Comparing GFR Estimating Equations Using Cystatin C and Creatinine in Elderly Individuals

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ABSTRACT

Current guidelines recommend reporting eGFR using the Chronic Kidney Disease epidemiology Collaboration (CKD-EPI) equations unless other equations are more accurate, and recommend the combination of creatinine and cystatin C (eGFRcr-cys) as more accurate than either eGFRcr or eGFRcys alone. However, preferred equations and filtration markers in elderly individuals are debated. In 805 adults enrolled in the community-based Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study, we measured GFR (mGFR) using plasma clearance of iohexol, standardized creatinine and cystatin C, and eGFR using the CKD-EPI, Japanese, Berlin Initiative Study (BIS), and Caucasian and Asian pediatric and adult subjects (CAPA) equations. We evaluated equation performance using bias, precision, and two measures of accuracy. We first compared the Japanese, BIS, and CAPA equations with the CKD-EPI equations to determine the preferred equations, and then compared eGFRcr and eGFRcys with eGFRcr-cys using the preferred equations. Mean (SD) age was 80.3 (4.0) years. Median (25th, 75th) mGFR was 64 (52, 73) ml/min per 1.73 m², and the prevalence of decreased GFR was 39% (95% confidence interval, 35.8 to 42.5). Among 24 comparisons with the other equations, CKD-EPI equations performed better in 9, similar in 13, and worse in 2. Using the CKD-EPI equations, eGFRcr-cys performed better than eGFRcr in four metrics, better than eGFRcys in two metrics, and similar to eGFRcys in two metrics. In conclusion, neither the Japanese, BIS, nor CAPA equations were superior to the CKD-EPI equations in this cohort of community-dwelling elderly individuals. Using the CKD-EPI equations, eGFRcr-cys performed better than eGFRcr or eGFRcys.


CKD is common in elderly individuals,1,2 and there is debate about the accuracy of GFR estimates in this important subgroup of the population. Lower muscle mass and dietary protein intake in elderly individuals may lead to greater bias in eGFR using serum creatinine.3 Serum cystatin C is thought to not be influenced by muscle mass and dietary protein intake,4,5 and has been hypothesized to be a better filtration marker than creatinine, particularly in older adults. Several reports have demonstrated that eGFR based on the combination of both standardized serum creatinine and cystatin C is more accurate than eGFR based on either marker alone,6–8 but there are few studies using standardized serum creatinine and cystatin C to evaluate these equations in elderly individuals.9–13

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Current guidelines recommend using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations for GFR estimation in clinical practice or other equations using standardized creatinine and cystatin C if they are more accurate than the CKD-EPI equations.14 Among other equations, only the Japanese equations,15,16 the Berlin Initiative Study (BIS) equations,12 and the Caucasian and Asian pediatric and adult subjects (CAPA) equation18 are based on standardized creatinine and standardized cystatin C assays. A comparison of the performance of equations developed by these four groups in elderly individuals has not been reported.

In a cohort of older adults from Iceland, we evaluated all equations in estimating the prevalence of decreased GFR (measured GFR [mGFR]<60 ml/min per 1.73 m²) and their performance in estimating mGFR. We first compared the performance of the Japanese, BIS, and CAPA equations with the CKD-EPI equations. We then compared the performance of eGFRcr and eGFRcrs with creatinine and cystatin C (eGFRcr-cys) using the preferred equations. Our goal was to provide data to assist clinical laboratories in deciding which equations to use for eGFR reporting and to assist practitioners in deciding which filtration marker to measure for GFR estimation in elderly individuals.

