RESEARCH LETTER

Performance of Serum β2-Microglobulin– and β-Trace Protein–Based Panel Markers and 2021 Creatinine- and Cystatin-Based GFR Estimating Equations in Pakistan

To the Editor:

Estimating glomerular filtration rate (GFR) using the creatinine-based equation (eGFRcr) as an initial test and the cystatin C-based equations (eGFRcys or eGFRcr-cys) as a confirmatory test is recommended.[1] Because 2009 CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) eGFRcr and 2012 CKD-EPI eGFRcr-cys included a term for race, which is a social and not biological construct, and these equations overestimated eGFR in Black individuals, the equations were refitted in 2021 without the term for Black race in the United States. The new 2021 CKD-EPI eGFRcr-cys equation was more accurate than the new equations without race with either creatinine or cystatin alone in both Black and non-Black individuals.[2] β2-Microglobulin (B2M) and β-trace protein (BTP), alternate filtration markers less influenced by race, are being considered for use in a panel including cystatin C (3-marker) or creatinine and cystatin C (4-marker).[3] Previously we reported that 2009 eGFRcr overestimated measured GFR (mGFR) in an adult population in Pakistan and that the calibrated equation CKD-EPI eGFRcr-PK eliminated bias and improved accuracy.[4] We also showed that, unlike its performance in other populations,[5] 2012 CKD-EPI eGFRcys exhibited substantial bias in Pakistanis and that 2012 eGFRcr-cys was no better than eGFRcr-PK.[6] In this study, we aimed to evaluate the performance of the 3- and 4-marker B2M and BTP panels, 2021 CKD-EPI eGFRcr, and 2021 CKD-EPI eGFRcr-cys. The primary reference comparator was CKD-EPI eGFRcr-PK, which is currently used in Pakistan. We also explored factors other than GFR that influence B2M and BTP levels.

In a cross-sectional study, B2M and BTP were measured among 557 Pakistani participants (≥40 years; 49.7% men).[7] mGFR was calculated using urinary inulin clearance.[8] A detailed description of the study methods is provided in Item S1, and a study flowchart is provided in Figure S1.

We compared bias (median difference in mGFR and eGFR), precision (interquartile range of differences), and accuracy (percentage of eGFR within 30% of mGFR and root mean square logarithmic [base e] error) between mGFR and eGFR. We used linear regression models to assess the associations between non-GFR determinants and log-transformed (base e) B2M and BTP, adjusting for mGFR and mGFR measurement error (≤2.5%, Table 1). The strength of significant associations was defined as intermediate and strong if the absolute percentage difference in B2M or BTP levels was 5%-10% and >10%, respectively.

For the 557 participants, the mean (standard deviation) age was 51 (10) years. The median value (interquartile range) of mGFR was 91 (74-110) mL/min/1.73 m^2. As shown in Table 2, both the 3- and 4-marker BTM and B2P panels exhibited a large positive bias and did not improve precision or accuracy (both P > 0.05) relative to eGFRcr-PK. The 3- and 4-marker panels exacerbated bias (P < 0.001) and did not improve precision or accuracy over 2012 eGFRcys or 2012 eGFRcr-cys. 2021 eGFRcr and eGFRcr-cys did not improve precision or accuracy over eGFRcr-PK. Results remained consistent when stratified by eGFR level (Tables S1 and S2).

In Table 1, non-GFR determinants with intermediate and strong associations with higher BTP included male sex, history of heart disease, and lower waist circumference. Determinants of higher B2M included male sex, higher total body fat, and lower serum albumin. Except for sex, determinants associated with cystatin C and creatinine differed from those of BTP and B2M.

These results suggest that neither the 3- nor 4-marker B2M and BTP panels, nor the race-free 2021 eGFRcr or 2021 eGFRcr-cys equations, were better than eGFRcr-PK. The 3- and 4-marker panels did not improve the performance of 2012 eGFRcys and eGFRcr-cys. We also observed that non-GFR determinants of BTP and B2M differed from those of cystatin C and creatinine. History of heart disease had a strong and intermediate association with higher BTP and cystatin C, respectively, but not with B2M or creatinine. Higher albumin levels were intermediately associated with lower BTP and B2M, but not with cystatin C or creatinine. To date, the 3- and 4-marker panels have been assessed predominantly among Europid and US Black populations.[3] Other eGFR equations containing BTP and B2M were assessed in Europid, Black, and Chinese populations with inconsistent performance compared with eGFRcr-cys.[9,10] The 3-marker BTM and B2P panel was more accurate than the 2012 eGFRcys, but not the 2012 eGFRcr-cys, and the 4-marker BTM and B2P panel was comparable to 2012 eGFRcr-cys.[3]

