Accuracy of GFR Estimating Equations in Patients with Discordances between Creatinine and Cystatin C-Based Estimations

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ABSTRACT

Background Cystatin C is recommended as a confirmatory test to eGFR when more precise estimates are needed for clinical decision making. Although eGFR on the basis of both creatinine and cystatin ($eGFR_{cr\text{-}cys}$) is the most accurate estimate in research studies, it is uncertain whether this is true in real-world settings, particularly when there are large discordances between eGFR based on creatinine ($eGFR_{cr}$) and that based on cystatin C ($eGFR_{cys}$)

Methods We included 6185 adults referred for measured GFR (mGFR) using plasma clearance of iohexol in Stockholm, Sweden, who had 9404 concurrent measurements of creatinine, cystatin C, and iohexol clearance. The performance of $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ was assessed against mGFR with median bias, P_{30}, and correct classification of GFR categories. We stratified analyses within three categories: $eGFR_{cys}$ at least 20% lower than $eGFR_{cr}$ ($eGFR_{cys} < eGFR_{cr}$), $eGFR_{cys}$ within 20% of $eGFR_{cr}$ ($eGFR_{cys} \approx eGFR_{cr}$), and $eGFR_{cys}$ at least 20% higher than $eGFR_{cr}$ ($eGFR_{cys} > eGFR_{cr}$).

Results $eGFR_{cr}$ and $eGFR_{cys}$ were similar in 4226 (45%) samples, and among these samples all three estimating equations performed similarly. By contrast, $eGFR_{cr\text{-}cys}$ was much more accurate in cases of discordance. For example, when $eGFR_{cys} < eGFR_{cr}$ (47% of samples), the median biases were 15.0 (overestimation), −8.5 (underestimation), and 0.8 ml/min per 1.73 m^2 for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$, respectively; P_{30} was 50%, 73%, and 84%, respectively; and correct classification was 38%, 45%, and 62%, respectively. When $eGFR_{cys} > eGFR_{cr}$ (8% of samples), the median biases were −4.5, 8.4, and 1.4 ml/min per 1.73m^2. The findings were consistent among individuals with cardiovascular disease, heart failure, diabetes mellitus, liver disease, and cancer.

Conclusions When $eGFR_{cr}$ and $eGFR_{cys}$ are highly discordant in clinical practice, $eGFR_{cr\text{-}cys}$ is more accurate than either $eGFR_{cr}$ or $eGFR_{cys}$.

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INTRODUCTION

eGFR is a key parameter to inform clinical decisions in medicine and plays a central role in the diagnosis, prognosis, and management of patients with chronic kidney disease. Currently, eGFR is most commonly estimated using serum creatinine ($eGFR_{cr}$). However, recent statements from leading kidney organizations stress the need to facilitate increased, routine, and timely use of cystatin C in health care as an additional filtration marker.[1,2] Research studies demonstrate that eGFR on the basis of both creatinine

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and cystatin C ($eGFR_{cr\text{-}cys}$) is more accurate than $eGFR_{cr}$ when compared with measured GFR (mGFR) using plasma clearance of iohexol, an accepted reference method.[3,4] However, less is known about the accuracy of the combined equation in real-world clinical practice because worldwide implementation of cystatin C testing has been slow, despite recommendations for use since 2012. Sweden is unique in this aspect: Since the early 2000s, measurements of serum cystatin C—as well as mGFR—have been widely available without subspecialty consultation.[5,6]

Both creatinine and cystatin C have non-GFR determinants which may result in inaccurate estimates in certain clinical settings. Recent evidence suggests that there are often large discrepancies between eGFR solely based on creatinine ($eGFR_{cr}$) and that based on cystatin C ($eGFR_{cys}$).[7–10] Studies have suggested that intraindividual discrepancies between $eGFR_{cys}$ and $eGFR_{cr}$ are associated with adverse outcomes,[7,9,11] with a worse prognosis for individuals who have an $eGFR_{cys}$ that is lower than $eGFR_{cr}$. However, it is unknown whether this difference reflects better estimation of GFR or merely captures additional confounders (*e.g.*, inflammation). Few studies have investigated which GFR estimating equation best approximates mGFR when $eGFR_{cr}$ and $eGFR_{cys}$ differ.[12,13] Thus, there is uncertainty as to which estimating equation should guide clinical practice in situations of large discordances between $eGFR_{cr}$ and $eGFR_{cys}$.

To address this question using real-world clinical data, we analyzed more than 9000 simultaneous assessments of serum creatinine, cystatin C, and mGFR in 6185 individuals from an independent cohort not involved in the development of the new Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. We assessed the performance of $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ against mGFR within three categories: $eGFR_{cys}$ at least 20% lower than $eGFR_{cr}$ ($eGFR_{cys} < eGFR_{cr}$), $eGFR_{cys}$ within 20% of $eGFR_{cr}$ ($eGFR_{cys} \approx eGFR_{cr}$), and $eGFR_{cys}$ at least 20% higher than $eGFR_{cr}$ ($eGFR_{cys} > eGFR_{cr}$).

Significance Statement

Large discordances between eGFR on the basis of creatinine ($eGFR_{cr}$) or cystatin C ($eGFR_{cys}$) are common in clinical practice. However, which GFR estimating equation ($eGFR_{cr}$, $eGFR_{cys}$, or $eGFR_{cr\text{-}cys}$) is most accurate in these settings is not known. In this real-world study of 9404 concurrent measurements of creatinine, cystatin C, and iohexol clearance, all three equations performed similarly when $eGFR_{cr}$ and $eGFR_{cys}$ were similar (45% of cases). However, with large discordances (55% of cases), $eGFR_{cr\text{-}cys}$ was much more accurate than either alone. These findings were consistent among individuals with cardiovascular disease, heart failure, diabetes mellitus, liver disease, and cancer who have been underrepresented in research cohorts. Thus, when $eGFR_{cr}$ and $eGFR_{cys}$ are largely discordant in clinical practice, $eGFR_{cr\text{-}cys}$ is more accurate than $eGFR_{cr}$ or $eGFR_{cys}$.

METHODS

Data Source and Study Population

We used data from the Stockholm Creatinine Measurements (SCREAM) project,[14] a health care utilization cohort from the region of Stockholm, Sweden, with data collected between 2006 and 2019. A single health care provider in the Stockholm region provides universal and tax-funded health care to 20%–25% of the population of Sweden. Using unique personal identification numbers,[15] SCREAM linked regional and national administrative databases that hold complete information on demographics, health care utilization, dispensed drugs,[16] diagnoses,[17] vital status,[18] kidney replacement therapy,[19] and completed laboratory tests. The Regional Ethical Review Board in Stockholm approved the study (2017/793-31); informed consent was not deemed necessary because all data were deidentified at the Swedish Board of Health and Welfare.