RESULTS

Characteristics of the Study Population and Distributions of mGFR and eGFR

Table 1 shows the demographic and clinical characteristics of participants. Mean (SD) age was 80.3 (4.0) years, 45.9% of participants were women, and 23.6% had diabetes. Figure 1 shows the distributions of mGFR and eGFR and the prevalence of decreased GFR for each. Median (25th, 75th) mGFR was 64 (52.73) ml/min per 1.73 m², with a wide range (12–112 ml/min per 1.73 m²). The estimated prevalence (95% confidence interval [95% CI]) of decreased GFR (GFR<60 ml/min per 1.73 m²) was 39.0% (35.8 to 42.5). For the CKD-EPI equations, compared with mGFR, the distribution of eGFRcr was shifted to the right, eGFRcrs was shifted to the left, and eGFRcr-cys was similar. Accordingly, prevalence estimates for decreased GFR were lower, higher, and similar using eGFRcr, eGFRcrs, and eGFRcr-cys, respectively, compared with mGFR. For the Japanese equations, compared with mGFR, the distributions of eGFRcr, eGFRcrs, and eGFRcr-cys were all shifted to the left, most for eGFRcr, least for eGFRcrs, and intermediate for eGFRcr-cys. Accordingly, prevalence estimates for decreased GFR were high for all compared with mGFR, more for eGFRcr, less for eGFRcrs, and intermediate for eGFRcr-cys. For the BIS equations, compared with mGFR, the distributions of eGFRcr and eGFRcr-cys were shifted to the left and similar to each other, and prevalence estimates for decreased GFR were high. For the CAPA equation, the distribution of eGFRcrs was similar to mGFR, as was the prevalence estimate for decreased GFR.

Comparisons of Equation Performance

Table 2 compares the CKD-EPI, Japanese, BIS, and CAPA equations for creatinine, cystatin C, and the combination in the whole cohort. Of the 24 comparisons of CKD-EPI equations to the Japanese, BIS, and CAPA equations in the whole cohort, performance of the CKD-EPI equations was better in 9, similar in 13, and worse in 2. For the three creatinine-based equations (eight comparisons in the whole cohort), the CKD-EPI equation had lower bias compared with the Japanese and BIS equations, had greater accuracy (higher percentage of eGFR within 30% of mGFR [P30] and lower root mean squared error [RMSE]) than the Japanese equation, but had similar RMSE and lower P30 than the BIS equation. All three equations had similar precision. For the three cystatin C-based equations (eight comparisons in the whole cohort), the CKD-EPI equation had lower bias and RMSE than the Japanese equation, but had similar RMSE and higher bias than the CAPA equation. All three equations had similar interquartile ranges (IQRs) and P30. For the three equations based on creatinine and cystatin C (eight comparisons in the whole cohort), the CKD-EPI equation had lower bias than the Japanese and BIS equations, and lower RMSE and similar
P30 compared with the Japanese equation. There were no differences in P30 and RMSE between the CKD-EPI and BIS equations, and no difference in precision among the three equations. The CKD-EPI equations also had lower or similar bias across the range of eGFR (Figure 2) and across all subgroups (Supplemental Tables 1–3).

Table 2. Comparison the performance of the CKD-EPI, Japanese, BIS, and CAPA equations in the entire cohort

<table>
<thead>
<tr>
<th>Equation</th>
<th>Bias Median Difference</th>
<th>Precision IQR</th>
<th>Accuracy P30</th>
<th>Accuracy MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFRcr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>-2.7 (-3.3 to -2.1)</td>
<td>12.1 (11.2 to 13.4)</td>
<td>91.7 (89.9 to 93.4)</td>
<td>0.17 (0.16 to 0.18)</td>
</tr>
<tr>
<td>Japanese</td>
<td>10.5 (9.8 to 11.2)</td>
<td>10.9 (9.7 to 12.1)</td>
<td>86.3 (83.9 to 88.6)</td>
<td>0.25 (0.24 to 0.26)</td>
</tr>
<tr>
<td>BIS</td>
<td>5.7 (5.1 to 6.4)</td>
<td>11.9 (10.6 to 12.7)</td>
<td>95.8 (94.4 to 97.1)</td>
<td>0.18 (0.17 to 0.19)</td>
</tr>
<tr>
<td>eGFRcys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>1.9 (1.3 to 2.8)</td>
<td>11.4 (10.6 to 12.4)</td>
<td>93.8 (92.0 to 95.4)</td>
<td>0.17 (0.16 to 0.18)</td>
</tr>
<tr>
<td>Japanese</td>
<td>4.6 (3.8 to 5.6)</td>
<td>11.2 (10.2 to 12.3)</td>
<td>92.8 (90.9 to 94.5)</td>
<td>0.19 (0.18 to 0.20)</td>
</tr>
<tr>
<td>CAPA</td>
<td>0.1 (-0.7 to 0.6)</td>
<td>11.8 (10.8 to 12.9)</td>
<td>94.4 (92.8 to 95.9)</td>
<td>0.16 (0.15 to 0.17)</td>
</tr>
<tr>
<td>eGFRcr-cys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD-EPI</td>
<td>-0.6 (-1.2 to 0.1)</td>
<td>10.2 (9.0 to 11.1)</td>
<td>96.1 (94.8 to 97.4)</td>
<td>0.14 (0.13 to 0.15)</td>
</tr>
<tr>
<td>Japanese</td>
<td>8.2 (7.7 to 8.7)</td>
<td>9.0 (8.2 to 10.0)</td>
<td>93.0 (91.2 to 94.8)</td>
<td>0.20 (0.20 to 0.21)</td>
</tr>
<tr>
<td>BIS</td>
<td>5.3 (4.9 to 6.1)</td>
<td>9.6 (8.6 to 10.4)</td>
<td>97.9 (96.8 to 98.8)</td>
<td>0.15 (0.15 to 0.16)</td>
</tr>
</tbody>
</table>

