Estimating Glomerular Filtration Rates by Use of Both Cystatin C and Standardized Serum Creatinine Avoids Ethnicity Coefficients in Asian Patients with Chronic Kidney Disease

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BACKGROUND: The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is most accurate for estimating glomerular filtration rate (GFR) but requires an adjustment for African-American patients. Estimation equations are also improved with the use of serum cystatin C combined with standardized creatinine. Combination equations have been derived by the CKD-EPI and Chinese investigators. We investigated whether these cystatin C–based equations improve estimation adequately, so that adjustments for ethnicity are not required in a multiethnic Asian population with chronic kidney disease (CKD).

METHODS: This was a cross-sectional study of 232 stable CKD patients who underwent GFR measurements using 3-sample plasma clearances of 99mTc-DTPA, and for whom serum cystatin C and creatinine were quantified.

RESULTS: For all patients, the median biases with cystatin C equations were generally greater than with the CKD-EPI equation, and precision and root mean square error (RMSE) were not significantly better. However, the combination serum creatinine and cystatin C equation improved the precision, RMSE, and percentage of estimated GFR to within 15% and 30% of the measured GFR (57.3% vs 50.0%, 88.4% vs 82.8%, respectively). The derived ethnicity coefficients for the combination equation were all (1.009–1.082) but small, suggesting that coefficients are not required. The Chinese-specific equations were more biased and performed more poorly than the CKD-EPI equation.

CONCLUSIONS: The use of a cystatin C and creatinine combination equation for estimating GFR in a multiethnic Asian population with CKD does not require ethnicity coefficients because the derived coefficients are very close to each other.

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The equations commonly used for estimating glomerular filtration rate (GFR)3 are the Modification of Diet in Renal Disease (MDRD) study equation and, more recently, the Chronic Kidney Disease–Epidemiology Collaborative Group (CKD-EPI) equation (1, 2). These equations rely on serum creatinine in combination with the demographic variables age, sex, and ethnicity to determine estimated GFR (eGFR). Because the equations were derived from a US population, they are directly applied to white American patients and adjusted for African-American ethnicity by a coefficient. In practice, using ethnicity-based coefficients is problematic in multiethnic populations and patients of mixed parentage (3). Ethnicity coefficients may be partly affected by true differences in body composition, in particular the amount of muscle mass (4). Coefficients may also be affected by errors in serum creatinine calibration, differences in the constituent populations of the equations, and different reference GFR measurement methods (5). It has been suggested that GFR estimations are improved with serum cystatin C–based equations, either alone or in combination with serum creatinine and other demographic variables (6–8). These equations, however, were not vali-

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6 Nonstandard abbreviations: GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease–Epidemiology Collaborative Group; eGFR, estimated GFR; mGFR, measured GFR; DTPA, diethylene triamine pentaacetic acid; cysC, serum cystatin C; ScysC, standardized serum cystatin C; RMSE, root mean square error; NKF, National Kidney Foundation; KDOQI, Kidney Disease Outcomes Quality Initiative.
dated in multiethnic Asian CKD populations outside of the original derivation populations, and the question exists whether GFR estimation is improved using cystatin C–based equations so that adjustments for ethnicity are not required. In an earlier study, we determined that GFR estimation with the CKD-EPI equation is more accurate than the MDRD equation in a multiethnic Asian CKD population for a wider GFR range (9). In this study, we compared the accuracy of the CKD-EPI equation to several serum cystatin C–based equations and evaluated derived ethnicity coefficients in a multiethnic Asian population with chronic kidney disease.

Methods

This was a parallel substudy of the Asian Kidney Disease Study approved by the institution review board. Briefly, we recruited patients with CKD presenting to the outpatient nephrology clinics in the National University Hospital, Singapore. The inclusion criteria were (a) age >21 years, (b) serum creatinine with an estimated or measured GFR (mGFR) (MDRD, Cockcroft–Gault (10), or creatinine clearance) of 10–90 mL/min, (c) stable CKD defined as 2 serum creatinines measured >60 days apart of <20% difference, and (d) the definition of CKD followed the clinical practice guidelines (11, 12). Patients were excluded if they had any of the following: (a) inability to consent, (b) physical conditions that render phlebotomy for blood samples difficult, (c) inability to collect urine samples successfully, (d) acute kidney function deterioration, amputation, edema, pleural effusion or ascites, skeletal muscle atrophy, or any condition that potentially interferes with the accuracy of the measurement of GFR. The patients were recruited consecutively with stratified sampling by 4 ethnic groups (Chinese, Malay, Indian, and other) and further by sex. Participants performed self-directed 24-h urine collections and underwent GFR measurement the next day, with blood and spot urine samples collected at the same time. All samples were processed within 4 h and stored at −80 °C. On completion of the recruitment of patients, stored serum samples were assayed for serum cystatin C concentration.

