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A New Panel-Estimated GFR, Including β₂-Microglobulin and β-Trace Protein and Not Including Race, Developed in a Diverse Population

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Rationale and Objective: Glomerular filtration rate (GFR) estimation based on creatinine and cystatin C (eGFR$_{cr-cys}$) is more accurate than estimated GFR (eGFR) based on creatinine or cystatin C alone (eGFR$_{cr}$ or eGFR$_{cys}$, respectively), but the inclusion of creatinine in eGFR$_{cr-cys}$ requires specification of a person's race. β₂-Microglobulin (B2M) and β-trace protein (BTP) are alternative filtration markers that appear to be less influenced by race than creatinine is.

Study Design: Study of diagnostic test accuracy.

Setting and Participants: Development in a pooled population of 7 studies with 5,017 participants with and without chronic kidney disease. External validation in a pooled population of 7 other studies with 2,245 participants.

Tests Compared: Panel eGFR using B2M and BTP in addition to cystatin C (3-marker panel) or creatinine and cystatin C (4-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 [⁵¹Cr]EDTA.

Results: Mean measured GFRs were 58.1 and 83.2 mL/min/1.73 m², and the proportions of Black participants were 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 with equations without age and sex, but addition of race did not further improve the performance. In validation, the 4-marker panels were more accurate than the 3-marker panels ($P < 0.001$). The 3-marker panel without race was more accurate than eGFR$_{cys}$ (percentage of estimates greater than 30% different from measured GFR [$1 - P_{30}$] of 15.6% vs 17.4%; $P = 0.01$), and the 4-marker panel without race was as accurate as eGFR$_{cr-cys}$ ($1 - P_{30}$ of 8.6% vs 9.4%; $P = 0.2$). 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 4-marker panel eGFR is as accurate as eGFR$_{cr-cys}$ without requiring specification of race. A more accurate race-free eGFR could be an important advance.

Clinical assessment of kidney function is part of routine medical care for adults.[1] Glomerular filtration rate (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

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than serum concentrations of endogenous filtration markers alone in each demographic group. Most clinical laboratories report estimated GFR (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$_{cr-cys}$) are recommended as confirmatory tests for eGFR$_{cr}$,[3] but there are limitations to this approach. eGFR$_{cys}$ is not more accurate than eGFR$_{cr}$, and, although eGFR$_{cr-cys}$ is more accurate than 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 US Congress to algorithms that include race.[6-9] The use of Black race in the 2009 Chronic Kidney Disease (CKD) Epidemiology Collaboration (CKD-EPI) creatinine equation leads to a 16% higher eGFR$_{cr}$ for the same level of creatinine as other people,[10] which could worsen care for Black patients 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 with measured GFR (mGFR) 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 clinical and demographic factors, particularly race.[14] Like cystatin C, β₂-microglobulin (B2M) and β-trace protein (BTP) are low-molecular-weight proteins that are filtered by the glomeruli and degraded by the tubules.[15,16]

PLAIN-LANGUAGE 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$_{cr-cys}$) is more accurate than eGFR based on creatinine or cystatin C alone, but the inclusion of creatinine in eGFR$_{cr-cys}$ requires specification of a person's race. β$_2$-Microglobulin and β-trace protein are alternative filtration markers that appear to be less influenced by race than creatinine is. In a pooled dataset of 7 studies (5,017 participants), new estimating equations were developed based on the combinations of these markers with and without age or sex and race. In a separate pooled dataset of 7 studies (2,245 participants), an equation that used all 4 markers, including age and sex but not race, was as accurate as eGFR$_{cr-cys}$. A more accurate race-free eGFR could be an important advance.

