ORIGINAL ARTICLE

Standardization of serum creatinine is essential for accurate use of unbiased estimated GFR equations: evidence from three cohorts matched on renal function

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ABSTRACT

Background. Differences in the performance of estimated glomerular filtration rate (eGFR) equations have been attributed to the mathematical form of the equations and to differences between patient demographics and measurement methods. We evaluated differences in serum creatinine (SCr) and eGFR in cohorts matched for age, sex, body mass index (BMI) and measured GFR (mGFR).

Methods. White North Americans from Minnesota ($n = 1093$) and the Chronic Renal Insufficiency Cohort (CRIC) ($n = 1548$) and White subjects from the European Kidney Function Consortium (EKFC) cohort ($n = 7727$) were matched for demographic patient characteristics (sex, age $\pm$ 3 years, BMI $\pm$ 2.5 kg/m^2) and renal function (mGFR $\pm$ 3 ml/min/1.73 m^2). SCr was measured with isotope dilution mass spectrometry (IDMS)-traceable assays in the Minnesota and EKFC cohorts and with non-standardized SCr assays recalculated to IDMS in the CRIC. The Minnesota cohort and CRIC shared a common method to measure GFR (renal clearance of iothalamate), while the EKFC cohort used a variety of exogenous markers and methods, all with recognized sufficient accuracy. We compared the SCr levels and eGFR predictions [for Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and EKFC equations] of patients fulfilling these matching criteria.
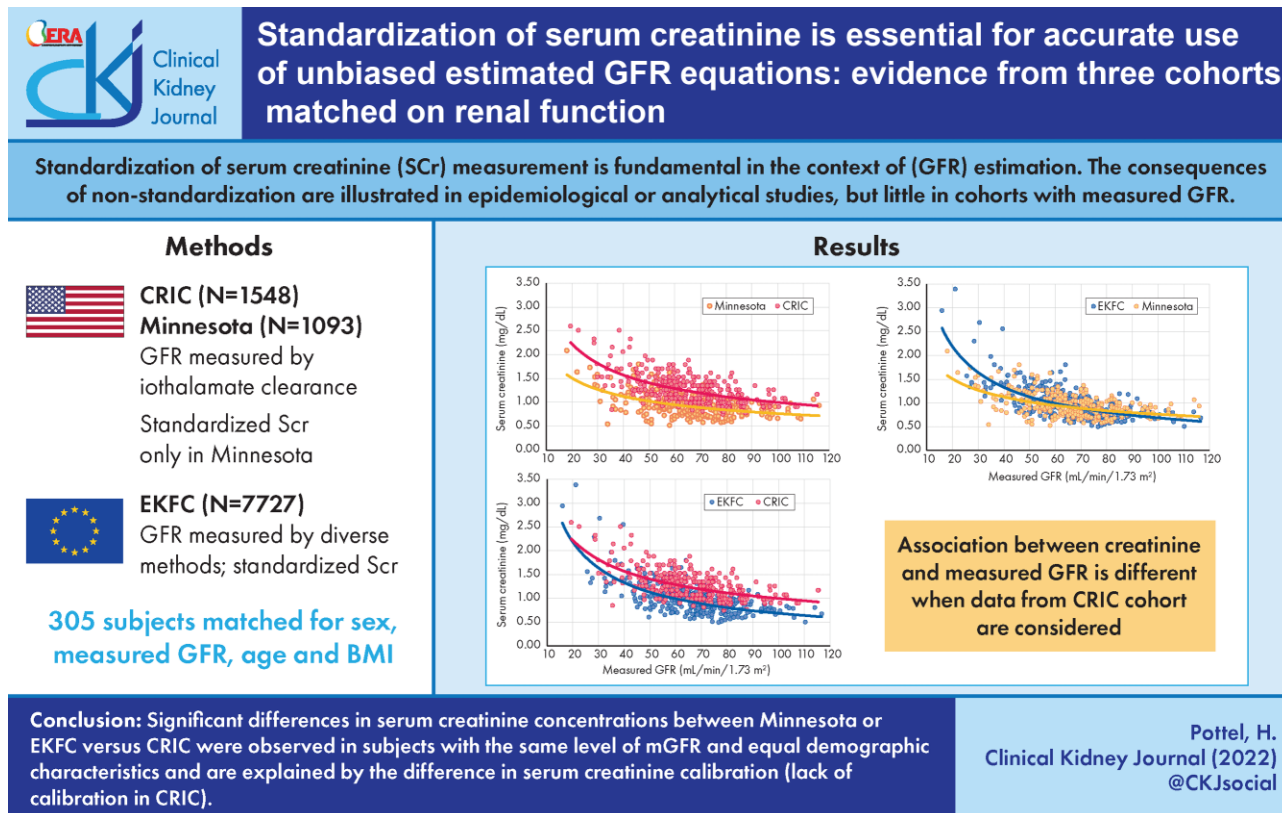
Results. For 305 matched individuals, mean SCr (mg/dL) was not different between the Minnesota and EKFC cohorts (females 0.83 $\pm$ 0.20 versus 0.86 $\pm$ 0.23, males 1.06 $\pm$ 0.23 versus 1.12 $\pm$ 0.37; $P > .05$) but significantly different from the CRIC [females 1.13 $\pm$ 0.23 ($P < .0001$), males 1.42 $\pm$ 0.31 ($P < .0001$)]. The CKD-EPI equations performed better than the EKFC equation in the CRIC, while the opposite was true in the Minnesota and EKFC cohorts.