We included all adult patients who received iohexol clearance testing between January 1, 2007, and December 31, 2018 (Supplemental Figure 1). Eligible individuals were required to have a serum creatinine and cystatin C test in the 30 days before or after the iohexol clearance measurement. All creatinine tests were standardized to isotope dilution mass spectrometry traceable methods and standardization of cystatin C occurred after 2010.[20] Patients on dialysis and those who had implausible mGFR values (<0 or >150 ml/min per 1.73 m^2) were excluded. When a patient had multiple concurrent iohexol-creatinine-cystatin C tests during follow-up, we included all measurements to increase statistical efficiency.

GFR Measurement

Iohexol clearance was analyzed at the central laboratory, Department of Clinical Chemistry, at Karolinska University Hospital in Stockholm, with clearance procedures performed by indication at specialist departments in the region of Stockholm following systematic protocols.[21] In brief, iohexol clearance was measured using single-point plasma clearance of iohexol and expressed per 1.73 m^2 body surface area. A total of 5 ml of iohexol (currently omnipaque 300 mg I/mL, GE Healthcare) was administered with an intravenous injection, followed by a 10 ml normal saline flush. Blood samples (5 ml) for plasma clearance measurement were obtained from the contralateral arm to the injection, with the timing based on the eGFR: approximately 4 hours for eGFR >40 ml/min per 1.73 m^2, within approximately 6–8 hours for eGFR 15–40 ml/min per 1.73 m^2 and after approximately 24 hours for eGFR <15 ml/min per 1.73 m^2. The exact times of iohexol injection and blood sampling were recorded, and samples were centrifuged before transport if the transport to the central laboratories could not take place on the same day. Serum iohexol concentration was determined by ultra-high-performance liquid chromatography separation and ultraviolet detection. The performance of the iohexol method was monitored through internal controls and an external quality assurance program for iohexol standardization across the country by the Government-run monitoring company Equalis (Uppsala, Sweden). Bird *et al.*[22] compared single-sample versus multisample GFR using

both iohexol and ^{51}Cr-EDTA as indicators (19). They found that single-sample iohexol at 3 and 4 hours was highly correlated with multisample iohexol (correlation coefficients of 0.97 and 0.99, respectively), with a mean difference (SD) of −3.0 (7.1) and 0.52 (4.3) and 95% limits of agreement of −17.2 to 11.2 and −8.1 to 9.1, respectively. Furthermore, compared with multisample ^{51}Ct-EDTA, the mean difference (SD) was 1.1 (9.4) for single-sample iohexol at 3 hours, 4.5 (8.9) for single-sample iohexol at 4 hours, and 4.0 (7.9) for multisample iohexol.

GFR Estimating Equations, Discordance between $eGFR_{cr}$ and $eGFR_{cys}$ and Covariates

$eGFR_{cr}$ and $eGFR_{cr\text{-}cys}$ were calculated using the 2021 CKD-EPI equations and $eGFR_{cys}$ with the 2012 CKD-EPI equation.[3,23] We assessed the performance of each equation in the overall population and according to the magnitude of discordance between $eGFR_{cr}$ and $eGFR_{cys}$. Discordance was calculated as $(eGFR_{cys}-eGFR_{cr})/eGFR_{cr}$ and categorized into $eGFR_{cys}<eGFR_{cr}$, $eGFR_{cys}\approx eGFR_{cr}$, and $eGFR_{cys}>eGFR_{cr}$. A measurement fell within $eGFR_{cys}<eGFR_{cr}$ when $eGFR_{cys}$ was more than 20% *lower* than $eGFR_{cr}$; $eGFR_{cys}\approx eGFR_{cr}$ if the difference between eGFR values was within 20% of $eGFR_{cr}$; and within $eGFR_{cys}>eGFR_{cr}$ when $eGFR_{cys}$ was more than 20% *higher* than $eGFR_{cr}$. We chose $eGFR_{cr}$ as denominator because it is currently the most commonly used eGFR measure worldwide. $(eGFR_{cys}-eGFR_{cr})/eGFR_{cr}$ can thus be interpreted as the percentual difference from $eGFR_{cr}$, with a negative number meaning lower $eGFR_{cys}$ than $eGFR_{cr}$ and positive number meaning a higher $eGFR_{cys}$ than $eGFR_{cr}$. We chose 20% as threshold on the basis of previous analyses that show meaningfully elevated risks for outcomes[10] and to allow for a significant proportion with $eGFR_{cys}<eGFR_{cr}$ and $eGFR_{cys}>eGFR_{cr}$. In addition, we also assessed continuous percentage differences between $eGFR_{cys}$ and $eGFR_{cr}$. For each individual, we extracted the following covariates: age, sex, body mass index (BMI), cardiovascular disease (CVD) (composite of myocardial infarction, other ischemic heart disease, heart failure, stroke, other cerebrovascular disease, arrhythmia, and peripheral vascular disease), hypertension, cancer, liver disease, and whether the individual had a kidney transplant or was a kidney donor (definitions in Supplemental Table 1).

Analysis

In our main analysis, we analyzed all measurements when patients had multiple concurrent iohexol-creatinine-cystatin C tests. The performance of all equations compared with mGFR was evaluated using the following metrics: bias, P_{30}, interquartile range (IQR), and correct classification of GFR categories. Bias was defined as the median difference between eGFR and mGFR (eGFR−mGFR). P_{30} was defined as the proportion of eGFRs within 30% of mGFR (P_{30}). A P_{30} value of 80%–90% is considered to be acceptable for GFR evaluation in many circumstances, and a P_{30} value of 90% or higher is preferred; these values correspond to approximately 60%–70% agreement and more than 70% agreement of eGFR with mGFR in GFR categories, respectively. We also reported P_{10}, defined as the proportion of eGFRs within 10% of mGFR. IQR was defined as the magnitude of the IQR of the differences between mGFR and eGFR and is a measure of precision. Correct classification of GFR categories was defined as agreement of eGFR and mGFR categories using the Kidney Disease Improving Global Outcomes (KDIGO) GFR categories (<15, 15–29, 30–44, 45–59, 60–89, and ≥90 ml/min per 1.73 m^2). Among patients with large discordances between $eGFR_{cr}$ and $eGFR_{cys}$, we also assessed the proportion that would be correctly and incorrectly reclassified across KDIGO GFR categories when using $eGFR_{cr\text{-}cys}$ instead of $eGFR_{cr}$ or $eGFR_{cys}$. The 95% confidence intervals (CIs) for each metric were calculated using the bootstrap method (10.000 bootstrap samples). All analyses were performed using R version 3.6.2 (R Foundation for Statistical Computing).[24]

