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ORIGINAL ARTICLE

How to estimate kidney function in kidney transplant recipients with mild to moderate kidney impairment: comparison of estimated glomerular filtration (eGFR) values between creatinine-based GFR equations and cystatin C-based GFR equations for Japanese population

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Abstract
Background With the recent increase in renal transplantations in Japan, accurate assessment of renal function is required.
Methods This study included 73 patients who had undergone renal transplantation at Nagoya Daini Red Cross Hospital at least 6 months previously and had stable renal function for >3 months. Glomerular filtration rates (GFRs) were measured by inulin clearance (mGFR) and compared with estimated cystatin C-based GFRs (eGFRcys), estimated creatinine-based GFRs (eGFRcre) and their average values (eGFRave).
Results mGFR was 43.3 ± 14.1 mL/min/1.73 m^2, eGFRcre was 39.6 ± 11.7, eGFRcys was 56.0 ± 17.1, and eGFRave was 47.8 ± 13.7 mL/min/1.73 m^2. Serum cystatin C was 1.39 ± 0.37 mg/L and serum creatinine was 1.58 ± 0.51 mg/dL. The correlation coefficients between mGFR and eGFRcre, eGFRcys, and eGFRave were 0.768, 0.831, and 0.841, respectively ($P < 0.001$, for all).The intraclass correlation coefficients were 0.754, 0.816, and 0.840, respectively ($P < 0.001$, for all).The mean differences between measured and estimated GFR values were 3.74 mL/min/1.73 m^2 with a root-mean square error (RMSE) of 9.06 for eGFRcre, +12.64 with RMSE of 9.48 for eGFRcys, and +4.45 with RMSE of 7.86 for eGFRave. Bland–Altman plots showed that eGFRcys overestimated GFR values compared with mGFR values in most cases and that eGFRave overestimated GFR values in 53 of 73 cases, whereas eGFRcre underestimated the values in 53 of 73 cases.
Conclusion eGFRave may be the best marker to estimate kidney function in Japanese renal transplant recipients with mildly reduced or normal kidney function.

Keywords Kidney transplant recipients · Inulin clearance · GFR · Creatinine · Cystatin C

Abbreviations

GFR	Glomerular filtration rate
eGFRcre	Estimated creatinine-based GFR
eGFRcys	Estimated cystatin C-based GFR
eGFRave	Averaged value of eGFRcre and eGFRcys
mGFR	Measured GFR
Cys-C	Cystatin-C
SD	Standard deviation
ICC	Intraclass correlation coefficients
RMSE	Root-mean square error
Cin	Inulin clearance
CKD	Chronic kidney disease

Introduction

Renal transplantations are increasing in Japan because graft survival has been longer due to the improvement of

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immunosuppressive agents. Thus, renal transplantation has become a more common form of renal replacement therapy.

Creatinine (Cre) is the most common clinical marker of kidney function, but it is often affected by age, gender, weight and muscle mass. In contrast, cystatin C (Cys C) which is a low-molecular-weight protein and cysteine protease inhibitor, is produced by nucleated cells at a constant production rate, freely filtered without tubular secretion or reabsorption and catabolized in the proximal tubule. In a previous meta-analysis, Cys C was evaluated in patients with various renal diseases and determined as a superior renal marker to Cre [1]. Furthermore Cys C-based formulas may provide better diagnostic performance than Cre-based equations for glomerular filtration rate (GFR) calculations after renal transplantation [2].

The Japanese Society of Nephrology recently developed equations for estimated Cre-based GFR (eGFRcre) and Cys C-based GFR (eGFRcys) [3, 4]. However, it is not known whether these equations are applicable to Japanese renal transplant recipients. Previous studies have indicated that Cys C is a more reliable GFR marker than Cre for assessing kidney function after renal transplantation in populations other than Japanese [1]. We performed measurement of inulin clearance, the gold standard method, to compute measured GFR (mGFR) values and compared mGFR with eGFRcre, eGFRcys and averaged values of eGFRcre and eGFRcys (eGFRave) to determine the best method for assessing kidney function in renal transplant recipients.