Conclusion. Significant differences in SCr concentrations between the Minnesota and EKFC cohorts versus CRIC were observed in subjects with the same level of mGFR and equal demographic characteristics and can be explained by the difference in SCr calibration.

LAY SUMMARY

Standardization of serum creatinine (SCr) measurement is fundamental for estimating glomerular filtration rate (GFR). We used data with GFR measured by a reference method from three cohorts: Chronic Renal Insufficiency Cohort (CRIC, $n = 1548$), Minnesota cohort ($n = 1093$) and European Kidney Function Consortium cohort (EKFC; $n = 7727$). In the EKFC and Minnesota cohorts, SCr was measured by standardized methods, although SCr 'calibration' was more debatable in the CRIC. GFR was measured by the same method in the CRIC and Minnesota cohort. Then we matched 305 White subjects for sex, measured GFR ($\pm$3 ml/min/1.73 m^2), age ($\pm$3 years) and body mass index ($\pm$2.5 kg/m^2). From these matched subjects we showed that the association between SCr and measured GFR was quite similar between subjects from the Minnesota and EKFC cohorts, but different between the CRIC and EKFC cohort and between the Minnesota cohort and CRIC. These differences lead to discrepancies in the analysis of the performance of different creatinine-based equations.

GRAPHICAL ABSTRACT

Standardization of serum creatinine is essential for accurate use of unbiased estimated GFR equations: evidence from three cohorts matched on renal function

Standardization of serum creatinine (SCr) measurement is fundamental in the context of (GFR) estimation. The consequences of non-standardization are illustrated in epidemiological or analytical studies, but little in cohorts with measured GFR.

Methods

CRIC (N=1548)
Minnesota (N=1093)
GFR measured by iothalamate clearance
Standardized Scr only in Minnesota

EKFC (N=7727)
GFR measured by diverse methods; standardized Scr

305 subjects matched for sex, measured GFR, age and BMI

Results

Association between creatinine and measured GFR is different when data from CRIC cohort are considered

Conclusion: Significant differences in serum creatinine concentrations between Minnesota or EKFC versus CRIC were observed in subjects with the same level of mGFR and equal demographic characteristics and are explained by the difference in serum creatinine calibration (lack of calibration in CRIC).

