Original Article

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Conversion methods for modified Jaffe reaction assays of serum creatinine

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Abstract

Background: Modifications in Jaffe serum creatinine (sCr) assays question the suitability of the results for direct comparison.

Methods: sCr in adult in-patients was routinely measured either by SRM 909-standardized/noncompensated (method A) or isotope dilution mass spectrometry traceable/compensated method (reference). We converted values by method A into values by the reference using a formula provided by the manufacturer [Beckman Coulter (BC)] and traditional equating methods.

Results: The BC-based conversion and linear equating resulted in underestimated sCr values, whereas equipercentile equating (EE) provided sCr with not significantly different distribution from the reference values. Proportions of patients with renal impairment did not differ between the reference and EE-converted sCr, as opposed to BC-recalculated values. Three percent of patients were classified into better renal function category when applying BC versus EE conversion.

Conclusions: Equipercentile equation was a more accurate method for recalculation of sCr obtained from different Jaffe reaction assays than the linear equating or the BC linear formula. This study emphasizes the importance of the derivation sample specificity when applying research results to other real-world populations.

Keywords: conversion, isotope dilution mass spectrometry standardization, Jaffe reaction, serum creatinine

Introduction

Serum creatinine (sCr) remains a key parameter for the estimation of the glomerular filtration rate (eGFR), commonly used to assess kidney function.[1] Because the limitations in the analytical performance of sCr measurement methods are well known,[2,3] a continuous effort is being made to improve assays' accuracy and reduce within and between instrument variability. In the Jaffe method, the most frequently used in clinical practice,[4] bias arising from nonspecificity for creatinine has been reduced by compensating for chromogenic interferences of the reaction.[5] Also, given the lack of calibration standardization traceable to a single accurate standard for sCr results, most manufacturers have progressively implemented isotope dilution mass spectrometry

(IDMS). Modifications of the methodology contribute to more reliable results[6]; however, to a certain extent, they may impair the longitudinal evaluation of kidney function, in particular in the identification and monitoring of chronic renal disease.[7] Although manufacturers provide specific conversion formulas for making sCr values comparable, the derivation sample from which these rules come from may not be necessarily representative of other settings.[8]

To examine methods for the conversion of sCr from the non-IDMS standardized and uncompensated into IDMS traceable and compensated values, we compared a linear formula provided by the assays manufacturer and traditional equation methods. Moreover, we investigated the effect of the variation in recalculated sCr values on eGFR level and on renal disease classification.

Methods

The study was approved by the Ethics Committee of Centro Hospitalar Universitário de São João, Porto, Portugal on January 12, 2016 (Comissão de Ética para a Saúde do Centro Hospitalar de S. João, reference number 365-15). Informed consents were waived due to the purely observational and retrospective nature of the study.

This retrospective analysis included all sCr measurements during hospitalizations of adult patients (age ≥18 years) admitted to Centro Hospitalar Universitário de São João, a tertiary hospital in Porto, Portugal between January 2012 and December 2015. During this period creatinine was determined by Jaffe's kinetic alkaline picrate method using Beckman Coulter Olympus AU5400 instrument (Beckman Coulter, Brea, CA). Until mid-September 2012, the hospital laboratory exclusively used an uncompensated and calibrated to standard reference material (SRM) 909b level 2 method (method A). Afterward, it was

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replaced with the compensated and calibrated to SRM 967 traceable to IDMS method (reference method). In the latter, the protein interferences with picrate were corrected by subtraction of a fixed value of 18 μmol/L of each result. In order to compare sCr values obtained from the 2 methods, the manufacturer proposed a formula: sCr reference = sCr method A × 1.04–17, based on linear regression analysis of 701 patients within creatinine concentration range 26.5 to 1024.0 μmol/L.

Blood was sampled and creatinine measured in routine practice. We automatically retrieved data from the hospital electronic medical record database including demographics, laboratory results, principal diagnosis coded according to the International Classification of Diseases 9th Revision. We used CKD-EPI formula[9] to calculate eGFR and defined renal function classes as follows: (1) eGFR≥ 60 mL/min/1.73 m² (normal/mild decrease), (2) 30 to 59 (moderate decrease), and (3) <30 (severe decrease).[10]

Given the large sample size, we estimated the magnitude of differences in baseline characteristics of patients between groups with different sCr measurement methods as the effect size using Cohen φ index for categorical and the analysis of variance test partial η^2 for continuous variables.[11] We considered a clinically relevant effect for φ ≥0.1 and η^2 ≥0.003.

