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Improved glomerular filtration rate estimation using New equations combined with standardized cystatin C and creatinine in Chinese adult chronic kidney disease patients

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ABSTRACT

Objectives: The newly developed glomerular filtration rate (GFR)-estimating equations developed by the CKD-EPI Collaboration and Feng *et al.* (2013) that are based on standardized serum cystatin C (ScysC), combined/not combined with serum creatinine (Scr), require further validation in China. We compared the performance of four new equations (CKD-EPI$_{cys}$, CKD-EPI$_{cr-cys}$, Feng$_{cys}$, and Feng$_{cr-cys}$ equations) with the CKD-EPI creatinine equation (CKD-EPI$_{cr}$) in adult Chinese chronic kidney disease (CKD) patients to clarify their clinical application.

Design and Methods: GFR was measured using the dual plasma sampling ^{99m}Tc-DTPA method (mGFR) in 252 adult CKD patients enrolled from four centres. Scr and ScysC were measured by standardized assays in a central laboratory. Each equation's performance was assessed using bias, precision, accuracy, agreement, and correct classification of the CKD stage.

Results: The measured GFR was 46 [25–83] mL/min per 1.73 m^2. The CKD-EPI$_{cys}$, CKD-EPI$_{cr-cys}$ and Feng$_{cys}$ equations provided significantly higher accuracy (P_{15}: 38.9%, 39.7%, and 38.9%) than the CKD-EPI$_{cr}$ equation (29.8%). The CKD-EPI$_{cr-cys}$ and Feng$_{cr-cys}$ equations presented higher precision (IQR of the difference, 16.4 and 17.3 mL/min per 1.73 m^2, respectively) and narrower acceptable limits in Bland–Altman analysis (56.6 and 50.8 mL/min per 1.73 m^2, respectively) than single marker-based equations. The CKD-EPI$_{cr-cys}$ equation achieved the highest overall correct proportion (61.5%) in classification of CKD stages.

Conclusions: Combining ScysC and Scr measurements for GFR estimation improves diagnostic performance. The Scr–ScysC equation showed better performance than equations based on either marker alone. The CKD-EPI$_{cr-cys}$ equation showed the best performance for GFR estimation in Chinese adult CKD patients.

Introduction

Chronic kidney disease (CKD) has become a major health problem worldwide. The prevalence of CKD in USA is reported to be 13% [1]; in Europe, it ranges from 5% to 35% [2]. The latest epidemiological survey in China revealed that overall prevalence of CKD in China is 10.8%, and the number of CKD patients has reached approximately 119.5 million [3]. Early recognition and diagnosis of CKD is crucial to its timely treatment that can delay its progression and prevent CKD-related cardiovascular and metabolic disorders. Glomerular filtration rate (GFR), the best overall index to reflect kidney function, is central to the diagnosis and classification of CKD [4]. Currently, the 'gold standard' for GFR determination is to measure the clearance of exogenous substances, such as inulin, iohexol, ^{51}Cr-EDTA, ^{99m}Tc-DTPA, and ^{125}I-iothalamate. These techniques are time-consuming, labour-intensive, expensive, and require the administration of substances that make them incompatible with routine monitoring in clinical practice [5].

To measure GFR conveniently, certain equations based on serum creatinine (Scr) and demographic characteristics have been developed. The Cockcroft–Gault (CG) equation [6] and the abbreviated Modification of Diet in Renal Disease (MDRD) equation [7] were recommended for use by the Kidney Disease Outcomes Quality Initiative (K/DOQI)

Abbreviations: eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CKD, chronic kidney disease; MDRD, Modification of Diet in Renal Disease; Scr, serum creatinine; ScysC, serum cystatin C; CysC, cystatin C; DTPA, diethylene triamine pentacetate acid; K/DOQI, Kidney Disease Outcomes Quality Initiative; PETIA, particle-enhanced turbidimetric immunoassay; PENIA, particle-enhanced nephelometric immunoassay.