LABORATORY TESTS

Patients were allowed a light, no-protein breakfast and underwent a GFR determination (British Nuclear Medicine Society guidelines) by 3-sample plasma clearance of 99mTc-DTPA by use of an intravenous bolus of Technescan diethylene triamine pentaacetic acid (DTPA) (Mallinkrodt Medical BV) (13). GFR was calculated by the slope-intercept method, normalized to body surface area, with the result corrected using the Brochner-Mortensen equation (14). Body surface area was calculated using the du Bois equation (15). The measured GFR is comparable to that obtained by urinary clearance of inulin (16–18). Serum creatinine was measured by an enzymatic method and calibrated with materials traceable to standardized creatinine (Siemens Advia), and serum cystatin C (cysC) was measured by particle-enhanced immunonephelometry on the BN Prospec platform (Dade Behring) in batches in 2009. All assays were performed in a central clinical laboratory accredited by the College of American Pathologists. The mean CV for the 2-year period of 2009–2010 for creatinine was 1.55% for both level 1 and 2 controls (1.27 and 5.92 mg/dL, respectively); range 0.80%–3.22%. The accuracy program for creatinine (CAP LN24) had a range of −4.6%–3.9% across 4 programs (goal for total error 10%). The CV for cystatin C results was 5.2%–6.2%. Cystatin C is not a clinical service test and we did not subscribe to an evaluation program, hence we were unable to determine any bias. We standardized serum cystatin C (ScysC) using adjustment equation 2: ScysC = 1.12 × cysC (19).

GFR ESTIMATION

We estimated GFR using the CKD-EPI equation (2):

\[
eGFR_1 = 141 \times \min (\text{Scr}/\kappa, 1)^{1.19} \times \max (\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\alpha \times \text{Age}} \times 1.018 \text{ [if female]}, \]

where Scr is standardized serum creatinine, \(\kappa\) is 0.7 for females and 0.9 for males, \(\alpha\) is −0.329 for females and −0.411 for males, min indicates the minimum of Scr/\(\kappa\) or 1, and max indicates the maximum of Scr/\(\kappa\) or 1. We had shown that this is the most accurate serum creatinine–based GFR-estimating equation for a multiethnic Asian CKD population. We used the serum cystatin C–based GFR estimating equations developed by the same group (CKD Epidemiology Collaboration) for CKD patients (8, 19):

\[
eGFR_2 = 127.7 \times (\min (\text{Scr}/\kappa, 1)^{1.17} \times \text{Age}^{-0.13} \times 0.91, \text{if female}) \times 1.06, \text{if African-American};
\]

\[
eGFR_3 = 177.6 \times \text{Scr}^{-0.65} \times (\min (\text{Scr}/\kappa, 1)^{0.57} \times \text{Age}^{-0.20} \times 0.82, \text{if female}) \times 1.11, \text{if African-American};
\]

where Scr is standardized serum creatinine and ScysC is standardized serum cystatin C concentration in mg/L (1 mg/L = 74.9 nmol/L). In Chinese patients, we also estimated GFR by the proposed Chinese-specific equations (7):

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Chinese eGFR4 = 86 × cysC−1.132;
Chinese eGFR5 = 176 × Scr−0.607 × cysC−0.638
× age−0.171 (× 0.85, if female);
Chinese eGFR6 = 169 × Scr−0.608 × cysC−0.63
× age−0.157 (× 0.83, if female).

STATISTICS
We compared the eGFR (CKD-EPI) to the mGFR using the following performance measures. The bias for eGFR was defined as the median difference between estimated and measured GFR (eGFR – mGFR) and precision as the interquartile range of this difference. Accuracy was defined as the percentage of GFR estimates within 15%, 30%, and 50% of the mGFR (P15, P30, and P50). We further assessed bias by examining root mean square error (RMSE). To calculate 95% CIs, we generated 2000 bootstrap samples to obtain the estimated and measured GFR (eGFR – mGFR) and eGFR was defined as the median difference between eGFR vs. mGFR.