We converted creatinine values by method A into expected values of reference method using (1) the linear formula provided by Beckman Coulter (BC) and (2) traditional equating methods: linear and equipercentile.[12] In the linear equating method, sCr values by method A were converted so as to have the same mean and standard deviation as reference sCr values. In the

equipercentile equating (EE) method the distribution of sCr of method A was set equal to the distribution of sCr of reference method, that is, values of method A and reference method with the same percentile rank were considered to be equivalent.

We based the analysis on the presumption of the equal distribution of true creatinine values between the 2 populations; thus, we assumed that observed between-group differences in sCr values arose from the measurement method.

We applied the Wilcoxon rank-sum test to compare the distribution of sCr values of method A and the converted values to those of the reference method, with a significance level of 1% assumed. All analyses were performed using R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

During the 4-year study period, there were 150,842 adult admissions, of which 101,888 hospitalizations (67.5%) had at least one sCr determined. Creatinine was measured 91,237 times by method A until 18 September 2012 and 445,519 by the reference method afterward. Overall, patients of the 2 subgroups did not differ in demographics and baseline clinical characteristics (no effect size in compared features) (Table 1).

The reference method provided results with higher precision than method A (2 decimals vs 1) (Fig. 1A). The median of creatinine by method A was 70.7 μmol/L [25th–75th percentile (P25–P75): 53.0–114.9] and 70.72 μmol/L (P25–P75: 52.16–108.73) for the reference method, with significant differences in the distribution of creatinine values (P value <.001), and with

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Table 1

Baseline characteristics of patients with determined serum creatinine concentration by the measurement method

	Method A N = 17,850	Reference method N = 84,038	Effect size*
Age (yr), median (P25–P75)	65.0 (51.0–77.0)	65.0 (52.0–77.0)	5.8×10^{-5}
Male, %	52.3	52.4	0.002
Admission department, %			
Medical	46.8	46.1	
Surgical	46.3	47.1	
Intensive/intermediate care unit	6.9	6.8	0.009
Emergency admission (vs elective), %	64.6	63.9	0.005
No. of sCr/admission, median (P25–P75)	3 (1–6)	3 (1 - 6)	7.7×10^{-6}
Principal diagnosis, %			
Circulatory system disease	23.2	23.2	
Hematology/oncology	12.4	12.6	
Infectious disease	3.8	3.5	
Endocrine/metabolic disease	4.7	4.5	
Respiratory disease	12.0	11.3	
Gastrointestinal disease	10.8	11.2	
Genitourinary system disease	7.4	7.4	
Injury and poisoning	7.1	7.3	
Other	18.6	19.0	0.012
Chronic kidney disease	13.5	13.3	0.003
Admission characteristics			
Hemoglobin (g/dL), mean (SD)	12.0 (2.2)	12.2 (2.3)	6.5×10^{-7}
BUN (mg/dL), median (P25–P75)	19.2 (14.5–28.0)	18.7 (14.0–27.1)	0.0001
Sodium (mEq/L), mean (SD)	137.1 (4.6)	137.3 (4.7)	0.0005
Potassium (mEq/L), mean (SD)	4.2 (0.6)	4.2 (0.6)	5.8×10^{-6}
Need for a higher level of care, %	9.4	10.2	0.009
In-hospital mortality, %	6.2	6.2	0.001
Length of stay (days)†, median (P25–P75)	7 (4–12)	7 (4–11)	1.4×10^{-5}

Method A, uncompensated and calibrated to SRM 909b level 2 method. Reference method, compensated and calibrated to SRM 967 traceable to IDMS method.

BUN = blood urea nitrogen; P25–P75 = 25th–75th percentile; sCr = serum creatinine; SD = standard deviation.

* Effect size estimated as φ for categorical and η^2 for continuous variables. All provided values support no clinically relevant differences between groups.

† Deaths not included.