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INTRODUCTION

Estimation of the glomerular filtration rate (GFR) is of paramount importance for the diagnostic follow-up of chronic kidney disease (CKD) and for epidemiological studies on the prevalence and risk factors for CKD [1, 2]. GFR estimation is generally performed by equations using biomarkers and demographic parameters such as age, sex and sometimes race [3–5]. The most studied and used equations are thus far based on serum creatinine (SCr) as a biochemical factor, even if other biomarkers (like cystatin C) can be used alone or in combination with creatinine [4, 6]. Many formulas for estimating GFR have been published in the literature in the last 2 decades. Interestingly, the prediction performance of these equations differs across settings. Age range, measured GFR (mGFR) and percentage of males and females in the different cohorts can explain these differences, although the same clinical criteria (including age and sex) and the same biomarker values are applied in these formulas. For instance, from a recent multicentric study, the mean bias of the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in a European cohort ($n = 7727$) was -4.2 ml/min/1.73 m^2, but $+4.0$ ml/min/1.73 m^2 in the American Chronic Renal Insufficiency Cohort (CRIC) ($n = 1548$) [5]. Different factors such as the reference method used to measure GFR (i.e. iohexol versus iothalamate and plasma versus urine clearance) can explain why such differences are observed [7–11]. However, in the example mentioned above, the opposite systematic dif-

ference between the two cohorts clearly raises questions about another factor, i.e. the standardization of SCr measurement [12–18]. Indeed, SCr can be measured by two different analytical methodologies, namely the Jaffe and enzymatic methods [19, 20]. These methods are proposed by different manufacturers on different analytical platforms. Before the introduction of the Standard Reference Material 967 in 2007 [a commutable international standard provided by the US National Institute of Standards and Technology (NIST)] and the launch of the Creatinine Standardization Program (which obliged manufacturers to align with this standard), there was no standardization of SCr measurements. Since then, most manufacturers have used the NIST standards to calibrate their SCr assays, and since 2017, one can assert that most creatinine assays are correctly calibrated against NIST Standard 967 [such methods are generally called isotope dilution mass spectrometry (IDMS) traceable methods] [21], even if there is still doubt for some Jaffe assays (although IDMS traceability is claimed by all manufacturers) [20]. No retrospective analysis has ever evaluated the impact of SCr standardization (or lack thereof) on different cohorts that have been used to establish some estimated GFR (eGFR) formulas.

Therefore, in the current analysis we investigated the standardization of SCr by comparing the relationship between eGFR and mGFR in three well-known cohorts, two from the USA [CRIC ($n = 1548$) and Minnesota ($n = 1093$)] and one from Europe [European Kidney Function Consortium (EKFC) (n = 7727)] [4, 5].

Briefly, we compared eGFR and mGFR and investigated whether differences in eGFR (by different equations) still persisted after matching subjects in the different cohorts for age, sex, body mass index (BMI) and mGFR. Because subjects were matched, we hypothesize that if differences still exist, it is most likely due to a difference in creatinine standardization.

MATERIALS AND METHODS

Cohorts and measurement methods

Three different cohorts of patients with mGFR, age, sex, BMI and SCr available were used in this study.

The CRIC Study contains renal data of White North Americans ($n = 1548$) [22, 23]. mGFR was obtained by renal clearance of iothalamate. SCr was measured in the CRIC participating centres. At the time of measuring SCr in this cohort, the Creatinine Standardization Program had not yet been instituted. Consequently, Joffe et al [24]reported that there was substantial variability in SCr assays across the laboratories and over time. Yet, to circumvent this issue, the investigators decided to harmonize SCr by 'recalibrating' the 13 different analytical platforms used in the CRIC laboratories on the Cleveland Clinic laboratory method with a library of five large-volume plasma specimens from apheresis patients. The Cleveland Clinic laboratory was chosen as the 'reference' laboratory because it had served as the central laboratory for the Modified Diet in Renal Disease (MDRD) Study [25, 26]. Consequently, all the participating laboratories provided results that were harmonized on the Cleveland Clinic laboratory standards. Finally, all these harmonized results underwent a second harmonization on the Roche Cobas enzymatic method, which had been shown to be standardized against the IDMS reference method. The equation allowing the harmonization on the Roche assay was Roche SCr $= 0.1032 + 0.8913 \times$ CRIC SCr [22, 27, 28].

