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Expressing the CKD-EPI Cystatin C Equations for Estimating GFR with Standardized Serum Cystatin C Values

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Assays traceable to high-level a reference materials are critical factors in ensuring accuracy of GFR estimates[1]. Serum cystatin C (Scys) is being proposed as a filtration marker that can be used with or as an alternative to serum creatinine (Scr) in equations to estimate GFR. The Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) published equations for estimating GFR from Scys in 2008 using serums assayed by the Cleveland Clinic Research Laboratory (CCRL) in 2003 using the Siemens-Dade-Behring (SDB) particle-enhanced immunonephelometric assay (PENIA) with the SDB BN II nephelometer[2]. At that time, Scys assays traceable to an internationally accepted reference material were not available. To foster consistent Scys results, the International Federation for Clinical Chemists (IFCC) Working Group for the Standardization of serum cystatin C and the Institute for Reference Materials and Measurements (IRMM) have collaborated for the production and characterization of a certified reference material (CRM)[3, 4]. This material, ERM-DA471/ IFCC, was made available to laboratories by IRMM in fall 2010. Here we report on the re-expression of previously reported equations to estimate GFR from Scys for use with Scys values traceable to the new IRMM CRM

The CKD-EPI Central Laboratory moved to the University of Minnesota (UMN) in 2009. At the time, comparisons between Scys measurement results at CCRL and UMN were made using serum calibration panels, which had been created in 2003 at CCRL and stored frozen at −70°[5]. The calibration panel included 40 reference sera pooled from at least 10 mixed-sex donors known to have serum creatinine values covering the range of 0.5 to 5.0 mg/dL. The panel was assayed for Scys in triplicate at CCRL in 2003 using the SDB measurement

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procedure as described above, and at the UMN in 2009 on a panel with one previous freeze thaw cycle using the same SDB PENIA reagent on a SDB ProSpec nephelometer. Conversion factors between the laboratories were determined using Deming linear regression[6,7]. To ensure stability over time, the calibration panel was retested on a previously unthawed panel at UMN in January 2011.

ERM-DA471/IFCC CRM was reconstituted at UMN as instructed in its Certificate of Analysis yielding an aqueous buffer solution with a cystatin C concentration 5.48 mg/L (uncertainty 0.15 mg/L)[3]. Lower concentration solutions of the reference material were prepared by dilution of the neat reconstituted reference material with volumetric additions of PENIA reagent buffer to create target values of 1.37 mg/L, 2.74 mg/L, and 4.11 mg/L. The instrument PENIA reagent buffer was considered to have zero concentration. Accuracy of volumetric additions was checked by weight. The materials were assayed in duplicate on two separate days. Regression equations were used to compare the mean concentrations assigned by the SDB PENIA reagents on the ProSpec at UMN compared to the calculated values based on the certified value of the reference material. Intercepts that were very small and non-significant ($p > 0.05$) were dropped from the regression. The calibration factor converts Scys results from UMN in 2009 (Scys-UMN'09) reported values to ERM-DA471/IFCC traceable values, which was then incorporated into the GFR estimating equation.

Mean (SD, range) level of Scys measured in the calibration panel was 2.01 (0.74, 0.78-3.64) mg/L in CCRL in 2003, 1.67 (0.58, 0.68-2.97) mg/L in UMN in 2009, and 1.67 (0.59, 0.65-2.98) mg/L in UMN 2011. The mean (SD) difference between Scys values from CCRL in 2003 (Scys-CCRL'03) and Scys-UMN'09 was -0.34 (0.16) mg/L (p-value <0.01). The regression of the Scys-UMN'09 on Scys-CCRL '03 showed a slope (SE) of 0.789 (0.009) and intercept of 0.083 (0.019) mg/L (Table 1, Equation 1 and Supplemental Figure 1).

The SDB ProSpec PENIA method resulted in lower values for Scys for the ERM-DA471/IFCC CRM than calculated based on the material's certified value. For all samples combined, the intercept (SE) was 0.043 (0.031) mg/L ($p=0.3$), and the slope was 1.109 ($p<0.001$), $R^2 = 0.9996$. After dropping the non- significant intercept, the slope was 1.120 ($P<0.001$), $R^2=0.9999$ (Table 1, Equation 2). The Scys-CCRL'03 were expressed as standardized Scys values (Table 1, Equations 3-4), and the published CKD-EPI Scys equations (Table 1, Equations 5-7) were re-expressed (Table 1, Equations 8-10).