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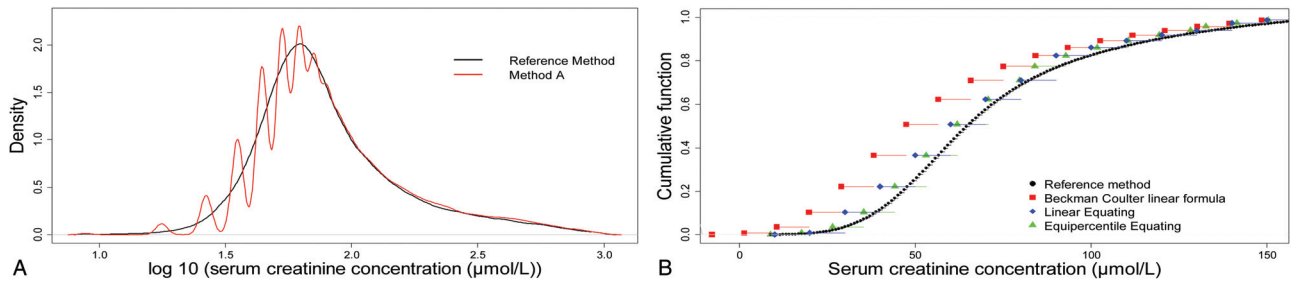www.portobiomedicaljournal.com

Figure 1. (A) Probability distribution function of serum creatinine concentration measured by method A and the reference method. (B) Cumulative density function for serum creatinine (sCr) values by the reference method versus Beckman Coulter-, Linear Equating-, and Equipercentile Equating-converted values. The smoothed density function of the reference method reflects sCr values with 2 decimal digits, while the "step-like" converted values represent sCr truncated to tenths as obtained by method A.

discrepancies more pronounced in concentrations below 70 μ mol/L.

Table 2 shows exemplary sCr values by method A and corresponding values obtained from different types of conversion. The linear equation resulted in the formula:

$$sCr \text{ reference} = sCr \text{ method A} \times 0.95 + 1.12.$$

Recalculation by the BC linear formula provided values distinctly lower than other types of conversion, with larger differences for lower sCr. In contrast, in the equating methods, there was a greater discrepancy between converted and original values along with the increase in sCr concentration.

Whereas the distributions of the converted sCr values did not improve toward the distribution of reference values for BC formula and linear equating (P value $<.001$ for both), the distribution of values converted by EE did not differ from those of the reference method (P value $=.0556$; Fig. 1B). The median eGFR was 86.96

(P25–P75: 51.35–106.23), 95.48 (P25–P75: 56.63–120.00) and 86.17 (P25–P75: 51.71–105.50) mL/min/1.73 m^2 for reference, BC and EE method, respectively (in comparison with the reference: $\eta^2 = 0.013$ for BC and $\eta^2 = 9.3 \times 10^{-6}$ for EE). These corresponded to eGFR-based categories of renal function of 70.0% of measurements in normal/mild decrease class, 16.9% in moderate decrease class and 13.1% in severe decrease class, according to the reference method. For recalibrated sCr the proportions were, respectively: 73.5%, 13.5%, and 13.0% for BC ($\phi = 0.036$) and 69.6%, 17.5%, and 12.9% for EE ($\phi = 0.006$). There were 3.0% more patients classified into better renal function category when using the BC formula compared to EE conversion categories: 397 patients with normal-mild decrease according to BC and a moderate decrease in EE, and 5 patients with moderate versus severe decrease, respectively. Ten patients were reclassified in the opposite direction changing from severe decrease category when using BC formula to moderate decrease function class according to EE values.

Discussion

The conversion of unstandardized and noncompensated sCr into IDMS traceable and compensated values using the EE method was the only approach that yielded sCr values whose distribution approximated the distribution of the reference values. Consequently, there were no differences in the proportions of eGFR-based renal function categories calculated from reference and EE-converted values. In practice, it means that applying the equipercentile equation recalculation is more accurate than the linear equating method or the BC equation.