The EFKC cohort (only adults, $n = 7727$) contains multiple European cohorts. The mGFR was obtained with renal clearance (on inulin) or plasma clearance methods [iohexol, chromium-51 ethylenediaminetetraacetic acid (^{51}Cr-EDTA)]. All SCr results were obtained with SCr assays that were traceable to the IDMS reference method (in Kent, all SCr results were obtained directly with IDMS) [5].

The Minnesota cohort ($n = 1093$) combines the Genetic Epidemiology Network of Arteriopathy (GENOA, $n = 687$) and Epidemiology of Coronary Artery Calcification (ECAC, $n = 406$) cohorts [29]. In Minnesota, the same measurement method (iothalamate renal clearance) was used for mGFR as in the CRIC Study and the patients were White North Americans. SCr was assayed using the IDMS-traceable enzymatic Roche Cobas method (Creatinine Plus, Roche Diagnostics, Indianapolis, IN, USA) [30].

To study the impact of potential differences in SCr calibration on eGFR, we compared the results for different GFR estimating equations, i.e. the 2009 CKD-EPI [age, sex, race (ASR)], the 2021 CKD-EPI [age, sex (AS)] and the EKFC equation [3–5].

Statistics

Patients of Minnesota ($n = 1093$) were matched to CRIC patients ($n = 1548$) and the matched pairs were then matched to European patients ($n = 7727$) in a 1:1:1 ratio, based on the following criteria:

- sex,
- age $\pm$ 3 years,

Table 1. Demographic and renal characteristics of the matched cohorts

Sex	Characteristics	Minnesota	CRIC	EKFC
Female ($n = 140$)	Age	64.3 ± 7.0	63.6 ± 7.2	64.4 ± 7.1
	BMI	29.1 ± 5.2	29.0 ± 5.2	28.8 ± 4.9
	mGFR	64.3 ± 16.1	64.1 ± 15.8	64.2 ± 16.1
	SCr[a]	0.83 ± 0.20	1.13 ± 0.23	0.86 ± 0.30
Male ($n = 165$)	Age	65.1 ± 7.7	63.9 ± 7.2	65.4 ± 7.8
	BMI	29.5 ± 3.2	29.5 ± 3.0	29.0 ± 3.0
	mGFR	67.7 ± 17.5	67.5 ± 17.4	67.6 ± 17.7
	SCr[a]	1.06 ± 0.23	1.42 ± 0.31	1.12 ± 0.37

Values presented as mean $\pm$ SD.
[a]Mean SCr is not significantly different between the Minnesota and EKFC cohorts but is highly significantly different ($P < .0001$, t-test) between the CRIC and the other two cohorts.

- BMI $\pm$ 2.5 kg/m^2 and
- mGFR $\pm$ 3 ml/min/1.73 m^2.

Descriptive statistics [mean $\pm$ standard deviation (SD)] was used for age, BMI and mGFR in each sex subgroup to demonstrate the success of the matching procedure.

We plotted SCr versus mGFR (the controlling variable) for the matched patients in Europe, Minnesota and CRIC, together with the fitted power function SCr $= A \times$ mGFR^{-B}, which allowed easy visual comparison of the systematic shift, if present. We further plotted the distribution of 'paired' differences (ideally these distributions should be Gaussian and centred around zero) and the paired differences against mGFR [ideally the patterns should cluster around zero, show no mGFR dependency and show homoscedasticity (having the same scatter)].

Regarding the impact on eGFR results, bias (eGFR − mGFR), interquartile range (IQR = 75th percentile – 25th percentile) and P10/P30 accuracy (the percentage of subjects with eGFR within 10%/30% of mGFR) with 95% confidence intervals (CIs) were calculated in the cohort of matching patients.

RESULTS

Matching Minnesota with CRIC with EKFC

We were able to match 305 subjects from the Minnesota, CRIC and EKFC cohorts. Table 1 presents descriptive statistics for the variables used as matching criteria and for SCr, which allow evaluation of successful matching. The mean SCr was roughly the same in the Minnesota and EKFC cohorts, but much higher in the CRIC, both in males and females.

