kidney disease stage 1 to stage 5, respectively; rGFR, reference GFR; $eGFR_1$, GFR estimated by equation based solely on cysC; $eGFR_2$, GFR estimated by equation based on a combination of cysC and Pcr; $eGFR_3$, GFR estimated by modified MDRD equation; $eGFR_4$; GFR estimated by the composite equation constituted from modified MDRD equation and the solely cysC-based equation; units for GFR were all $ml\,min^{-1}\,1.73\,m^{-2}$.

accuracy of $eGFR_2$ and $eGFR_4$ were somewhat improved compared with that of $eGFR_3$, but without statistical significance. The 50% accuracy was comparable among the four equations. As for $eGFR_2$ and $eGFR_4$, both of them showed the similar difference, absolute difference, precision and accuracy, but $eGFR_2$ had less bias compared with $eGFR_4$ (Table 2).

The performances of $eGFR_1$, $eGFR_2$, $eGFR_3$, and $eGFR_4$ in each stage of CKD were analyzed. In CKD stages 1–2, the differences between $eGFR_1$, $eGFR_2$, and rGFR were significantly less than those of $eGFR_3$ and $eGFR_4$; In CKD stage 3, $eGFR_1$ showed the smallest difference, and the differences were comparable for $eGFR_2$, $eGFR_3$, and $eGFR_4$; In CKD stages 4–5, the $eGFR_1$ showed the largest difference, and the results were comparable for $eGFR_2$, $eGFR_3$, and $eGFR_4$. The $eGFR_2$ showed smaller absolute difference in each CKD stages. Generally, $eGFR_2$ gave the best results among four equations in each CKD stage (see Figures 3 and 4).

The misclassification of CKD stage for four equations

The stage misclassification of CKD by $eGFR_1$, $eGFR_2$, $eGFR_3$, and $eGFR_4$ was compared. In CKD stage 1, $eGFR_1$, $eGFR_2$, and $eGFR_4$ showed smaller percent of CKD stage misclassification compared with $eGFR_3$ (χ^2-test, $P<0.05$). The percentage of stage 1 patients who were misdiagnosed as stage 2 by $eGFR_3$ was 55.2%; this number significantly decreased to 42.1, 42.1, and 44.7% by $eGFR_1$, $eGFR_2$, and $eGFR_4$, respectively. In CKD stage 2, there were also smaller percent of subjects misclassified as in CKD stage 3 by $eGFR_2$ and $eGFR_4$, compared with those of $eGFR_1$ and $eGFR_3$. The detailed data of misclassification results for patients in stages 3, 4, and 5 are shown in Table 3. Generally speaking, $eGFR_2$ and $eGFR_4$ showed similar performance, that is, lower misclassification in each stage of CKD.

The final equation

For more precise GFR prediction, all 567 patients were involved; using the similar methods in model 2, the final equations based on a combination of cysC and Pcr were remodeled as follows after smearing adjustment ($R^2=0.92$, standard deviation of residual $=0.096$, $P<0.001$ for model fitting):

$$eGFR_5 = 169\times Pcr^{-0.608}\times cysC^{-0.63} \times Age^{-0.157}\,(Female\times 0.83) \quad (5)$$

The scatter plot of both $eGFR_2$ and $eGFR_5$ (estimated GFR with Equation (5)) against rGFR was also plotted, and the results showed that $eGFR_2$ and $eGFR_5$ agree with each other perfectly (figure not shown).

DISCUSSION

In patients with near-normal kidney function, both the original MDRD equations in Western CKD patients and the modified MDRD equations in Chinese CKD patients underestimated reference GFR.[9,10] The clinical applicability of Pcr-based estimate equations in this patient population has increasingly been questioned, because the underestimation might result in an unnecessary investigation and/or referral to nephrologists, excessive monitoring, and interventions.

In the last decade, dozens of paper have compared the applicability of cysC with Pcr, in GFR estimation in different stages of CKD. Perlemoine *et al.*,[23] Christensson *et al.*,[24] and Mussap *et al.*[25] claimed that plasma cysC is more sensitive in detecting early or mild diabetic nephropathy patients; similar conclusions also were obtained in patients with non-diabetic patients,[26,27] in patients with renal transplants,[28–30] and in healthy adults.[31] Moreover, after a 4-year follow-up longitudinal study, Perkins *et al.*[32] demonstrated 100/cysC could accurately detect GFR change trends in diabetes patients with normal or elevated GFR.