Materials and methods

Study design and subjects

The study design was cross-sectional at a single center. Inclusion criteria were (1) age ≥20 years, (2) kidney

Table 1 Characteristics of the patients

Sex (male/female)	54/19
Age (years)	49.1 ± 12.8
Height (cm)	164 ± 8
Body weight (kg)	60.7 ± 14.1
Serum creatinine (mg/dL)	1.58 ± 0.51
Serum cystatin C (mg/L)	1.45 ± 0.38
eGFRcre (mL/min/1.73 m^2)	39.6 ± 11.7
eGFRcys (mL/min/1.73 m^2)	53.3 ± 16.4
eGFRave (mL/min/1.73 m^2)	47.8 ± 13.7
Measured GFR (mL/min/1.73 m^2)	43.3 ± 14.1

Data were expressed as means ± standard deviation or numbers

GFR glomerular filtration rate, *eGFRcre* estimated creatinine-based GFR, *eGFRcys* estimated cystatin C-based GFR, *eGFRave* average of eGFRcre and eGFRcys

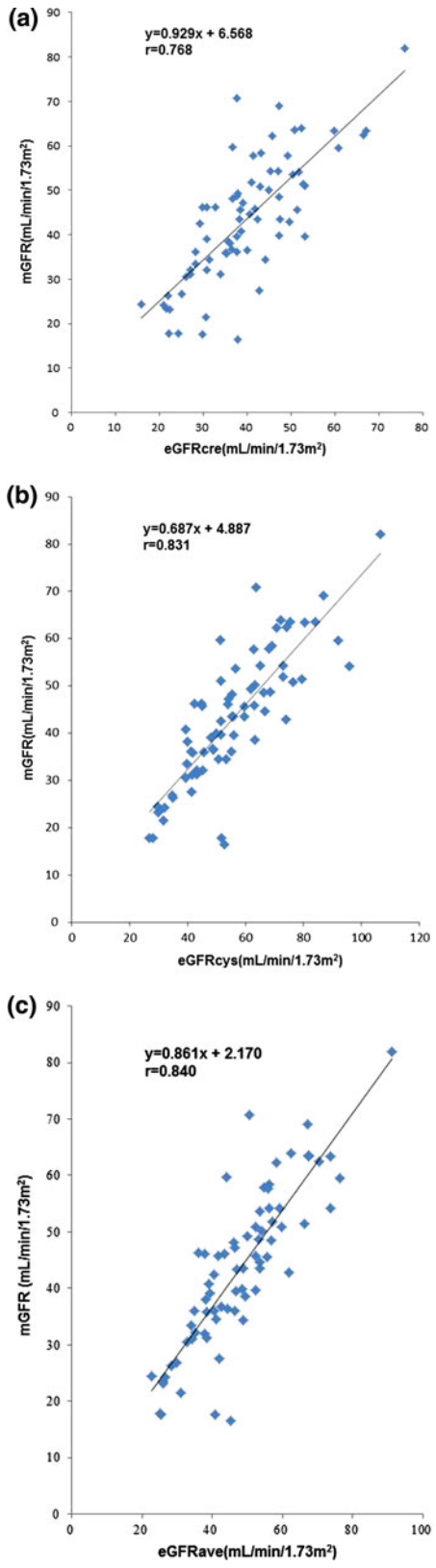

Fig. 1 Relationship between mGFR and other GFR measurement methods. **a** Relationship between eGFRcre and mGFR, **b** relationship between eGFRcys and mGFR, **c** relationship between eGFRave and mGFR

function relatively stable as assessed by using serum Cre values for >3 months, (3) renal transplantation >6 months previously, and (4) written informed consent from the patient to participate in this study. Exclusion criteria were (1) acute kidney injury, (2) acute rejection within 3 months, (3) inulin allergy, and (4) abnormal bladder function. All patients took calcineurin inhibitors (cyclosporine or tacrolimus), anti-metabolite agents (mycophenolic acid or mizoribine), and prednisolone (5 mg/day) according to our protocol. Cyclosporine and tacrolimus concentrations were adjusted to an area under the curve [AUC] (0–4 h) of 2000 ng/mL for cyclosporine and an AUC (0–4 h) of 50 ng/dL for tacrolimus. All patients gave written informed consent to participate in this study and the study was approved by the Ethics Committee of Nagoya Daini Red Cross Hospital, Nagoya, Japan.

