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A New Panel Estimated GFR, Including β_2-Microglobulin and β-Trace Protein and Not Including Race, Developed in a Diverse Population

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Article Information

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Abstract

Rationale and Objective: GFR estimation based on creatinine and cystatin C ($eGFR_{cr\text{-}cys}$) is more accurate than eGFR based on either creatinine or cystatin C alone ($eGFR_{cr}$ or $eGFR_{cys}$), but the inclusion of creatinine in $eGFR_{cr\text{-}cys}$ requires specification of a person's race. Beta-2-microglobulin (B2M) and beta-trace protein (BTP) are alternative filtration markers that appear to be less influenced by race than creatinine.

Study Design: Study of diagnostic test accuracy.

Setting and Participants: Development in pooled population of seven studies with 5017 participants with and without chronic kidney disease. External validation in a pooled population of seven other studies with 2245 participants.

Tests compared: Panel eGFR using B2M and BTP in addition to cystatin C (three-marker panel) or creatinine and cystatin C (four-marker panel) with and without age and sex or race.

Outcomes: GFR measured as the urinary clearance of iothalamate, plasma clearance of iohexol, or plasma clearance of Cr-EDTA

Results: Mean measured GFR was 58.1 and 83.2 ml/min/1.73m^2 and the proportion of blacks was 38.6% and 24.0%, in the development and validation populations, respectively. In development, addition of age and sex improved the performance of all equations compared to equations without age and sex, but addition of race did not further improve the performance. In validation, the four-marker panels were more accurate than the three-marker panels ($p<0.001$). The three-marker panel without race was more accurate than $eGFR_{cys}$ [1- P_{30} of 15.6 vs 17.4% ($p=0.014$)], and the four-marker panel without race was as accurate as $eGFR_{cr\text{-}cys}$ [1- P_{30} of 8.6 vs 9.4% ($p=0.17$)]. Results were generally consistent across subgroups.

Limitations: No representation of participants with severe comorbid illness and from geographic areas outside of North America and Europe.

Conclusions: The four-marker panel eGFR is as accurate as $eGFR_{cr\text{-}cys}$, without requiring specification of race. A more accurate race-free eGFR could be an important advance.

Lay summary

Assessment of glomerular filtration rate (GFR) is critical for many aspects of medical practice. GFR estimation based on creatinine and cystatin C together ($eGFR_{cr\text{-}cys}$) is more accurate than eGFR based on either creatinine or cystatin C alone, but the inclusion of creatinine in $eGFR_{cr\text{-}cys}$ requires specification of a person's race. Beta-2-microglobulin (B2M) and beta-trace protein (BTP) are alternative filtration markers that appear to be less influenced by race than creatinine. In a pooled dataset of 7 studies (5017 participants), we developed new estimating equations based on the combinations of these markers, with and without age or sex and race. In a separate pooled dataset of 7 studies (2245 participants) we showed that the equation that used all four markers, age

and sex, but not race, was as accurate as $eGFR_{cr\text{-}cys}$. A more accurate race-free eGFR could be an important advance.

Keywords

Glomerular filtration rate; creatinine; cystatin C; beta trace protein; beta-2-microglobulin; estimating equations; race

Introduction

Clinical assessment of kidney function is part of routine medical care for adults[1]. GFR estimates incorporate clinical and demographic factors (age, sex and race) that explain some of the variation of markers unrelated to GFR, and are more accurate and useful than serum concentrations of endogenous filtration markers alone in each demographic group. Most clinical laboratories report estimated glomerular filtration rate (eGFR) when serum creatinine is measured ($eGFR_{cr}$)[2]. eGFR based on cystatin C ($eGFR_{cys}$) or the combination of creatinine and cystatin C ($eGFR_{cr\text{-}cys}$) are recommended as confirmatory tests for $eGFR_{cr}$[3], however, there are limitations of this approach. $eGFR_{cys}$ is not more accurate than $eGFR_{cr}$, and although $eGFR_{cr\text{-}cys}$ is more accurate than either $eGFR_{cr}$ or $eGFR_{cys}$, it is not independent of $eGFR_{cr}$. Further, in some populations, neither marker provides accurate estimates because the demographic and clinical factors do not accurately account for the non-GFR determinants[4,5].

There is increased scrutiny around use of race in GFR estimation, including current attention by the United States Congress to algorithms that include race [6–9]. The use of Black race in the 2009 CKD-EPI creatinine equation leads to a 16% higher $eGFR_{cr}$ for the same level of creatinine compared to other people,[10] which could worsen care for Blacks because of delayed referral for specialist care, dialysis and transplantation, and may represent an example of race-based medicine[6,7]. Conversely, omission of the Black race coefficient leads to lower $eGFR_{cr}$ compared to measured GFR and could worsen care because of contraindications to life-saving drugs and contrast-imaging procedures[11,12]. Thus, accurate GFR estimates matter in Black people; there is an urgent need to have more accurate GFR estimating equations that do not require a coefficient for race[6,7,12,13].