Previously, we observed that 2012 eGFRcys exhibited a large bias in the Pakistani population.[6] Unlike our decision to modify 2009 eGFRcr to account for the bias, presumably due to the lower muscle mass and protein intake in Pakistani compared with Europid populations in which 2009 eGFRcr was developed, we elected not to calibrate 2012 eGFRcys in Pakistan because the source of bias was unknown and because of the uncertainty of the calibrated equation robustness across the country.[6] The usefulness of B2M and BTP in improving eGFR in Pakistan remains limited. Future studies are needed to validate our findings in South Asia and the South Asian diaspora elsewhere to explore unidentified non-GFR determinants of cystatin C, B2M, and BTP and to evaluate the feasibility of modifying these equations to improve GFR estimation. Additional research should include cost-effectiveness analyses of filtration markers other than creatinine for broad applications, especially for low-resource countries.

Table 1. General Linear Model Analysis of IQR-Standardized mGFR and Non-GFR Determinants on Natural Log-Transformed (Base e) Filtration Markers (N = 557)

eGFR Determinants of Interest	IQR	Mean Percent Change			
		B2M (95% CI)	BTP (95% CI)	Cystatin C (95% CI)	Creatinine (95% CI)
Measured GFR (mGFR)	36.6	-47.8 (-51.1, -44.2)[a]	-52.4 (-55.5, -49.0)[a]	-42.6 (-45.1, -40.0)[a]	-50.0 (-53.0, -46.9)[a]
Age (year)	13.0	0.002 (-3.67, 3.82)	3.56 (-2.63, 10.2)	3.02 (-0.14, 6.30)	-1.00 (-4.41, 2.52)
Sex (men vs women)	-	10.1 (2.33, 17.2)[a]	20.7 (9.22, 30.8)[a]	13.1 (7.07, 18.7)[a]	24.8 (17.8, 31.2)[a]
Smoking (yes vs no)	-	5.04 (-0.36, 10.7)	4.91 (-3.61, 14.2)	5.14 (0.72, 9.74)[b]	-0.66 (-5.37, 4.28)
Body mass index (kg/m^2)	6.6	0.63 (-4.35, 5.88)	7.10 (-0.98, 15.8)	4.49 (0.36, 8.80)[c]	12.4 (7.03, 18.1)[a]
Waist circumference (cm)	15.0	0.12 (-4.04, 4.46)	-7.39 (-13.2, -1.10)[b]	0.35 (-2.88, 3.69)	-3.07 (-6.98, 1.01)
Total body fat (kg)	10.6	6.40 (3.70, 9.18)[b]	3.81 (0.22, 7.52)[c]	2.65 (0.67, 4.67)[c]	-0.19 (-2.93, 2.62)
Lean body mass (kg)	14.7	2.55 (-1.46, 6.73)	-1.43 (-7.17, 4.67)	1.08 (-2.05, 4.31)	7.33 (2.71, 12.1)[b]
History of heart disease (yes vs no)	-	7.25 (-1.13, 16.3)	11.6 (1.64, 22.5)[a]	8.32 (2.36, 14.6)[b]	6.62 (-0.42, 14.2)
Serum albumin (g/dL)	0.4	-7.39 (-9.84, -4.88)[b]	-6.49 (-10.1, -2.69)[b]	-3.93 (-6.05, -1.77)[c]	-0.60 (-2.85, 1.71)
LDL cholesterol (mmol/L)	37.0	-3.61 (-6.06, -1.10)[c]	-2.25 (-5.98, 1.63)	-2.10 (-4.14, -0.02)[c]	-1.22 (-3.63, 1.26)
Dietary protein intake (g/day)	19.0	0.39 (-0.34, 1.14)	0.18 (-0.49, 0.85)	0.27 (-0.05, 0.59)	-0.25 (-0.90, 0.41)
Urine creatinine (mg/kg/d)	6.4	-1.82 (-10.2, 7.43)	9.90 (-4.72, 26.8)	0.67 (-5.47, 7.21)	17.5 (8.11, 27.7)[a]
R^2 for the multivariable model with mGFR measurement error		78.2%	64.2%	81.4%	80.5%
R^2 for mGFR with mGFR measurement error		74.6%	59.5%	75.3%	65.4%
R^2 for mGFR without mGFR measurement error		73.4%	58.5%	74.2%	64.4%

Note: Mean percent change in serum B2M, BTP, cystatin C, and creatinine levels for an IQR-standardized increment in an eGFR determinant variable, calculated as $100 \times (e^{\beta\text{-coefficient}} - 1)$ using error-in-variables regression models assuming log-transformed mGFR with ≤2.5% measurement error. The general linear model included all variables presented in the table and corrected for mGFR measurement error. Strength of association for statistically significant results is indicated as follows. Abbreviations: B2M, β2-microglobulin; BTP, β-trace protein; GFR, glomerular filtration rate; IQR, interquartile range; LDL, low-density lipoprotein; mGFR, measured glomerular filtration rate.
[a]strong (absolute average percent difference in B2M/BTP levels >10%).
[b]intermediate (absolute average percent difference in B2M/BTP levels 5–10% inclusive).
[c]weak (absolute average percent difference in B2M/BTP levels <5%). The same code was applied for cystatin C and creatinine. R^2 was based on all eGFR determinants presented in the table.