Although recommended goals for bias in analytical performance are not being reached consistently by all manufacturers,[6,13] effective IDMS standardization and modifications in the Jaffe method have decreased the analytical component of creatinine variability[5,14] and corrected estimations of eGFR.[15] The conversion of the results of premodification assays to standardized measures is expected to approximate to the true value of creatinine, hence may contribute to more accurate diagnosis and monitoring of the course of renal disease. The recalculation method is, however, crucial since even small differences in sCr can create major shifts in the distribution of the eGFR.[16] In the critical range 88.4 to 132.6 μmol/L, which puts patients around the threshold of 60 mL/min/1.73 m^2 of eGFR, depending on sex and age, such variation may lead to clinical misinterpretation when the creatinine-based eGFR is used for CKD staging.[13] In our study, potentially underestimated sCr

<table>
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Table 2

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Exemplary serum creatinine values (μmol/L) determined by method A and corresponding values for different methods of conversion.

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<tr><td rowspan="2">**sCr (method A)**</td><td colspan="3">**Converted sCr values**</td></tr>
<tr><td>**Beckman Coulter Formula**</td><td>**Linear equating**</td><td>**Equipercentile equating**</td></tr>
<tr><td>9.0</td><td>−7.6</td><td>9.69</td><td>8.84</td></tr>
<tr><td>10.0</td><td>− 6.6</td><td>10.65</td><td>8.84</td></tr>
<tr><td>15.0</td><td>−1.4</td><td>15.41</td><td>8.84</td></tr>
<tr><td>20.0</td><td>3.8</td><td>20.17</td><td>22.10</td></tr>
<tr><td>30.0</td><td>14.2</td><td>29.70</td><td>30.94</td></tr>
<tr><td>40.0</td><td>24.6</td><td>39.22</td><td>39.78</td></tr>
<tr><td>50.0</td><td>35.0</td><td>48.74</td><td>48.62</td></tr>
<tr><td>60.0</td><td>45.0</td><td>58.27</td><td>57.46</td></tr>
<tr><td>70.0</td><td>55.8</td><td>67.79</td><td>66.30</td></tr>
<tr><td>80.0</td><td>66.2</td><td>77.31</td><td>83.98</td></tr>
<tr><td>90.0</td><td>76.6</td><td>86.84</td><td>88.40</td></tr>
<tr><td>100.0</td><td>87.0</td><td>96.36</td><td>97.24</td></tr>
<tr><td>120.0</td><td>107.8</td><td>115.41</td><td>114.92</td></tr>
<tr><td>150.0</td><td>139.0</td><td>143.97</td><td>137.02</td></tr>
<tr><td>180.0</td><td>170.2</td><td>172.54</td><td>167.96</td></tr>
<tr><td>200.0</td><td>191.0</td><td>191.60</td><td>185.64</td></tr>
<tr><td>300.0</td><td>295.0</td><td>286.84</td><td>278.46</td></tr>
<tr><td>500.0</td><td>503.0</td><td>477.32</td><td>481.78</td></tr>
<tr><td>1000.0</td><td>1023.0</td><td>953.52</td><td>976.82</td></tr>
</table>

Method A, uncompensated and calibrated to SRM 909b level 2 method.
sCr = serum creatinine.

values obtained through the BC formula resulted in higher eGFR and 3% fewer patients with kidney impairment when compared with EE values–based classification. The narrow range of sCr and unknown characteristics of the derivation sample raises the question of the adequacy of using the BC formula in our population of acutely hospitalized patients. Indeed, the accuracy of the Jaffe reaction varies significantly according to interfering substances, and population examined.[8]

In our study available data determined the choice of the equating methods as the alternative to manufacturer's formula; the 2 analytical methods for sCr were used exclusively and consecutively in the hospital laboratory. Examined conversion methods aimed at reducing bias in sCr measurement by method A, however, did not address the imprecision of the Jaffe method. More specific and precise enzymatic based methods are preferred; nevertheless, the cheaper Jaffe method is still used widely in clinical practice.[4] Our results suggest that EE equation is more appropriate for conversion than BC equation when comparing sCr results from pre- and postmodification assays; however, our findings refer to a single hospital and may be not valid for other settings. In the context of the continuous improvement of analytical methods and the ever-increasing use of large databases from routine procedures for clinical practice and research, potential variability, that is, changing the meaning of parameter values, including sCr, must be recognized and addressed.

Conclusion

The conversion methods evaluated provided discordant sCr values, determining the proportion and severity of renal failure in studied patients. sCr values obtained from modified Jaffe assays may become comparable if the conversion method is chosen with good judgment and caution. This study emphasizes the importance of the derivation sample specificity when applying research results to other real-world populations.

Acknowledgments

Conflicts of interest

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