A recently published paper by Macdonald *et al.*[33] showed that cysC level was not independent of lean body mass. Also,

Table 3 | Percentages of CKD stage misclassification by Pcr- and cysC-based equations in CKD stages 1–5

	CKD staged by rGFR				
	CKD stage 1	CKD stage 2	CKD stage 3	CKD stage 4	CKD stage 5
Classification based on $eGFR_1$					
CKD stage 1	**57.9***	19.1	3.5	0	0
CKD stage 2	42.1	**59.6**	20.7	0	0
CKD stage 3	0	21.3	**50**	24.1	0
CKD stage 4	0	0	25.8	**62.1**	47.4
CKD stage 5	0	0	0	13.8	**52.6**
Classification based on $eGFR_2$					
CKD stage 1	**57.9***	10.6	0	0	0
CKD stage 2	42.1	**74.5**	17.1	0	0
CKD stage 3	0	14.9	**69.1**	20.7	0
CKD stage 4	0	0	13.8	**62.1**	26.3
CKD stage 5	0	0	0	17.2	**73.6***
Classification based on $eGFR_3$					
CKD stage 1	**44.8**	10.6	1.8	0	0
CKD stage 2	55.2	**68.1**	13.8	**0**	0
CKD stage 3	0	21.3	**81**	24.1	0
CKD stage 4	0	0	3.4	**62.1**	36.8
CKD stage 5	0	0	0	13.8	**63.2**
Classification based on $eGFR_4$					
CKD stage 1	**55.3***	10.6	0	0	0
CKD stage 2	44.7	**74.5**	15.6	0	0
CKD stage 3	0	14.9	**74.1**	20.7	0
CKD stage 4	0	0	10.3	**68.9**	36.8
CKD stage 5	0	0	0	10.4	**63.2**

CKD, chronic kidney disease; CKD1 to CKD5 stood for chronic kidney disease stage 1 to stage 5, respectively; cysC, cystatin C; $eGFR_1$, GFR estimated by equation based solely on cysC; $eGFR_2$, GFR estimated by equation based on a combination of cysC and Pcr; $eGFR_3$, GFR estimated by modified MDRD equation; $eGFR_4$; GFR estimated by the composite equation constituted from modified MDRD equation and the solely cysC-based equation; Pcr, plasma creatinine; rGFR, reference GFR ml min^{-1} 1.73 m^{-2}; units for GFR were all ml min^{-1} 1.73 m^{-2}.

Bouvet *et al.*[34] compared equations based on either Pcr, cysC, or a combination of them, in 100 children and young adults (age range: 1.4–22.8 years old, mean GFR level: 95 ml min^{-1} 1.73 m^{-2}); the results showed that the GFR estimation was significantly improved if the equation included both Pcr and cysC.

These results support our hypothesis that an equation that included Pcr, cysC, age, and gender might improve the performance of GFR-estimating equations based Pcr or cysC alone. Such a composite equation was also constituted from modified MDRD equation for Chinese and the solely cysC-based equation. In fact, we think that the composite equation is inconvenient to apply in clinical practice. Thus, we expected that a single equation based on a combination of cysC and Pcr would give more accurate GFR estimation. Indeed, our combined equation yielded considerable performance improvement compared with those of Pcr- or cysC-based equations, and slight superiority to the composite equation in each stage of CKD, particularly in patients with near-normal kidney function. Statistically speaking, it gave the best model fit; the R^2 was higher than the Pcr-based modified MDRD equation and the equation based solely on cysC, and it also showed the smallest standard error of residual, the smallest difference and absolute difference, and the highest accuracy.

In this study, stage misclassification was reduced by the equation based on a combination of cysC and Pcr, especially in early stages of CKD. The misclassification of CKD stage 1 into stage 2 (eGFR $<$ 90 ml min^{-1} 1.73 m^{-2}) by the combined Equation (2) was decreased from 55.2 to 42.1% compared with modified MDRD equation, and CKD stage 2 into stage 3 (eGFR $<$ 60 ml min^{-1} 1.73 m^{-2}) was also decreased from 21.3 to 14.9%. These will help clinicians to estimate GFR and make diagnosis more correctly, and assure clinicians to make proper clinical action plan for CKD patients, and avoid unnecessary clinical interventions. Because the final combined Equation (5) was based on all patients and was assumed to be more accurate than the combined Equation (2), we recommended that the combined Equation (5) be used in clinical practice.