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guidelines in 2002. However, subsequent validation studies demonstrated that neither equation provided satisfactory results in various patient populations [8,9]. To minimise such limitations, including imprecision and systematic underestimation of measured GFR with the MDRD equation, a Chronic Kidney Disease Epidemiology Collaboration creatinine equation (CKD-EPI$_{cr}$ equation) was developed based on standardized Scr [10]. Accumulating evidences demonstrated that the CKD-EPI$_{cr}$ equation performed better than the CG and MDRD equations and could be applicable in clinical nephrology [11,12]. In 2012, the K/DOQI guidelines recommended the use of the CKD-EPI$_{cr}$ equation to report estimated GFR (eGFR) in adults determined using serum creatinine levels, as measured by an assay calibrated to the isotope dilution mass spectrometry reference method [13]. Recent studies have suggested that the CKD-EPI$_{cr}$ equation may be the most appropriate creatinine-based equation for determining GFR in Chinese CKD patients [14–17].

However, the accuracy of creatinine-based equations is not satisfactory because the Scr concentration is easily affected by factors other than GFR [18]. Under such circumstances, the use of cystatin C (CysC) as an alternative marker has received great attention. CysC is a cysteine proteinase inhibitor with a molecular weight of 13 kDa that is produced by all nucleated cells at a constant rate and is considered to be close to the 'ideal' endogenous marker: it is freely filtered by the glomerulus and catabolized in the proximal tubular epithelial cells without being secreted [19]. Unlike Scr, CysC is not be easily affected by gender or muscle mass; its concentration in serum/plasma depends on the GFR [19]. As it has been reported to be possibly superior to Scr in GFR estimation, several equations based on serum cystatin C (ScysC) have been proposed between 2000 and 2010 [20]. However, the measurement of CysC varies across centres owing to the lack of international standardization, and thus, these equations were not widely used. In the fall of 2010, certified CysC reference material (ERM-DA471/IFCC) for calibrating laboratory assays was released [21], resulting in the measurement of CysC in different laboratories becoming comparable and traceable. This provides a foundation for the evaluation of equations in various populations.

In 2012, Inker *et al.* reported two equations for estimated GFR [22], one based on standardized ScysC values (CKD-EPI$_{cys}$ equation) and the other based on standardized ScysC combined with standardized Scr values (CKD-EPI$_{cr\text{-}cys}$ equation). As reported, the CKD-EPI$_{cr\text{-}cys}$ equation was accurate compared to the equations based on either marker alone [22]. However, participants in the development study were mostly of western origin; therefore, it is crucial to validate the performance of the equations in ethnically diverse groups. Almost simultaneously, two GFR equations based on standardized ScysC (Feng$_{cys}$ equation) and combined with Scr (Feng$_{cr\text{-}cys}$ equation) were also developed using a population of 788 Chinese CKD patients [23].

Under these circumstances, several issues need to be resolved in China, as follows: (i) Are equations based on standardized ScysC better than those based on Scr? (ii) Are Scr–ScysC combined equations better than equations based on either marker alone? (iii) Among CKD-EPI$_{cr}$, CKD-EPI$_{cys}$, CKD-EPI$_{cr\text{-}cys}$, Feng$_{cys}$, and Feng$_{cr\text{-}cys}$ equations, which is the optimal equation for the Chinese adult CKD population? In this study, we assessed the performance of the four newly developed equations in Chinese adult CKD populations and compared their performance against the CKD-EPI$_{cr}$ equation, which is presently considered as the best choice for estimating GFR in China [14–17].

Materials and methods

Participant selection

Overall, 252 adult CKD patients (aged 18–90 years) were enrolled in four major medical centres (North China, Beijing; East China, Shanghai; Central China, Changsha; and Northeast China, Dalian) from September 2007 to December 2010. The study was performed in accordance with the declaration of the Ethics Review Board for Human Studies of Peking Union Medical College Hospital. Written informed consent was provided by all the participants after education with regard to the potential benefits, risks, and study procedures. The diagnosis of CKD was according to the criteria provided by K/DOQI guidelines [4].

