Kidney Medicine Original Research

GFR Estimation Using a Panel of Filtration Markers in Shanghai and Beijing

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Rationale & Objectives: Estimated glomerular filtration rate (eGFR) using creatinine and cystatin C ($eGFR_{cr\text{-}cys}$) may be less accurate compared to measured GFR (mGFR) in China than in North America, Europe, and Australia due to variation across regions in their non-GFR determinants. The non-GFR determinants of β_2-microglobulin (B2M) and β-trace protein (BTP) differ from those of creatinine and cystatin C. Thus, the average eGFR using all 4 markers ($eGFR_{avg}$) could be more accurate than $eGFR_{cr\text{-}cys}$ in China.

Study Design: Diagnostic test study.

Setting & Participants: 1,066 participants in Shanghai and Beijing with creatinine and cystatin C and 666 participants with all 4 filtration markers.

Tests Compared: Index tests were previously developed equations for eGFR using creatinine, cystatin C, B2M, and BTP and combinations. The reference test was mGFR using plasma clearance of iohexol. We compared the performance of $eGFR_{avg}$ to $eGFR_{cr\text{-}cys}$ using the proportion of participants with errors in eGFR >30% of mGFR ($1 - P_{30}$) and root mean square error (RMSE) of the regression of eGFR on mGFR on the logarithmic scale. We also compared classification and reclassification of mGFR categories using $eGFR_{avg}$ compared to $eGFR_{cr\text{-}cys}$.

Outcomes: Accuracy was significantly better for $eGFR_{avg}$ ($1 - P_{30}$ of 10.4% and RMSE of 0.214) compared to $eGFR_{cr\text{-}cys}$ ($1 - P_{30}$ of 13.8% and RMSE of 0.232; $P = 0.004$ and $P = 0.006$, respectively). However, improvements in accuracy did not generally translate into significant improvement in classification or reclassification of mGFR categories.

Limitations: Study population may not be generalizable to clinical settings other than large urban medical centers in China.

Conclusions: A panel of endogenous filtration markers including B2M and BTP in addition to creatinine and cystatin C may improve GFR estimation in China. Further study is necessary to determine whether GFR estimation using B2M and BTP can be improved and whether these improvements lead to useful clinical applications.

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Current guidelines from Kidney Disease: Improving Global Outcomes (KDIGO) recommend using estimated glomerular filtration rate (eGFR) from creatinine ($eGFR_{cr}$) as the initial test and from cystatin C ($eGFR_{cys}$) or the combination of creatinine and cystatin C ($eGFR_{cr\text{-}cys}$) as a confirmatory test for clinical assessment of kidney function.[1] GFR estimation from all endogenous filtration markers is limited by determinants of serum concentrations of the markers other than GFR (non-GFR determinants), which may vary across populations. In principle, if the non-GFR determinants of each marker are not strongly correlated, a panel of markers can improve GFR estimates by reducing the error from each marker.[2] The non-GFR determinants of creatinine (muscle mass and diet) differ from those of cystatin C (adiposity, smoking, inflammation, and others); thus, $eGFR_{cr\text{-}cys}$ is more accurate than either $eGFR_{cr}$ or $eGFR_{cys}$.

Like cystatin C, β_2-microglobulin (B2M) and β-trace protein (BTP) are low-molecular-weight protein filtration markers, but their non-GFR determinants differ from creatinine and cystatin C.[3,4] Prior studies in community-based elderly populations or populations with chronic kidney disease (CKD) suggest that the non-GFR determinants are associated with smoking and C-reactive protein (for B2M), sex (for BTP), and urine protein excretion (for both B2M and BTP), independent of GFR.[3,4] Thus, eGFR using B2M and BTP in addition to creatinine and cystatin C could be more accurate than $eGFR_{cr\text{-}cys}$.

As a performance measure for accuracy of GFR estimating equations, Kidney Disease Outcome Quality Initiative (KDOQI) guidelines recommended that 90% of eGFR be within 30% of measured GFR (mGFR; P_{30}>90%, equivalent to $1 - P_{30}$ of <10%, where P_{30} is the percentage of eGFRs that are within 30% of mGFR).[5] The KDIGO guidelines recommend the CKD Epidemiology Collaboration (CKD-EPI) equations for GFR estimation in North America, Europe, and Australia. In these regions, $1 - P_{30}$ can be as low as 10% using assays traceable to international standards.[6-8] In other regions, the CKD-EPI equations may be less accurate, particularly because of differences in non-GFR determinants of creatinine; thus, confirmatory testing may be more important in these regions.[9] In China, the CKD-EPI equations are often used for clinical practice, research, and public health,[10,11] although some studies suggest that the equations may be less accurate there ($1 - P_{30}$ of 27% for $eGFR_{cr}$ and $eGFR_{cys}$ and 23% for $eGFR_{cr\text{-}cys}$ in 2 studies using standardized creatinine and cystatin C).[12,13]