A panel of endogenous filtration markers could improve the accuracy of GFR estimation by reducing the impact of the non-GFR determinants of each marker and by obviating the need for clinical and demographic factors, and in particular race[14]. Like cystatin C, beta-2-microglobulin (B2M) and beta-trace protein (BTP) are low molecular weight proteins that are filtered by the glomeruli and degraded by the tubules. [15,16] Like cystatin C, they have been shown to be useful in estimating GFR, are less influenced by age, sex and race than creatinine, and are more strongly associated with death and cardiovascular disease compared to creatinine or $eGFR_{cr}$[17–25]. We previously reported that a four-marker panel eGFR including creatinine, cystatin C, B2M and BTP was not more accurate than $eGFR_{cr\text{-}cys}$ in a combined population of 3 US cohorts with CKD, but the panel was more accurate than $eGFR_{cr\text{-}cys}$ in two Chinese cohorts including participants with and without CKD where $eGFR_{cr}$ was less accurate than in the US cohorts[26,27]. We hypothesized that the advantage of

a panel eGFR would be more apparent in diverse populations with and without CKD. The current analysis aimed to evaluate whether including B2M and BTP in a panel eGFR would enable performance comparable to or better than currently recommended equations without the need for creatinine or race.

Methods

Data Sources

Collaborators provided data from research studies and clinical populations (Tables S2a and S2b)[10,26,28–46]. GFR was measured using urinary or plasma clearance of exogenous filtration markers (Table S1). We allocated the datasets into development vs external validation such that each dataset representation of CKD and non-CKD studies and sufficient representation of Black people. We included 7 studies with a total of 5017 participants in the Development Population. We randomly divided this dataset into separate datasets for initial development (n=3,363) and internal validation (n=1,654) (Table S2a, Figure S1). We included 7 additional studies with a total of 2,245 participants in the External Validation Population (Table S2b). We calibrated all methods to urinary clearance of iothalamate (the reference method used for development of the reference equations[10,47]) by reducing the assigned value of other methods by 5%, based on a systematic comparison of all methods (Table S2a)[48]. The institutional review boards of all participating institutions approved each study or the current analysis. For GFR measurements done for research studies, informed consent was obtained by the participating studies at the time of the measurements.

Laboratory Methods

Table S1 describes the analytical methods used for all endogenous filtration markers. We calibrated serum creatinine assays or measured serum creatinine on the Roche enzymatic method (Roche-Hitachi P-Module instrument with Roche Creatininase Plus assay, Hoffman-La Roche, Ltd., Basel, Switzerland), traceable to National Institute Standardized Technology (NIST) creatinine standard reference material 967[49]. We calibrated serum cystatin C assays or measured serum cystatin C on the Siemens Dade Behring Nephelometer (Table S1), traceable to International Federation for Clinical Chemists (IFCC) Working Group for the Standardization of Serum Cystatin C and the Institute for Reference Materials and Measurements (IRMM) certified reference materials[50,51]. We measured B2M on the Siemens Prospec from 2011–2013, the Roche Mod P from 2013–2015, the Roche COBAS from 2015 to 2019. We measured BTP on the Siemens ProSpec from 2013 to 2019. Stability of the assays over time was evaluated using pooled QC material and calibration panels[52].

Development and Validation of Equations

Our a priori hypothesis is that additional endogenous filtration markers can contribute to greater accuracy of GFR estimates because of diminished contribution from non GFR determinants of each marker, potentially eliminating the need for both creatinine and race coefficient. As such, we developed new equations using both B2M and BTP rather than either alone; with creatinine (hereafter referred to as four marker panels) and without creatinine (hereafter referred to as three marker panels); and tested with and without a race coefficient. We selected the 2009 CKD-EPI creatinine equation, 2012 CKD-EPI cystatin C

equation and 2012 CKD-EPI creatinine cystatin C equation as reference equations since they are recommended by current guidelines[3,10,47]. Since all new and reference equations were developed by the CKD-EPI research group, we refer to reference equations only by the filtration marker and publication year.

As in previous work, we pre-specified a process for developing and validating equations[26,47]. In brief, we used least squares linear regression to relate log transformed measured GFR to log of the filtration markers, with or without age and sex or race communities. For each marker, we used nonparametric smoothing splines to characterize the shape of the relationship of log measured GFR with log filtration marker, and then approximated the smoothing splines by piecewise linear splines to represent observed non-linearity. We used the spline for creatinine and cystatin C that we had previously developed[10,47]. For comparison of the magnitude of the race coefficient across markers, we developed equations for each marker alone with and without use of age and sex or race.