Comparison of SCr results in the matched cohort

Figures 1–3 present SCr versus mGFR for the matching patients in the different cohorts. The difference between SCr results in the whole mGFR range is confirmed between the CRIC and Minnesota cohort in Figure 1, between the CRIC and EKFC cohort in Figure 2, whereas SCr concentrations were similar between the EKFC and Minnesota cohorts in Figure 3.

Figure 4 presents the distributions of the paired differences and the paired differences against average mGFR for the matched patients.
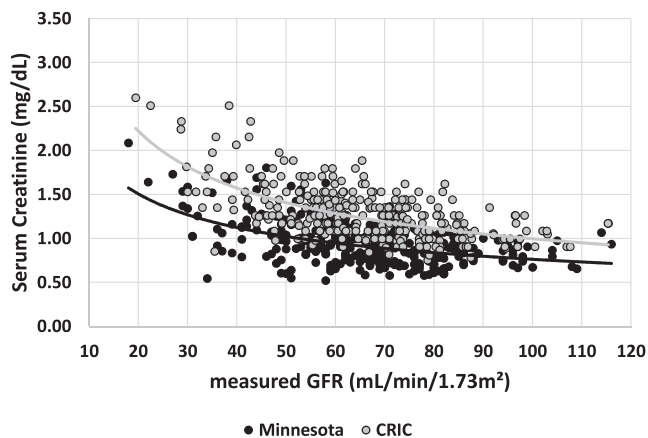
FIGURE 1: SCr versus mGFR for the matched patients of the Minnesota cohort and CRIC.
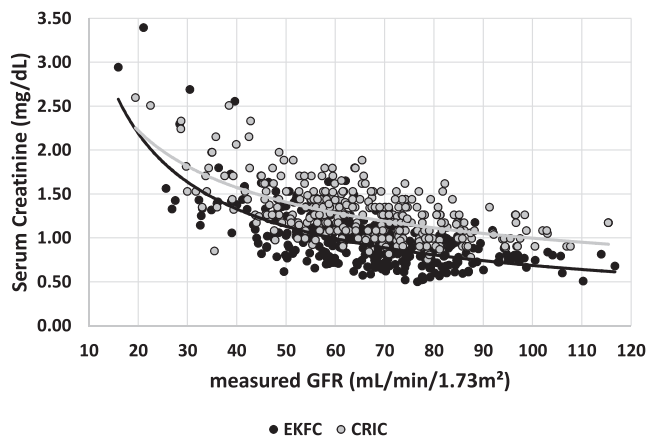

FIGURE 2: SCr versus mGFR for the matched patients of the EKFC cohort and CRIC.
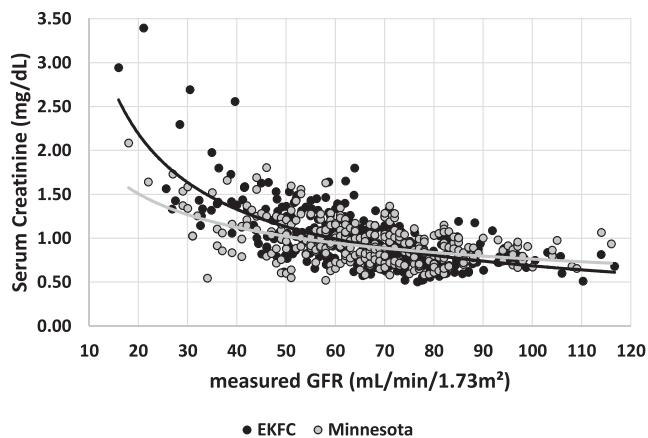

FIGURE 3: SCr versus mGFR for the matched patients of the EKFC and Minnesota cohorts.

Comparison of the performance of eGFR equations in the matched cohort

The performance of the CKD-EPI (ASR), CKD-EPI (AS) and EKFC$_{Crea}$ equations in the matched patients based on the Minnesota, CRIC and EKFC results is shown in Table 2.