Prior studies of eGFR using BTP alone or in combination with creatinine and cystatin C have demonstrated variable performance,[14-16] but there are fewer data available for the performance of eGFR using BTP in combination with B2M. In a study by the CKD-EPI group of patients in North America with CKD and mean mGFR of 48 mL/min/1.73 m^2, eGFR using B2M ($eGFR_{B2M}$) or BTP ($eGFR_{BTP}$) was not more accurate than $eGFR_{cr}$ or $eGFR_{cys}$, eGFR using the combination of B2M and BTP ($eGFR_{B2M\text{-}BTP}$) was not more accurate than $eGFR_{cr\text{-}cys}$, and a panel of markers comprised of the average of $eGFR_{B2M\text{-}BTP}$ and $eGFR_{cr\text{-}cys}$ ($eGFR_{avg}$) was not more accurate than $eGFR_{cr\text{-}cys}$.[17] We hypothesized that the addition of B2M and BTP to $eGFR_{cr\text{-}cys}$ might improve GFR estimation more in populations in which $eGFR_{cr\text{-}cys}$ is less accurate. We evaluated the performance of previously developed GFR estimating equations using B2M and BTP in 2 urban populations in China.

METHODS

The design is a diagnostic test study using cross-sectional analysis in a pooled database, with eGFR as the index test and mGFR as the reference test. The study was approved by the institutional review boards of all participating institutions (Tufts Health Sciences IRB 12315; Ethics Committee of Clinical Research of Peking University First Hospital 2013[616]; Ethics Committee of People's Hospital of Peking University 2014PHB098-01; Clinical Trial Ethics Committee Shanghai Ruijin Hospital (2014) L.L.S.NO. (20); University of Minnesota ARDL lab 1307E38081). Written informed consent was provided by all study participants.

Populations

The study populations at the Shanghai Ruijin Hospital (SRH), Shanghai; the Peking University First Hospital (PUFH), Beijing; and the Peking University People's Hospital (PUPH), Beijing, China, included hospitalized patients, outpatients, or healthy volunteers 18 years or older. Exclusion criteria included acute illness associated with acute changes in GFR and use of medications known to inhibit tubular secretion of creatinine, such as cimetidine or trimethoprim. A total of 1,088 participants had measurements of GFR from June 2013 to November 2016; there were 811 from SRH and 277 from PUFH and PUPH. In Shanghai, 422 participants had measurements of creatinine and cystatin C only, and 389 participants had measurements of all filtration markers (creatinine, cystatin C, B2M, and BTP). In Beijing, all participants had measurements of all filtration markers. For analyses of eGFR based on creatinine and cystatin C, we included all 1,088 participants. For analyses of eGFR based on B2M and BTP, we included 666 participants with measurements of all filtration markers.

Measured GFR

GFR was measured at SRH, PUFH, and PUPH as plasma clearance of iohexol, a method with acceptable accuracy compared with inulin clearance.[18] We used samples collected 2 to 5 hours after bolus intravenous iohexol administration, calculated GFR using the slope-intercept method corrected by the Brochner Mortensen coefficients, and indexed the results to 1.73 m^2 body surface area.[19] At SRH, 2 postbolus samples were collected in 422 participants and 3 postbolus samples were collected in 389 participants. Among participants with 3 samples, mGFR did not differ significantly whether computed using only 2 samples (first and last) or all 3 samples (Deming regression point estimate for intercept of 0.16 mL/min/1.73 m^2, for slope of 0.99, for correlation coefficient of 0.997). At PUFH and PUPH, 3 samples were collected in all participants.

Plasma iohexol concentrations were measured in frozen samples at SRH, PUFH, and PUPH using high-performance liquid chromatography (Table S1) and were compared with measurements using the same method at the University of Minnesota (UMN), which has been found to have acceptable accuracy with proficiency testing samples from the Equalis program for external quality assessment (Equalis AB, Uppsala, Sweden).[20] Measurements performed at SRH, PUFH, and PUPH were comparable to measurements on the same samples performed at UMN (Deming regression point estimates for intercepts of −0.04 to 0.69 mg/dL, for slopes of 0.93 to 1.05, and for correlation coefficients of 0.996 to 0.999; Table S2).

Estimated GFR

We used GFR estimating equations developed by CKD-EPI for use with standardized creatinine and standardized cystatin C and for use with B2M and BTP performed at UMN (Table S3).[6-8,17] We considered single-marker equations ($eGFR_{cr}$, $eGFR_{cys}$, $eGFR_{BTP}$, and $eGFR_{B2M}$), 2-marker equations ($eGFR_{cr\text{-}cys}$ and $eGFR_{B2M\text{-}BTP}$), and a 4-marker equation ($eGFR_{avg}$, the average of $eGFR_{cr\text{-}cys}$ and $eGFR_{B2M\text{-}BTP}$). We compared the performance of the CKD-EPI equations with other equations developed more recently for use with standardized creatinine and cystatin C.[21-24] We developed "best-fit" equations using linear regression with age and sex in the combined study population with all 4 markers and in the Shanghai and Beijing subgroups, including a 4-marker equation ($eGFR_{all}$), to illustrate "optimal" performance of the markers. As sensitivity analysis, we used the traditional strategy for the development of new estimating equations, specifically, to evaluate the performance of the "best-fit" equations developed in the Shanghai participants in the Beijing participants. We did not consider this strategy for the primary analysis because we considered the sample size and diversity of the 2 study populations not to be satisfactory for this purpose. For newly developed equations, mGFR and serum concentrations of filtration markers were log transformed as previously described.[6-8,17]