Patients were excluded from the study if any of the following conditions were present: (a) acute kidney injury; (b) receiving haemodialysis or peritoneal dialysis; (c) general oedema, pleural effusion, ascites, or severe heart failure; (d) severe malnutrition, absence of limbs, or ketoacidosis; (e) receiving cimetidine or trimethoprim; (f) received glucocorticoid therapy in the previous 3 months; (g) hyperthyroidism or hypothyroidism; or (h) leukaemia or cancer.

Measurement of GFR

The ^{99m}Tc-DTPA plasma clearance rate was used to measure GFR (mGFR) using the two-sample method [24]. The staff from the four hospitals participating underwent training for these procedures before the initiation of the study. ^{99m}Tc-DTPA 296 MBq was injected into the elbow median cubital vein via an intravenous bolus injection. Blood was collected, and radioactivity measurements (P_1 and P_2) were performed at 2 h (T_1) and 4 h (T_2), respectively. GFR was corrected for the standard body surface area by multiplying the measured value by 1.73 and dividing it by the patient's body surface area, derived from the Du Bois formula [25]. GFR was calculated as follows:

$$\mathrm{GFR} = \{D\ \ln(P_1/P_2)/(T_2-T_1)\}\exp\{[(T_1 \ln P_2)-(T_2 \ln P_1)]/(T_2-T_1)\} \times 1.73/\mathrm{BSA}$$

$$\mathrm{BSA}\left(m^2\right) = 0.007184 \times \text{body weight}^{0.425} \times \text{height}^{0.725}$$

wherein D is the radioactive count for the injected drugs, T_1 and T_2 are the first and second blood collection times from the contralateral arm following the intravenous bolus injection of ^{99m}Tc-DTPA, respectively, and while P_1 and P_2 are the radioactive counts in blood plasma at T_1 and T_2, respectively. The units of weight and height were kg and cm, respectively.

The Brochner-Mortensen method [26] was used for correcting for the systematic error of the slope–intercept technique. The corrected GFR was calculated as follows:

$$\mathrm{mGFR} = 0.990778 \times \mathrm{GFR} - 0.001218 \times \mathrm{GFR}^2$$

Estimation of GFR

Estimated GFR (eGFR) values were calculated separately using the CKD-EPI$_{cr}$, CKD-EPI$_{cys}$, CKD-EPI$_{cr\text{-}cys}$, Feng$_{cys}$, and Feng$_{cr\text{-}cys}$ equations, and their corresponding results were labelled as eGFR$_{CKD\text{-}EPIcr}$, eGFR$_{CKD\text{-}EPIcys}$, eGFR$_{CKD\text{-}EPIcr\text{-}cys}$, eGFR$_{Fengcys}$, and eGFR$_{Fengcr\text{-}cys}$, respectively, as presented in detail in Table 1.

Analytical methods

On the day of ^{99m}Tc-DTPA GFR measurement, a fasting blood sample was collected, centrifuged, and stored at −80 °C. General information of the patients, including age, gender, height, and weight, were documented. All the serum samples were transferred in the frozen state to the central laboratory at the Department of Laboratory Medicine, Peking Union Medical College Hospital, stored and tested via a standard procedure. Scr was measured by an isotope-dilution mass spectrometry-traceable enzymatic method (Roche-Hitachi P-Module instrument with Roche Creatininase Plus assay; Hoffman-La Roche, Basel, Switzerland). Accuracy of the creatinine assay was assessed using NIST SRM 967 I and II (National Institute of Standards and Technology Standard Reference Material). Bias for the creatinine assay with respect to NIST SRM 967 I and II was −0.75% and 1.96%, respectively. During the study period, the coefficients of variation were 0.7% and 0.6% at creatinine concentrations

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