Subgroup Analyses and Sensitivity Analyses

We assessed performance within subgroups of interest by assessing median bias. *A priori*-defined strata included age (< or ≥65 years), sex, BMI (< or ≥25 kg/m^2 and continuously), mGFR (< or ≥60 ml/min per 1.73 m^2), and the presence of CVD, heart failure, diabetes mellitus, liver disease, and cancer. Furthermore, we performed the following sensitivity analyses: First, we redefined our exposure as the raw difference between $eGFR_{cys}$ and $eGFR_{cr}$ and used the categories $eGFR_{cys}<eGFR_{cr}$ by more than 15 ml/min per 1.73 m^2, $eGFR_{cys}\approx eGFR_{cr}$ (values do not differ by more than 15 ml/min per 1.73 m^2) and $eGFR_{cys}>eGFR_{cr}$ by more than 15 ml/min per 1.73 m^2. The threshold of 15 ml/min per 1.73 m^2 allows comparison with other studies that have used these thresholds.[9,11] Second, we restricted our analysis to measurements of iohexol, creatinine, and cystatin C taken on the same day, instead of using a 30-day window. Third, we used the first measurement for each patient rather than all measurements. Fourth, to eliminate the possible interference of nonstandardized cystatin C tests, we restricted the analysis to measurements performed after 2011.[20] Fifth, we combined sensitivity analyses two to four and restricted the analysis to the first measurement per patient using standardized cystatin C measurements and where iohexol, creatinine, and cystatin C were measured on the same day. Finally, we assessed the performance of the arithmetic mean of $eGFR_{cr}$ and $eGFR_{cys}$ (which is used in Sweden), rather than $eGFR_{cr\text{-}cys}$.[25]

RESULTS

Baseline Characteristics

We included 6185 individuals contributing 9404 mGFR measurements (Supplemental Figure 2). Of these 9404 measurements, 77.1% had mGFR, creatinine, and cystatin C measured on the same day. Of the 9404 measurements, 89.9% had creatinine or cystatin C measured within 7 days of mGFR,

Table 1. Baseline characteristics of 6185 persons (with 9404 observations) referred to iohexol clearance testing in Stockholm during 2007–2018, overall and stratified by discordance between $eGFR_{cr}$ and $eGFR_{cys}$

Baseline Characteristics	Overall	$eGFR_{cys}$<$eGFR_{cr}$ [a]	$eGFR_{cys}$≈$eGFR_{cr}$ [a]	$eGFR_{cys}$>$eGFR_{cr}$ [a]
		$eGFR_{cys}$ >20% Lower Than $eGFR_{cr}$	$eGFR_{cys}$ Within 20% of $eGFR_{cr}$	$eGFR_{cys}$ >20% Higher Than $eGFR_{cr}$
No. of measurements, *n* (%)	9404 (100)	4465 (47)	4226 (45)	713 (8)
No. of unique individuals, *n* (%)	6185 (100)	2927 (47)	3174 (51)	650 (11)
Mean age (SD), yr	56 (17)	60 (16)	53 (18)	50 (16)
Age ≥65 yr, *n* (%)	3569 (38)	2101 (47)	1310 (31)	158 (22)
Female sex, *n* (%)	3751 (40)	1681 (38)	1760 (42)	310 (43)
Mean BMI (SD), kg/m^2	26 (8)	26 (5)	26 (8)	26 (19)
BMI ≥25 kg/m^2, *n* (%)[b]	3887 (41)	1938 (43)	1665 (39)	284 (40)
GFR evaluations, median (IQR)				
Cr, μmol/L[c]	94 (74–128)	96 (75–130)	89 (71–120)	114 (93–239)
Cys, mg/L	1 (1–2)	2 (1–2)	1 (1–1)	1 (1–2)
$eGFR_{cr}$, ml/min per 1.73 m^2	74 (50–97)	71 (48–94)	80 (54–101)	62 (25–79)
$eGFR_{cys}$, ml/min per 1.73 m^2	56 (35–84)	42 (28–59)	77 (50–99)	84 (34–107)
$eGFR_{cr\text{-}cys}$, ml/min per 1.73 m^2	65 (42–90)	54 (36–73)	81 (53–103)	74 (29–96)
mGFR, ml/min per 1.73 m^2	62 (41–84)	53 (36–71)	74 (51–92)	69 (27–89)
mGFR categories, *n* (%)				
≥90	1755 (19)	379 (8)	1211 (29)	165 (23)
60 to <90	3245 (35)	1412 (32)	1579 (37)	254 (36)
45 to <60	1677 (18)	1001 (22)	602 (14)	74 (10)
30 to <45	1344 (14)	897 (20)	409 (10)	38 (5)
15 to <30	1020 (11)	647 (14)	308 (7)	65 (9)
<15	363 (4)	129 (3)	117 (3)	117 (16)
Medical history, *n* (%)				
CVD[d]	2787 (30)	1705 (38)	942 (22)	140 (20)
Heart failure	974 (10)	682 (15)	253 (6)	39 (5)
DM	2490 (26)	1549 (35)	844 (20)	97 (14)
Cancer	2664 (28)	1431 (32)	1123 (27)	110 (15)
Liver disease	2521 (27)	1694 (38)	788 (19)	39 (5)
Kidney transplantation	350 (4)	212 (5)	132 (3)	6 (1)
Kidney donor	284 (3)	14 (0)	214 (5)	56 (8)

BMI, body mass index; IQR, interquartile range; Cr, creatinine; cys, cystatin C; mGFR, measured GFR; CVD, cardiovascular disease; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; RASi, renin-angiotensin system inhibition (angiotensin-converting enzyme inhibitor or angiotensin receptor blocker); NSAIDs, nonsteroidal anti-inflammatory drugs.

[a]Adds up to >100% because an individual can contribute multiple measured GFR measurements and therefore contribute to all three strata. Note that the numbers in Table 1 represent the number of measurements, not the number of unique individuals.

[b]Body mass index was missing for 1640 (17%) in the overall group, 691 (15%) in the $eGFR_{cys}$<$eGFR_{cr}$ group, 828 (20%) in the $eGFR_{cys}$≈$eGFR_{cr}$ group, and 121 (17%) in the $eGFR_{cys}$>$eGFR_{cr}$ group.

[c]To convert plasma creatinine from μmol/L to mg/dl, multiply by 0.0113.