Assays for endogenous filtration markers were performed in frozen samples using methods shown in Table S1. Serum creatinine and cystatin C assays were

performed at SRH and UMN and were traceable to international reference materials, Standard Reference Material (SRM) 967 (National Institutes of Standards and Technology, Gaithersberg, MD) and ERM-DA471/International Federation of Clinical Chemistry and Laboratory (IFCC; Institute for Reference Materials and Measurements, Geel, Belgium), respectively.[24,25] For participants at SRH, measurements in the subgroup with creatinine and cystatin C only were performed at SRH (n = 422), and measurements in the subgroup with all filtration markers were performed at UMN (n = 389). Measurements of serum creatinine and cystatin C performed at SRH were comparable to measurements on the same samples performed at UMN (for creatinine, Deming regression point estimates for intercepts of −0.04 and 0.17 mg/dL, for slopes of 0.99 and 0.91, and correlations of 0.992 to 0.999; for cystatin C, Deming regression point estimates for intercepts of −0.12 and −0.03 mg/L, for slopes of 1.086 and 1.16, and for correlations of 0.984 to 0.996 for cystatin C; Table S2). Thus, no adjustments in the measured concentrations were made. For participants at PUFH and PUPH, measurements for all endogenous filtration markers were performed at UMN.

Statistical Analysis

Population characteristics were described using mean and standard deviation (SD) or percentage. Subgroups were defined by mGFR or eGFR (≥90, 60-89, 45-59, 30-44, and <30 mL/min/1.73 m^2), age (≤40, 40-64, and >65 years), sex, body mass index (BMI; <20, 20-25, 26-30, and >30 kg/m^2), clinical diagnosis of diabetes (yes or no), and location (Shanghai or Beijing). Pearson correlations were computed for endogenous filtration markers with mGFR and with each other and for partial correlations of filtration markers with each other after adjustment for mGFR.

Metrics for comparison of equation performance include bias, precision, 2 measures of accuracy, classification by GFR subgroups, and reclassification of mGFR subgroups by eGFR. For comparisons among estimating equations, $eGFR_{cr}$ and $eGFR_{cr\text{-}cys}$ using the CKD-EPI equations were used as the reference equations because they are recommended by clinical practice guidelines. Bias was assessed as the median difference between mGFR and eGFR (mGFR − eGFR, a positive value indicates an underestimate of mGFR and a negative value indicates an overestimate of mGFR). Precision was assessed as the interquartile range of the difference between mGFR and eGFR. Accuracy was assessed as the percentage of eGFR within 30% of mGFR (P_{30}) and reported as $1 - P_{30}$ as a measure of large errors, and as root mean squared error (RMSE) for the regression of log mGFR on log eGFR as an overall measure of goodness of fit. Unit for RMSE is log GFR. The 95% confidence intervals (CIs) around the median difference, interquartile range of the difference, $1 - P_{30}$, and RMSE were calculated using bootstrap method (500 bootstraps). For comparisons of $1 - P_{30}$ and RMSE, we computed P values using McNemar and signed rank tests for paired comparisons, respectively, and considered $P < 0.05$ significant without consideration of multiple comparisons. Classification of equations was assessed by evaluating the concordance for eGFR and mGFR by GFR categories and by area under the receiver operating characteristic curve for detecting mGFR threshold of 60 mL/min/1.73 m^2. Improvement in participant classification to mGFR ≥ 60 mL/min/1.73 m^2 by eGFR was evaluated using net reclassification index statistic.

To limit the number of hypothesis tests for comparisons among the CKD-EPI equations, we focused on comparisons of accuracy, classification, and reclassification of $eGFR_{cr\text{-}cys}$ versus $eGFR_{cr}$ and of $eGFR_{avg}$ versus $eGFR_{cr\text{-}cys}$. For best-fit equations, we focused on $eGFR_{all}$ rather than $eGFR_{avg}$. We did not perform statistical testing for the sensitivity analysis. For comparison of performance in subgroups, we focused on bias because bias in subgroups is a cause of imprecision and inaccuracy in the overall cohort. As in previous studies, for comparison of other equations using creatinine and cystatin C to the CKD-EPI equations, we used bias, precision, and accuracy and considered nonoverlapping CIs as significant because of multiple comparisons.[26-28]

RESULTS

Demographic and Clinical Characteristics

The study population included 1,088 participants, 811 from Shanghai and 277 from Beijing (Table 1). There were 45% women, mean (SD) age was 46 (16) years, BMI was 24.2 kg/m^2, 15% had diabetes, and mean (SD) mGFR was 64 (33) mL/min/1.73 m^2. Compared with participants from Shanghai, participants from Beijing had similar mean BMI, but a nominally larger proportion of women, younger mean age, fewer participants with diabetes, and higher mean GFR. Of the total, 666 participants had measurements of all 4 filtration markers (creatinine, cystatin C, B2M, and BTP; Table S4). Among participants from Shanghai, those with measurements of creatinine and cystatin C only had similar characteristics to those with measurements of all markers (Table S5).