In the initial development dataset we compared the new equations to the reference equations fit to this population ($eGFR_{cys}$ for three-marker panels, and $eGFR_{cr\text{-}cys}$ for four-marker panels. Equations that demonstrated improved performance, defined by 3% relative lower RMSE compared to the reference equation were brought into internal validation for verification of the statistical significance of demographic factors. Development and internal validation datasets were combined into one population (called the “Development Population” hereafter) to derive final coefficients.

In the external validation population (hereafter called the “Validation Population”), we compared the new equations to each other and the reference equations. For comparison of the magnitude of the coefficients for the filtration markers, we derived standardized coefficients by re-expressing the equations subtracting each participant’s value from the mean and dividing by the standard deviation, performed separately for each spline term. We compared performance of equations in the overall population and in subgroups, and final equations were selected based on ranking of RMSE overall and within subgroups and clinically significant differences.

Metrics for Equation Performance

We assessed bias as the median of the difference between measured and estimated GFR, and precision as the inter-quartile range (IQR) for the differences[10,53]. We assessed accuracy as root mean square error (RMSE) and as the percentage of estimates greater than 30% different from measured GFR (1- P_{30} respectively). Confidence intervals were calculated by bootstrap methods (2000 bootstraps)[54]. We focus our assessment of the significance of the differences among the new equations and the reference equations for accuracy (1-P_{30} using McNemar’s test and RMSE using the signed rank test) rather than bias, which may be more affected by differences in measurement methods and by regression to the mean. Accuracy metrics incorporate both bias and precision, and 1-P_{30} specifically reflects large errors, which are clinically relevant. We also assessed performance in subgroup of race communities (Black people vs others), eGFR (<30, 30-<60, 60-<89, and >90 ml/min/1.73 m^2), age (< 40, 40–65 and > 65 years), sex, body mass index (BMI) (<20, 20-<25, 25-<30,

and ≥30 kg/m^2) and presence or absence of diabetes. Race was ascertained by the investigators or study participants at the time of data collection in each study.

Results

Clinical Characteristics

In the development population, mean (standard deviation) measured GFR was 58.1 (29.7) mL/min/1.73m^2 (range 3.0 to 186.0 mL/min/1.73m^2) (Table 1). The mean (standard deviation, range) age was 55.7 (15.9, 18–92) years, 43.8% female, and 38.6% were Black people. In the validation population, mean (standard deviation) measured GFR was 83.2 (27.4) mL/min/1.73m^2 (range 8.0 to 184.0 mL/min/1.73m^2), the mean (SD, range) age 52.8 (12.8, 18–91) years old and 29% were female. Black people were in 5 of the 7 development cohorts (> 5% in 3 of the 7 cohorts and 39% overall) and in all of validation cohorts (> 5% in 5 of the 7 cohorts and 24% overall) (Table 1). Clinical characteristics of the participants in each study are shown in Table S2.

Development

As expected, all filtration markers were correlated negatively with measured GFR and positively with each other for cystatin C and B2M (Table S3). After adjusting for measured GFR, the correlations among filtration markers ranged from 0.508 (95% confidence intervals [CI] 0.487, 0.528) for creatinine and BTP to 0.774 (95% CI 0.763, 0.785) for cystatin C and B2M (Table S3).

We identified a spline for BTP, with a knot at 0.6 mg/L. In single-marker equations, race coefficients deviated further from 1.0 for equations with creatinine and BTP [1.160 (95% CI: 1.146, 1.174) and 0.861 (95% CI 0.848, 0.874, respectively] compared to those for cystatin C and B2M [0.991 (95% CI 0.979, 1.003) and 0.974 (95% CI 0.960, 0.987), respectively] (Table S4). The coefficient for race in the four-marker panel was significantly smaller than for the $eGFR_{cr\text{-}cys}$ [1.052 (95% CI: 1.040, 1.064) vs. 1.08 (95% CI 1.067, 1.093)].

In the overall population, regardless of the inclusion or exclusion of age and sex or race, four-marker panels were more accurate than the corresponding three-marker panels (Table S5). Addition of age and sex improved the performance of the three-marker and four-marker panels compared to panels without age and sex, but the addition of race did not further improve performance (Table S5). Results were generally similar in subgroups of people from Black vs other communities.