Bias in the Minnesota and EKFC patients is much smaller for EKFC$_{Crea}$ than for CKD-EPI equation, while the opposite is true for the CRIC patients. Bias in the Minnesota and EKFC data is positive, while it is negative in the CRIC data. P10/P30 accuracy for EKFC$_{Crea}$ is much higher in the Minnesota and EKFC cohorts than in the CRIC, while the opposite is true for the CKD-EPI equation.

DISCUSSION

To compare the relationship between SCr and mGFR between two different populations, an ideal study would use identical methods to measure SCr and GFR, identical methods to identify and recruit study patients with similar demographic characteristics and the same statistical approach. With our original matching approach, we aimed to approximate equivalence with such an ideal study.

The impact of SCr calibration has been illustrated in the past in epidemiological studies (e.g. when comparing the prevalence of CKD with calibrated and uncalibrated results) [16, 31, 32] and in analytical studies (comparing different assays with SCr determined by mass spectrometry) [19, 20]. Here we used cohorts with mGFR and, based on the matching analysis, could show that SCr concentrations were different between the Minnesota and EKFC cohorts and CRIC, most likely due to differences in SCr calibration, which may have a great impact on the performance of equations. However, comparing the CRIC data with the EKFC and Minnesota data is not straightforward. Indeed, there are several differences in the methodologies and patient characteristics of the three studies that need to be considered besides calibration. First, measured GFR in the CRIC and Minnesota cohort is obtained via the clearance of non-radiolabeled iothalamate assayed with capillary electrophoresis from timed plasma and urine samples, whereas mGFR in the EKFC cohort was obtained with iohexol, inulin or ^{51}Cr-EDTA, and mostly using plasma clearance. So there is a clear difference in GFR measurement methods between the CRIC, Minnesota and EKFC cohorts [7, 8, 11, 33]. Second, there are differences in the preparation of patients prior to the measurement of GFR (e.g. fasting state in Minnesota, after a light protein meal in CRIC). Third, there might be population-specific differences between White North Americans (CRIC and Minnesota) and White Europeans due to differences in nutritional habits, muscle mass etc. Fourth, the health status (e.g. diabetes status, cardiovascular risk factors) was probably different between the three cohorts. Thus we cannot exclude that matching for age, sex, BMI and mGFR is sufficient to obtain cohorts with similar properties.