Data are presented with 95% CIs. Nonoverlapping 95% CIs were considered to represent differences. Bias was calculated as the median value of (mGFR – eGFR). IQR is the interquartile range of the difference between mGFR and eGFR. RMSE is the root mean squared error for the regression of log mGFR on log eGFR. Units for GFR, bias, and IQR are ml/min per 1.73 m². To convert GFR from ml/min per 1.73 m² to ml/s per 1.73 m², multiply by 0.0167.

*No different than CKD-EPI.
†Better than CKD-EPI.
‡Worse than CKD-EPI.

Figure 1. Distribution and CKD prevalence of mGFR and eGFR. (A) CKD-EPI equations. (B) Japanese equations. (C) BIS equations. (D) CAPA equation. Distributions of mGFR and eGFR are demonstrated using kernel density plots. CKD stages 3–5 are defined as GFR < 60 ml/min per 1.73 m².
Comparisons of Filtration Markers Using the CKD-EPI Equations

Supplemental Table 4 compares the performance of filtration markers using the CKD-EPI equations in the entire cohort. eGFRcr and eGFRcys performed similarly according to all four metrics. Compared with eGFRcr, eGFRcr-cys performed better for all four metrics. Compared with eGFRcys, eGFRcr-cys performance was better for two metrics (bias and RMSE) and similar for two metrics (IQR and P30). eGFRcr-cys had similar or lower bias compared with eGFRcr and eGFRcys across all subgroups (Figure 3).

Other Comparisons

For the Japanese equations, eGFRcr-cys was better than eGFRcr for three of four metrics, but better than eGFRcys in only one metric and worse in one; for the BIS equations, eGFRcr-cys was better than eGFRcys in two of four metrics (Supplemental Tables 5 and 6). Comparing all combinations of equations and filtration markers, the CKD-EPI eGFRcr-cys was better, similar, and worse than other combinations in 18, 14, and 0 of 32 comparisons, respectively, and no equation performed better than the CKD-EPI eGFRcr-cys (Supplemental Table 7).

Figure 2. Median bias in eGFR by level of eGFR. Bias is calculated as the median value of (mGFR−eGFR). Dashed line indicates the 95% CIs.
DISCUSSION

Accurately estimating GFR in elderly individuals is important for detection and staging of CKD. There is debate about the preferred filtration markers and estimating equations for this purpose. Filtration markers differ in their non-GFR determinants and equations using the same filtration markers differ in their modeling of surrogates for the non-GFR determinants (age, sex, race, and body size). In this study, we sought to determine whether the Japanese, BIS, or CAPA equations are preferable to the CKD-EPI equations for reporting eGFR by clinical laboratories and whether the combination of creatinine and cystatin C is preferable to either alone for estimating GFR and determining the prevalence of decreased GFR in elderly individuals. Our results showed that neither the Japanese, BIS, nor CAPA equations were consistently superior to the CKD-EPI equations in the whole cohort or in subgroups defined by age, sex, body mass index (BMI), diabetes status, and mGFR levels. Among the CKD-EPI equations, eGFRcr and eGFRcys had similar accuracy but were biased in the opposite directions. By contrast, eGFRcr-cys was less biased, more precise, and more accurately estimated the prevalence of decreased GFR. Indeed, no equation performed better than the CKD-EPI eGFRcr-cys.