External Validation

Table 2 shows the equations for the three-marker and four-marker panels we are recommending (See Table S6 for additional formulas which might be of interest in research studies including equations which used either of the two novel markers). Variables in the three-marker panel include cystatin C, B2M, BTP, age and sex. Variables in the four-marker panel include creatinine, cystatin C, B2M, BTP, age and sex. Standardized coefficients for creatinine were less negative (weaker) for the four-marker panel compared to the $eGFR_{cr}$ and $eGFR_{cr\text{-}cys}$ [−0.208 (95% CI −0.219,−0.196) vs −0.558 (95% CI −0.558,−0.565) and

−0.282 (95% CI −0.296, −0.268), respectively]. The new equations had less bias compared to 2015 B2M and BTP equations developed in CKD populations (Table S7).

$eGFR_{cr\text{-}cys}$ (equation 5) was more accurate than both $eGFR_{cr}$ (equation 1) and $eGFR_{cys}$ (equation 2) (Tables 3 and S8). $eGFR_{cr}$ were more accurate than the $eGFR_{cys}$ equation and the four-marker panels (equations 6 and 7) were more accurate than the three-marker panels (equations 3 and 4) ($p<0.001$)]. The three-marker panel without race (equation 4) was more accurate than $eGFR_{cys}$ (equation 2) [1-P_{30} of 15.6 vs 17.4 ($p=0.014$)]. The four-marker panel without race (equation 7) was as accurate as $eGFR_{cr\text{-}cys}$ (equation 5) [1- P_{30} of 8.6 vs 9.4% ($p=0.17$)]. The addition of race to the three-marker (equation 3) and four-marker panel (equation 6) led to small further improvements in accuracy (1-P_{30} of 14.8 and 8.4%, respectively), and the four-marker panel with race (equation 6) was nominally significantly more accurate than $eGFR_{cr\text{-}cys}$ (equation 5) ($p=0.048$). Comparisons of RMSE were generally consistent. Results were generally consistent across subgroups in Black vs. other peoples (Figure 1 and Table S8), and across subgroups of eGFR, age, sex, diabetes and BMI (Figures S2 –S6). Results using non-calibrated measured GFR were generally more accurate than with calibrated measured GFR. Using non-calibrated measured GFR, the 4-marker panel without race was more accurate than $eGFR_{cr\text{-}cys}$ (Table S9).

$eGFR_{cr\text{-}cys}$ (equation 5) was unbiased, but $eGFR_{cr}$ (equation 1) overestimated and $eGFR_{cys}$ (equation 2) underestimated measured GFR. There was differential bias by race groups for $eGFR_{cr}$, $eGFR_{cys}$ and $eGFR_{cr\text{-}cys}$. The three-marker panels (equations 3–4) and four-marker panels (equations 6–7) underestimated measured GFR, but improved the differential bias between race groups (Figure 1 and Table S8).

Discussion

Accurate assessment of GFR is essential for detection, staging, and assessment of progression, management, prognostication, and drug dosage adjustment in chronic kidney disease. Estimated GFR using creatinine and cystatin C are widely used, but the inclusion of demographic variables in GFR estimating equations, particularly specification of race, has raised concerns about serious negative consequences for delivery of care and reinforcing implicit bias[6,7,12,13]. Availability of rigorously developed, more accurate GFR estimating equations that do not require specification of race could improve their utility and broad acceptance[6,7]. Our main findings are that the addition of BTP and B2M to cystatin C in a three marker panel without race improved the precision and accuracy compared to $eGFR_{cys}$ and the addition of BTP and B2M to creatinine and cystatin C in a four marker panel without race was more accurate than the three- marker panel and as accurate as $eGFR_{cr\text{-}cys}$ which includes race. More accurate equations that can be used as a confirmatory or alternative test to $eGFR_{cr}$ that do not require use of creatinine or race could be a major advance.

The serum concentrations of all endogenous filtration markers are influenced by their non-GFR determinants, including their generation, tubular reabsorption and secretion, and extra-renal elimination, all of which lead to error in GFR estimates[1,55]. Serum creatinine is affected by muscle mass and diet, and drugs that inhibit tubular secretion of creatinine or

extra-renal elimination of creatinine. Demographic characteristics such as age, sex and race have been used as surrogates for some of the non-GFR determinants in GFR estimating equations, but they represent average values for the relationship between the marker and its non-GFR determinants and can lead to error in individuals, and bias and imprecision in populations with variation in non-GFR determinants of the marker that differ from the development population. Importantly, race is a social versus a biological construct. Prior studies have suggested that genetic measures of ancestry might be a better tool to account for the possible variation in creatinine generation by Black ancestry[56]. We do not advocate use of ancestry markers at this time as it would require their measurement for GFR estimation and would add complexity to the implementation of eGFR reporting. Moreover, it would not explain the observed geographical variation with the use of the current race coefficient between Black people in the United States and Europe vs Africa[57–62]. The panel eGFR equations reported here are a further advance as they do not require consideration of race or ancestry. Further work is required to determine if the new equations presented here are more robust across geographical regions.