[d]Cardiovascular disease was defined as a composite of myocardial infarction, other ischemic heart disease, heart failure, stroke, other cerebrovascular disease, arrhythmia, and peripheral vascular disease.

and 10.1% had creatinine or cystatin C measured between 7 and 30 days of mGFR. The mean (SD) age was 56 (17) years, with 38% aged 65 years or older and 40% female (Table 1). The median mGFR was 62 ml/min per 1.73 m^2, $eGFR_{cr}$ 74 ml/min per 1.73 m^2, $eGFR_{cys}$ 56 ml/min per 1.73 m^2, and $eGFR_{cr\text{-}cys}$ 65 ml/min per 1.73 m^2 (distributions shown in Supplemental Figure 3). The median (IQR) discordance between $eGFR_{cys}$ and $eGFR_{cr}$ was −18.3% (−35.3% to −0.6%), showing that most patients had $eGFR_{cys}$ that was lower than their $eGFR_{cr}$ (Supplemental Figure 4). In total, 47% of observations were in the category $eGFR_{cys}$<$eGFR_{cr}$ by more than 20%, 45% had $eGFR_{cys}$≈$eGFR_{cr}$, and 8% had $eGFR_{cys}$>$eGFR_{cr}$ by more than 20%. Individuals in the category $eGFR_{cys}$<$eGFR_{cr}$ were older, had lower levels of mGFR, and were more likely to have CVD, heart failure, diabetes, cancer, and liver disease than individuals with $eGFR_{cys}$≈$eGFR_{cr}$ or $eGFR_{cys}$>$eGFR_{cr}$ (Table 1).

Performance of Equations Stratified by Discordance between $eGFR_{cr}$ and $eGFR_{cys}$

Overall, the median biases for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ were 8.7, −2.3, and 2.5 ml/min per 1.73 m^2, respectively, with P_{30} of 68.8%, 80.7%, and 86.4% (Table 2). Furthermore, IQR was lowest for $eGFR_{cr\text{-}cys}$ (Table 2, Figure 1).

When stratifying by discordance between $eGFR_{cys}$ and $eGFR_{cr}$, all three equations displayed similar performance when $eGFR_{cys}$≈$eGFR_{cr}$, with little differences in bias, P_{30}, and correct classification. For instance, the median biases were 4.5, 2.1, and 5.0 ml/min per 1.73 m^2 for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$, respectively (Table 2, Figure 1). By contrast, when $eGFR_{cys}$ and $eGFR_{cr}$ were substantially different, $eGFR_{cr\text{-}cys}$ had better performance than $eGFR_{cr}$ and $eGFR_{cys}$. Specifically, among observations with $eGFR_{cys}$<$eGFR_{cr}$, $eGFR_{cr}$ overestimated and $eGFR_{cys}$ underestimated mGFR (Figure 1, Supplemental

Table 2. Bias, P_{30}, interquartile range and correct classification of different Chronic Kidney Disease Epidemiology Collaboration eGFR equations, overall and stratified by the magnitude and direction of the discordance between $eGFR_{cr}$ and $eGFR_{cys}$

Metrics	Total Population	$eGFR_{cys}$<$eGFR_{cr}$[a]	$eGFR_{cys}$≈$eGFR_{cr}$[a]	$eGFR_{cys}$>$eGFR_{cr}$[a]
		$eGFR_{cys}$ >20% Lower Than $eGFR_{cr}$	$eGFR_{cys}$ Within 20% of $eGFR_{cr}$	$eGFR_{cys}$ >20% Higher Than $eGFR_{cr}$
$eGFR_{cr}$[b]				
Bias, median difference (ml/min per 1.73 m^2)[c]	8.7 (8.4–9.0)	15.0 (14.6–15.5)	4.5 (4.1–4.8)	−4.5 (−5.3 to −3.8)
P_{30} (%)[b]	68.8 (67.8–69.7)	49.7 (48.3–51.2)	86.0 (84.9–87.0)	85.9 (83.2–88.3)
IQR, ml/min per 1.73 m^2[d]	18.6 (0.2–18.8)	17.5 (7.0–24.5)	15.1 (−2.0 to 13.1)	12.3 (−13.0 to −0.7)
Correct classification (%)[e]	52.6 (51.6–53.6)	38.1 (36.7–39.5)	66.5 (65.1–67.9)	61.9 (58.3–65.4)
$eGFR_{cys}$[b]				
Bias, median difference (ml/min per 1.73 m^2)[c]	−2.3 (−2.6 to −2.0)	−8.6 (−9.0 to −8.3)	2.1 (1.7–2.4)	8.4 (7.3–10.0)
P_{30}[f]	80.7 (79.9–81.5)	72.9 (71.6–74.2)	90.4 (89.5–91.3)	71.8 (68.4–75.1)
IQR, ml/min per 1.73 m^2[d]	15.6 (−10.5 to 5.1)	14.2 (−16.5 to −2.3)	13.8 (−4.0 to 9.9)	16.5 (2.5–19.0)
Correct classification[e]	57.4 (56.4–58.4)	45.4 (43.9–46.8)	69.2 (67.8–70.5)	62.9 (59.4–66.6)
$eGFR_{cr-cys}$[b]				
Bias, median difference (ml/min per 1.73 m^2)[c]	2.5 (2.2–2.7)	0.7 (0.4–1.0)	5.0 (4.6–5.4)	1.8 (1.2–2.5)
P_{30}[b]	86.4 (85.7–87.1)	84.3 (83.2–85.4)	88.3 (87.3–89.3)	87.8 (85.4–90.2)
IQR, ml/min per 1.73 m^2[d]	13.6 (−3.5–10.0)	12.6 (−5.5–7.1)	14.5 (−1.4–13.2)	12.3 (−2.6–9.6)
Correct classification[e]	65.4 (64.5–66.4)	61.6 (60.2–63)	68.4 (67–69.7)	71.9 (68.6–75.2)

Cr, creatinine; cys, cystatin C; IQR, interquartile range; mGFR, measured GFR.
[a]A measurement fell within $eGFR_{cys}$<$eGFR_{cr}$ when $eGFR_{cys}$ was more than 20% *lower* than $eGFR_{cr}$; $eGFR_{cys}$≈$eGFR_{cr}$ if the difference between eGFR values was within 20% of $eGFR_{cr}$; and within $eGFR_{cys}$>$eGFR_{cr}$ when $eGFR_{cys}$ was more than 20% *higher* than $eGFR_{cr}$.
[b]eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration 2012 and 2021 equations.
[c]Bias was expressed as the median difference in eGFR minus measured GFR (95% confidence interval). A negative bias indicates underestimation of the measured GFR, and a positive bias indicates overestimation of the measured GFR.
[d]Interquartile range is defined as the interquartile range and a measure of precision (the dispersion of individual errors around the median bias).
[e]Correct classification of GFR categories was defined as agreement of eGFR and measured GFR categories using the Kidney Disease Improving Global Outcomes GFR categories (<15, 15–29, 30–44, 45–59, 60–89 and ≥90 ml/min per 1.73 m^2).
[f]P_{30} was defined as the percentage of individuals with eGFRs within 30% of measured GFR (95% confidence interval).