In CKD stages 5, 4, and 3, the performance of the equation based on a combination of cysC and Pcr, was only slightly superior to the modified MDRD equation. This may be due to the fact that the non-renal clearance of cysC in humans is substantially higher in humans with moderately/severely reduced kidney function.[13] Thus, plasma cysC might be unsuitable as a GFR marker in advanced kidney failure. Since it is currently more expensive to measure cysC than Pcr (cysC is about US $8.5, while Pcr is approximately US $0.75), and the modified MDRD equation can provide

acceptable accuracy in CKD stage 5, 4, and 3 we recommended the modified MDRD equation in advanced kidney failure.

There are also some limitations of this study. First, rGFR measurement by ^{99m}Tc-DTPA plasma clearance method was still used in this study. This method can provide enough accuracy in GFR measurement, and was recommended by the Nephrourology Committee of Society of Nuclear Medicine.[35,36] It is, however, different from the renal clearance of ^{125}I-iothalamate that used in the MDRD study. Studies have shown that dual blood sampling overestimates GFR at lower levels (by an average of 0.5 ml min^{-1} when GFR = 10 ml min^{-1}) and underestimates GFR at higher levels (by an average of 20 ml min^{-1} when GFR = 100 ml min^{-1}),[37] whereas the renal clearance of ^{125}I-iothalamate overestimated renal clearance of inulin by 3–5 ml min^{-1} at low levels of GFR and by 15–25 ml min^{-1} in healthy subjects.[38–41]

Second, there are several methods to measure plasma cysC in clinical laboratories. The use of different assay systems and calibrators had been reported to probably contribute to the differences in the reference values of plasma cysC,[42] which could cause problems when cysC-based equation for GFR was used. In order to achieve maximal diagnostic performance, the difference assay systems would require slightly different prediction equations. In this study, we used an immunonephelometry assay method to measurement plasma cysC that had higher accuracy than other method in cysC measurement.[43]

Third, the equation is based on data from Chinese CKD patients, and so it is not clear if it is applicable to other racial population. Subjects with certain clinical condition were excluded from the study, such as patients with edema or on steroid. The developed equation will not be suitable for this group of patients.

In summary, we added cysC into Pcr-based, GFR-estimating equation, which achieved a better agreement with reference GFR, compared with modified MDRD equation, especially in early detection of CKD. We also suggest that a better understanding of factors that affect cysC and Pcr levels independent of GFR (such as in patients with edema, skeletal muscle atrophy, malnutrition, amputation can cause the levels of Pcr to decrease, and hyperthyroidism, malignant tumor and acute inflammatory conditions may lead to the levels of cysC to increase) could potentially improve the proper use of GFR-estimating equation in clinical practice.

MATERIALS AND METHODS

Subjects and design

This was part of the protocol of Chinese eGFR cooperate study program.[9] Nine renal institutes of university hospital located in nine different geographic regions of China participated in this study from June 2004 to September 2005. The same patient inclusion and exclusion criteria were used in all of the participating renal institutes as described previously.[9] Briefly, patients with CKD aged more than 18 years were eligible for inclusion. CKD was diagnosed and classified according to Kidney Disease Outcome Quality Initiatives clinical practice guideline.[7] Patients with acute kidney function deterioration, edema, skeletal muscle atrophy, pleural effusion or ascites, malnutrition, amputation, heart failure, ketoacidosis, hypothyroidism or hyperthyroidism, malignant tumor, and acute inflammatory conditions were excluded. Patients who were currently taking high-dose steroids, cimetidine, trimethoprim, or who were on any kind of renal replacement therapy, were also excluded. From the total 684 patients in the previous study, 573 cases with available cysC results were included in this study.

The nine participating renal institutes used the same data collecting method and the same data collection form. The collected data included sex, age, body height, body weight, blood pressure, and GFR. Fasting plasma of the selected patients were taken and stored at −30°C before analysis, and all of the blood samples were transferred a the single laboratory (Department of Laboratory, Peking University First Hospital) in freezed condition for analysis of cysC and Pcr.

This study was approved by the local Ethics Committee and written informed consent was obtained from all patients enrolled into the study.

Methods

GFR measurement. rGFR was measured using ^{99m}Tc-DTPA plasma clearance method as described previously.[9] Among the nine participating renal institutes, efforts had been made to make the inter-institute variance as small as possible, including staff training and ^{99m}Tc-DTPA drug selection (radiochemical purity greater than 95%, percentage of ^{99m}Tc-DTPA bound to plasma protein less than 5%). The identical operational procedures were followed by all the nine participating centers, including patients' preparation, intravenous injection, plasma sampling time point and procedure, and radioactivity measurement.[10]

rGFR was calculated using dual plasma sampling method,[35,36] standardized by body surface area,[44] and resulted in the rGFR equation:

$$\mathrm{rGFR\,(ml\,min^{-1}/1.73m^{-2})} = \{D\ln(P_1/P_2)/(T_2 - T_1)\}\exp\{[(T_1\ln P_2) - (T_2\ln P_1)]/(T_2 - T_1)\}\times 0.93\times 1.73/\mathrm{BSA}.$$

where D is dosage of drug injected, T_1 is time of first blood sampling ($\sim$2 h), P_1 is plasma activity at T_1, T_2 is time of second blood sampling ($\sim$4 h), and P_2 is plasma activity at T_2. The units of measurement were counts per minute, per milliliter for D, P_1, and P_2, and minutes for T_1 and T_2. BSA is the abbreviated form of body surface area.

