Research Paper

Measurement of glomerular filtration rate using endogenous d-serine clearance in living kidney transplant donors and recipients

Masataka Kawamura, Atsushi Hesaka, Ayumu Taniguchi, Shigeaki Nakazawa, Toyofumi Abe, Makoto Hirata, Ruyi Sakate, Masaru Horio, Shiro Takahara, Norio Nonomura, Yoshitaka Isaka, Ryoichi Imamura, Tomonori Kimura, *

Department of Urology, Osaka University Graduate School of Medicine, Suita, Japan

Department of Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan

Reverse Translational Project, Center for Rare Disease Research, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka, Japan

Department of Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan

Laboratory of Rare Disease Resource library, Center for Rare Disease Research, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN)

Department of Nephrology, Kansai Medical Hospital, Osaka, Japan

KAGAMI Project, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka, Japan

Department of Renal Transplantation, Kansai Medical Hospital, Osaka, Japan

Abstract

Background: Endogenous molecules that provide an unbiased and a precise evaluation of kidney function are still necessary. We explored the potential of clearance of o-serine, a rare enantiomer of serine and a biomarker of kidney function, as a measure of glomerular filtration rate (GFR).

Methods: This was a cross-sectional observational study of 200 living kidney transplant donors and recipients enrolled between July 2019 and December 2020 in a single Japanese center, for whom GFR was measured by clearance of inulin (C-in). Clearance of o-serine (C-oSer) was calculated based on blood and urine levels of o-serine, as measured by two-dimensional high-performance liquid chromatography. Analytical performance was assessed by calculating biases. Utilizing data from 129 participants, we developed equations for C-in based on C-oSer and C-cre using a linear regression model, and the performance was validated in 68 participants.

Findings: The means of C-in and C-oSer were 66.7 and 55.7 mL/min/1.73 m² of body surface area, respectively, in the entire cohort. C-oSer underestimated C-in with a proportional bias of 22.0% (95% confidence interval, 14.2–29.8%) and a constant bias of -1.24 (-5.78–2.30), whereas the proportional bias was minor to that of C-cre (34.6% [31.1–38.2%] and 2.47 (-1.18–6.13) for proportional and constant bias, respectively). Combination of C-oSer and C-cre measured C-in with an equation of 0.391/C-cre + 0.418/C-oSer + 0.418/C-cre + 3.852, which reduced the proportional bias (6.5% [-0.2–13.1%] and -4.30 [-8.87–0.28] for proportional and constant bias, respectively). In the validation dataset, this equation performed well with median absolute residual of 3.5 [2.3–4.8] and high ratio of agreement (ratios of 30% and 15% different from C-in [P30 and P15] of 98.5 [91.4–100] and 89.7 [80.0–95.2], respectively).

Interpretation: The smaller proportional bias compared to that of C-cre is an advantage of C-oSer as a measure of GFR with precision and minor biases and can support important clinical decisions.

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1. Introduction

Evaluation of kidney function is essential in daily clinical practice. The measured glomerular filtration rate (mGFR) using exogenous inulin has been considered as the gold standard of kidney function [1], though...
Acids are mirror-image enantiomers of serine, and L-amino acids have been shown to exist in the body. Among D-amino acids, D-serine represented kidney function and disease activity. With the development of measurement techniques and equipment, D-serine has been regarded as exclusively present in the human body until recently. The clearance of D-serine (C-DSer) agreed well with C-in with its minor proportional bias against C-in to that with C-cre. The combination of C-DSer and C-cre measured C-in with an equation of 0.391 × C-DSer + 0.418 × C-cre + 3.852. This endogenous factor-based equation can measure the GFR with a high performance.

Evidence before this study

Current evaluation of kidney function has several limitations: a labor-intensive procedure for clearance of inulin (C-in), a major proportional bias for clearance of creatinine (C-cre), and imprecise estimation of estimated GFR (eGFR). Endogenous molecules that potentiate the precise assessment of kidney function with small biases are still necessary for important clinical decisions, including drug administration design, transplant donor selection, and chronic kidney disease (CKD) stage classification. The blood level of D-serine, a rare enantiomer of serine, reflects the prognosis of the kidney and correlates well with C-in.