Table 2 compares the assigned values for Scys-CCRL'03, Scys-UMN'09 and standardized Scys and corresponding estimated GFR. Using the Scys-UMN'09 values in the original estimating equations resulted in substantially higher values for estimated GFR than would have been obtained using Scys-CCRL'03 values. The eGFR values calculated standardized Scys and the re-expressed equations are nearly identical to those that would have been obtained using Scys-CCRL'03 values and the original equation.

In conclusion, drift in the Dade Behring Scys assay was observed between CCRL in 2003 and UMN in 2009. Similar drift has been observed in other laboratories using SDB measurement procedures, suggesting that this is due to subtle changes in their reagents or calibrators (personal communication Russell Tracy, PhD), Availability of CRM should

facilitate better comparability of Scys results among laboratories and measurement procedures manufactured by different invitro diagnostic manufacturers. Re-expression of the CKD-EPI Scys equations for use with ERM-DA471/IFCC-traceable Scys measurements will allow more consistent and accurate GFR estimates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

List of equations

Steps in standardization of cystatin C	
Equation 1	Scys-UMN'09= 0.083 + 0.789 * Scys-CCRL'03
Equation 2	standardized Scys = 1.12 * Scys-UMN'09
Equation 3*	standardized Scys = 0.093 + 0.884 * Scys-CCRL'03
Equation 4**	Scys-CCRL'03= −0.105 + 1.13 * standardized Scys
CKD-EPI equations for non-standardized Scys	
Equation 5	$eGFR = 76.7 * (Scys)^{-119}$
Equation 6	$eGFR = 127.7 * (Scys)^{-117} * age^{-013} * (0.91 \text{ if female}) * (1.06 \text{ if black})$
Equation 7	$eGFR = 177.6 * \text{standardized Scr}^{-0.65} * (Scys)^{-0.57} * age^{-0.20} * (0.82 \text{ if female}) * (1.11 \text{ if black})$
CKD-EPI equations re-expressed for standardized Scys^	
Equation 8	$eGFR = 76.7 * (-0.105 + 1.13 * \text{standardized Scys})^{-1.19}$
Equation 9	$eGFR = 127.7 * (-0.105 + 1.13 * \text{standardized Scys})^{-1.17} * age^{-0.13} * (0.91 \text{ if female}) * (1.06 \text{ if black})$
Equation 10	$eGFR = 177.6 * \text{standardized Scr}^{-0.65} * (-0.105 + 1.13 * \text{standardized Scys})^{-0\ 57} * age^{-0.20} * (0.82 \text{ if female}) * (1.11 \text{ if black})$

UMN, University of Minnesota; CCRL, Cleveland Clinic Research laboratory; ScysC, serum cystatin C; Scr, serum creatinine

Standardized serum creatinine (standardized Scr), refers to creatinine measured with assays that are traceable to the certified reference materials for creatinine whose value was assigned using isotope dilution mass spectroscopy.

*Combination of equation 1 and 2

**Re-expression of equation 3

^Cystatin C assays traceable to the reference materials produced and characterized from the International Federation for Clinical Chemists (IFCC) Working Group for the standardization of Scys and the Institute for Reference Materials and Measurements (IRMM). The reference material was produced by adding recombinant cystatin C into a serum pool from blood donors, with the assigned value established by dry mass analysis.

Table 2

Values for Cystatin C and Estimated GFR before and after standardization of the Cystatin C assay and re-expression of the GFR estimating equation

Cystatin Values *Mg/L*			Estimated GFR using equation 5 *ml/min per 1.73m²*			Estimated GFR using equation 8 *ml/min per 1.73m²*
Scys-CCRL'03	Scys-UMN'09*	Standardized Scys**	Scys-CCRL'03	Scys-UMN'09	Standardized Scys	Standardized Scys
0.5	0.48	0.53	175	185	162	175
0.9	0.79	0.89	87	101	88	87
1.0	0.87	0.98	77	90	79	77
1.1	0.95	1.07	68	81	71	69
1.3	1.11	1.24	56	68	59	56
1.5	1.27	1.42	47	58	51	47
2.0	1.66	1.86	34	42	37	34
2.5	2.06	2.30	26	33	28	26

* Converted from Scys-CCRL'03 using equation 1

** Converted from Scys-UMN '09 using equation 2. Cystatin C traceable to the reference materials produced and characterized from the International Federation for Clinical Chemists (IFCC) Working Group for the standardization of Scys and the Institute for Reference Materials and Measurements (IRMM)