PERFORMANCE IN CHINESE-SPECIFIC EQUATIONS
In the Chinese subpopulation, the Chinese-specific equations developed by Ma et al. (7) had greater bias than the CKD-EPI equation (19) and the serum cystatin C-based equations (Table 3). Nonetheless, the performance of the cystatin-based equations improved with the addition of demographic variables and had the best performance in combination with serum creatinine. Compared to the CKD-EPI equation, the cystatin-based equations did not consistently show improvements in all of the accuracy performance parameters; however, P15 and P30 were higher using eGFR3.

PERFORMANCE IN NON-CHINESE POPULATIONS
In the non-Chinese groups, bias was greater using the US cystatin C–based equations (eGFR1, eGFR2, and eGFR3) compared to CKD-EPI (Table 4). Again, performance improved with the addition of demographic variables, with the best performance obtained when combined with serum creatinine. Whereas RMSE was not better than with the CKD-EPI equation, precision was marginally improved with equation eGFR3 in both Malays and Indians and others.

DERIVED ETHNICITY COEFFICIENTS
For all patients, we derived “Asian” coefficients for equations eGFR2 and eGFR3 of 1.077 (95% CI 1.046 – 1.108) and 1.055 (95% CI 1.026 – 1.086).
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1.091, "Indians and others" coefficients of 1.043 (0.995–0.004) for equations eGFR2 and eGFR3. We derived Malay coefficients of 1.123 (1.057–1.190, respectively. We also derived Chinese coefficients of 1.064 (1.019 –1.110, respectively. We note that the generated ethnicity coefficients for all ethnic groups were all >1. Taken together with the persistence of a lower (but still >1) coefficient for African-Americans for equations eGFR2 and eGFR3 (1.06 and 1.11, respectively), our results suggest that cystatin C adjusts estimated GFR down-}

The best performance is obtained using the combination biomarker equation (eGFR3) with demographic variables. The P30 accuracy performance parameter recommended by the NKF KDOQI guidelines is similar between eGFR3 (88.4%) and the CKD-EPI equation (82.8%) and is close to that achieved by other validation studies of the MDRD study equations (12, 20). The P15 accuracy is increased by 7.3% in eGFR3 compared to the CKD-EPI equation. This improvement in accuracy of GFR estimation and, consequently, correctness of CKD staging, while requiring only a spot serum test, is attractive for both clinical care and research.

The derived ethnicity coefficients for eGFR3 equation range from 1.009 to 1.082. We note that the generated ethnicity coefficients for all ethnic groups were still >1. Taken together with the persistence of a lower (but still >1) coefficient for African-Americans for equations eGFR2 and eGFR3 (1.06 and 1.11, respectively), our results suggest that cystatin C adjusts estimated GFR down-

Discussion

Our study shows that the serum cystatin C–based GFR-estimating equations for CKD patients that incorporate demographic variables and/or standardized serum creatinine perform as well as the CKD-EPI equation. The equations having serum cystatin C alone (eGFR1 and eGFR2) are not better than with the CKD-EPI equation.
ward (8). We previously argued that unless there is a consistent, >10% bias between measured and estimated GFR, it would be difficult to attribute derived ethnicity coefficients to true ethnic differences in GFR (9). This is because the bias may be a result of differences arising from the constituting derivation population, biomarker assay calibration, GFR measurement method, and physiologic variability. A study population with more high-GFR patients can be expected to have greater variability and differences in measured GFR. Therefore, ethnicity coefficients may arise due to chance, particularly if the study population is small. Serum creatinine calibration is an important source of bias and may be the cause of the ethnicity coefficient (9, 21, 22). Physiologic variability and dif-
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Table 3. Comparison of equations in Chinese CKD patients (n = 94).