Added value of this study

The smaller proportional bias compared to that of C-cre is an advantage of C-DSer as a measure of C-in. Combinational assessment by the clearance of two endogenous molecules, D-serine and creatinine, is likely to provide precise and unbiased measures for GFR by endogenous molecules, and may support important clinical decisions, such as adjustment of drug dosages, diagnosis of CKD, and suitability as a living donor for kidney transplantation.

This procedure is labor intensive, time consuming, and expensive [2]. Therefore, estimation of GFR using endogenous molecules has widely been used. Clearance of creatinine (C-cre) correlates strongly with GFR, while this method has a major proportional bias and overestimates GFR due to kidney tubular secretion of creatinine into urine. The estimated GFR (eGFR), calculated using a combination of age, sex, race, and serum levels of creatinine and/or cystatin C [3,4], is convenient and useful for screening chronic kidney disease (CKD). Most equations for eGFR show relatively small bias; however, their precisions are not good enough to provide a correct estimation for the individual patient [5]. Endogenous molecules that potentiate the precise assessment of kidney function with small biases is still necessary for important clinical decisions, including drug administration design, transplant donor selection, and CKD stage classification [6,7].

\[ \frac{V}{S_x} = 0.886 \times C_{DSer} + 0.047 \times C_{cre} + 1.34 \]

D-serine is a candidate biomarker of kidney function. L- and D-amino acids are mirror-image enantiomers of serine, and L-amino acids have been regarded as exclusively present in the human body until recently. With the development of measurement techniques and equipment [8,9], D-amino acids have been shown to exist in the body. Among D-amino acids, D-serine represented kidney function and disease activity. A higher level of D-serine in blood is associated with earlier progression to end-stage kidney diseases necessitating kidney replacement therapy [10]. Additionally, the blood level of D-serine correlates well with the measured GFR (mGFR) [11]. These studies suggest the possibility of using D-serine for the estimation of GFR.

The clearance of D-serine in the body, D-serine is synthesized in the brain through chiral conversion from L-serine by serine racemase [12,13] or uptaken from food. D-Serine circulates the bloodstream in the body and is delivered to the kidney. D-Serine is then filtered through the glomerulus and excreted in the urine [14]. When kidney function is impaired, the urinary excretion of D-serine decreases, and its blood level increases [11,14,15]. Since the kidney precisely regulates blood level of D-serine, we hypothesized that the kidney function could be measured by the clearance of D-serine (C-DSer).

In this cross-sectional observational study of a prospective cohort, we explored the potential agreement of C-DSer with GFR. We also developed a method to measure GFR using the combination of C-DSer and C-cre. This endogenous factor-based method can provide a more convenient method to measure GFR.

2. Methods

2.1. Study design and participants

This is a cross-sectional observational study of a prospective cohort. Participants in this prospective cohort study were adults aged 20 or older who were potential living kidney donors, post kidney donors, and kidney transplant recipients. We recruited 200 participants from three centers in Japan, and blood and urine samples were collected at Kansai Medical Hospital between July 2019 and December 2020. Analytical performance of C-DSer as a measure of GFR was assessed by calculating biases. Exclusion criteria was cases with urine output of less than 20 mL per 30 minutes. This study was conducted in compliance with the Declaration of Helsinki, the Ethical Guidelines for Medical Research Involving Human Subjects, and the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism". Approval for all facilities was obtained from the Central Ethics Review Committee of Osaka University (#16330). Written informed consent for this study was obtained from all participants. This study adhered to the STROBE guidelines.

2.2. Clearance of inulin, creatinine and d-serine, and equations for GFR estimation

Clearance of inulin (C-in) was calculated from serum and urine inulin concentrations and urine volume using standardized methods described previously [16]. Inulin (1%; Fuji Yakuin, Saitama, Japan) was administered intravenously using an infusion pump under fasting, medication-suspended, and hydrated conditions. The infusion rate was 300 mL/h for 0–30 min and 100 mL/h for 30–120 min. To maintain urine output during clearance measurements, participants were given 500 mL of water 30 min before the start of inulin administration and 60 mL of water at each urine collection time point. Participants urinated completely 30 minutes after the start of administration and urine was collected every 30 minutes (30, 60, 90, and 120 minutes after the start of administration). Clearance was calculated as follows: \[ \text{U}_x \times \frac{1}{C_{DSer}} \times \frac{1}{C_{cre}} \times V \]