Correlations Among Filtration Markers

Point estimates for correlations of creatinine, cystatin C, B2M, and BTP with mGFR were 0.90, 0.89, 0.88, and 0.84, respectively (Table S6). Point estimates for partial correlations of endogenous filtration markers after adjusting for mGFR ranged from 0.59 to 0.29.

Performance of GFR Estimating Equations

Participants With Measurements of Creatinine and Cystatin C Only

Among the 1,088 participants (Table 2, upper panel), accuracy of the CKD-EPI equations was not optimal (for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$, $1 - P_{30}$ was 23.5%, 28.1%, and 17.7%, respectively, and RMSE was 0.285,

Table 1. Demographic and Clinical Characteristics of the Study Population From 2 Large Urban Chinese Populations, 2013-2016

Population	Overall	Shanghai	Beijing
	1,088 (100%)	811 (74.5%)	277 (25.5%)
Female sex	491 (45.1%)	353 (43.5%)	138 (49.8%)
Age, y	45.8 (15.6)	46.9 (15.7)	42.8 (15.2)
≤40	429 (39.4%)	303 (37.4%)	126 (45.5%)
41-65	527 (48.4%)	398 (49.1%)	129 (46.6%)
>65	132 (12.1%)	110 (13.6%)	22 (7.9%)
BMI, kg/m^2	24.2 (4.1)	24.0 (4.0)	24.7 (4.3)
≤24	557 (51.2%)	424 (52.3%)	133 (48.0%)
24-28	368 (33.8%)	280 (34.5%)	88 (31.8%)
>28	163 (15.0%)	107 (13.2%)	56 (20.2%)
Diabetes	167 (15.4%)	133 (16.4%)	34 (12.4%)
mGFR, mL/min/1.73 m^2	64.2 (33.3)	59.6 (32.5)	77.5 (32.1)
<60	513 (47.2%)	423 (52.2%)	90 (32.5%)
60-89	315 (29.0%)	233 (28.7%)	82 (29.6%)
≥90	260 (23.9%)	155 (19.1%)	105 (37.9%)
Creatinine, mg/dL	1.63 (1.38)	1.79 (1.48)	1.17 (0.93)
Cystatin C, mg/L	1.74 (1.15)	1.89 (1.21)	1.32 (0.83)
B2M,[a] mg/L	3.85 (3.67)	4.37 (3.87)	3.12 (3.23)
BTP,[a] mg/L	1.32 (1.02)	1.53 (1.07)	1.03 (0.86)

Note: Values for categorical variables are given as number (percent); values for continuous variables are given as mean (standard deviation).
Abbreviations: B2M, β$_2$-microglobulin; BMI, body mass index; BTP, β-trace protein; mGFR, measured glomerular filtration rate.
[a]n = 666 overall, 398 Shanghai, 277 Beijing.

0.328, and 0.269, respectively). Accuracy was better for $eGFR_{cr\text{-}cys}$ than $eGFR_{cr}$ (significantly lower $1-P_{30}$ and RMSE). Accuracy of $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ varied across subgroups from Shanghai and Beijing (Table S7).

Participants With Measurement of All Markers

Among the 666 participants (Table 2, lower panel), $eGFR_{cr}$ significantly underestimated mGFR (median, −4.3 [95% CI, −5.8 to −3.5] mL/min/1.73 m^2), whereas $eGFR_{cys}$ significantly overestimated mGFR (median, 3.5 [95% CI, 2.4 to 4.2] mL/min/1.73 m^2) and $eGFR_{cr\text{-}cys}$ was unbiased (median, 0.1 [95% CI, −0.8 to 1.0] mL/min/1.73 m^2]. Both $eGFR_{B2M}$ and $eGFR_{BTP}$ significantly underestimated mGFR (median values of 4.0 [95% CI, 2.1-5.8] and 12.5 [95% CI, 11.2-15.8] mL/min/1.73 m^2, respectively). Consequently, $eGFR_{B2M\text{-}BTP}$ and $eGFR_{avg}$ significantly underestimated mGFR (median values of 7.1 [95% CI, 5.6-8.5] and 2.7 [95% CI, 1.9-3.6] mL/min/1.73 m^2, respectively). Although accuracy was significantly better for $eGFR_{cr\text{-}cys}$ ($1-P_{30}$ of 13.8% and RMSE of 0.232) than $eGFR_{cr}$ ($1-P_{30}$ of 20.7% and RMSE of 0.254), accuracy was significantly better for $eGFR_{avg}$ ($1-P_{30}$ of 10.4% and RMSE of 0.214) compared with $eGFR_{cr\text{-}cys}$. Comparisons of accuracy between $eGFR_{cr\text{-}cys}$ versus $eGFR_{cr}$ and $eGFR_{avg}$ versus $eGFR_{cr\text{-}cys}$ were generally similar in subgroups from Shanghai and Beijing, although not all differences were significant (Table S7). Among subgroups defined by age, sex, BMI, diabetes, and eGFR, bias across subgroups was generally similar for $eGFR_{avg}$ (Fig 1).