Serum creatinine is the endogenous filtration marker that has been most widely used to estimate GFR. Because of age-related decline in muscle mass and dietary protein intake in elderly individuals, there is concern that creatinine may not be the preferred filtration marker for GFR estimation. In particular, lower muscle mass or protein intake could lead to systematic overestimation of mGFR and underestimation of CKD prevalence when using eGFRcr. Cystatin C, an alternative endogenous filtration marker, which is not affected by muscle mass and dietary protein intake, has been hypothesized to be superior to creatinine, particularly in elderly individuals. In populations that include younger adults, the combination of the two markers has been shown to provide more precise and accurate estimates than either alone. Two prior studies in elderly participants suggest that the combination of creatinine and cystatin C provide the most accurate estimates. Our results using the CKD-EPI equations are consistent with these studies. We also showed that the combined equation provided the most accurate estimate of prevalence of mGFR<60 ml/min per 1.73 m².

In principle, the improved accuracy of eGFRcr-cys over eGFRcr and eGFRcys reflects the smaller effects of the non-GFR determinants of each marker when they are used in combination rather than when they are used alone. Our findings and those of others demonstrate the importance of non-GFR determinants of both cystatin C and creatinine in elderly individuals. Although few studies have directly assessed the non-GFR determinants of serum cystatin C, several reports suggest that fat mass and inflammation are two potential factors. Both of these factors are more common in elderly individuals. For example, body fat mass increases with age and there is high prevalence of cardiovascular disease and other chronic diseases among elderly persons, which are associated with inflammation. These factors may account, in part, for the systematic underestimation of GFR and overestimation of CKD prevalence that we observed using eGFRcys using the CKD-EPI equations.

The Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend that clinical laboratories report eGFR in adults using the CKD-EPI equations, unless there are alternative equations using standardized assays that have been shown to improve accuracy of GFR estimation. The CKD-EPI equations were developed in a North American and European study population with a wide age range, and they are currently recommended for eGFR reporting in all age groups of adults in North America, Europe, and Australia without modification.

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**Figure 3.** Comparison of bias of the CKD-EPI equations. Bias is calculated as the median difference between mGFR and eGFR. Bars indicate the 95% CIs. N indicates sample size.
BIS equations were developed in a European elderly population, leading to speculation that the BIS equations would be more appropriate for eGFR reporting in elderly individuals. However, in general, we found similar or better performance of the CKD-EPI equations across the range of metrics tested and in most subgroups. Other studies that have compared the CKD-EPI and BIS equations using standardized serum creatinine and cystatin C assays report that they show generally similar performance to each other across the range of metrics, although there is some variation among studies. The CAPA equation was developed in northern European adults and children and Japanese adults. A previous report has demonstrated similar performance of the CAPA and CKD-EPI cystatin C equations in the CKD-EPI external validation data set. We observed similar results in this elderly population, although the CAPA equation had even lower bias than the CKD-EPI equation. Overall, our findings in this European elderly population demonstrate that the CKD-EPI equations perform as well as or better than the BIS and CAPA equations in most comparisons. On the basis of these findings, we would not conclude that either the BIS or CAPA equations are superior to the CKD-EPI equations or preferred for eGFR reporting in elderly individuals in this setting. Instead, using the CKD-EPI equation for adults of all ages would facilitate eGFR reporting by clinical laboratories.