Inulin clearance

Inulin clearance (Cin) was measured in 73 renal transplant recipients. Cin was calculated from serum and urine concentration and urine flow rate. Each patient received 500 ml of water orally 30 min before the infusion. Inulin (1 %) was administered by means of a continuous intravenous infusion for 2 h (300 mL/h for the first 30 min and 100 mL/h for the remaining 90 min) under overnight fasting, but hydrated conditions. During the inulin infusion, serum samples were collected 4 times at 0 (blank), 45, 75, and 105 min for inulin, and urine samples were collected between 30 and 60, 60 and 90, and 90 and 120 min for inulin after completely emptying the bladder at 30 min from the start of the inulin infusion. The patients were hydrated with 60 mL of water at 30, 60, and 90 min after the start of the inulin infusion to maintain urine flow. Inulin samples were assayed by means of an enzymatic method using a kit (Diacolor Inulin; Toyobo Co., Osaka, Japan). The mean value of 3 measurements was used to determine Cin [3].

Serum Cre and Cys C

Serum Cre was measured by the IDMS-traceable enzymatic method in our hospital. Cys C was measured using a kit from Alfresa Pharma Company in a single laboratory and multiplied by 0.96 for standardization [5].

eGFRcre, eGFRcys, and eGFRave

eGFRcre was calculated using the equation issued by the Japanese Society of Nephrology [3]. The formula is $194 \times (\text{age})^{-0.287} \times (\text{serum Cre})^{-1.094}$, including a correction factor of 0.739 for women. eGFRcys was calculated using the equation issued by the Japanese Society of Nephrology [4]. The formula for men is $(104 \times \text{serum Cys-C}^{-1.019} \times 0.996^{\text{age}}) - 8$ and the formula for women is $(104 \times 0.929 \times \text{serum Cys-C}^{-1.019} \times 0.996^{\text{age}}) - 8$ eGFRave was calculated as the average value of eGFRcre and eGFRcys.

Statistical analyses

Data are expressed as mean ± standard deviation (SD) or numbers. The correlation, consistency between mGFR and estimated GFRs were analyzed by intraclass correlation coefficients (ICCs) and Pearson's correlation coefficients, and differences between mGFR and errors were investigated by Bland–Altman plotting method. Two-sided P values <0.05 were considered statistically significant. All statistical analyses were performed using PASW Statistics ver. 18.

Results

Table 1 shows the characteristics of the 73 patients enrolled in this study. Bladder function was evaluated before renal transplantation, and was almost normal.

Figure 1 shows the relationships between mGFR and eGFRcre, eGFRcys, and eGFRave. There were highly significant correlations between mGFR and each of the other measurements ($P < 0.001$, for each). ICCs are described in Table 2. ICCs demonstrated agreement of two values by two different measurement methods. The mean differences with a root-mean square error (RMSE) from mGFR are also described in Table 2. Bland–Altman plots

Table 2 Relationship between measured GFR and estimated GFR

Estimated GFR	Correlation parameters					Mean differences	Accuracy with 30 %
	ICC	95 %CI	P values	Pearson's r	P values		
eGFRcre	0.754	0.635–0.838	<0.001	0.768	<0.001	−3.74	82.2
eGFRcys	0.816	0.722–0.881	<0.001	0.831	<0.001	+12.64	52.1
eGFRave	0.840	0.757–0.897	<0.001	0.841	<0.001	+4.45	86.3

