Calibration of the Siemens Cystatin C Immunoassay Has Changed over Time

To the Editor:

The Siemens cystatin C immunoassay has been widely used in clinical research, particularly in the US. In recent years, however, the results obtained with the method appear to have changed.

The glomerular filtration rate (GFR) is generally accepted as the best overall indicator of kidney function and is an important measure for assessing kidney disease. Several studies have shown cystatin C to be superior to creatinine for estimation of the GFR (1), which is usually expressed as the relative GFR [in units of mL·min⁻¹·(1.73 m²)⁻¹]. This practice has led to the development of formulas to convert cystatin C measurements in milligrams per liter to a calculated GFR in these units, without the need for demographic coefficients (2, 3). The formulas were developed from studies that compared cystatin C concentrations with measured GFRs by using such exogenous markers as iohexol, diethylenetriamine pentaacetic acid or ⁵¹Cr-EDTA clearance (4).

We were concerned that the calibration of the Siemens cystatin C method had changed during the last 5 years, because we noted that the cystatin C concentrations of participants in a longitudinal cohort improved substantially over time. The study investigators considered these results implausible because the GFR is known to decrease with age. The aim of the present study was to determine whether the Siemens cystatin C method has changed its calibration during the last 5 years and, if so, to quantify the magnitude of the change.

We used our laboratory’s current routine method from Gentian as a comparative method. To ensure that the comparative method had been stable, we verified that each lot of the Gentian reagent produced results within 3% of the originally assigned values for 10 patient serum pools prepared in 2005 and stored at −70 °C. Freshly collected and lithium heparin–treated samples obtained from routine cystatin C requests were then used to compare the 2 cystatin C methods. New sets of patient samples were used for each comparison between 2006 and 2010 (Table 1).

Plasma cystatin C was analyzed on an Architect ci8200® analyzer (Abbott Diagnostics) with reagents and calibrator from Gentian and on a BN ProSpec analyzer (Siemens Healthcare Diagnostics) with reagents and calibrator from Siemens.

A Deming regression analysis showed no significant difference between the 2 methods in 2006 (Table 1); however, both the y intercept and the slope differed significantly from 2007 onward. In 2005, the Gentian (x) and Siemens (y) methods showed strong agreement (y = 1.0507x + 0.0369 mg/L; r² = 0.994; n = 95). In March 2006, the 2 methods also gave comparable results (y = 1.005x − 0.026 mg/L; r² = 0.995; n = 92). In December 2007, the slope of the linear regression analysis had decreased by approximately 7% while maintaining a high r² value (y = 0.928x − 0.054 mg/L; r² = 0.997; n = 180). By October 2008, the bias was larger, and the slope was substantially different, despite a high correlation coefficient (y = 0.855x − 0.099 mg/L; r² = 0.997; n = 88). These results indicated a 15% decrease in the results obtained with the Siemens method. A retest of the same Siemens reagent lot in 2010 with the patient pools showed a similar slope (0.864). A final comparison in May 2010 showed a persistent bias and a lower slope compared with our control method (y = 0.8725x − 0.1133 mg/L; r² = 0.998; n = 112).

The observed change in calibration of the Siemens method increased GFR values. For instance, a cystatin C concentration corresponding to a GFR of 60 mL·min⁻¹·(1.73 m²)⁻¹ in 2005 increased to approximately 80 mL·min⁻¹·(1.73 m²)⁻¹ in 2010.

Owing to the nonlinear relationship between cystatin C and

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Table 1. Comparison of the Siemens and Gentian cystatin C methods by Deming regression analysis.

<table>
<thead>
<tr>
<th>Year</th>
<th>Bias</th>
<th>95% CI</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>y Intercept, mg/L</td>
<td>0.03</td>
<td>−0.01 to 0.07</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.99</td>
<td>0.97–1.02</td>
<td>0.014</td>
</tr>
<tr>
<td>2007</td>
<td>y Intercept, mg/L</td>
<td>0.06</td>
<td>0.04–0.08</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>1.08</td>
<td>1.06–1.09</td>
<td>0.007</td>
</tr>
<tr>
<td>2008</td>
<td>y Intercept, mg/L</td>
<td>0.12</td>
<td>0.09–0.15</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>1.17</td>
<td>1.15–1.19</td>
<td>0.011</td>
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<tr>
<td>2010</td>
<td>y Intercept, mg/L</td>
<td>0.13</td>
<td>0.11–0.15</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>1.14</td>
<td>1.13–1.16</td>
<td>0.007</td>
</tr>
</tbody>
</table>
the estimated GFR, the bias in cystatin C concentrations in patients with a normal kidney function and those with a moderately decreased kidney function had the greatest effect on the estimated GFR. Therefore, it is essential that the calibration of the cystatin C method be consistent, because small changes in cystatin C will introduce a large bias in GFR estimates. The Siemens direct carbohydrate-deficient transferrin method was reported to have a downward shift in calibration between 2006 and 2007 (5). Both Siemens methods are based on particle-enhanced nephelometry, and the downward shifts in calibration for the 2 methods coincided in time and approximate magnitude.

The results of this study indicate that a downward shift in calibration for the Siemens cystatin C method occurred between March 2006 and December 2008. Cystatin C–based equations to estimate GFR that were derived from results obtained from older lots of reagent and calibrator cannot be used with the current Siemens method. The results emphasize the need for an international cystatin C reference material, which has recently been developed by the Institute for Reference Materials and Measurements.

Siemens, when contacted, declined to comment on this letter.

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