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 than creatinine or eGFR$_{cr}$.[17-25] We previously reported that a 4-marker panel eGFR including creatinine, cystatin C, B2M, and BTP was not more accurate than eGFR$_{cr-cys}$ in a combined population of 3 US cohorts with CKD, but the panel was more accurate than eGFR$_{cr-cys}$ in 2 Chinese cohorts including participants with and without CKD in which 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 present analysis aimed to evaluate whether the inclusion of 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 (Table S1).[10,26,28-46] GFR was measured using urinary or plasma clearance of exogenous filtration markers. We allocated the datasets into development versus external validation, such that each dataset represented CKD and non-CKD studies and showed sufficient representation of Black people. We included 7 studies with a total of 5,017 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; Fig S1; Table S1). We included 7 additional studies with a total of 2,245 participants in the external validation population (Table S1). For the

calibrated mGFR values shown in Table S1, 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.[48] The institutional review boards of all participating institutions approved each study or the present 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 S2 describes the analytical methods used for all endogenous filtration markers. We calibrated serum creatinine assays to, or measured serum creatinine with, the Roche enzymatic method (Roche-Hitachi Modular P instrument with Roche Creatininase Plus assay; Hoffman-La Roche Ltd), traceable to National Institute Standardized Technology creatinine standard reference material 967.[49] We calibrated serum cystatin C assays to or measured serum cystatin C on a Siemens Dade Behring Nephelometer (Table S2), traceable to the International Federation for Clinical Chemists Working Group for the Standardization of Serum Cystatin C and the Institute for Reference Materials and Measurements–certified reference materials.[50,51] B2M was measured on a Siemens Prospec from 2011 to 2013, a Roche Modular P from 2013 to 2015, and a Roche COBAS from 2015 to 2019. BTP was measured on a Siemens ProSpec from 2013 to 2019. Stability of the assays over time was evaluated using pooled quality-control 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 creatinine and race coefficients. As such, we developed new equations using both B2M and BTP rather than either alone, with creatinine (hereafter referred to as 4-marker panels) and without creatinine (hereafter referred to as 3-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 because they are recommended by current guidelines.[3,10,47] Because all new and reference equations were developed by the CKD-EPI research group, these equations are referred to by filtration marker and publication year.

As in previous work, we prespecified a process for developing and validating equations.[26,47] In brief, we used least-squares linear regression to relate logarithmically transformed mGFR to log-transformed filtration markers with or without age and sex or race coefficients. For each marker, we used nonparametric smoothing splines to

characterize the shape of the relationship of log-transformed mGFR with log-transformed filtration marker and then approximated the smoothing splines by piecewise linear splines to represent observed nonlinearity. We used the spline for creatinine and cystatin C 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 the use of age and sex or race.

In the initial development dataset, we compared the new equations versus the reference equations fit to this population (eGFR$_{cys}$ for 3-marker panels and eGFR$_{cr-cys}$ for 4-marker panels). Equations that demonstrated improved performance, defined by 3% relative lower root mean squared error (RMSE) compared with 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 versus 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, which was 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 interquartile range for the differences.[10,53] We assessed accuracy as RMSE and as the percentage of estimates greater than 30% different from mGFR $(1 - P_{30})$. CIs were calculated by bootstrap methods (2,000 bootstraps).[54] In assessing the significance of the differences among the new equations and the reference equations we focused on accuracy $(1 - P_{30}$ by McNemar's test and RMSE by 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 bias and precision, and $1 - P_{30}$ specifically reflects large errors, which are clinically relevant. Performance in subgroups was also assessed, including race communities (Black people vs others) and subgroups based on eGFR (<30, 30-<60, 60-<89, and >90 mL/min/1.73 m^2), age (<40, 40-65, and >65 y), sex, body mass index (<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 mGFR was 58.1 ± 29.7 (standard deviation) mL/min/1.73 m^2 (range, 3.0-186.0 mL/min/1.73 m^2; Table 1). The mean age was 55.7 ± 15.9 years (range, 18-92 years); 43.8% were female, and 38.6% were Black. In the validation population, mean mGFR was 83.2 ± 27.4 mL/min/1.73 m^2 (range, 8.0-184.0 mL/min/1.73 m^2), the mean age was 52.8 ± 12.8 years (range, 18-91 years), and 29% were female. Black people were included in 5 of the 7 development cohorts (>5% in 3 of the 7 cohorts and 39% overall) and in all 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 S1.