Figure 5). The median biases were 15.0, −8.5, and 0.8 ml/min per 1.73 m^2 for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr-cys}$, respectively; P_{30} was 49.7%, 72.9%, and 84.3%, respectively; P_{10} was 15.8%, 24.0%, and 40.2%, respectively; and correct classification of GFR categories was 38.1%, 45.4%, and 61.6%, respectively (Table 2, Supplemental Table 2). By contrast, among observations with $eGFR_{cys}$>$eGFR_{cr}$, the pattern was reversed, with $eGFR_{cr}$ underestimating and $eGFR_{cys}$ overestimating mGFR (Supplemental Figure 5). The median biases were −4.5, 8.4, and 1.8 for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr-cys}$, respectively; P_{30} was 85.9%, 71.8%, and 87.8%, respectively; P_{10} was 34.1%, 25.9%, and 43.9%, respectively; and correct classification of GFR categories was 61.9%, 62.9%, and 71.9%, respectively (Table 2, Supplemental Table 2).

The median bias for $eGFR_{cr}$ and $eGFR_{cys}$ was larger than the median bias for $eGFR_{cr-cys}$ whenever the discrepancy between $eGFR_{cys}$ and $eGFR_{cr}$ was >10% (Supplemental Figure 6). Correct classification of GFR categories for each GFR category is shown in Supplemental Tables 3 and 4. Consistent with the overall classification results, $eGFR_{cr-cys}$ showed better classification than $eGFR_{cr}$ or $eGFR_{cys}$ among observations with $eGFR_{cys}$<$eGFR_{cr}$ or $eGFR_{cys}$>$eGFR_{cr}$. For instance, among those with $eGFR_{cys}$<$eGFR_{cr}$, correct classification for $eGFR_{cr-cys}$ was 50.5%, 56.5%, 66.5%, and 55.8% for mGFR categories of 45–59, 30–44, 15–29, and <15 ml/min per 1.73 m^2, respectively.

Among patients with $eGFR_{cys}$<$eGFR_{cr}$, using $eGFR_{cr-cys}$ instead of $eGFR_{cr}$ reclassified 38.1% to a correct GFR category and incorrectly reclassified 25.5%, with a net difference of 12.6%. Furthermore, $eGFR_{cr-cys}$ reclassified more participants correctly than incorrectly than $eGFR_{cys}$, with a net difference of 8.6% (Table 3, Supplemental Table 5). Similar findings were obtained in the stratum of patients with $eGFR_{cys}$>$eGFR_{cr}$.

Performance of Equations in Subgroups

The findings were consistent in subgroups of age, sex, BMI, mGFR, CVD, diabetes, heart failure, liver disease and cancer, with $eGFR_{cr-cys}$ having the smallest bias across observations with $eGFR_{cys}$<$eGFR_{cr}$ (Figure 2, Supplemental Table 6). Although the bias for $eGFR_{cr-cys}$ was small among the subgroup of patients with heart failure (N=974), P_{30} was low. For instance, P_{30} for $eGFR_{cr-cys}$ was 76.5% among heart failure patients who had $eGFR_{cys}$<$eGFR_{cr}$. P_{30} for $eGFR_{cr-cys}$ was acceptable among patients with liver disease (N=2521) or cancer (N=2664), being close to or >85%. Analysis by continuous BMI showed that when $eGFR_{cys}$≈$eGFR_{cr}$, $eGFR_{cys}$ had the smallest bias, particularly at low BMI. When $eGFR_{cys}$<$eGFR_{cr}$, $eGFR_{cr-cys}$ performed best, also at extreme values of BMI >40 (Supplemental Figure 7).

Sensitivity Analyses

The findings were consistent when using raw differences between $eGFR_{cr}$ and $eGFR_{cys}$ (Supplemental Tables 7 and 8, Supplemental Figures 8 and 9). Furthermore, we observed similar findings when restricting to same-day measurements (Supplemental Table 9), when restricting to one measurement per patient (Supplemental Table 10), when restricting to