<table>
<thead>
<tr>
<th>Equation</th>
<th>Accuracy, % (95% CI)</th>
<th>Bias, mL·min⁻¹·(1.73 m²)⁻¹ (95% CI)</th>
<th>Precision, mL·min⁻¹·(1.73 m²)⁻¹ (95% CI)</th>
<th>RMSE (95% CI)</th>
<th>P15</th>
<th>P30</th>
<th>P50</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR1 (Scr+C)</td>
<td>1.3 (3.3 to 0.7)</td>
<td>-3.3 (-3.0 to 0.7)</td>
<td>9.0 (-2.3 to 17.8)</td>
<td>15.5 (1.55–16.4)</td>
<td>-2.5 (-4.1 to -0.9)</td>
<td>13.0 (8.4–17.6)</td>
<td></td>
</tr>
<tr>
<td>eGFR2 (Scr+C + age + sex + ethnicity)</td>
<td>3.3 (4.9 to 1.7)</td>
<td>-3.1 (-4.9 to -0.7)</td>
<td>8.0 (-1.2 to 16.9)</td>
<td>14.7 (12.6–17.7)</td>
<td>-2.3 (-4.1 to -0.8)</td>
<td>13.6 (8.4–17.6)</td>
<td></td>
</tr>
<tr>
<td>eGFR3 (Scr+C + age + sex + ethnicity)</td>
<td>6.6 (4.6–8.9)</td>
<td>-2.2 (-4.0 to -0.5)</td>
<td>13.8 (7.6–19.8)</td>
<td>14.7 (14.6–16.4)</td>
<td>-1.3 (-3.3 to 0.7)</td>
<td>11.8 (7.6–16.0)</td>
<td></td>
</tr>
<tr>
<td>eGFR4 (Scr+C)</td>
<td>15.3 (12.8–17.8)</td>
<td>14.1 (10.0–18.3)</td>
<td>15.8 (9.6–22.0)</td>
<td>27.5 (19.1–35.9)</td>
<td>-2.5 (-4.1 to -0.9)</td>
<td>13.0 (8.4–17.6)</td>
<td></td>
</tr>
<tr>
<td>eGFR5 (Scr+C + age + sex + ethnicity)</td>
<td>27.5 (19.1–35.9)</td>
<td>14.1 (10.0–18.3)</td>
<td>15.5 (10.4–20.6)</td>
<td>27.5 (19.1–35.9)</td>
<td>-2.5 (-4.1 to -0.9)</td>
<td>13.0 (8.4–17.6)</td>
<td></td>
</tr>
<tr>
<td>CKD-EPI GFR (Scr+C + age + sex + ethnicity)</td>
<td>27.5 (19.1–35.9)</td>
<td>14.1 (10.0–18.3)</td>
<td>15.5 (10.4–20.6)</td>
<td>27.5 (19.1–35.9)</td>
<td>-2.5 (-4.1 to -0.9)</td>
<td>13.0 (8.4–17.6)</td>
<td></td>
</tr>
</tbody>
</table>

a Scr, standardized serum creatinine.

Our data also suggests that in multiethnic CKD populations, it would be possible to increase precision differ from GFR measurement techniques may result in up to 20% differences (13, 17, 23). We previously showed that varying the protocol for the calculation of GFR measurements by the slope-intercept method reduced a derived Chinese coefficient for the MDRD study equation by 2% (9). Moreover, with the exception of the Indian and other groups, equation eGFR3 yielded ethnicity coefficients for the Malay and Chinese groups that were marginally higher than 1 within their 95% CIs, further supporting the idea that ethnicity adjustment is not required for the combination biomarker equation in multiethnic Asian populations. It is unlikely that a large, representative, economical study will be performed to develop “true” ethnicity coefficients. Like other studies, our ethnicity coefficients were derived mathematically (9, 21, 24). The proliferation of such coefficients will not be practical or practicable in many cosmopolitan cities and countries. Our study is the first to suggest that a multiple biomarker approach to GFR-estimating equations may obviate ethnicity coefficients because the adjustment quanta are small. Therefore, in the absence of external validation, we do not recommend the use of our derived ethnicity coefficients. Our study results do suggest that equation eGFR3, however, can be used without considering ethnicity coefficients in Asians with CKD both within and outside the US.

