Assessment of glomerular filtration rate (GFR) is essential for clinical practice. GFR is difficult to measure [measured GFR (mGFR)]; instead it is estimated [estimated GFR (eGFR)] by use of the serum concentration of endogenous filtration markers. However, all endogenous filtration markers are affected by factors other than GFR (non-GFR determinants), including generation, renal tubular reabsorption and secretion, and extrarenal elimination. Creatinine, a 113-Da breakdown product of muscle metabolism, was first identified in 1847 and proposed as a filtration marker in 1926 (1, 2). Creatinine-based eGFR (eGFRcr) is computed from serum creatinine concentration in combination with age, sex, and race as surrogates for creatinine generation by muscle. Cystatin C, a 13 300-Da serum protein produced by virtually all nucleated cells, was first identified in 1979 and proposed as a filtration marker in 1985 (3–5). Cystatin C is less influenced by muscle mass than creatinine and has often been proposed to be more accurate than creatinine for estimation of measured GFR in subgroups of the population, including vegetarians and those with muscle wasting, chronic disease, or limb amputation. The non-GFR determinants of cystatin C are not well known, and cystatin C–based eGFR (eGFRcys) is not more accurate for routine GFR estimation than eGFRcr. eGFR based on the combination of creatinine and cystatin C (eGFRcr-cys) is more accurate than either alone, reflecting the lesser influence of non-GFR determinants of either marker when both are used (6). eGFRcys is also more accurate in predicting prognosis than either eGFRcr-cys or eGFRcr, possibly reflecting opposite influences of non-GFR determinants of the markers (for example, muscle wasting leading to lower serum creatinine and inflammation leading to higher serum cystatin C) (7).

Standardized reference materials are available for both creatinine and cystatin C, and estimating equations have been developed for use with standardized creatinine and cystatin C by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (6, 8–11). Current clinical practice guidelines recommend measuring serum creatinine and reporting eGFRcr by use of the CKD-EPI 2009 equation as the initial test and measuring serum cystatin C and reporting eGFRcys and eGFRcr-cys by use of the CKD-EPI 2012 equations as a confirmatory test (12). Use of a single equation for each filtration marker (or combination) facilitates widespread implementation of eGFR reporting, but other GFR-estimating equations are recommended if they are more accurate than the CKD-EPI equations. Creatinine is routinely measured in acute and chronic illness with eGFRcr reported by 90% of clinical laboratories in the US (13). Limitations to more widespread use of cystatin C include lack of agreement among commercial assays despite introduction of the standard, few evaluations of the CKD-EPI 2012 equations in large clinical populations, and incomplete understanding of the non-GFR determinants of cystatin C. In this issue of Clinical Chemistry, Grubb et al. (14) make significant progress in addressing the first 2 of these issues.

Anders Grubb and colleagues are to be credited with landmark achievements in this field (3–5). More recently, Grubb led the Working Group for the Standardization of Cystatin C (WG-SCC), which was established and supported by the International Federation of Clinical Chemistry and the Institute for Reference Materials and Measurements and resulted in the development of the reference materials (9, 10). The current article has 2 aims, to improve agreement among commercial assays despite introduction of the standard, few evaluations of the CKD-EPI 2012 equations in large clinical populations, and incomplete understanding of the non-GFR determinants of cystatin C. In this issue of Clinical Chemistry, Grubb et al. (14) make significant progress in addressing the first 2 of these issues.

For the first aim, the WG-SCC cooperated with 7 diagnostic companies to evaluate 6 assays (2 are identical). Linear regression was used to relate assigned values for the 6 assays in 800–1000 plasma samples or 10 plasma pools. Correlations were excellent (R² from 0.993 to 1.000), but there was heterogeneity in the comparisons, with slope coefficients ranging from

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Nonstandard abbreviations: GFR, glomerular filtration rate; mGFR, measured GFR; eGFR, estimated GFR; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; WG-SCC, Working Group for the Standardization of Cystatin C; CAPA, Caucasian and Asian Pediatric and Adult subjects.
0.932 to 1.058 and intercept coefficients ranging from −0.1105 to 0.0388. The difference in eGFRcys between the highest and lowest assigned values for cystatin C after adjustment was judged to be negligible (approximately 5% compared with the mean). A major strength of the study is the articulation of the differences among measurement procedures and the need for harmonization among clinical laboratory measurement procedures for cystatin C. Ideally, diagnostic companies will use this information to calibrate their measurement procedures relative to the international standard, so that GFR estimation by use of equations expressed for standardized cystatin C truly are assay independent.