In regions other than North America, Europe, and Australia, KDIGO recommends modifications to the CKD-EPI creatinine equations or alternative creatinine-based equation if they are shown to improve accuracy of GFR estimates. The creatinine-based Japanese equations were developed specifically for GFR estimation in Japan, where muscle mass and dietary protein intake are lower than in North America and Europe, leading to biased eGFRcr using the CKD-EPI equation. As such, the Japanese creatinine equation demonstrated a large bias in this Icelandic study population. By contrast, given the lesser effects of muscle mass and diet on cystatin C than creatinine, ethnic or regional coefficients might not be necessary for GFR estimation using cystatin C. Horio et al. previously noted that the CKD-EPI cystatin C equation performed well in their Japanese validation population. Similarly, in a study of multiethnic Asian population in Singapore, the CKD-EPI cystatin C equation was unbiased without modification for ethnicity. The CAPA equation was developed using the same Japanese study population as the Japanese equation. We showed that the Japanese and CAPA cystatin C equations performed reasonably well in this Icelandic study population, but not consistently better than the CKD-EPI cystatin C equation. These findings confirm that cystatin C appears to be robust to ethnic and geographic differences. Further studies are necessary to determine the accuracy of the CKD-EPI cystatin C equation in regions other than East Asia.

Strengths of our study include use of a large elderly cohort, measurement of serum creatinine and cystatin C using standardized assays, and a prespecified, rigorous statistical analysis including point estimates and 95% CIs for testing the performance of equations in overall cohort and subgroups defined by demographic and clinical characteristics and mGFR levels. Our study has limitations. First, our study population is Icelandic and does not include blacks and Asians; thus, we may not necessarily extend our results to other ethnic groups. Second, use of different exogenous filtration markers in the development and validation populations may cause bias in eGFR in the validation population. Some of the differences in bias among equations observed in this study and between our study and others may reflect differences in GFR measurements across the study population. Third, measurement error in mGFR may contribute to imprecision in eGFR, making it difficult to detect differences among equations.

In conclusion, we have demonstrated that neither the Japanese, BIS, nor CAPA equations were overall superior to the CKD-EPI equations in elderly individuals and would not be preferable for eGFR reporting. Using the CKD-EPI equations, eGFRcr-cys is less biased, more precise, and more accurate than eGFRcr and eGFRcys in elderly persons. If accurate GFR estimates are required in elderly individuals, we suggest using eGFRcr-cys.

**CONCISE METHODS**

**Study Population**

The Age, Gene/Environment Susceptibility (AGES)-Reykjavik study originates from the Reykjavik Study, a community-based cohort established in 1967 to prospectively study cardiovascular disease in Iceland. Between 2002 and 2006, a total of 5764 male and female individuals participated in detailed follow-up evaluations. The second visit of the AGES-Reykjavik study was a repeat examination of 3411 participants between 2007 and 2011, and 805 of them were enrolled in the substudy to measure GFR (AGES-Kidney). The study was approved by the Icelandic Bioethics Committee (approval number VSN-00-063) and the institutional review boards of the National Institute on Aging and Tufts Medical Center. All participants gave written informed consent.

**Laboratory Methods**

GFR was measured using plasma clearance of iohexol and was expressed per 1.73 m² body surface area. Details of the GFR measurement procedure can be found in the Supplemental Methods. Briefly, 5 ml of iohexol was administered over a period of 30 seconds followed by a 10-ml normal saline flush. Blood samples for plasma clearance measurements were taken from a second catheter at approximately 120, 180, 240, and 300 minutes, with the exact times recorded. Plasma clearance of iohexol was calculated using the Brochner-Mortensen equation. Serum creatinine was measured using the Roche Hitachi-P-Module instrument with the Roche Creatinine Plus assay (coefficient of variation was 2.2% for creatinine assay), which is traceable to National Institute Standardized Technology creatinine standard reference material 909b. Serum cystatin C was measured on the Siemens BN100 Nephelometer using a particle-enhanced immunonephelometric assay (coefficient of variation of 2.7% and 5.5% for intra- and inter-assay imprecision, respectively), with assays traceable to International Federation for Clinical Chemists Working Group for the Standardization of Serum Cystatin C and the Institute for Reference Materials and Measurements certified reference materials.
GFR Estimation Equations

eGFR was computed from equations developed by CKD-EPI, the Japanese group, BIS, and CAPA.\textsuperscript{6,15–18} Supplemental Table 8 shows these equations in detail.