where \( x \) is a substrate such as inulin, creatinine, or D-serine, \( U_x \) is the urinary concentration of \( x \), \( V \) is the flow rate, and \( S_x \) is the serum concentration of \( x \). Each clearance was corrected for body surface area using the formula of Dubois and Dubois [17]. The mean clearance values at each time point (30–60, 60–90, 90–120) were used for analysis. Blood was collected in the middle of each urine collection time (45, 75, and 105 minutes after dosing). Serum and urine inulin concentrations were colorimetrically determined using Diacol inulin kit (Toyobo, Osaka, Japan). The measurement is continuously calibrated using reference control material. Creatinine in serum and urine was measured enzymatically (Determin L CRE, Hitachi Chemical, Tokyo, Japan), and serum cystatin C (sCys) was measured using an immunological turbid metric assay (Nescoat GC Cystatin C, Alfresa Pharma, Osaka, Japan). The serum creatinine and cystatin C assays were continuously calibrated using human pooled serum (L-Consera EX, Nissui Pharmaceutical, Tokyo, Japan) and cystatin C standard (Nescoat Cystatin C Standard, Alfresa Pharma), respectively. The measurements were surveyed by the Japanese Association of Medical Technologist's quality control survey project and data standardization project system.
2.3. d-Serine quantification

Blood and urine samples to measure d-serine were collected at the same time as inulin clearance measurements. The preparation of samples and quantification of serine enantiomers by a two-dimensional high-performance liquid chromatography (2D-HPLC) system were performed as previously described [8,9]. Briefly, 20-fold volumes of methanol were added to the sample and an aliquot (10 μL of the supernatant obtained from the methanol homogenate) was placed in a brown tube. After drying the solution under reduced pressure, 20 μL of 200 mM sodium borate buffer (pH 8.0) and 5 μL of fluorescence labeling reagent (40 mM 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) in anhydrous MeCN) were added, and then heated at 60°C for 2 min. An aqueous 0.1% (v/v) TFA solution (75 μL) was added, and 2 μL of the reaction mixture was subjected to the 2D-HPLC.

The enantiomers of serine were quantified using the 2D-HPLC platform. The NBD-derivatives of the amino acids were separated using a reversed-phase column (Singularity RP column, 1.0 mm i.d. × 50 mm; provided by KAGAMI Inc., Osaka, Japan) with the gradient elution using aqueous mobile phases containing MeCN and formic acid. To separately determine d- and l-serine, the fractions of serine were automatically collected using a multi-loop valve, and transferred to the enantioselective column (Singularity CSP-001S, 1.5 mm i.d. × 75 mm; KAGAMI Inc.). Then, d- and l-serine were separated in the second dimension by the enantioselective column. The mobile phases are the mixed solution of MeOH-MeCN containing formic acid, and the fluorescence detection of the NBD-amino acids was carried out at 530 nm with excitation at 470 nm using two photomultiplier tubes.

Target peaks were quantified by scaling the standard peak shape [20]. In this method, the shape of a peak was used for identification of the substrate, whereas the magnitude of the intensity was used for quantification. Prior to the quantification, peak sections of various concentrations of standard serine enantiomers (d-serine, 199-08822; l-serine, 634-25661; Fujifilm Wako Chemical Corporation, Osaka, Japan) were obtained as standard shapes for calibration. The obtained data points were multiplied by a constant so that the peak shape was the same as that of the reference peak generated based on 1 pmol injection of the serine enantiomers. Using the obtained constant, the calibration lines were made against the amounts of the injected amino acid. From the chromatogram of a sample, target shapes of serine enantiomers were identified based on the elution time and the shape of the peak. Four kinds of peak sections were assigned for each enantiomer, and the peak section, where the interference from intrinsic substances was not severe, was selected for quantification. The peak shape obtained by the standard amino acid enantiomer was superimposed to the obtained peak sections, and the magnification constant best fitted to the target peak was identified. The concentration of the target enantiomer was calculated by using the identified magnification constant and the calibration lines. The peak shape method potentiated quantification within a few seconds. The fully automatic 2D-HPLC system required less than 10 minutes for the measurements of d-serine, including separation, identification, and quantification steps.