However, in the matched analysis for mGFR, age, BMI and sex, we observed that SCr concentrations in the Minnesota and EKFC cohorts were similar but the SCr concentrations in the CRIC were systematically higher compared with both the Minnesota and EKFC results. The Minnesota cohort and CRIC used the same mGFR measurement method and are both in White North American subjects. Therefore these results suggest that the observed systematic differences in SCr between the Minnesota and EKFC cohorts on one side and the CRIC on the other side, once again in patients sharing the same demographic characteristics and the same mGFR level, may be explained by differences in the calibration of SCr assays. Indeed, it should be emphasized that the Minnesota and EKFC cohorts used assays standardized to the IDMS gold standard method. The way the CRIC SCr values have been calibrated is more debatable. Indeed, SCr was measured in the CRIC before the standardization program and large variability was observed between the different laboratories participating
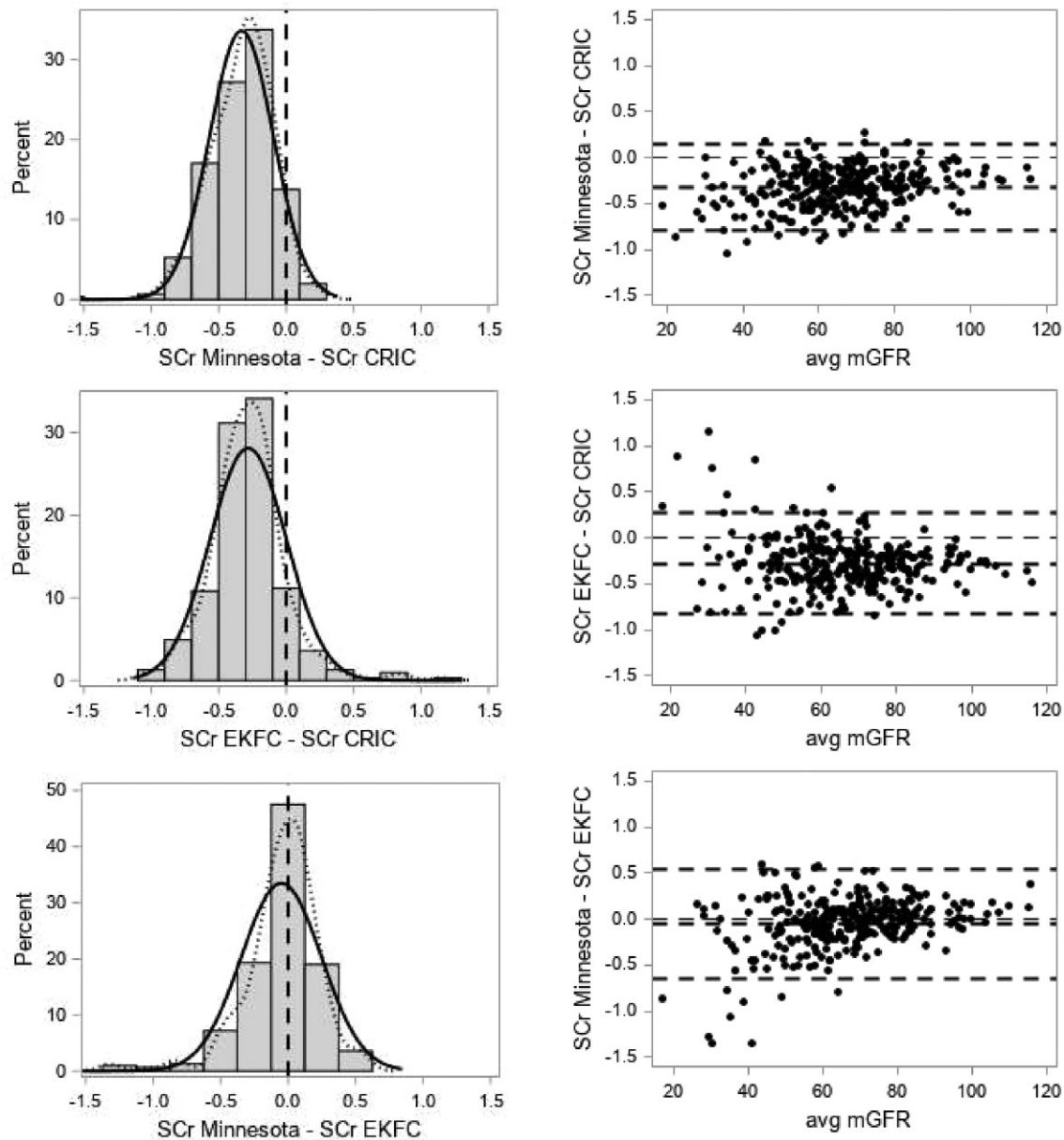
FIGURE 4: (Left panel) Distributions of paired differences of SCr (solid curve: normal; dotted curve: kernel; vertical dashed line is drawn at zero difference). **(Right panel)** Paired differences against average mGFR in the matching patients. The horizontal dashed lines are at zero difference, mean difference and the 95% limits of agreement.

to the CRIC Study [24]. This variability was corrected by 'recalibrating' the 13 different analytical platforms used in the CRIC laboratories on the Cleveland Clinic laboratory method with a library of five large-volume plasma specimens from apheresis patients [25, 26]. As a consequence, the results were harmonized (which does not mean that the results were calibrated), but, to the best of our knowledge, no study has been performed to verify the commutability of the apheresis plasma samples with all the methods used by the 13 laboratories. The calibration of these harmonized results required a second calibration with the Roche Cobas enzymatic method, which had been shown to be standardized against the IDMS reference method. Thus this is not a direct calibration to IDMS, but an indirect one, which is far from ideal, as previously shown [15, 32]. Moreover, it remains unclear on which samples the final calibration equation in the CRIC was

obtained and if the method used to measure the CRIC SCr was the Beckman CX3 analyser, which had initially been used at the Cleveland Clinic [24, 26, 27].