For the second aim, Grubb et al. pooled data on mGFR and cystatin C concentrations in 4960 individuals [3495 Swedish adults, 763 Japanese adults, and 702 Swedish and Dutch children, referred to as CAPA (Caucasian and Asian pediatric and adult subjects)]. GFR was measured with different methods in each group; 2 groups used 1-sample plasma clearance methods that are more suitable for clinical practice than research. Cystatin C was assayed by use of different methods but adjusted according to the regressions developed in the first aim. The new equation was developed in a randomly selected sample consisting of two thirds of the study population and internally validated and compared with the CKD-EPI 2012 cystatin C equation in the remaining one third. The new equation includes age, a single coefficient (exponent) for serum cystatin C (−1.069), and a value for extrarenal elimination of cystatin C [7 mL · min⁻¹ · (1.73 m²)⁻¹ across the range of GFR]. By contrast, the CKD-EPI equation was developed in white and African-American adults in North America and Europe and includes age and sex and 2 coefficients for serum cystatin C (−1.328 and −0.499 for cystatin C >0.8 and ≤0.8 mg/L, respectively) (6). Performance of the CAPA and CKD-EPI equations was equivalent in the 3 groups. For both equations, bias and precision differed across the groups, but accuracy was judged to be acceptable in all. The authors concluded that the CAPA equation is as accurate as the CKD-EPI equation, and the CAPA equation may be preferred because it does not require specification of sex and takes into account extrarenal elimination. Strengths of the study are inclusion of Asians as well as whites and inclusion of children as well as adults. Weaknesses are absence of other racial and ethnic groups, heterogeneity in GFR measurement procedures, inference rather than measurement of extrarenal elimination of cystatin C, and most important, absence of an external validation population.

Confirmation of findings of any new estimating equation in an external population is really required for true evaluation of validity. The findings reported by Grubb et al. of the performance of the CKD-EPI equation in the internal validation dataset for the CAPA population can provide valuable information regarding the validity of the CKD-EPI equation, but do not provide information regarding the validity of the CAPA equation. To overcome this limitation, we compared the performance of the CAPA and CKD-EPI equations in the study populations used for development and validation of the CKD-EPI equation (Fig. 1) (6, 14, 15). In the CKD-EPI external validation population (Fig. 1A), performance of the 2 equations was equivalent overall. Both equations underestimated mGFR at higher measurements; however, the CKD-EPI equation was less biased in subjects with mGFR 90–119 mL · min⁻¹ · (1.73 m²)⁻¹, likely because of the inclusion of the second coefficient for serum cystatin C concentrations <0.8 mg/L. The small difference in bias between men and women for the CAPA equation, but not for the CKD-EPI equation, suggests that the absence of a term for sex in the CAPA equation is more of a disadvantage than an advantage. Evaluation of the CAPA equation in the CKD-EPI internal validation population (Fig. 1B) revealed similar findings. In addition, there was no difference in bias between blacks and nonblacks. On the basis of these findings, in addition to the findings reported by Grubb et al. in this issue (14), we would conclude that the CAPA equation is not more accurate than the CKD-EPI 2012 cystatin C equation and we believe it is not preferred for eGFRcys reporting. In addition, we would conclude that GFR estimation based on cystatin C may not require specification of race (at least for North American and European whites, African Americans, or Japanese) and may not require different equations for adults and children. Further research would help confirm these conclusions.

We believe that cystatin C has come of age. Laboratory assays are improving, and GFR-estimating equations based on cystatin C have wide application. We suggest that cystatin C should be measured more often and should be reported as eGFRcys and as eGFRcr-cys if creatinine is measured concomitantly. Avoiding the need for specification of race and confounding by muscle mass and more accurate prognosis are major advantages of eGFRcys over eGFRcr. However, eGFRcys is not more accurate than eGFRcr for routine use, and without knowledge of the non-GFR determinants of cystatin C and reduction in cost of assays, we do not foresee “new” cystatin C replacing “old” serum creatinine as the initial test for GFR estimation in the near future. At the present time, it is most useful as a confirmatory test, especially in combination with creatinine, where eGFR based on both filtration markers simultaneously provides greater accuracy than either alone.

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Further research is required to identify subgroups of the population in which the non-GFR determinants of creatinine lead to inaccuracy of eGFRcr and appropriate diagnostic testing strategies for these subgroups. Identification of non-GFR determinants of cystatin C is required for better clinical interpretation of eGFRcys, for estimation of both GFR and prognosis. Finally, the search for additional filtration markers should continue, since use of a panel of endogenous filtration markers has the potential to minimize the effect of non-GFR determinants of each marker alone, even without full knowledge of the non-GFR determinants of each marker.
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