2.4. Training and validation of equations

We divided entire cohort 2:1 into training and validation dataset. In the training dataset, the least squares regression line for d-serine and creatinine clearance against inulin clearance were calculated by simple regression analysis, and the coefficient for the combined equation was calculated by multiple regression analysis. We compared the usefulness of those equations and eGFR equations in the validation dataset. The coefficients of determination (R²), bias, root-mean-square error (RMSE), and accuracy were calculated as a metrics for comparison. Bias of the equation was expressed as the median of the absolute value of the difference between calculated GFR and C-in. Precision was assessed as the interquartile range (IQR) for the true difference between calculated GFR and C-in. RMSE for C-in calculated using the equation was the square root of (sum of squared differences / n). Accuracy was expressed as the percentage of participants whose calculated GFR was within less than 30, 15, or 7.5% of C-in (P30, P15, and P7.5). 95% confidence intervals were calculated using bootstrap resampling with a normal distribution approximation.

2.5. Statistical analysis

Data were expressed as mean ± standard deviation, or as count and ratio (%). The normal distribution of each variable was confirmed using q plots, and Pearson’s correlation coefficient was calculated. Comparison between methods was performed using Deming regression through the evaluation of slope and intercept of the regression line. Agreement between methods was visualized using Bland-Altman plots. Bias of each equation against C-in was compared using signed-rank test. Accuracy between equations for C-in was compared using McNemar’s test. Sample size analysis was performed based on the assumption that the combination of C-dSer and C-cre agrees with GFR better than C-cre alone. Suppose that the bias of C-cre against C-in was 5, which was reduced to 80% by the combination with standard deviation for the difference was 50%, the number needed for the test based on error probability of 0.05 and power of 0.85 was 34. Since we intended to divide participants into 2:1 for training and validation set and set 20% as a margin, the total number for participants required was 163. Subgroup analyses were performed for sex, transplantation-related status, age, and GFR. Statistical significance was defined as p < 0.05. JMP® pro 15.0, GraphPad Prism 5.0, and STATA 15.0 were used for statistical analyses and data visualization.

2.6. Role of funding source

This study was funded by Japan Society for the Promotion of Science (JSPS, grant number 17H04188), Japan Agency of Medical Research and Development (AMED, JP20gm5010001), Osaka Kidney Bank (OKF19-0010), Shiseido Co., Ltd and KAGAMI Inc. The funders had no role in study design, data collection, analysis, interpretation, or writing of the report.

3. Results

The background demographics of the participants are shown in Table 1 and Supplementary Table 2. The total number of eligible participants was 197, after having excluded 3 participants with low urine output. These participants consisted of 125 potential living kidney donors, 27 post-donors, and 45 transplant recipients. In the overall cohort, the mean values of serum creatinine, cystatin C, and plasma d-serine were 0.86 mg/dL, 1.1 mg/L, and 2.3 μM, respectively. The mean clearances of inulin, creatinine, and d-serine were 66.7, 98.2, and 55.7 mL per minutes per 1.73 m² of body surface area, respectively. The levels and clearances of d-serine in plasma and serum were almost identical (Supplementary Figure S1), and we used plasma level of d-serine in the analysis.
In order to examine whether C-DSer is reliable as a measure of C-in, we first analyzed the analytical performance (Figure 1). In the total cohort population, C-DSer was significantly and strongly correlated with C-in (R = 0.91, \( P < 0.0001 \)) like C-cre (R = 0.93). C-DSer underestimated C-in with a proportional bias of 22.0% (95% Confidence interval, 14.2/\( C0 \) 29.8%) and a constant bias of -1.24 (-5.78/\( C0 \) 3.31), whereas this proportional bias was minor to that of C-cre (34.6% [-31.1/\( C0 \) 38.2%] and 2.47 (-1.18/\( C0 \) 6.13) for a proportional and a constant bias, respectively) Table 2. The Brand-Altman plot confirmed the smaller bias of C-DSer in the measurement of C-in than that of C-cre (Figure 2).

Since the small proportional bias in the measurement of GFR is the advantage of C-DSer, we developed C-DSer-based methods to measure GFR. For this purpose, we divided entire cohort into 129 participants for training cohort and 68 for validation (2:1) randomly (Table 1). The training and validation dataset were indifferent in the distributions and proportions of all variables. Table 3 shows the coefficients of the equation calculated based on training dataset by regression analysis for C-oSer, C-cre, and their combination. The values and the regression lines for each equation are shown in the Supplementary Figure S2. The equation for C-in based on the combination of C-oSer and C-cre was expressed as: \( 0.391 \times \text{C-DSer} + 0.418 \times \text{C-cre} + 3.852 \). This equation greatly reduced the proportional bias (6.5% [-0.2/\( C0 \) 13.1%] and -4.30 [-8.87/\( C0 \) 0.28] for proportional and constant bias, respectively; Supplementary Table 3).