GFR glomerular filtration rate, *ICC* intraclass correlation coefficient, *eGFRcre* estimated creatinine-based GFR, *eGFRcys* estimated cystatin C-based GFR, *eGFRave* average of eGFRcre and eGFRcys

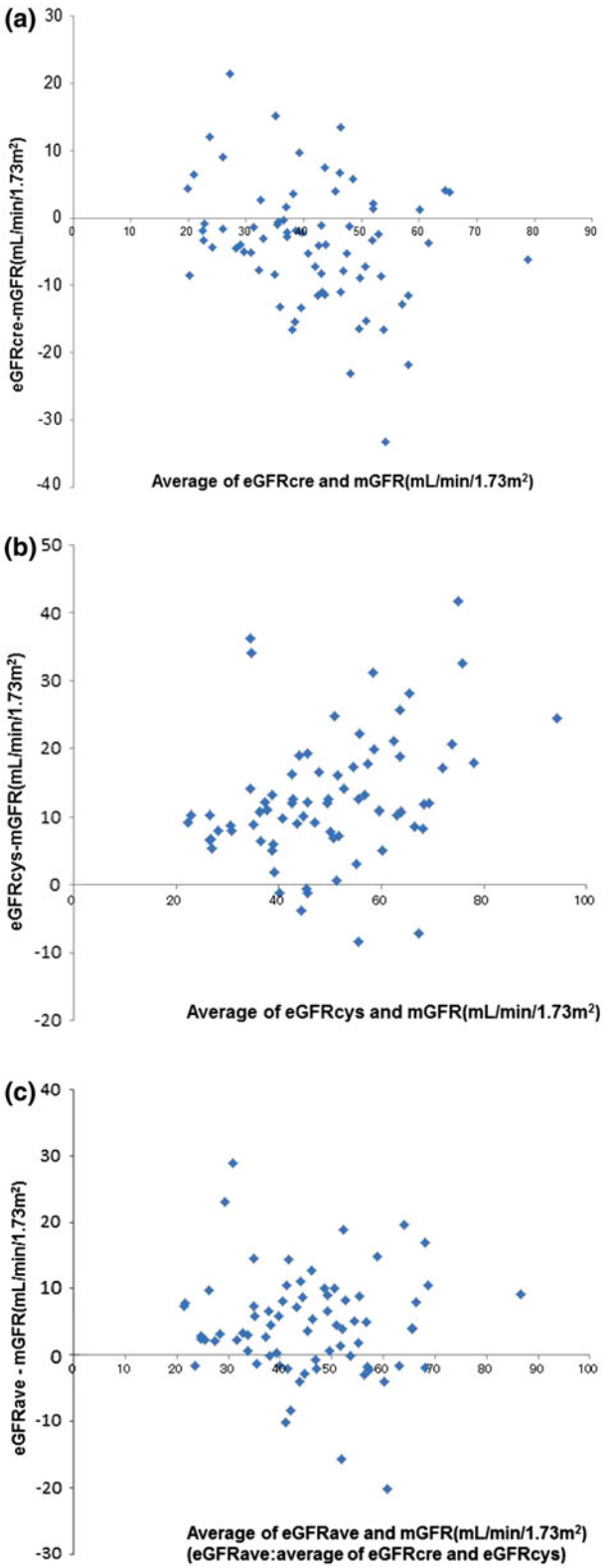

Fig. 2 Bland–Altman plots of mGFR and other GFR measurement methods. **a** Relationship between eGFRcre and mGFR. This showed that eGFRcre tended to underestimate mGFR. **b** Relationship between eGFRcys and mGFR. This showed that, in most cases, eGFRcys overestimated mGFR. **c** Relationship between eGFRave and mGFR. This showed that eGFRave had the best relationship with mGFR

showed that eGFRcys overestimated GFR in most cases and eGFRave overestimated GFR in 53 of 73 cases. In contrast, eGFRcre underestimated GFR in 53 of 73 cases (Fig. 2).