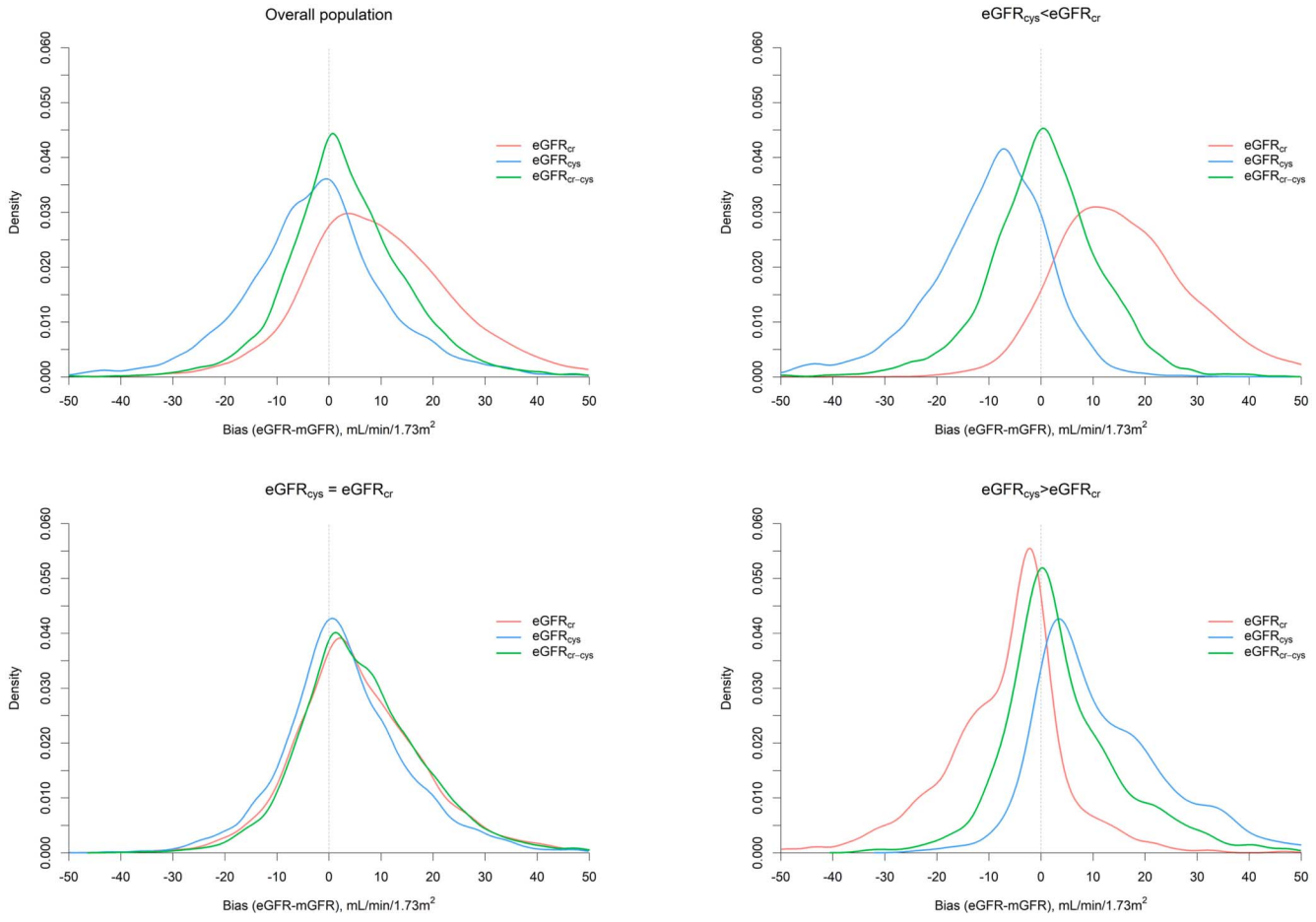

Figure 1. Density plot of bias for three eGFR equations, overall and stratified by discordance between $eGFR_{cr}$ and $eGFR_{cys}$. A measurement fell within $eGFR_{cys}$<$eGFR_{cr}$ when $eGFR_{cys}$ was more than 20% *lower* than $eGFR_{cr}$; $eGFR_{cys}$≈$eGFR_{cr}$ if the difference between eGFR values was within 20% of $eGFR_{cr}$ and within $eGFR_{cys}$>$eGFR_{cr}$ when $eGFR_{cys}$ was more than 20% *higher* than $eGFR_{cr}$. Figure 1 can be viewed in color online at www.jasn.org.

standardized cystatin C measurements (Supplemental Table 11), when combining the previous three sensitivity analyses (Supplemental Table 12), or when using the arithmetic mean of $eGFR_{cr}$ and $eGFR_{cys}$ instead of $eGFR_{cr\text{-}cys}$ (Supplemental Table 13).

DISCUSSION

In this large study of >9000 closely spaced serum creatinine, cystatin C, and mGFR tests in a real-world clinical setting, we found that eGFR on the basis of both creatinine and cystatin C was the most accurate estimate of mGFR overall, similar to what has been established in research cohorts.[3] Interestingly, this observation also held when there were large discordances between $eGFR_{cr}$ and $eGFR_{cys}$. Similar to previous observations, we found that discordant eGFR values occurred commonly, with $eGFR_{cys}$ more than 20% lower than $eGFR_{cr}$ nearly half of the time. In situations of large discordances, $eGFR_{cr}$ and $eGFR_{cys}$ both showed substantial bias, albeit in opposite directions, as well as low P_{30} and low correct classification compared with mGFR. By contrast, the combined equation $eGFR_{cr\text{-}cys}$ performed well and would be acceptable for clinical decision making in many circumstances. Our findings were robust across multiple sensitivity analyses and consistent in subgroups of age, BMI, mGFR, and comorbidities known to affect the accuracy of serum creatinine and cystatin C levels. These findings have clinical implications, providing real-world evidence to guide GFR-based clinical decision making in situations of discordances between $eGFR_{cr}$ and $eGFR_{cys}$.

Our observation that $eGFR_{cys}$ was more than 20% lower than $eGFR_{cr}$ in 47% of cases in routine clinical settings is novel. Previous observations have been made in research cohorts[7,9,11] and generally show lower magnitude of discrepancies. This may be explained by the indications of iohexol clearance testing and the more common presence of comorbidities in routine clinical settings.[26] We show that in situations of large discordances between $eGFR_{cr}$ and $eGFR_{cys}$, both equations were similarly but oppositely biased and that $eGFR_{cr\text{-}cys}$ best

Table 3. Correct and incorrect reclassification from GFR categories based on $eGFR_{cr}$ or $eGFR_{cys}$ to categories based on $eGFR_{cr\text{-}cys}$ in patients with $eGFR_{cys}$<$eGFR_{cr}$ and $eGFR_{cys}$>$eGFR_{cr}$

Reclassification	Replacing GFR Categories Based on $eGFR_{cr}$ by $eGFR_{cr\text{-}cys}$		Replacing GFR Categories Based on $eGFR_{cys}$ by $eGFR_{cr\text{-}cys}$	
	$eGFR_{cys}$<$eGFR_{cr}$ $eGFR_{cys}$ >20% Lower Than $eGFR_{cr}$	$eGFR_{cys}$>$eGFR_{cr}$ $eGFR_{cys}$ >20% Higher Than $eGFR_{cr}$	$eGFR_{cys}$<$eGFR_{cr}$ $eGFR_{cys}$ >20% Lower Than $eGFR_{cr}$	$eGFR_{cys}$>$eGFR_{cr}$ $eGFR_{cys}$ >20% Higher Than $eGFR_{cr}$
Participants, *n*	4465	713	4465	713
Total reclassified, *n* (%)	2838 (63.6)	284 (39.8)	2407 (53.9)	161 (22.6)
Correctly reclassified, *n* (%)	1700 (38.1)	174 (24.4)	1396 (31.3)	108 (15.1)
Incorrectly reclassified, *n* (%)	1138 (25.5)	110 (15.4)	1011 (22.6)	53 (7.4)
Net difference, %	12.6	9.0	8.6	7.7

approximated mGFR. A previous study similarly found that the bias for $eGFR_{cr}$ and $eGFR_{cys}$ progressively increased for larger discordances between $eGFR_{cr}$ and $eGFR_{cys}$, whereas bias remained small when using the arithmetic mean of $eGFR_{cr}$ and $eGFR_{cys}$.[12]. Both creatinine and cystatin C are affected by non-GFR determinants. Although serum creatinine may be influenced by muscle mass, diet, physical activity, and certain drugs, serum cystatin C is influenced by obesity, smoking, inflammation, and thyroid disorders.[27–32] A large difference between $eGFR_{cr}$ and $eGFR_{cys}$ arises when serum creatinine, cystatin C, or both are influenced by their non-GFR determinants, leading to biased approximation of true GFR. Combining both markers improves precision by reducing errors that are due to variation in the non-GFR determinants of each marker.[33] Grubb proposed an alternative hypothesis for the discrepancies between $eGFR_{cys}$ and $eGFR_{cr}$ called the