Development

As expected, all filtration markers were correlated negatively with mGFR and positively with each other (Table S3). After adjusting for mGFR, the correlations among filtration markers ranged from 0.508 (95% 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).

Table 1. Participant Characteristics in Study Populations

Characteristic	Development (n = 5,017)	External Validation (n = 2,245)
Age, y	55.7 ± 15.9	52.8 ± 12.8
Age category		
<40 y	893 (17.8%)	331 (14.7%)
40-65 y	2,689 (53.6%)	1,570 (69.9%)
>65 y	1,435 (28.6%)	344 (15.3%)
Female sex	2,198 (43.8%)	652 (29.0%)
Black race	1,934 (38.6%)	539 (24.0%)
BMI, kg/m^2	29.0 ± 6.1	27.5 ± 5.4
BMI category		
<20 kg/m^2	131 (2.6%)	82 (3.7%)
20-<25 kg/m^2	1,212 (24.2%)	692 (30.9%)
25-<30 kg/m^2	1,870 (37.3%)	878 (39.2%)
≥30 kg/m^2	1,804 (36.0%)	588 (26.3%)
Diabetes	1,296 (27.4%)	731 (34.7%)
mGFR, mL/min/1.73 m^2	58.1 ± 29.7	83.2 ± 27.4
mGFR category		
<30 mL/min/1.73 m^2	858 (17.1%)	52 (2.3%)
30-<60 mL/min/1.73 m^2	2,091 (41.7%)	414 (18.4%)
60-<90 mL/min/1.73 m^2	1,387 (27.7%)	846 (37.7%)
≥90 mL/min/1.73 m^2	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 (Fig S1). Values for categorical variables are given as number (percentage), for continuous variables, mean ± standard deviation.
Abbreviations: BMI, body mass index; B2M, β₂-microglobulin; BTP, β-trace protein; mGFR, measured glomerular filtration rate.

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 with 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 4-marker panel was significantly lower than for eGFR$_{cr-cys}$ (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, 4-marker panels were more accurate than the corresponding 3-marker panels (Table S5). Addition of age and sex improved the performance of the 3-marker and 4-marker panels compared with 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 versus other communities.

External Validation

Table 2 shows the equations for the 3-marker and 4-marker panels we are recommending (Table S6 provides additional formulas that might be of interest in research studies including equations using either of the 2 novel markers). Variables in the 3-marker panel include cystatin C, B2M, BTP, age, and sex. Variables in the 4-marker panel include creatinine, cystatin C, B2M, BTP, age, and sex. Standardized coefficients for creatinine were less negative (ie, weaker) for the 4-marker panel compared with eGFR$_{cr}$ and eGFR$_{cr-cys}$ (−0.208 [95% CI, −0.219 to −0.196] vs −0.558 [95% CI, −0.558 to −0.565] and −0.282 [95% CI, −0.296 to −0.268], respectively). The new equations had less bias than 2015 B2M and BTP equations developed in CKD populations (Table S7).

eGFR$_{cr-cys}$ (equation 5) was more accurate than eGFR$_{cr}$ (equation 1) and eGFR$_{cys}$ (equation 2; Tables 3 and S8). eGFR$_{cr}$ was more accurate than eGFR$_{cys}$, and the 4-marker panels (equations 6 and 7) were more accurate than the

Table 2. Variables and Coefficients in 2020 Equations in Development and Internal Validation Population