We then examined the performance of the established equation in validation dataset (Table 4). C-oSer performed very well, which became further better when C-oSer was used in combination with C-

### Table 1

Characteristics of the participants.

| Participants | Total (n = 197) | Training (n = 129) | Validation (n = 68) | \( P \) |
|--------------|----------------|-------------------|--------------------|-----|
| Potential living donor | 125 (64.5) | 83 (64.3) | 42 (61.8) | 0.87 |
| Post-donor | 27 (13.7) | 18 (14.0) | 9 (13.2) | 0.64 |
| Transplant recipient | 45 (22.8) | 28 (21.7) | 17 (25.0) | 0.76 |
| Age, y | 60.1 \( \pm \) 12.4 | 60.4 \( \pm \) 12.7 | 59.5 \( \pm \) 11.8 | 0.062 |
| Male | 84 (42.6) | 56 (43.4) | 22 (41.2) | 0.76 |
| Body mass index, kg/m² | 23.2 \( \pm \) 3.5 | 23.5 \( \pm \) 3.7 | 22.6 \( \pm \) 2.9 | 0.26 |
| Body surface area, m² | 1.63 \( \pm \) 0.18 | 1.64 \( \pm \) 0.18 | 1.62 \( \pm \) 0.19 | 0.50 |
| Diabetes | 10 (5.1) | 7 (5.4) | 3 (4.4) | 0.76 |
| Hypertension | 34 (17.3) | 22 (17.1) | 12 (17.7) | 0.92 |
| Hyperlipidemia | 35 (17.8) | 20 (15.5) | 15 (22.1) | 0.26 |
| Hemoglobin, g/dL | 12.7 \( \pm \) 1.7 | 12.8 \( \pm \) 1.6 | 12.5 \( \pm \) 1.8 | 0.28 |
| Serum albumin, g/dL | 4.0 \( \pm \) 0.3 | 4.0 \( \pm \) 0.3 | 4.0 \( \pm \) 0.3 | 0.94 |
| Serum creatinine, mg/dL | 0.86 \( \pm \) 0.36 | 0.87 \( \pm \) 0.37 | 0.85 \( \pm \) 0.33 | 0.73 |
| Serum cystatin C, mg/L | 1.1 \( \pm \) 0.4 | 1.1 \( \pm \) 0.4 | 1.1 \( \pm \) 0.4 | 0.56 |
| Plasma o-serine, \( \mu \text{M} \) | 2.3 \( \pm \) 0.8 | 2.3 \( \pm \) 0.8 | 2.3 \( \pm \) 0.3 | 0.61 |
| Inulin clearance, mL/min/1.73 m² | 66.7 \( \pm \) 20.2 | 64.4 \( \pm \) 20.5 | 67.2 \( \pm \) 19.9 | 0.80 |
| Creatinine clearance, mL/min/1.73 m² | 98.2 \( \pm \) 30.1 | 98.0 \( \pm \) 30.9 | 98.7 \( \pm \) 28.7 | 0.87 |
| o-Serine clearance, mL/min/1.73 m² | 55.7 \( \pm \) 16.9 | 55.3 \( \pm \) 17.2 | 56.4 \( \pm \) 16.4 | 0.66 |
| eGFR_c, mL/min/1.73 m² | 67.5 \( \pm \) 19.7 | 67.9 \( \pm \) 20.8 | 66.8 \( \pm \) 17.5 | 0.73 |
| eGFR_cys, mL/min/1.73 m² | 71.1 \( \pm \) 21.6 | 70.1 \( \pm \) 21.9 | 72.8 \( \pm \) 21.2 | 0.42 |

Data, n (%) or mean \( \pm \) SD. eGFR_c and eGFR_cys denote creatinine- and cystatin C-based estimated glomerular filtration rates (eGFRs), respectively, based on the Japanese formula\(^2,18\). \( P \) values were given for the difference between the training and validation cohorts.

### Table 2

Comparison between clearances of o-serine and creatinine as a surrogate for inulin clearance.

|                   | Slope (95% CI) | Intercept (95% CI) |
|-------------------|----------------|--------------------|
| o-Serine clearance | 1.220 (1.142–1.298) | -1.24 (-5.78–3.31) |
| Creatinine clearance | 0.654 (0.618–0.689) | 2.47 (-1.18–6.13) |

Slopes and intercepts of Deming regression lines are shown. CI, confidence interval.