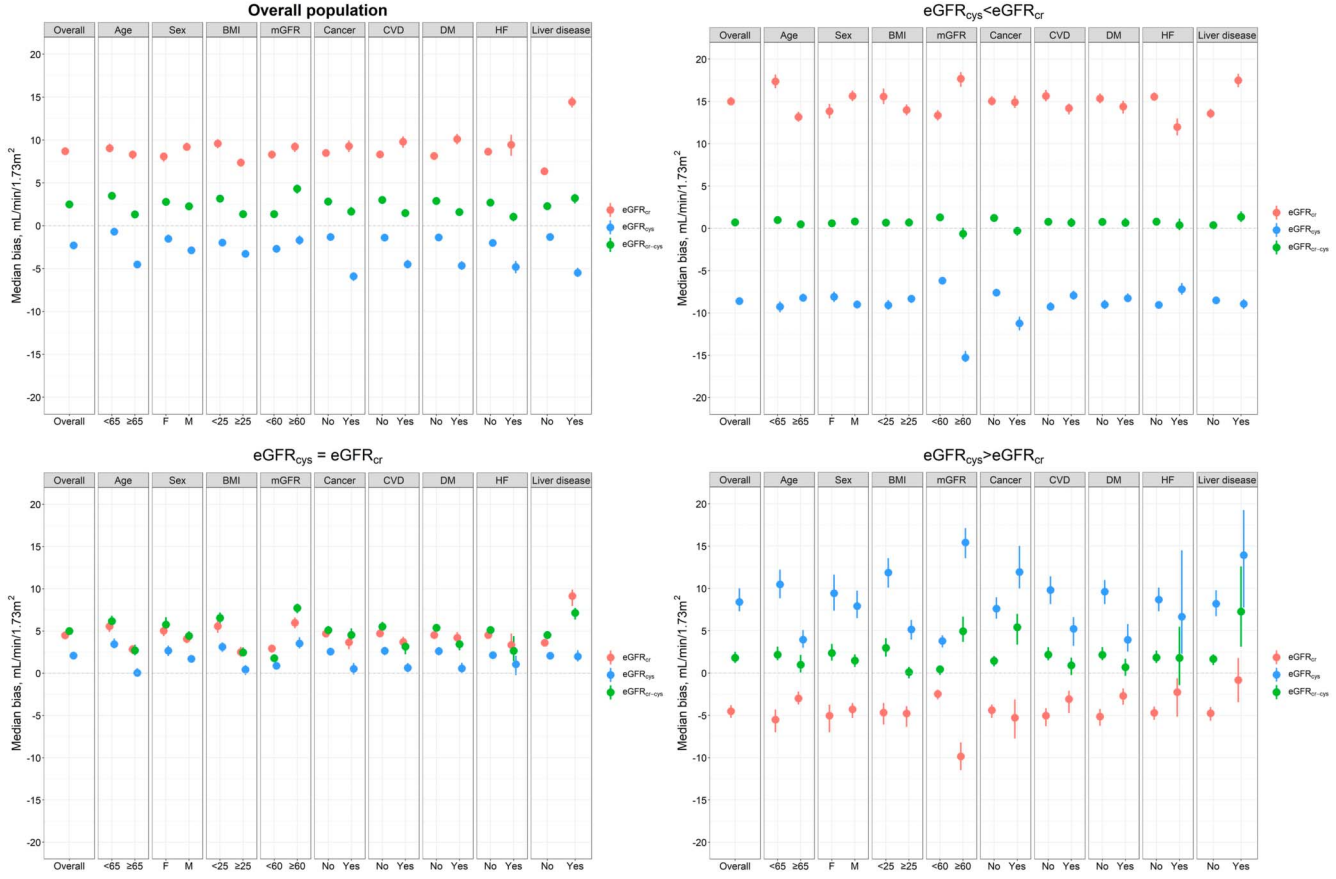

Figure 2. Bias of $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ across subgroups, overall and stratified by the extent of discordance between $eGFR_{cr}$ and $eGFR_{cys}$. A measurement fell within $eGFR_{cys}$<$eGFR_{cr}$ when $eGFR_{cys}$ was more than 20% *lower* than $eGFR_{cr}$; $eGFR_{cys}$≈$eGFR_{cr}$ if the difference between eGFR values was within 20% of $eGFR_{cr}$ and within $eGFR_{cys}$>$eGFR_{cr}$ when $eGFR_{cys}$ was more than 20% *higher* than $eGFR_{cr}$. BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; HF, heart failure; mGFR, measured glomerular filtration rate. Figure 2 can be viewed in color online at www.jasn.org.

shrunken pore syndrome, where an $eGFR_{cys}/eGFR_{cr}$ ratio <0.6 or 0.7 reflects selective impairment of filtration of cystatin C and other middle molecular weight macromolecules (approximately 10k–30k daltons).[34,35] Furthermore, the use of $eGFR_{cr\text{-}cys}$ instead of $eGFR_{cr}$ or $eGFR_{cys}$ reclassified more patients correctly than incorrectly. Such reclassification can have potential implications for drug dosing, initiation, and discontinuation which are based on GFR thresholds, including sodium-glucose cotransporter two inhibitors, renin-angiotensin system inhibitors, direct oral anticoagulants, and metformin.[36,37]

Our findings hold significant clinical implications that $eGFR_{cr\text{-}cys}$ best approximates mGFR in a variety of settings, including those when there are large discrepancies between $eGFR_{cr}$ and $eGFR_{cys}$. A strength of our study is the evaluation of GFR equation's performance among patients with comorbid conditions known to affect serum creatinine or cystatin-C levels, including heart failure, liver disease, and cancer. These patients have been excluded or minimally included in preceding research cohorts. Among people with liver disease and cancer, $eGFR_{cr\text{-}cys}$ had acceptable performance. However, among heart failure patients, $eGFR_{cr\text{-}cys}$ had low P_{30} (approximately 75%), regardless of the magnitude of discrepancy between $eGFR_{cr}$ and $eGFR_{cys}$. It is recommended to measure GFR through clearance of exogenous filtration markers rather than using GFR estimating equations when more accurate assessment of GFR is needed for decision making, such as in evaluating candidacy for living kidney donation and dosing for cancer chemotherapy.[1,26] Other clinical scenarios where measuring GFR may be indicated because $eGFR_{cr}$ may not be valid are reviewed elsewhere.[38]