Sex	Scr, mg/dL	Scys, mg/L	SBTP, mg/L	Equation for Estimating GFR
2020 Cystatin C–B2M-BTP Equation[a]				
Female	−	≤0.8	≤0.6	$110 \times (Scys/0.8)^{-0.876} \times SB2M^{-0.205} \times (SBTP/0.6)^{0.038} \times 0.999^{age}$
			>0.6	$110 \times (Scys/0.8)^{-0.876} \times SB2M^{-0.205} \times (SBTP/0.6)^{-0.243} \times 0.999^{age}$
		>0.8	≤0.6	$110 \times (Scys/0.8)^{-0.697} \times SB2M^{-0.205} \times (SBTP/0.6)^{0.038} \times 0.999^{age}$
			>0.6	$110 \times (Scys/0.8)^{-0.697} \times SB2M^{-0.205} \times (SBTP/0.6)^{-0.243} \times 0.999^{age}$
Male	−	≤0.8	≤0.6	$120 \times (Scys/0.8)^{-0.876} \times SB2M^{-0.205} \times (SBTP/0.6)^{0.038} \times 0.999^{age}$
			>0.6	$120 \times (Scys/0.8)^{-0.876} \times SB2M^{-0.205} \times (SBTP/0.6)^{-0.243} \times 0.999^{age}$
		>0.8	≤0.6	$120 \times (Scys/0.8)^{-0.697} \times SB2M^{-0.205} \times (SBTP/0.6)^{0.038} \times 0.999^{age}$
			>0.6	$120 \times (Scys/0.8)^{-0.697} \times SB2M^{-0.205} \times (SBTP/0.6)^{-0.243} \times 0.999^{age}$
2020 Creatinine–Cystatin C–B2M-BTP Equation[b]				
Female	≤0.7	≤0.8	≤0.6	$123 \times (Scr/0.7)^{-0.243} \times (Scys/0.8)^{-0.519} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$123 \times (Scr/0.7)^{-0.243} \times (Scys/0.8)^{-0.519} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
		>0.8	≤0.6	$123 \times (Scr/0.7)^{-0.243} \times (Scys/0.8)^{-0.423} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$123 \times (Scr/0.7)^{-0.243} \times (Scys/0.8)^{-0.423} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
Female	>0.7	≤0.8	≤0.6	$123 \times (Scr/0.7)^{-0.471} \times (Scys/0.8)^{-0.519} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$123 \times (Scr/0.7)^{-0.471} \times (Scys/0.8)^{-0.519} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
		>0.8	≤0.6	$123 \times (Scr/0.7)^{-0.471} \times (Scys/0.8)^{-0.423} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$123 \times (Scr/0.7)^{-0.471} \times (Scys/0.8)^{-0.423} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
Male	≤0.9	≤0.8	≤0.6	$131 \times (Scr/0.9)^{-0.295} \times (Scys/0.8)^{-0.519} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$131 \times (Scr/0.9)^{-0.295} \times (Scys/0.8)^{-0.519} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
		>0.8	≤0.6	$131 \times (Scr/0.9)^{-0.295} \times (Scys/0.8)^{-0.423} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$131 \times (Scr/0.9)^{-0.295} \times (Scys/0.8)^{-0.423} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
Male	>0.9	≤0.8	≤0.6	$131 \times (Scr/0.9)^{-0.471} \times (Scys/0.8)^{-0.519} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$131 \times (Scr/0.9)^{-0.471} \times (Scys/0.8)^{-0.519} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$
		>0.8	≤0.6	$131 \times (Scr/0.9)^{-0.471} \times (Scys/0.8)^{-0.423} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.004} \times 0.996^{age}$
			>0.6	$131 \times (Scr/0.9)^{-0.471} \times (Scys/0.8)^{-0.423} \times SB2M^{-0.103} \times (SBTP/0.6)^{-0.177} \times 0.996^{age}$

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

Abbreviations: BTP, β-trace protein; B2M, β$_2$-microglobulin; max, maximum of the 2 listed terms; min, minimum of the 2 listed terms; SBTP, serum β-trace protein; SB2M, serum β$_2$-microglobulin; Scr, serum creatinine; Scys, serum cystatin C.