Other equations using creatinine and cystatin C did not perform consistently better than CKD-EPI equations (Tables S8a and 8b). As expected, best-fit equations generally performed better than the CKD-EPI equations (Table S9). Among best-fit equations, $eGFR_{cr\text{-}cys}$ was more accurate than $eGFR_{cr}$, but $eGFR_{B2M}$ and $eGFR_{BTP}$ were not more accurate than $eGFR_{cr}$, and $eGFR_{all}$ was generally not more accurate than $eGFR_{cr\text{-}cys}$. The accuracy of best-fit equations varied among subgroups by location. Point estimates from the sensitivity analysis (Table S10) were consistent with those for the best-fit equations in Beijing.

Point estimates for concordance among eGFR and mGFR categories and for area under the receiver operating characteristic curve for detecting an mGFR threshold of 60 mL/min/1.73 m^2 were higher for $eGFR_{cr\text{-}cys}$ than

Table 2. Performance of GFR Estimating Equations in the Study Population With Creatinine and Cystatin C Only and in the Study Population With All Filtration Markers

Equations	Median Bias (95% CI)	IQR (95% CI)	$1-P_{30}$ (95% CI)	RMSE (95% CI)
Creatinine and Cystatin C Only (N= 1,088)				
$eGFR_{cr}$	−2.8 (−3.8 to −1.9)	17.7 (16.1 to 19.2)	23.5 (20.9 to 25.9)	0.285 (0.263 to 0.310)
$eGFR_{cys}$	5.0 (4.1 to 5.5)	16.3 (15.4 to 17.8)	28.1 (25.6 to 30.8)	0.328 (0.308 to 0.353)
$eGFR_{cr\text{-}cys}$	1.8 (1.0 to 2.5)	15.6 (14.0 to 17.0)	17.7 (15.3 to 20.2)[a]	0.269 (0.248 to 0.295)[b]
All Filtration Markers (N= 666)				
$eGFR_{cr}$	−4.3 (−5.8 to −3.5)	16.7 (14.7 to 18.5)	20.7 (17.7 to 24.0)	0.254 (0.233 to 0.279)
$eGFR_{cys}$	3.5 (2.4 to 4.2)	16.5 (14.6 to 18.0)	23.6 (20.6 to 26.9)	0.292 (0.272 to 0.313)
$eGFR_{B2M}$	4.0 (2.1 to 5.8)	20.4 (18.3 to 22.6)	23.3 (20.2 to 26.8)	0.277 (0.258 to 0.297)
$eGFR_{BTP}$	12.5 (11.2 to 15.8)	27.8 (25.2 to 30.3)	41.6 (37.8 to 45.8)	0.381 (0.363 to 0.400)
$eGFR_{cr\text{-}cys}$	0.1 (−0.8 to 1.0)	14.3 (12.9 to 15.6)	13.8 (11.6 to 16.4)[c]	0.232 (0.212 to 0.254)[d]
$eGFR_{B2M\text{-}BTP}$	7.1 (5.6 to 8.5)	20.2 (18.5 to 23.0)	23.1 (19.9 to 26.4)	0.279 (0.264 to 0.297)
$eGFR_{avg}$	2.7 (1.9 to 3.6)	13.5 (12.0 to 15.3)	10.4 (7.7 to 12.8)[e]	0.214 (0.196 to 0.235)[f]

Abbreviations: $1-P_{30}$, errors in estimated glomerular filtration rate >30% of measured glomerular filtration rate; CI, confidence interval; eGFR, estimated glomerular filtration rate; $eGFR_{avg}$, average eGFR using creatinine, cystatin C, β$_2$-microglobulin, and β-trace protein; $eGFR_{B2M}$, eGFR using β2-microglobulin; $eGFR_{BTP}$, eGFR using β-trace protein; $eGFR_{B2M\text{-}BTP}$, eGFR using β2-microglobulin and β-trace protein; $eGFR_{cr}$, eGFR using creatinine; $eGFR_{cys}$, eGFR using cystatin C; $eGFR_{cr\text{-}cys}$, eGFR using creatinine and cystatin C; IQR, interquartile range; RMSE, root mean squared error.
$eGFR_{cr\text{-}cys}$ versus $eGFR_{cr}$: [a]$P < 0.001$, [b]$P = 0.02$, [c]$P < 0.001$, [d]$P = 0.004$; $eGFR_{avg}$ versus $eGFR_{cr\text{-}cys}$: [e]$P = 0.006$, [f]$P = 0.004$.