Previous studies have shown that on a population level, $eGFR_{cys}$ is more strongly associated with adverse outcomes than $eGFR_{cr}$.[39] Moreover, intraindividual differences in eGFR by creatinine vs. cystatin C predict adverse outcomes,[7,9,11] with patients having a large discrepancy between $eGFR_{cr}$ and $eGFR_{cys}$ having a worse prognosis than individuals with no or small discrepancies. Our findings suggest that the stronger associations observed with $eGFR_{cys}$ do not reflect kidney function *per se* but rather non-GFR determinants of cystatin. Similarly, the strong associations between eGFR discrepancies and outcomes are likely to be explained by confounding of both $eGFR_{cr}$ and $eGFR_{cys}$ by non-GFR determinants.

Strengths of our study include its large sample size and unique setting, involving patients from a country with routine cystatin C testing and access to mGFR assessments. Inhabitants of Sweden enjoy universal tax-funded health care, which minimizes selection bias from disparate access to health care. Furthermore, this was an independent cohort not involved in the development of the novel CKD-EPI equations. Our study may better capture the performance of GFR estimating equations in routine clinical practice compared with research cohorts that include relatively healthy persons who are more likely to have predictable muscle mass and fewer comorbid conditions. Our study also has limitations. First, we lacked information on race. According to Swedish Government annual statistics,[40] only approximately 2.5% of the included cohort were born in African countries; thus, our findings may be limited in terms of generalizability to other world regions. Second, the subgroups of comorbidity were defined according to International Classification of Diseases-10 codes. Although these have been shown to have good positive predictive value,[17] they are not sensitive nor do they capture the severity of conditions. Third, we did not assess other developed eGFR equations, which should be the topic of future work. Fourth, certain specific subgroups may have been underrepresented in our cohort, including healthy individuals who are body builders or individuals with marked muscle wasting (*e.g.*, spinal cord injury with paraplegia and advanced neuromuscular diseases). Fifth, we also included unstandardized cystatin C measurements. However, results were virtually identical in sensitivity analyses that only included standardized cystatin C measurements. Finally, we used single-sample iohexol as the gold standard, which has shown small bias but slightly lower precision than multisample iohexol. However, any inaccuracy in the gold standard would equally affect the different CKD-EPI eGFR equations.

In conclusion, $eGFR_{cr\text{-}cys}$ best approximates mGFR in routine clinical care, even when large discordances between $eGFR_{cr}$ and $eGFR_{cys}$ are found. When available, $eGFR_{cr\text{-}cys}$ should be used to guide clinical decisions.

DISCLOSURES

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Consultancy: Xeltis BV; Advisory or Leadership Role: Advisory Board Next-kidney; and Other Interests or Relationships: Chair Thematic Working Group Vascular Tissue Engineering at TERMIS, Member guideline committee Dutch Society of Nephrology, and presidentelect Vascular Access Society. All remaining authors have nothing to disclose.

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DATA SHARING STATEMENT

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. The data may be shared on reasonable request for academic research collaborations that fulfill GDPR as well as national and institutional ethics regulations and standards by contacting Juan-Jesus Carrero (juan.jesus.carrero@ki.se).

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://links.lww.com/JSN/E405.

Supplemental Table 1. Definition of study covariates.

Supplemental Table 2. P_{10} of different CKD-EPI eGFR equations stratified by the magnitude and direction of the discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

Supplemental Table 3. Agreement between mGFR and eGFR categories stratified by discordance between $eGFR_{cr}$ and $eGFR_{cys}$: given a certain eGFR category, what is the probability an individual is in a certain mGFR category.

Supplemental Table 4. Agreement between mGFR and eGFR categories stratified by discordance between $eGFR_{cr}$ and $eGFR_{cys}$: given a certain mGFR category, what is the probability an individual is in a certain eGFR category.

Supplemental Table 5. Correct and incorrect reclassification from GFR categories based on $eGFR_{cr}$ or $eGFR_{cys}$ to categories based on $eGFR_{cr\text{-}cys}$, in patients with $eGFR_{cys}<eGFR_{cr}$ and $eGFR_{cys}>eGFR_{cr}$.

Supplemental Table 6. Bias, P_{30}, and correct classification of $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ across subgroups stratified by discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

Supplemental Table 7. Baseline characteristics of included individuals (n=6185) with measured GFR measurements (n=9404) in Stockholm during 2007–2018 stratified by raw discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

Supplemental Table 8. Bias, P_{30}, and correct classification of $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ overall and stratified by raw discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

Supplemental Table 9. Sensitivity analysis restricting to same-day measurements of iohexol, creatinine, and cystatin C (N=7252).

Supplemental Table 10. Sensitivity analysis restricting to first measurement for each patient (N=6185).

Supplemental Table 11. Sensitivity analysis restricting to measurements taken after 2011 (*i.e.*, after standardization of cystatin C) (N=7277).

Supplemental Table 12. Sensitivity analysis restricting to the first measurement per patient, with standardized cystatin C measurements and where iohexol, creatinine, and cystatin C were measured on the same day (N=3828).

Supplemental Table 13. Sensitivity analysis using the average of $eGFR_{cr}$ and $eGFR_{cys}$ instead of $eGFR_{cr\text{-}cys}$.

Supplemental Figure 1. Study design diagram.

Supplemental Figure 2. Flow chart of included participants in the study.

Supplemental Figure 3. Density plot of mGFR, $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ in the overall population and stratified by discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

Supplemental Figure 4. Density plot of discordance between $eGFR_{cr}$ and $eGFR_{cys}$ in the overall population.

Supplemental Figure 5. Comparison of mGFR and eGFR among patients with (A) $eGFR_{cys}<eGFR_{cr}$, (B) $eGFR_{cys}\approx eGFR_{cr}$, and (C) $eGFR_{cys}>eGFR_{cr}$.

Supplemental Figure 6. Median bias for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ across the range of discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

Supplemental Figure 7. Density plot of bias for the three eGFR equations stratified by absolute discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

Supplemental Figure 8. Bias of different CKD-EPI eGFR equations across subgroups stratified by absolute discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

Supplemental Figure 9. Bias of different CKD-EPI eGFR equations across subgroups stratified by absolute discordance between $eGFR_{cr}$ and $eGFR_{cys}$.

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