[a]The 2020 Cystatin C–B2M-BTP Equation can be expressed as a single equation: $120 \times min(Scys/0.8,1)^{-0.876} \times max(Scys/0.8,1)^{-0.697} \times B2M^{-0.205} \times min(SBTP/0.6,1)^{0.038} \times max(SBTP/0.6,1)^{-0.243} \times 0.999^{age}$ [×0.922 if female].

[b]The 2020 Creatinine–Cystatin C–B2M-BTP Equation can be expressed as a single equation: $131 \times min(Scr/k,1)^{\alpha} \times max(Scr/k,1)^{-0.471} \times min(Scys/0.8,1)^{-0.519} \times max(Scys/0.8,1)^{-0.423} \times SB2M^{-0.103} \times min(SBTP/0.6,1)^{-0.004} \times max(SBTP/0.6,1)^{-0.177} \times 0.996^{age}$ [× 0.937 if female], where k is 0.7 for women and 0.9 for men, α is −0.243 for women and −0.295 for men.

Table 3. Performance of GFR Estimating Equations in the External Validation Dataset

	Equation Description		Performance in Overall Population[a]	
	Filtration Markers	**Demographics**	**1 − P$_{30}$ (95% CI)**	**RMSE (95% CI)**
Equation with creatinine only				
1. 2009 CKD-EPI creatinine[10]	Creatinine	Age, sex, race	11.8% (10.5%-13.2%)	0.199 (0.193-0.206)
Equations with cystatin C				
2. 2012 CKD-EPI cystatin C[47]	Cystatin C	Age, sex	17.4% (15.9%-18.9%): ↑ vs eq. 1	0.262 (0.250-0.274): ↑ vs eq. 1
3. Present study (2020)	Cystatin C, B2M, BTP	Age, sex, race	14.8% (13.4%-16.2%): ↓ vs eq. 2	0.256 (0.243-0.268): ↓ vs eq. 2
4. Present/recommended (2020)	Cystatin C, B2M, BTP	Age, sex	15.6% (14.2%-17.1%): ↓ vs eq. 2	0.259 (0.247-0.271): ↓ vs eq. 2
Equations with creatinine and cystatin C				
5. 2012 CKD-EPI creatinine–cystatin C[47]	Creatinine, cystatin C	Age, sex, race	9.4% (8.2%-10.6%): ↓ vs eq. 1	0.199 (0.191-0.206): ↔ vs eq. 1
6. Present study (2020)	Creatinine, cystatin C, B2M, BTP	Age, sex, race	8.4% (7.3%-9.5%): ↔ vs eq. 5; ↓ vs eq. 3	0.195 (0.187-0.203): ↓ vs eqs. 5, 3
7. Present/recommended (2020)	Creatinine, cystatin C, B2M, BTP	Age, sex	8.6% (7.5%-9.8%): ↓ vs eq. 5; ↔ vs eq. 4	0.197 (0.188-0.205): ↓ vs eqs. 5, 4

Abbreviations: BTP, β-trace protein; B2M, β$_2$-microglobulin; GFR, glomerular filtration rate; RMSE, root mean square error.
[a]1 − P$_{30}$ and RMSE are measures of accuracy; values in parentheses are 95% confidence intervals. 1 − P$_{30}$ is the percentage of estimates greater than 30% different from measured GFR. For comparisons, ↑ indicates a higher value (ie, worse performance) than the comparator equation at $P \leq 0.05$, ↓ indicates a lower value (ie, better performance) than the comparator equation at $P \leq 0.05$, and ↔ indicates a comparable value (ie, performance neither better nor worse) than the comparator equation ($P > 0.05$).