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**Figure 1.** Scatter plots between clearances of inulin, o-serine and creatinine.

Correlations between inulin clearance and (A) o-serine clearance and (B) creatinine clearance. Black line shows Deming regression line, and gray-dotted line shows identical line. R, Pearson's correlation coefficient.
Figure 2. Bland-Altman plots of \( \Delta \)-serine clearance and creatinine clearance for inulin clearance. (A) \( \Delta \)-serine clearance and (B) creatinine clearance. The solid black line and gray area show the mean of the difference and 95% limits of agreement.

Table 3

| Parameter for equation | Equation for estimating GFR | 95% CI of coefficient |
|------------------------|-----------------------------|-----------------------|
| \( \Delta \)-serine clearance | \( 1.085 \times C-\Delta\text{Ser} + 6.434 \) | \( 0.998 - 1.172 \) |
| Creatinine clearance | \( 0.618 \times C-\text{cre} + 5.889 \) | \( 0.577 - 0.660 \) |
| Combination of \( \Delta \)-serine and creatinine clearances | \( 0.391 \times C-\Delta\text{Ser} + 0.418 \times C-\text{cre} + 3.852 \) | \( 0.210 - 0.572 \) |

C-\( \Delta \)Ser and C-\text{cre}, clearances of \( \Delta \)-serine and creatinine, respectively. CI, confidence interval.

Table 4

| Equations | \( R^2 \) | Bias (95% CI) | IQR (95% CI) | RMSE (95% CI) | P30% (95% CI) | P15% (95% CI) | P7.5% (95% CI) |
|-----------|---------|---------------|--------------|--------------|--------------|--------------|---------------|
| Combination | 0.90    | 3.5 (2.3–4.8) | 6.5 (4.8–9.6) | 6.2 (5.1–7.5) | 98.5 (91.4–100.0) | 89.7 (80.0–95.2) | 64.7 (52.8–75.0) |
| \( \Delta \)-serine clearance | 0.83    | 5.6 (3.5–7.2)* | 10.7 (7.1–14.8) | 8.2 (6.9–9.6) | 97.1 (89.3–99.8) | 79.4 (68.2–87.4)* | 45.6 (34.3–57.4)* |
| Creatinine clearance | 0.87    | 4.3 (3.2–5.7)* | 8.9 (6.5–12.8) | 7.3 (6.0–8.7) | 97.1 (89.3–99.8) | 83.8 (73.1–90.9)* | 51.5 (39.8–63.0)* |
| eGFR_cys | 0.65    | 7.2 (5.6–11.9)* | 16.6 (11.4–19.9) | 12.8 (10.6–15.4) | 88.2 (78.2–94.2)* | 58.8 (47.0–69.8)* | 36.8 (26.3–48.7)* |
| eGFR_cys | 0.55    | 6.9 (5.3–9.4) | 14.1 (10.2–18.8) | 13.7 (10.4–17.3) | 85.3 (74.8–92.0)* | 64.7 (52.8–75.0)* | 33.8 (23.7–45.7)* |

The combination denotes an equation based on a combination of clearances of \( \Delta \)-serine and creatinine. eGFR_cys denotes cystatin-C-based estimated glomerular filtration rate (eGFR), respectively, based on the Japanese formula.\(^2,18\) Performance of conventionally-used eGFRs were shown as references. Bias, absolute value of residual. IQR, interquartile range of difference; RMSE, root mean square error. Accuracy was calculated as the ratios that differed from inulin clearance by less than 30%, 15%, and 7.5% (P30, P15, and P7.5). CI, confidence interval. *Statistically significant versus combination equation.

In the present study, we demonstrated that C-\( \Delta \)Ser is a reliable endogenous measure of GFR. C-\( \Delta \)Ser correlates well with GFR and has the advantage of a small proportional bias for the measurement of C-in over C-cre. An equation based on C-\( \Delta \)Ser enables the precise measurement of the GFR, and the equation based on the combination of C-cre and C-\( \Delta \)Ser achieved precise GFR measurement with minor biases. Measurement of endogenous \( \Delta \)-serine will provide key information for precision medicine.