Statistical Analyses

Approach

Equations by research group were compared by filtration markers. The Japanese, BIS, and CAPA equations were compared with the CKD-EPI equations, because the KDIGO guidelines recommend using the CKD-EPI equation as a reference for evaluation of new equations. Filtration markers were first compared for the preferred research group equations. eGFR\textsubscript{cr} and eGFR\textsubscript{cys} were compared with eGFR\textsubscript{rc}–cys, because prior publications have shown that the eGFR\textsubscript{rc}–cys performs better than eGFR\textsubscript{cr} and eGFR\textsubscript{cys}.\textsuperscript{6–8} Additional analyses included comparison of filtration markers for other research group equations as appropriate, and comparisons of all combinations of research group equations and filtration markers to the best performing combination.

Prevalence of Decreased GFR (GFR<60 ml/min per 1.73 m\textsuperscript{2})

The distribution of mGFR and eGFR for all equations was demonstrated using kernel density plots generated using the \textit{density} function. The prevalence of decreased GFR was estimated using mGFR and eGFR for all equations.

Equation Performance

The performance of all equations compared with mGFR was evaluated using metrics for bias, precision, and accuracy. Bias was assessed as the median difference between mGFR and eGFR (mGFR – eGFR). Precision was assessed as the IQR of the difference between mGFR and eGFR. Accuracy was assessed as the percentage of eGFR within 30% of mGFR (P\textsubscript{30}) as a measure of large errors, and the RMSE for the regression of log mGFR on log eGFR as an overall measure of goodness of fit.\textsuperscript{34} The 95% CIs around the median difference, IQR of the difference, P\textsubscript{30}, and RMSE were calculated using the bootstrap method (1000 bootstraps). For all four metrics, differences between equations were determined by nonoverlapping 95% CIs, allowing use of a consistent approach for comparison across all metrics, whereas \textit{P} values could only be determined for differences in bias and P\textsubscript{30}. In addition, for bias, important differences between the equations were determined by comparison of the absolute value of the median difference because magnitude, rather than the direction, is more clinically meaningful.\textsuperscript{35}

All four metrics were used to characterize performance in the overall cohort. Bias was used to compare performance in subgroups, because bias in subgroups is a cause of imprecision and inaccuracy. Subgroups were defined by demographic and clinical characteristics and level of mGFR. Subgroups were defined by age (<80, 80–84, and ≥85 years), sex (men versus women), diabetes status (yes versus no), BMI (<20, 20–24, 25–29, and ≥30 kg/m\textsuperscript{2}), and mGFR (<30, 30–59, 60–89, and ≥90 ml/min per 1.73 m\textsuperscript{2}). Diabetes was defined as either having fasting serum glucose >106.2 mg/dl, self-reporting a diabetes diagnosis, taking insulin injections or tablets for diabetes, or following a special diet for diabetes in past 5 years. We used mGFR instead of eGFR to define subgroups to avoid varying sample sizes across equations.

All analyses were performed using R software (version 2.15.3, http://www.r-project.org; Free Software Foundation Inc.).

ACKNOWLEDGMENTS

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DISCLOSURES

None.

REFERENCES


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CORRECTION


Please note the following correction in the above article published in the August 2015 issue of the Journal of the American Society of Nephrology. We regret that our article contained errors in the definition of diabetes and the proportion of participants with diabetes. The correct definition of diabetes was a fasting serum glucose >126 mg/dl, self-reporting a diabetes diagnosis, taking insulin injections or tablets for diabetes, or following a special diet for diabetes in the past 5 years. Using the correct classification of diabetes, the number of study participants with diabetes was 91 of 805 (11.3%) and the number (percent) of participants with diabetes by GFR category was 6 (21%), 35 (12%), 44 (9.6%), and 6 (18%) in GFR categories <30, 30–59, 60–89, and ≥90 ml/min/1.73 m², respectively (P trend 0.11) (Table 1). Using the correct classification for diabetes, there remained no substantive differences in the performance of GFR estimating equations among subjects with or without diabetes (Figure 3 and revised Supplemental Tables 1, 2, or 3; see supplemental tables for comparisons).