3-marker panels (equations 3 and 4; $P < 0.001$). The 3-marker panel without race (equation 4) was more accurate than eGFR$_{cys}$ (equation 2; 1 − P$_{30}$ of 15.6% vs 17.4%; $P = 0.01$). The 4-marker panel without race (equation 7) was as accurate as eGFR$_{cr-cys}$ (equation 5; 1 − P$_{30}$ of 8.6% vs 9.4%; $P = 0.2$). The addition of race to the 3-marker (equation 3) and 4-marker (equation 6) panels led to small further improvements in accuracy (1 − P$_{30}$ of 14.8% and 8.4%, respectively), and the 4-marker panel with race (equation 6) was nominally more accurate than eGFR$_{cr-cys}$ (equation 5), but this was of borderline significance ($P = 0.05$). Comparisons of RMSE were generally consistent. Results were generally consistent across subgroups in Black versus other people (Fig 1; Table S8) and across subgroups of eGFR, age, sex, diabetes, and body mass index (Figs S2-S6). Results that used noncalibrated mGFR were generally more accurate than those that used calibrated mGFR. Using noncalibrated mGFR, the 4-marker panel without race was more accurate than eGFR$_{cr-cys}$ (Table S9).

eGFR$_{cr-cys}$ (equation 5) was unbiased, but eGFR$_{cr}$ (equation 1) overestimated and eGFR$_{cys}$ (equation 2) underestimated mGFR. There was differential bias by race group for eGFR$_{cr}$, eGFR$_{cys}$, and eGFR$_{cr-cys}$. The 3-marker panels (equations 3, 4) and 4-marker panels (equations 6, 7) underestimated mGFR but improved the differential bias among race groups (Fig 1; Table S8).

Discussion

Accurate assessment of GFR is essential for detection, staging, and assessment of progression, management,

prognostication, and drug dosage adjustment in CKD. GFR estimates 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 reinforcement of 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 3-marker panel without race improved precision and accuracy compared with eGFR$_{cys}$ and the addition of BTP and B2M to creatinine and cystatin C in a 4-marker panel without race was more accurate than the 3-marker panel and as accurate as eGFR$_{cr-cys}$, which includes race. More accurate equations that can be used to confirm or replace 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 extrarenal elimination, all of which lead to error in GFR estimates.[1,55] Serum creatinine is affected by muscle mass, diet, and drugs that inhibit tubular secretion of creatinine or extrarenal 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
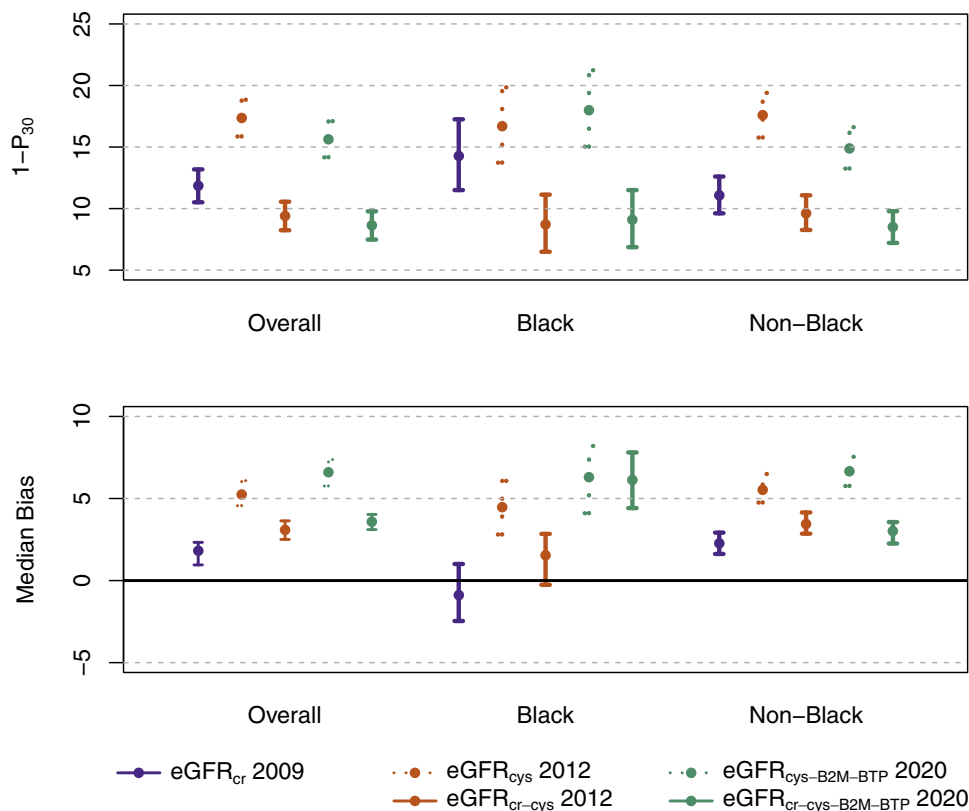
Figure 1. Performance of reference eGFR and panel eGFR equations in the external validation population and overall and by race. Accuracy as measured by $1 - P_{30}$ or the percentage of estimates greater than 30% different from measured GFR. The vertical bars indicate 95% CIs. Solid lines indicate equations 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 3- and 4-marker panels.