As illustrated in our study, the impact of SCr calibration on the global performance of different eGFR equations is important. Indeed, we have shown that the performance of the EKFC equation is better in the matched subjects from EKFC cohorts compared with the CKD-EPI equation (as expected, as the equation was developed in part with this cohort), but also in matched subjects from the Minnesota cohort, which is completely independent from both EKFC and CKD-EPI equation development. Because the CRIC was part of the development cohort for the CKD-EPI equation, the better performance of CKD-EPI equation in the CRIC compared with the EKFC cohort is not unexpected, but raises the question of the applicability of the CKD-EPI

Table 2. Bias, IQR (P25–P75), P10 and P30 statistics (with 95% CI) in the matched subjects ($n = 305$) for the CKD-EPI (ASR), CKD-EPI (AS) and EKFC equations

Variables	CKD-EPI (ASR)	CKD-EPI (AS)	EKFC$_{Crea}$
Minnesota			
Bias	9.4 (7.5–11.5)	14.0 (12.2–15.9)	4.8 (3.1–7.4)
IQR (P25–P75)	19.0 (0.0–19.0)	19.3 (4.3–23.6)	16.9 (−3.6–13.3)
P10 (%)	32.5 (27.2–37.7)	27.9 (22.8–32.9)	38.4 (32.9–43.8)
P30 (%)	75.7 (70.9–80.6)	64.6 (59.2–70.0)	82.3 (78.0–86.6)
CRIC			
Bias	−11.2 (−12.5 to −9.5)	−7.7 (−8.9 to −6.0)	−13.2 (−14.3 to −11.5)
IQR (P25–P75)	12.5 (−17.2 to −4.7)	12.8 (−14.1 to −1.3)	11.9 (−19.0 to −7.1)
P10 (%)	24.6 (19.7–29.5)	35.4 (30.0–40.8)	19.0 (14.6–23.4)
P30 (%)	84.6 (80.5–88.7)	89.5 (86.0–93.0)	81.3 (76.9–85.7)
EKFC			
Bias	8.0 (5.6–9.7)	12.2 (9.6–13.9)	3.9 (1.9–5.5)
IQR (P25–P75)	17.1 (−0.7–16.4)	18.1 (3.2–21.3)	15.2 (−4.1–11.1)
P10 (%)	39.7 (34.2–45.2)	31.8 (26.5–37.1)	45.9 (40.3–51.5)
P30 (%)	79.3 (74.8–83.9)	67.5 (62.3–72.8)	87.2 (83.4–91.0)

P25: 25th percentile; P75: 75th percentile.

equation in laboratories using an enzymatic, well-calibrated assay for SCr measurement [13, 14, 32].

The strength of the current study is the large cohorts available, allowing an original analysis based on matched subjects for age, sex, BMI and mGFR, simulating an ideal study setup to compare the SCr and GFR relationship between populations. The limitation is the absence of Black subjects in both the Minnesota and EKFC cohorts. However, there is no reason to believe that the issues of SCr calibration are different in White and Black populations. Although we aimed to match patients based on demographic characteristics (sex, age and BMI) and renal function (mGFR), it remains impossible to totally rule out that biases were affected by other population differences, health status, data cleaning or differences in protocols for mGFR [34, 35].

In conclusion, our results show that even after controlling for age, sex, BMI and mGFR, there were still fundamental differences in the relationship between SCr and mGFR in the studied cohorts. Better standardization of SCr and harmonization of GFR methods are needed for meaningful progress, as differences still exist in widely used epidemiological cohorts, which may have led to biased coefficients and impaired external validity of GFR equations developed based on such cohorts.

DATA AVAILABILITY STATEMENT

Data for the matched cohorts can be made available upon request to the corresponding author, after agreement with NIDDK (CRIC Cohort).

CONFLICT OF INTEREST STATEMENT

P.D. is member of the *CKJ* Editorial Board. The other authors declare no competing interests.

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