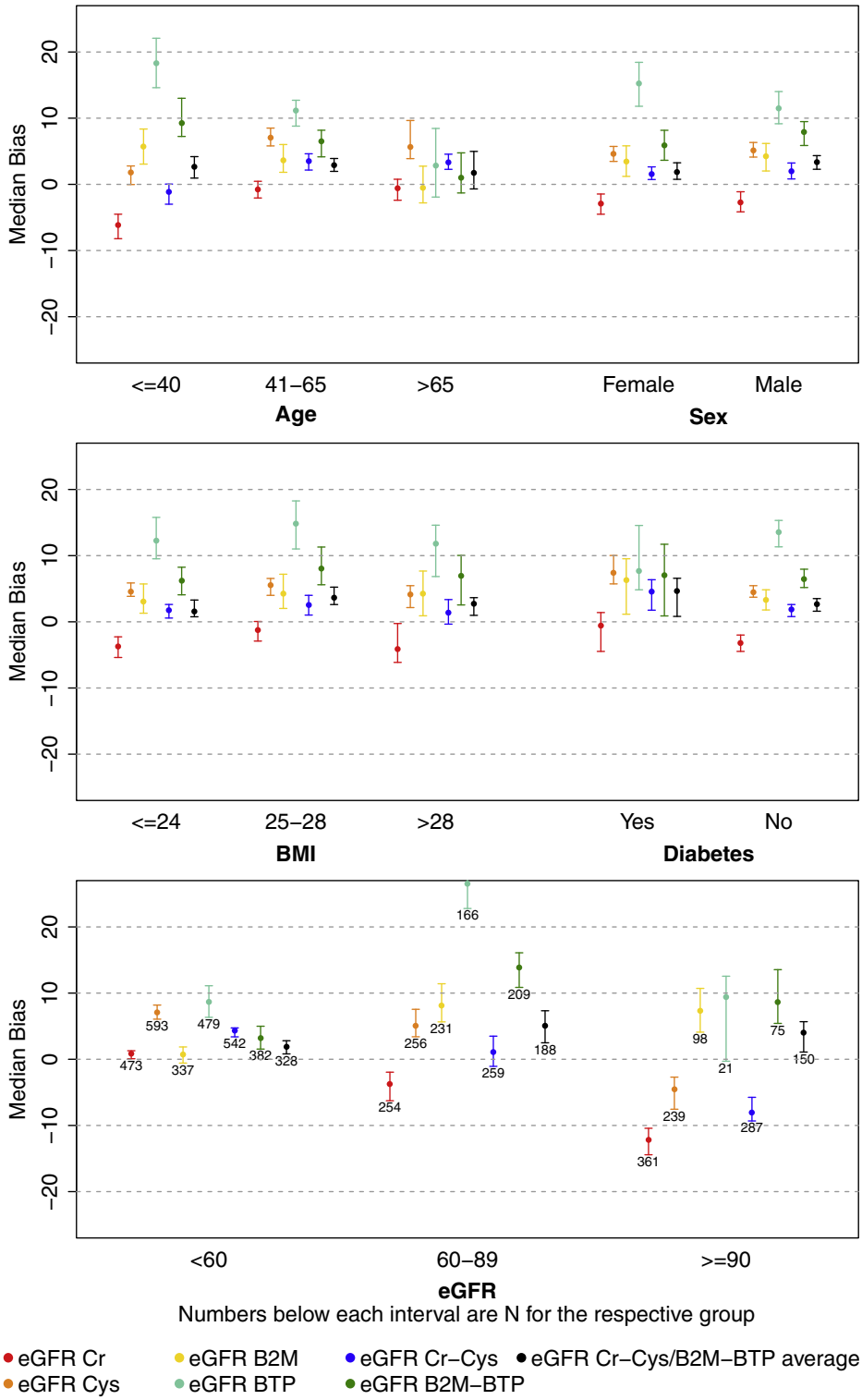

Figure 1. Performance of estimating equations in subgroups of the study population with all filtration markers with creatinine and cystatin C only (n = 1,088) and in the study population with all filtration markers (n = 666) according to age, sex, body mass index (BMI), diabetes, and estimated glomerular filtration rate (eGFR). Bias is defined as measured GFR (mGFR) minus eGFR. A positive value indicates underestimation of mGFR. Abbreviations: $eGFR_{B2M}$, eGFR using β2-microglobulin; $eGFR_{BTP}$, eGFR using β-trace protein; $eGFR_{B2M\text{-}BTP}$, eGFR using β2-microglobulin and β-trace protein; $eGFR_{cr}$, eGFR using creatinine; $eGFR_{cys}$, eGFR using cystatin C; $eGFR_{cr\text{-}cys}$, eGFR using creatinine and cystatin C.

Table 3. Classification of eGFR Versus mGFR Categories in the Study Population With Creatinine and Cystatin C Only and in the Study Population With All Filtration Markers

Equation	Concordance (95% CI)	AUROC for mGFR 60 (95% CI)
Creatinine and Cystatin C Only (N= 1088)		
$eGFR_{cr}$	0.75 (0.73-0.78)	0.90 (0.88-0.91)
$eGFR_{cr\text{-}cys}$	0.80 (0.77-0.82)[a]	0.91 (0.89-0.93)[b]
All Filtration Markers (N= 666)		
$eGFR_{cr}$	0.76 (0.73-0.79)	0.89 (0.87-0.92)
$eGFR_{cr\text{-}cys}$	0.82 (0.79-0.85)[c]	0.92 (0.90-0.94)[d]
$eGFR_{avg}$	0.82 (0.79-0.85)[e]	0.92 (0.90-0.94)[f]

Note: Concordance was determined using mGFR and eGFR categories ≥90, 60 to 89, 45 to 59, 30 to 44, and <30 mL/min/1.73 m^2. Concordance and AUROC vary from 0 to 1.00, with higher values indicating better classification. Abbreviations: AUROC, area under the receiving operating curve; CI, confidence interval; eGFR, estimated glomerular filtration rate; $eGFR_{avg}$, average eGFR using creatinine, cystatin C, β_2-microglobulin, and β-trace protein; $eGFR_{cr(cr\text{-}cys)}$, estimated glomerular filtration rate using creatinine (creatinine and cystatin C); mGFR, measured glomerular filtration rate.
$eGFR_{cr\text{-}cys}$ versus $eGFR_{cr}$: [a]$P = 0.02$, [b]$P = 0.2$, [c]$P = 0.01$, [d]$P = 0.01$; $eGFR_{avg}$ versus $eGFR_{cr\text{-}cys}$: [e]$P = 0.9$, [f]$P = 0.9$.