Guidelines recommend use of confirmatory tests for $eGFR_{cr}$ in clinical scenarios where a more precise and accurate estimate of GFR is required[3]. Serum cystatin C is less affected by race than creatinine, but is affected by obesity, inflammation, smoking and alterations in thyroid and adrenal hormones[63–69] and as such $eGFR_{cys}$ is not more accurate than $eGFR_{cr}$[47]. We have previously shown that a panel of multiple non-correlated filtration markers can result in a more accurate estimate and minimize the requirement for demographic factors by diminishing the impact of the non GFR determinants of each marker on the resulting GFR estimate[14]. Here, we show that the addition of B2M and BTP to cystatin C in the three-marker panel eGFR provided greater accuracy than $eGFR_{cys}$ but not $eGFR_{cr\text{-}cys}$, reflecting the important contribution of creatinine to GFR estimation in the populations included in this study. The addition of B2M and BTP to creatinine and cystatin C in the four-marker panel was more accurate than $eGFR_{cr\text{-}cys}$, and allowed elimination of race with similar performance of $eGFR_{cr\text{-}cys}$. Although the four-marker panel eGFR is also not independent of creatinine the magnitude of the creatinine coefficient is attenuated compared to the 2012 creatinine-cystatin C equation, thus reducing the contribution of creatinine to the four-marker panel eGFR. Overall, these findings are consistent with our hypothesis, and suggests a path forward to improved GFR estimation and without the need for specification of race.

Strengths of this study include its design, with separate large databases for development and validation of the new equations, a diverse development population including participants with and without CKD, higher measured GFR compared to our 2015 BTP and B2M equations, and a pre-specified rigorous statistical analytical plan for testing of all variables. The pooled development and validation databases in these diverse development populations allows for greater general applicability than our previous equations. Comparison of equations in a separate validation population overcomes limitations of differences among studies in patient characteristics and methods for measurement of GFR. We attempted to minimized differences by GFR measurement method by calibrating the measured GFR using a common method[48].

The major limitations of these and existing GFR estimating equations is their development in ambulatory populations without serious comorbidity and lack of representation from geographically diverse groups. Specifically, our study population does not include participants with acute or serious chronic comorbidity that may cause malnutrition and muscle wasting, which may potentially affect creatinine more than cystatin C, B2M and BTP, such that $eGFR_{cys}$ or the three-marker panel without creatinine could be preferred as alternative tests initial tests for GFR evaluation. It is possible that in these settings, where creatinine is likely to perform poorly, the three-marker panel might provide greater accuracy, or conversely, the non GFR determinants might have a greater contribution to the overall eGFR and lead to decreased accuracy. Further evaluation in these populations is required to consider these possibilities.

There are other limitations. First, the mean GFR in the development population is higher than in the CKD populations used to develop the 2015 equations, it is lower than in the development population for the 2009 creatinine and 2012 cystatin C equations and in the External Validation population in this study, thus regression to the mean is a likely explanation for the underestimation of measured GFR in the validation population in the current study. However, performance was consistent across range of GFR suggesting that this may not decrease generalizability. Another limitation is possible variation in measurement methods for endogenous filtration markers over time, even though we used a single laboratory for calibration or measurement in all studies, and had calibration panels and quality control samples to evaluate stability over time[52]. In addition, GFR is known to be measured with error, which may account for some of the observed imprecision[65].

Several steps would need to be done prior to implementation. First, clinical and laboratory practice guidelines should consider indications and preferred diagnostic strategies for laboratory testing and reporting panel eGFRs that include consideration of local public health priorities, clinical practice patterns, and cost/benefit analyses. Second, although our research laboratory has observed stability in filtration marker assays over a decade[52], variation in these assays among laboratories could lead to errors, with potential for errors compounded with each additional analyte. Thus manufacturers and clinical chemists would need to develop standards as have been developed for creatinine and cystatin C.[70] Finally, we suggest investigations into the cost effectiveness of these additional tests in clinical settings where GFR levels affect management decisions. Current attention by Congress suggests an avenue for advocacy for sensible cost structure for GFR confirmatory tests[8,9]