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 the 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 geographic variation with the use of the current race coefficient between Black people in the United States and Europe versus 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 geographic regions.

Guidelines recommend the use of confirmatory tests for eGFR$_{cr}$ in clinical scenarios in which a more precise and accurate estimate of GFR is required.[3] Serum cystatin C is less affected by race than creatinine is, 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 noncorrelated 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 3-marker panel eGFR provided greater accuracy than eGFR$_{cys}$ but not eGFR$_{cr-cys}$, reflecting the important contribution of creatinine to GFR estimation in the populations included in the present study. The addition of B2M and BTP to creatinine and cystatin C in the 4-marker panel resulted in better accuracy than eGFR$_{cr-cys}$ and allowed elimination of race with a similar performance to eGFR$_{cr-cys}$. Although the 4-marker panel eGFR is also not independent of creatinine, the magnitude of the creatinine coefficient is attenuated compared with the 2012 creatinine–cystatin C equation, thereby reducing the contribution of creatinine to the 4-marker panel eGFR. Overall, these findings are consistent with our hypothesis and suggest a path forward to improved GFR estimation 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 mGFR compared with the 2015 BTP and B2M equations, and a prespecified 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 the 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 minimize differences by GFR measurement method by calibrating the mGFR 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 a 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 3-marker panel without creatinine could be preferred as alternative initial tests for GFR evaluation. It is possible that, in these settings, in which creatinine estimation is likely to perform poorly, the 3-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 and lower than in the development populations for the 2009 creatinine and 2012 cystatin C equations and the external validation population in the present study, meaning regression to the mean is a likely explanation for the underestimation of mGFR in the validation population in the present study. However, performance was consistent across the range of GFRs, 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 taken before implementation of the panels recommended here. 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, even though our research laboratory has observed stability in filtration

marker assays over a period of 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 in which 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 3-marker and 4-marker panel eGFRs that use B2M and BTP but do not include race as confirmatory or alternative tests for $eGFR_{cr}$. The 4-marker panel eGFR is less dependent on creatinine and is as accurate as the 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 geographic locations.

Supplementary Material

Supplementary File 1 (PDF)

Figure S1: Flow chart showing the development of CKD-EPI pooled datasets.

Figure S2: Performance of eGFR equations in the overall population and by eGFR subgroups.

Figure S3: Performance of eGFR equations by age subgroups.

Figure S4: Performance of eGFR equations by body mass index subgroups.

Figure S5: Performance of eGFR equations by diabetes subgroups.

Figure S6: Performance of eGFR equations by sex subgroups.