Discussion

Many kidney transplant recipients are in chronic kidney disease (CKD) stage 3–4 T and, as such, accurate measurement for GFR is critical for the examination of new therapies and care of renal recipients. However, the study of assessing their kidney function has not been conducted in Japan.

Because various factors influence measured Cre values, Cys C is superior to Cre as a marker of kidney function and is considered to be effective in kidney transplant recipients. Poge et al. [2] showed that Cys C-based equations may provide better diagnostic performance than Cre-based equations in 118 patients after renal transplantation and White et al. [6] showed that Cys C-based methods were superior to Cre-based methods in a study of 119 post kidney transplant recipients. In contrast, Zahran et al. [7] reported that the Cre-based GFR equation was not inferior to the eGFRcys equation when estimating GFR in 103 renal transplant patients. Although caution is required because glucocorticoid medication in adult renal transplant patients is associated with increased Cys C in a dose-dependent manner, Risch et al. [8] said that this did not preclude the use of Cys C for detecting impaired renal function in renal transplant patients, since Cys C was more accurate than Cre and 24-h Cre clearance.

The Japanese Society of Nephrology announced the use of the Cre-based equation and the Cys C-based equation for GFR in Japanese patients. We often use eGFRcre clinically to estimate renal function, although it has been reported that eGFRcre underestimates GFR in healthy populations [3]. Kakuta eet al. [9] also reported that in living potential kidney donors, mean mGFR was 96.1 ± 14.7 mL/min/1.73 m^2 and mean eGFRcre was 72.6 ± 12.7 mL/min/1.73 m^2; thus, eGFRcre underestimated mean mGFR. Yazawa et al. [10] showed that in patients with mild kidney impairment after kidney donation, mean mGFR was 55.2 ± 10.3 mL/min/1.73 m^2 and mean eGFRcre was 49.4 mL/min/1.73 m^2. Although they did not compare mGFR with eGFRcre statistically, eGFRcre tended to underestimate mGFR. Our result also shows that eGFRcre tended to underestimate GFR. The subjects were mainly young people, and they would have higher muscle mass which would affect this result. Use of eGFRcys was proposed by Horio et al. [11] in 2012; however, they said it was possible that Cys C was not accurate in patients with very low GFR. In our study, there were no patients with

GFR <15 mL/min/1.73 m^2 by Cin. With regard to the combined Cre−Cys C equation, it was reported that this performed better than equations based on either of these markers alone and may be useful as a confirmatory test for CKD [12]. Horio et al. [13] also reported that the average of the Cre−Cys C equation was useful to estimate GFR in the Japanese population. There have been no previous studies comparing these two equations in Japanese kidney transplant recipients. The patients in the present study had mild to moderate renal dysfunction after kidney transplantation, and there were no patients with GFR <15 mL/min/1.73 m^2. Similar to studies of CKD patients, eGFRcre in our study was correlated with mGFR, but underestimated GFR in patients with better renal function. In contrast, eGFRcys overestimated GFR in most patients. The reasons for the difference remain unclear; however, the difference in the Cys C measurement method or low-dose prednisolone may have affected the results. Cys C was overestimated and eGFRcys usually underestimated GFR in patients receiving prednisolone. eGFRave showed the least bias and the highest accuracy in our study. We expected eGFRcys to be effective; however, the result showed that it could not be used independently. In clinical practice, we measure Cys C in various ways but we have to revise it when using the Cys C-based equation. We conclude that eGFRave is a more reliable equation than either eGFRcre or eGFRcys for Japanese transplant recipients.

Study limitations

There were several limitations in this study. The sample size of this study was small and confined to a single center and did not include subjects with severe renal impairment or children. Cys C was measured using the Alfresa Pharma Company kit, and this could have affected the results. Finally, all patients were receiving immunosuppressive drugs such as prednisolone which influence Cys C.

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Conflict of interest We declare no financial conflicts.

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