Measurement of kidney function is essential in daily clinical practice. Precise and unbiased measurement of kidney function is particularly crucial in the decision of the following situations: adjustment of drug dosages [21], diagnosis of CKD [22], and suitability as a living donor for kidney transplantation [23]. C-in is the gold standard measurement of GFR [1], and additional methods using a variety of radiotracers (\(^{99}\)mTc-DTPA, \(^{125}\)I-iothalamate, and \(^{51}\)Cr-EDTA) and nonradioactive (iohexol and iothalamate) tracers have been

**cre.** \( R^2 \) for combination of C-\( \Delta \)Ser and C-cre, C-\( \Delta \)Ser alone, C-cre alone were 0.90, 0.83, and 0.87, respectively. The combined equation of C-\( \Delta \)Ser and C-cre was the least biased with median absolute residual for C-in of 3.5 [95% confidence interval, 2.3–4.8] mL/min/1.73 m\(^2\). This bias was significantly less than any other equations, including C-cre (4.5 [3.2–5.7]; \( P = 0.002 \)). Regarding precision and RMSE, combined equation was the best; it had the lowest IQR, 6.5 [4.8–9.6] and RMSE, 6.2 [5.1–7.5]. In terms of accuracy, the combined equation agreed with C-in accurately (P\(_{15}\), 89.7 [80.0–95.2]). The combined equation provided the better accuracy of prediction within 30, 15, and 7.5% of C-in than other equations. For P\(_{15}\) and P\(_{7.5}\), the accuracy of combined equation was superior to that of C-cre (P = 0.04 and 0.02, respectively). The Brand-Altman plot showed the best agreement between the combination equation and C-in, which was consistent across GFR values and least biased (Figure 3, Supplementary Figure S3). Performance of the equations were consistent across the subgroups defined by sex, transplantation-related status, age, and GFR (Supplementary Figure S4). Similar performance was observed for the equation based on combination of clearances of serum \( \Delta \)-serine and creatinine (Supplementary Figure S5, Supplementary Table 4–6). Overall, the equations based on combination of clearances of intrinsic factors, \( \Delta \)-serine and creatinine, enabled the measurement of GFR with lower bias and higher precision.

4. **Discussion**

In the present study, we demonstrated that C-\( \Delta \)Ser is a reliable endogenous measure of GFR. C-\( \Delta \)Ser correlates well with GFR and has the advantage of a small proportional bias for the measurement of C-in over C-cre. An equation based on C-\( \Delta \)Ser enables the precise measurement of the GFR, and the equation based on the combination of C-cre and C-\( \Delta \)Ser achieved precise GFR measurement with minor biases. Measurement of endogenous \( \Delta \)-serine will provide key information for precision medicine.
The levels of molecules in blood and urine. Thus, C-DSer may relate
to the determinant of D-serine dynamics is tubular reabsorption. About 40 %
of agreement. The solid black line and gray area show the mean of the difference and 95% limits
of measurement. The results are database-specific, as the cohort consisted only of Japanese. To verify the result, it will be necessary to measure D-serine in multiple racial groups.

In conclusion, the combination of C-nSer and C-cre can measure the GFR with precision and minor biases. The current method using endogenous molecules may reduce clinical burdens for measuring GFR. The new method is precise across the tested range of GFR and has the potential to facilitate key clinical decisions in various clinical situations.

5. Contributors

Conceptualization, TK; data curation & formal analysis, MK, TK; funding acquisition; YI, TK; investigation, MK, AH, AT, SN, TA, MHi, RS, MHo, ST, RI and TK; project administration, NN, YI, RI, TK; validation, MK, AH, AT, SN, TA, MHi, RS, MHo, ST, NN, YI, RI and TK; visualization & writing – original draft, MK, TK; writing – review & editing, MK, AH, TK. All authors had full access to all the data, approved the manuscript, and are responsible for the decision to submit for publication.

Declaration of Competing Interest

A part of this study was funded by Shiseido Co., Ltd and KAGAMI Inc. TK has an equity in KAGAMI Inc. TK is an inventor on issued and applied patents (WO2020080484A1, PCT/JP2020/048977), which are related with this work. All other authors declare no competing interests.

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Data sharing statement

Anonymized or aggregated data will be shared upon legitimate requests from the academic researchers for research purpose depending on the nature of the request, the merit of the proposed research, and the intended use. The usage proposal will be reviewed by the steering committee for the approval.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101223.

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