In the Chinese subgroup, the Chinese-specific equations also showed improved performance when demographic variables were added (7). The bias and other accuracy parameters, however, were poorer than with the serum cystatin C–based equations derived from the US population. In fact, the straight application of the combination biomarkers equation (eGFR3) performed very well, yielding GFR estimates with similar or better precision and accuracy (P15 and P30). One possible reason is that our laboratory had the advantage of calibrating our serum cystatin C from frozen samples in 2005–2006 (26, 27). We previously showed that the CKD-EPI cystatin C study assayed serum cystatin C from frozen samples in 2005–2006 (8). Therefore, bias and any derived coefficients in this study may be caused by this assay drift. Moreover, our cystatin C assays also were performed before the availability (June 2010) of an international cystatin C reference material (ERM-DA471/IFCC). Fortunately, the timing of our study assays (2009) permits us to mathematically adjust our serum cystatin C results to standardized cystatin C (19).
Table 4. Performance of equations in non-Chinese CKD patients.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Bias, mL·min⁻¹·(1.73 m²)⁻¹ (95% CI)</th>
<th>Precision, mL·min⁻¹·(1.73 m²)⁻¹ (95% CI)</th>
<th>RMSE (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR1 (ScysC)</td>
<td>−2.4 (−5.2 to 0.3)</td>
<td>12.9 (8.9–17.0)</td>
<td>10.3 (7.3–14.1)</td>
</tr>
<tr>
<td>eGFR2 (ScysC)</td>
<td>1.5 (−6.1 to 1.4)</td>
<td>11.0 (7.4–15.8)</td>
<td>12.8 (7.3–17.3)</td>
</tr>
<tr>
<td>eGFR3 (ScysC)</td>
<td>1.9 (−6.1 to 1.1)</td>
<td>11.6 (7.4–15.8)</td>
<td>12.8 (7.3–17.3)</td>
</tr>
<tr>
<td>CKD-EPI GFR (Scr)</td>
<td>0.8 (−3.7 to 0.0)</td>
<td>12.2 (7.5–16.9)</td>
<td>14.2 (7.3–19.8)</td>
</tr>
<tr>
<td>Indian and others</td>
<td>1.9 (−0.9 to 3.3)</td>
<td>12.4 (7.6–17.2)</td>
<td>14.3 (7.6–20.9)</td>
</tr>
<tr>
<td>eGFR1 (ScysC)</td>
<td>1.2 (−2.4 to 4.8)</td>
<td>17.7 (10.9–24.5)</td>
<td>15.9 (11.1–20.7)</td>
</tr>
<tr>
<td>eGFR2 (ScysC)</td>
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<td>14.2 (7.3–19.8)</td>
</tr>
</tbody>
</table>

and percentage accuracy to within 15% and 30% of the measured GFR by using the equation for a combination of biomarkers (eGFR3) without considering ethnicity coefficients. If assay costs are not an impediment, the >7% increase in the proportion of patients achieving this margin of accuracy would improve the accuracy of screening programs and epidemiological studies of CKD, particularly for GFR in the range of 60–90 mL·min⁻¹·(1.73 m²)⁻¹. Why should the addition of serum cystatin C to serum creatinine improve GFR estimation, especially in a multiethnic Asian population? Prior studies have alluded to the fact that serum creatinine-based equations underestimate and serum cystatin C–based equations overestimate GFR, and the average yield the best estimate (6, 8, 28). This may be related to factors responsible for the generation of these biomarkers. Serum creatinine generation reflects the amount of lean muscle mass (4). Body mass index influences serum cystatin C generation (29–31). We suspect that the degree of ethnic and environmental influences on fat and muscle proportion is rendered less important once we average the GFR estimates using a combination of these 2 markers. It would be helpful to have a future study evaluate the contribution of fat mass to cystatin C concentrations between different ethnic populations.

One major limitation of our study is the use of a different GFR measurement technique from the previous study (8). Bias may be attributed to the use of whole-plasma clearance of 99mTc-DTPA, which can overestimate or underestimate GFR measured by urinary clearance of iothalamate (17, 23). Our method of calculating GFR may also result in a small difference despite using a technique similar to that used by the Beijing investigators (7). Our nephelometer was different, although cystatin C was calibrated with manufacturer-provided materials and used the same type of assay described in previous studies (7, 8). Whereas we had a representative population of CKD patients including some with diabetes, we did not have kidney transplant patients and healthy individuals. Therefore, the application of the cystatin C–based equations in other populations cannot be defined (8, 28). But we note that the cystatin C equations considered in this study were derived from CKD patients and excluded healthy people. As in other Asian studies, the various ethnicity coefficients were derived mathematically because we did not have comparator group of patients of European descent.

In summary, our study shows that a combined serum cystatin C and standardized creatinine GFR-estimating equation performs similarly to the CKD-EPI equation. In multiethnic Asian CKD populations, it is reasonable to use this equation without adjustments for ethnicity.
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Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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