Measurement of Pcr and plasma cysC. Pcr and plasma cysC measurement was performed in a single laboratory (Department of Laboratory, Peking University First Hospital); Pcr was measured by the Jaffe's kinetic method, described elsewhere,[10] on a Hitachi 7600 autoanalyzer (Hitachi Company, Chiyoda-ku, Tokyo, Japan); normal reference range was 0.72 to 1.48 mg dl^{-1} (64–131 μmol l^{-1}); plasma cysC was measured by automated particle-enhanced immunonephelometry method using a BN 100 nephelometer (Dada Behring, Marburg, Germany), the coefficients of variation were 2.1% at cysC concentration of 1.0 mg l^{-1} and 1.8% at cysC concentration of 4.0 mg l^{-1} during the study period; normal reference range was 0.50 to 1.17 mg l^{-1}.

Estimation of GFR with modified abbreviated MDRD equation for Chinese, and a composite equation constituted from modified MDRD equation and the solely cysC-based equation. Hitachi Pcr was put into modified abbreviated MDRD equation to estimate GFR

($eGFR_3$) ($R^2 = 0.86$, standard deviation of residual = 0.136, $P < 0.001$ for model fitting)[9]:

$$eGFR_3 = 175 \times Pcr^{-1.234} \times age^{-0.179} (female \times 0.79) \quad (3)$$

Estimate GFR using the composite equation was as follow:

$$eGFR_4 = \sqrt{(87 \times cysC^{-1.132}) \times [175 \times Pcr^{-1.234} \times Age^{-0.179} \times (Female \times 0.79)]} \quad (4)$$

New equation development methods and statistical analysis. First, from the selected 573 patients, six individuals were excluded (they were excluded because the values of cysC, Pcr, or rGFR were considered measurement error). The remaining 567 cases were used for further analysis. From these cases, 376 cases were randomly selected to be included in the regression model, the remaining 191 cases were used as validation set. We used an approach similar to that used to develop the MDRD equations, that is, the logarithmic-transformed rGFR was regressed on logarithmic-transformed plasma cysC, age, and sex in model 1, and logarithmic-transformed Pcr was added to model 1 to expand the model to model 2. Multiple stepwise regression was used; *P*-value less than 0.05 was used as inclusion criteria and a *P*-value greater than 0.10 was used as exclusion criteria. In the concern that retransforming back to the usual scale might induce bias, the predicted eGFR was adjusted using smearing method.[45] The smearing coefficients for these two models were calculated to be 1.04 and 1.02, respectively. The model fit (R^2) and standard deviation of residual among different predictive models were compared.

Second, eGFRs estimated using the equation based solely on cysC, the equation based on a combination of cysC and Pcr, the modified MDRD equation, and the composite equation were compared with rGFR using Bland–Altman analysis in the validation set. The difference between eGFR and rGFR was defined as eGFR minus rGFR, the absolute difference between eGFR and rGFR was defined as the absolute value of difference. The difference between eGFR and rGFR was regressed against the average of the two methods. The bias for eGFR was expressed as the area between the regression line and a common distance along the zero-difference line. Ninety-five percent limits of agreement were constructed around this regression line. The precision was expressed as the width between the 95% limits of agreement. Accuracy was measured as the percentage of estimated GFR not deviating more than 15, 30, and 50% from the rGFR. The accuracy of the estimating equation in different stages of CKD was compared with χ^2-test.

Quantitative variables were described as mean ± standard deviation or median. Because of skewed distribution, Spearman correlation and linear regression were used to describe the relationship between eGFRs and rGFR. The Wilcoxon signed ranks test was used to compare the difference and absolute difference for a certain stage of CKD. The results were considered to be significant if the *P*-value was less than 0.05. Microsoft Office Excel 2003 (Microsoft Corporation) and Medcalc for Windows, version 8.0 (Medcalc software, Mariekerke, Belgium) were used in statistical analysis.

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