$eGFR_{cr}$ and higher for $eGFR_{avg}$ than $eGFR_{cr\text{-}cys}$, but differences were not generally statistically significant (Table 3). Using an mGFR threshold of 60 mL/min/1.73 m^2, $eGFR_{cr\text{-}cys}$ did not generally lead to significant reclassification compared with $eGFR_{cr}$, and $eGFR_{avg}$ did not lead to significant reclassification compared to $eGFR_{cr\text{-}cys}$ (Table 4).

DISCUSSION

The main finding of our study in participants in Shanghai and Beijing is that GFR estimation from previously developed equations using a panel of endogenous filtration markers including B2M and BTP in addition to creatinine and cystatin C ($eGFR_{avg}$) was more accurate than from creatinine and cystatin C ($eGFR_{cr\text{-}cys}$). Because $eGFR_{cr\text{-}cys}$ is currently recommended as a confirmatory test for clinical assessment of GFR, our findings may have implications for clinical research and practice.

$eGFR_{B2M}$ and $eGFR_{BTP}$ were not more accurate than $eGFR_{cr}$ and $eGFR_{cys}$, consistent with the hypothesis that the improved accuracy of $eGFR_{avg}$ over $eGFR_{cr\text{-}cys}$ is not due to greater contribution to GFR estimation of B2M and BTP than creatinine or cystatin C, but reflects lesser contribution of non-GFR determinants of each filtration marker as more markers are added to the panel. These findings support the growing literature that GFR estimation can be improved by the use of a panel of filtration markers, even if they are not more strongly associated with mGFR than creatinine and cystatin C.[29,30] However, in this study, the improvement in accuracy did not generally translate into statistically significant improvement in classification or reclassification of mGFR categories by $eGFR_{avg}$ compared with $eGFR_{cr}$ or $eGFR_{cr\text{-}cys}$, and we would not recommend use of B2M or BTP in GFR estimating equations in clinical practice at this time. Possibly the improvement in accuracy was limited because the previously developed equations that we used for $eGFR_{B2M}$ and $eGFR_{BTP}$ were derived in a CKD population with lower mGFR than the study populations in Shanghai and Beijing, and both $eGFR_{B2M}$ and $eGFR_{BTP}$ significantly underestimated mGFR. Prior studies have also shown that equations derived from CKD populations underestimate mGFR in populations with higher mGFRs.[6-8] The underestimation was particularly evident for $eGFR_{BTP}$. We are not aware of estimating equations using B2M and BTP developed in study populations with higher mGFRs. Other limitations to clinical application of B2M and BTP at this time are that assays are not standardized across clinical laboratories and the additional cost of a 4-marker panel compared with measurement of creatinine and cystatin C.

The accuracy of the CKD-EPI equations using GFR_{cr}, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ in our study population was not optimal, similar to previous reports in China, and importantly, not as accurate as in study populations in North America, Europe, and Australia.[12,13] These results reinforce the need for confirmatory testing in China. Of interest, other equations were not more accurate than the CKD-EPI equations in this population, consistent with another recent report.[31] As expected, best-fit equations derived in this population showed generally better performance than the CKD-EPI equations but did not show further improvement by adding B2M and BTP to creatinine and cystatin C. Possibly, GFR estimation in China could be improved by developing alternative estimating equations based on creatinine or cystatin C, as has been done in some other Asian countries[9]; this might limit the potential improvement from adding B2M and BTP. Additional filtration markers, such as other metabolites or low-molecular-weight proteins, might also be helpful.

Our study has several strengths. We studied a large population from 2 urban locations with relevant clinical characteristics. We used consistent mGFR protocols in both locations, using an accepted GFR measurement method and assays for iohexol traceable to a reference laboratory. We used assays for creatinine and cystatin C traceable to international reference materials and assays for B2M and BTP traceable to the research laboratory in which estimating equations were developed. We used guideline-recommended equations for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$. We used accepted metrics for assessing the performance of GFR estimating equations and limited the number of comparisons to avoid false-positive results due to multiple comparisons.

Our study also has limitations. The study population included hospitalized patients and may not be generalizable to clinical settings other than large urban medical centers in China. The study populations from Shanghai and Beijing differed in mGFR, and we observed some differences in the performance of equations between the study populations in Shanghai and Beijing, which may have been the result of differences in measurement methods despite

Table 4. Reclassification of Participants Above or Below mGFR Threshold of 60 mL/min/1.73 m^2 Using $eGFR_{cr\text{-}cys}$ Versus $eGFR_{cr}$ and Using $eGFR_{avg}$ Versus $eGFR_{cr\text{-}cys}$ in the Study Population With Creatinine and Cystatin C Only and the Study Population With All Filtration Markers