In conclusion, we present three-marker and four-marker panel eGFRs using B2M and BTP, which do not include race as confirmatory or alternative tests for $eGFR_{cr}$. The four-marker panel eGFR is less dependent on creatinine and is as accurate as 2012 creatinine-cystatin C equation. An eGFR that does not require race and is less dependent on creatinine could provide more robust GFR estimates across a greater variety of populations. Further studies are required to understand how best to use these equations in clinical practice, especially in diverse clinical settings and geographical locations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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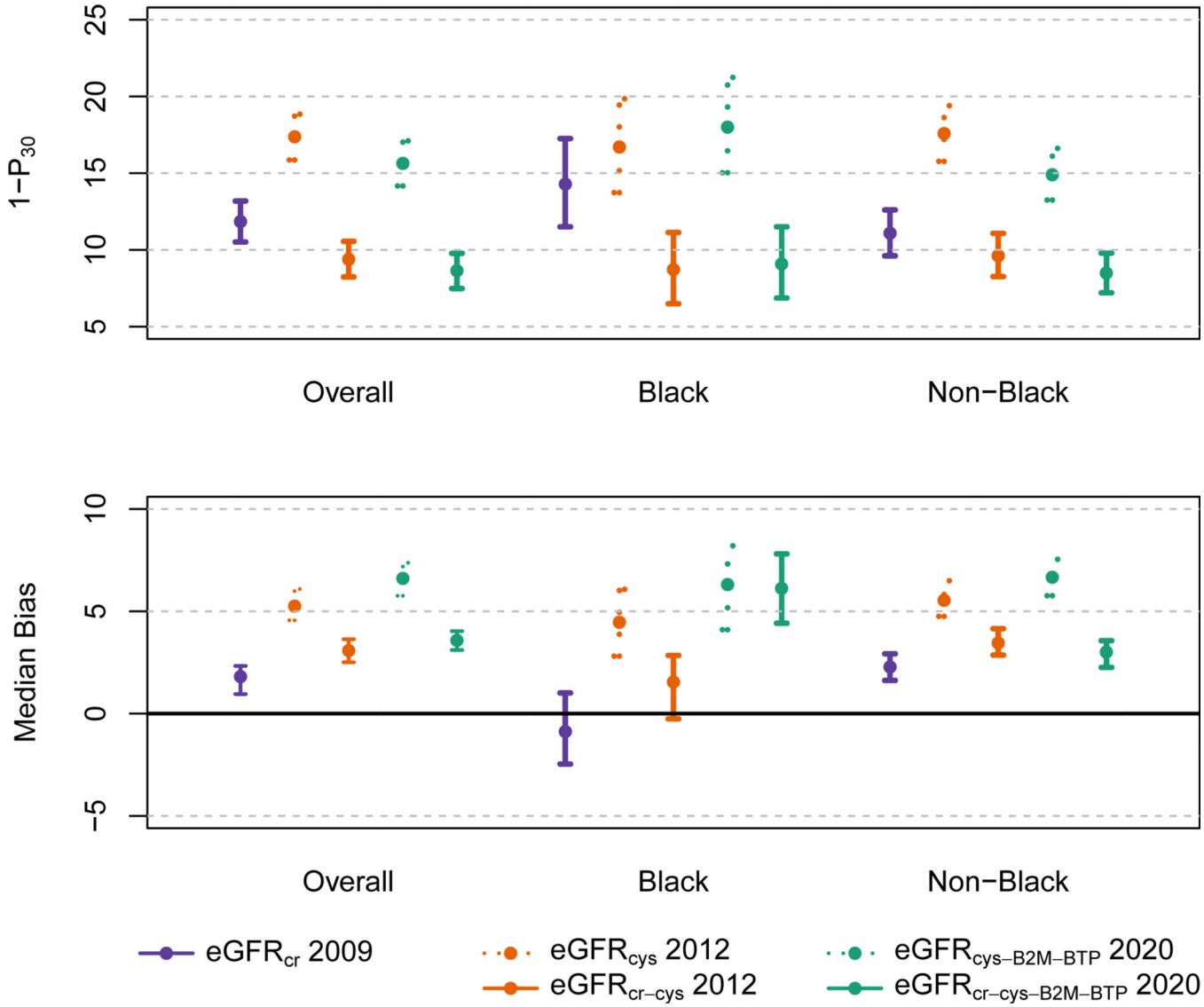

Figure 1: Performance of Reference eGFR and Panel eGFR Equations in the External Validation Population and Overall and by Race

Accuracy as measured by 1- P_{30} or the percentage of estimates greater than 30% of measured GFR. The vertical bars indicate 95% confidence intervals. Solid lines indicate equations that include creatinine. Purple indicates the 2009 CKD-EPI creatinine equation; Orange indicates the 2012 CKD-EPI cystatin C and creatinine-cystatin C equations; Green indicates the new 2020 CKD-EPI three and four marker panels.