Table S1: Characteristics of the development/internal validation and external validation population by study.

Table S2: Assays and quality control for creatinine, cystatin C, B2M, and BTP.

Table S3: Correlations and partial correlations of filtration markers and mGFR in combined development/internal validation and external validation populations.

Table S4: Exponentiated coefficients for the race coefficient in GFR estimating equations.

Table S5: Panel eGFR equation performance in development/internal validation population with and without inclusion of age, sex, and race.

Table S6: Variables and coefficients for other new equations that might be used in research developed in the development/internal validation population.

Table S7: Performance of 2020 B2M and BTP equations vs 2015 B2M and BTP equations in external validation population.

Table S8: New panel eGFR equation performance compared to reference equations in external validation overall and by race.

Table S9: New panel eGFR performance compared to reference equations in external validation population using noncalibrated mGFR.

Article Information

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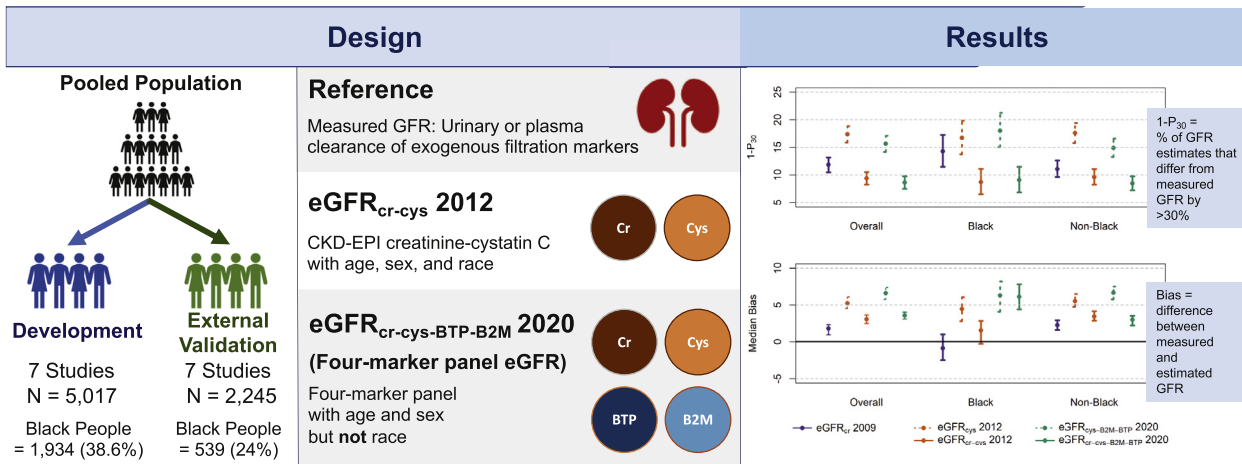
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A New Panel eGFR, Including Beta-2 Microglobulin and Beta Trace Protein and Not Including Race, Developed in a Diverse Population

Design

Pooled Population

Development
7 Studies
N = 5,017
Black People = 1,934 (38.6%)

External Validation
7 Studies
N = 2,245
Black People = 539 (24%)

Reference
Measured GFR: Urinary or plasma clearance of exogenous filtration markers

eGFR$_{cr-cys}$ 2012
CKD-EPI creatinine-cystatin C with age, sex, and race

Cr   Cys

eGFR$_{cr-cys-BTP-B2M}$ 2020
(Four-marker panel eGFR)
Four-marker panel with age and sex but **not** race

Cr   Cys
BTP   B2M

Results

1-P$_{30}$ = % of GFR estimates that differ from measured GFR by >30%

Bias = difference between measured and estimated GFR

eGFR$_{cr}$ 2009   eGFR$_{cys}$ 2012   e3FR$_{cys-B2M-BTP}$ 2020
eGFR$_{cr-cys}$ 2012   eGFR$_{cr-cys-B2M-BTP}$ 2020

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

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