		Total Group				Subgroup With mGFR<60			Subgroup With mGFR≥60		
	eGFR Category	N	Total Reclassified	Correctly Reclassified	Incorrectly Reclassified	Overall NRI (95% CI)	Correctly Reclassified	Incorrectly Reclassified	Net Difference	Correctly Reclassified	Incorrectly Reclassified	Net Difference
Creatinine and Cystatin C Only (N= 1,088)												
$eGFR_{cr\text{-}cys}$ vs $eGFR_{cr}$	Overall	1088	87 (8.0%)	48 (4.4%)	39 (3.6%)	0.8% (−0.9% to 2.6%)	42 (3.9%)	3 (0.3%)	3.6%	6 (0.6%)	36 (3.3%)	−2.8%
	30-89	535	83 (15.5%)	45 (8.4%)	38 (7.1%)	1.3% (−2.1% to 4.7%)	39 (7.3%)	3 (0.6%)	6.7%	6 (1.1%)	35 (6.5%)	−5.4%
	45-74	267	65 (24.3%)	38 (14.2%)	27 (10.1%)	4.1% (−1.8% to 10.0%)	32 (12.0%)	3 (1.1%)	10.9%	6 (2.2%)	24 (9.0%)	−6.7%
All Filtration Markers (N= 666)												
$eGFR_{cr\text{-}cys}$ vs $eGFR_{cr}$	Overall	666	52 (7.8%)	33 (5.0%)	19 (2.9%)	2.1% (−0.1% to 4.4%)	29 (4.4%)	1 (0.2%)	4.2%	4 (0.6%)	18 (2.7%)	−2.1%
	30-89	310	49 (15.8%)	30 (9.7%)	19 (6.1%)	3.5% (−1.0% to 8.2%)	26 (8.4%)	1 (0.3%)	8.1%	4 (1.3%)	18 (5.8%)	−4.5%
	45-74	157	37 (23.6%)	25 (15.9%)	12 (7.6%)	8.3% (0.1% to 16.0%)	21 (13.4%)	1 (0.6%)	12.7%	4 (2.5%)	11 (7.0%)	−4.5%
$eGFR_{avg}$ vs $eGFR_{cr\text{-}cys}$	Overall	666	25 (3.8%)	12 (1.8%)	13 (2.0%)	−0.2% (−1.8% to 1.5%)	9 (1.4%)	0 (0.0%)	1.4%	3 (0.5%)	13 (2.0%)	−1.5%
	30-89	329	25 (7.6%)	12 (3.6%)	13 (4.0%)	−0.3% (−3.0% to 3.6%)	9 (2.7%)	0 (0.0%)	2.7%	3 (0.9%)	13 (4.0%)	−3.0%
	45-74	165	24 (14.5%)	12 (7.3%)	12 (7.3%)	−0.0% (−6.3% to 6.3%)	9 (5.5%)	0 (0.0%)	5.5%	3 (1.8%)	12 (7.3%)	−5.5%

Note: Values expressed as number (percent) or percent unless noted otherwise. Unit for eGFR and mGFR is mL/min/1.73 m^2. Value for the NRI ranges from −200 to 200 and is the sum of the percentage of participants who have been correctly reclassified subgroup with mGFR >60 or <60.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; $eGFR_{B2M}$, eGFR using β2-microglobulin; $eGFR_{BTP}$, eGFR using β-trace protein; $eGFR_{B2M\text{-}BTP}$, eGFR using β2-microglobulin and β-trace protein; $eGFR_{cr}$, eGFR using creatinine; $eGFR_{cys}$, eGFR using cystatin C; $eGFR_{cr\text{-}cys}$, eGFR using creatinine and cystatin; CmGFR, measured glomerular filtration rate; N, sample size; NRI, net reclassification index.

our attempt to minimize them. The estimating equations that we used for B2M and BTP may not be optimal for the GFR range of the study population.

In conclusion, our study demonstrates that a panel of endogenous filtration markers including B2M and BTP in addition to creatinine and cystatin C may improve GFR estimation in China. Further study is necessary to determine whether GFR estimation using B2M and BTP can be improved and whether these improvements will lead to useful clinical applications.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1: Assay Methods

Table S2: Comparison of Assays in Shanghai and Beijing to University of Minnesota

Table S3: CKD-EPI GFR Estimating Equations Used in This Study

Table S4: Demographic and Clinical Characteristics of the Study Population With All Filtration Markers From Two Large Urban Chinese Populations, 2013-2016

Table S5: Demographic and Clinical Characteristics of the Shanghai Study Population With Creatinine and Cystatin C Only and With All Filtration Markers

Table S6: Correlations of Filtration Markers With Measured GFR and With Each Other and Partial Correlations Among Filtration Markers After Adjustment for Measured GFR

Table S7: Performance of GFR Estimating Equations in the Subgroups of the Study Population

Table S8a: Comparison of Performance of Newer GFR Estimating equations to the CKD-EPI Equations (N = 1,088)

Table S8b: Comparison of Performance of Newer GFR Estimating equations to the CKD-EPI Equations (N = 666)

Table S9: Performance of "Best-Fit" Equations in the Study Population and Subgroups With All Filtration Markers

Table S10: Performance of GFR Estimating Equations Developed in the Study Population With All Filtration Markers From Shanghai (N = 389) in the Study Population From Beijing (N = 277)

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