Table 1

Participant Characteristics in Study Populations

	Development (N=5017) ***N (%) or Mean (SD)***	**External Validation (N=2245)** ***N (%) or Mean (SD)***
Age, years	55.7 (15.9)	52.8 (12.8)
<40	893 (17.8)	331 (14.7)
40–65	2689 (53.6)	1570 (69.9)
>65	1435 (28.6)	344 (15.3)
Female	2198 (43.8)	652 (29.0)
Black people	1934 (38.6)	539 (24.0)
BMI, kg/m^2	29.0 (6.1)	27.5 (5.4)
<20	131 (2.6)	82 (3.7)
20-<25	1212 (24.2)	692 (30.9)
25-<30	1870 (37.3)	878 (39.2)
≥30	1804 (36.0)	588 (26.3)
Diabetes	1296 (27.4)	731 (34.7)
Measured GFR, $ml/min/1.73m^2$	58.1 (29.7)	83.2 (27.4)
<30	858 (17.1)	52 (2.3)
30-<60	2091 (41.7)	414 (18.4)
60-<90	1387 (27.7)	846 (37.7)
≥90	681 (13.6)	933 (41.6)
Creatinine, mg/dL	1.6 (0.9)	1.1 (0.5)
Cystatin C, mg/L	1.5 (0.6)	1.2 (0.5)
B2M, mg/L	3.8 (2.3)	2.6 (1.5)
BTP, mg/L	1.2 (0.8)	0.8 (0.4)

Development includes initial development and internal Validation (Figure S1). B2M, Beta 2-Microglobulin; BTP, Beta-Trace Protein.

Note: Values for categorical variables are given as number (percentage), for continuous variables, mean (standard deviation)

Table 2 :

Variables and Coefficients in 2020 Equations in Development and Internal Validation Population

Sex	Serum Creatinine mg/dl	Serum Cystatin C mg/L	Serum BTP mg/L	Equation for Estimating GFR
2020 Cystatin C-B2M-BTP Equation				
Female		≤0.8	≤0.6	$110 \times (Scys/0.8)^{-0.876} \times B2M^{-0.205} \times (SBTP/0.6)^{0.038} \times 0.999^{age}$
			>0.6	$110 \times (Scys/0.8)^{-0.876} \times B2M^{-0.205} \times (SBTP/0.6)^{-0.243} \times 0.999^{age}$
		>0.8	≤0.6	$110 \times (Scys/0.8)^{-0.697} \times B2M^{-0.205} \times (SBTP/0.6)^{0.038} \times 0.999^{age}$
			>0.6	$110 \times (Scys/0.8)^{-0.697} \times B2M^{-0.205} \times (SBTP/0.6)^{-0.243} \times 0.999^{age}$
Male		≤0.8	≤0.6	$120 \times (Scys/0.8)^{-0.876} \times B2M^{-0.205} \times (SBTP/0.6)^{0.038} \times 0.999^{age}$
			>0.6	$120 \times (Scys/0.8)^{-0.876} \times B2M^{-0.205} \times (SBTP/0.6)^{-0.243} \times 0.999^{age}$
		>0.8	≤0.6	$120 \times (Scys/0.8)^{-0.697} \times B2M^{-0.205} \times (SBTP/0.6)^{0.038} \times 0.999^{age}$
			>0.6	$120 \times (Scys/0.8)^{-0.697} \times B2M^{-0.205} \times (SBTP/0.6)^{-0.243} \times 0.999^{age}$
2020 Creatinine-Cystatin C-B2M-BTP Equation				
Female	≤0.7	≤0.8	≤0.6	$123 \times (Scr/0.7)^{-0.243} \times (Scys/0.8)^{-0.519} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$123 \times (Scr/0.7)^{-0.243} \times (Scys/0.8)^{-0.519} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.177}$ x0.996^{age}
		>0.8	≤0.6	$123 \times (Scr/0.7)^{-0.243} \times (Scys/0.8)^{-0.423} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$123 \times (Scr/0.7)^{-0.243} \times (Scys/0.8)^{-0.423} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
Female	>0.7	≤0.8	≤0.6	$123 \times (Scr/0.7)^{-0.471} \times (Scys/0.8)^{-0.519} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$123 \times (Scr/0.7)^{-0.471} \times (Scys/0.8)^{-0.519} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
		>0.8	≤0.6	$123 \times (Scr/0.7)^{-0.471} \times (Scys/0.8)^{-0.423} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$123 \times (Scr/0.7)^{-0.471} \times (Scys/0.8)^{-0.423} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
Male	≤0.9	≤0.8	≤0.6	$131 \times (Scr/0.9)^{-0.295} \times (Scys/0.8)^{-0.519} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$131 \times (Scr/0.9)^{-0.295} \times (Scys/0.8)^{-0.519} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
		>0.8	≤0.6	$131 \times (Scr/0.9)^{-0.295} \times (Scys/0.8)^{-0.423} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$131 \times (Scr/0.9)^{-0.295} \times (Scys/0.8)^{-0.423} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
Male	>0.9	≤0.8	≤0.6	$131 \times (Scr/0.9)^{-0.471} \times (Scys/0.8)^{-0.519} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$131 \times (Scr/0.9)^{-0.471} \times (Scys/0.8)^{-0.519} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
		>0.8	≤0.6	$131 \times (Scr/0.9)^{-0.471} \times (Scys/0.8)^{-0.423} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$