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SUPPLEMENTARY MATERIAL

Supplementary File 1 (PDF)

Figure S1: Flowchart of the study design.

Item S1: Detailed description of methods.

Table S1: Linear Regression Between Baseline Characteristics and Log-Transformed BTP and B2M Adjusting for Age, Sex, and Measured GFR (N = 557)

Table S2: Linear Regression Between Baseline Characteristics and Log-Transformed B2M and BTP Adjusting for All Non-GFR Determinants and Measured GFR (N = 557)

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Table 2. Performance of GFR Estimating Equations Compared with Measured GFR (N = 557)

Equation	Filtration Marker	Demographics	Bias,[a] Median Difference (95% CI) (mL/min/1.73 m²)	Precision,[b] IQR (95% CI) (mL/min/1.73 m²)	Accuracy,[c] P$_{30}$ (95% CI)	RMSLE[d] (95% CI)
2009 CKD-EPI eGFRcr	Creatinine	Age, sex, race	-6.76 (-9.10, -5.90)	22.6 (20.3, 25.4)	76.1 (72.4, 79.6)	0.289 (0.263, 0.323)
2014 CKD-EPI eGFRcr-PK	Creatinine	Age, sex, race	NA	22.7 (20.6, 25.8)	82.4 (79.0, 85.5)	0.265 (0.243, 0.297)
2021 CKD-EPI eGFRcr	Creatinine	Age, sex	-8.94 (-11.2, -8.10)	23.0 (20.5, 25.6)	73.8 (69.9, 77.4)	0.303 (0.277, 0.336)
2012 CKD-EPI eGFRcys	Cystatin C	Age, sex	12.7 (10.7, 15.2)	25.6 (23.2, 28.3)	73.3 (69.4, 76.9)	0.322 (0.303, 0.349)
2012 CKD-EPI eGFRcr-cys	Creatinine, Cystatin C	Age, sex, race	2.73 (1.16, 4.58)	21.2 (18.6, 24.3)	83.1 (79.8, 86.1)	0.253 (0.231, 0.285)
2021 CKD-EPI eGFRcr-cys	Creatinine, Cystatin C	Age, sex	-0.20 (-1.49, 1.78)	21.0 (18.2, 23.9)	82.8 (79.4, 85.8)	0.254 (0.231, 0.289)
2020 Cystatin C-B2M-BTP equation (3-marker panel)	Cystatin C, B2M, BTP	Age, sex	15.3 (13.6, 18.1)	26.7 (23.9, 29.0)	70.7 (66.8, 74.5)	0.331 (0.312, 0.355)
2020 Creatinine-Cystatin C-B2M-BTP equation (4-marker panel)	Creatinine, Cystatin C, B2M, BTP	Age, sex	5.12 (3.49, 7.20)	22.1 (19.5, 25.7)	81.3 (77.8, 84.5)	0.256 (0.235, 0.288)

Abbreviations: B2M, β2-microglobulin; BTP, β-trace protein; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; RMSLE, Root Mean Squared Logarithmic Error.

[a]Bias was expressed as the median difference in measured GFR minus estimated GFR (95% bootstrapped confidence interval). Negative bias indicates eGFR overestimation of measured GFR, and positive bias indicates eGFR underestimation of measured GFR. NA, not applicable because bias was expected to be zero (the equation was developed in the study population). A larger absolute value indicates greater bias.

[b]Precision was expressed as the interquartile range (IQR) of differences in measured GFR minus estimated GFR (95% bootstrapped confidence interval). A larger absolute value indicates poorer precision.

[c]P$_{30}$ was defined as the percentage of individuals with estimated GFR within 30% of measured GFR (95% bootstrapped confidence interval). The 95% CI on P$_{30}$ was calculated using the Clopper–Pearson (exact) method. A smaller P$_{30}$ indicates poorer accuracy.

[d]RMSLE was defined as the square root of the mean squared difference of measured GFR and estimated GFR on the logarithmic scale. A larger RMSLE indicates poorer accuracy.

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