Sex	Serum Creatinine mg/dl	Serum Cystatin C mg/L	Serum BTP mg/L	Equation for Estimating GFR
			>0.6	$131 \times (Scr/0.9)^{-0.471} \times (Scys/0.8)^{-0.423} \times B2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$

** 2020 Cystatin C- B2M-BPT equation can be expressed as a single equation: $120 \text{ X } \min(Scys/0.8,1)^{-0.876} \text{ X } \max(Scys/0.8,1)^{-0.697} \text{ X } B2M^{-0.205} \text{ X } \min(SBTP/0.6,1)^{0.038} \text{ X } \max(SBTP/0.6,1)^{-0.243} \text{ X } 0.999^{age}$ [X 0.922 if female], where Scys is serum cystatin C, B2M, Beta2-Microglobulin; BTP, Beta-Trace Protein

*** 2020 Creatinine-Cystatin C-B2M-BTP Equation can be expressed as a single equation $131 \text{ X } \min(Scr/k,1)^{\alpha} \text{ X } \max(Scr/k,1)^{-0.471} \text{ X } \min(Scys/0.8,1)^{-0.519} \text{ X } \max(Scys/0.8,1)^{-0.423} \text{ X } B2M^{-0.103} \text{ X } \min(SBTP/0.6,1)^{-0.004} \text{ X } \max(SBTP/0.6,1)^{-0.177} \text{ X } 0.996^{age}$ [X 0.937 if female] where Scr is serum creatinine Scys is serum cystatin C, SB2M is serum B2M, SBTP is serum BTP; B2M, Beta 2-Microglobulin; BTP, Beta-Trace Protein, k is 0.7 for females and 0.9 males, α is -0.243for females and -0.295for males, min indicates the minimum of Scr/k or 1, max indicates the maximum of Scr/k or 1

All equations were developed by the CKD-EPI research group.

Table 3:

Performance of Estimating Equations in the External Validation Dataset

Equation Description				Performance	
Group	Equation Number Year of Publication	Filtration markers	Demographics	Overall Population	
				1-P_{30}	RMSE
Equation with Creatinine only	1. Ref (2009)[10]	Creatinine	Age, sex, race	11.8 (10.5, 13.2)	0.199 (0.193, 0.206)
Equations with Cystatin C	2. Ref (2012)[47]	Cystatin C	Age, sex	17.4 [1] (15.9, 18.88)	0.262 [1] (0.250, 0.274)
	3. New (2020)	Cystatin C-B2M-BTP	Age, sex, race	14.8 [2] (13.4, 16.2)	0.256 [2] (0.243, 0.268)
	4. New/Recommended (2020)	Cystatin C-B2M-BTP	Age, sex	15.6 [2] (14.2, 17.1)	0.259 [2] (0.247, 0.271)
Equations with Creatinine and Cystatin C	5. Ref (2012)[47]	Creatinine -Cystatin C	Age, sex, race	9.4 [1] (8.2, 10.6)	0.199 [1] (0.191, 0.206)
	6. New (2020)	Creatinine - Cystatin C-B2M-BTP	Age, sex, race	8.4 [5, 3] (7.3, 9.5)	0.195 [5, 3] (0.187, 0.203)
	7. New/Recommended (2020)	Creatinine -Cystatin C-B2M-BTP	Age, sex	8.6 [5,4] (7.5, 9.8)	0.197 [5, 4] (0.188, 0.205)

1-P_{30} and RMSE are measures of accuracy. 1-P_{30} is computed as 100 percent minus the percent of estimates greater than 30% of the measured GFR; RMSE, root mean square error; B2M, Beta2-Microglobulin; BTP, Beta-Trace Protein; A, age; S, sex; R, race;

Reference equations are the 2009 CKD-EPI creatinine equation (1), 2012 CKD-EPI cystatin C equation (2), and 2012 CKD-EPI creatinine-cystatin C equation (3). The superscript number indicates the comparator equation, and formatting of the superscript informs direction of the comparison. No underline marking indicates that the equation is better than the comparator equation for a p-value ≤ 0.05; an underline marking indicates that the equation is worse than the comparator equation for p-value ≤ 0.05. Double underline indicates that the equation is neither better nor worse than the comparator equation P >0.05.