Does the ID-MS traceable MDRD equation work and is it suitable for use with compensated Jaffé and enzymatic creatinine assays?

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Abstract

Background. International recommendations suggest that measurement of serum creatinine should be supplemented with an estimate of glomerular filtration rate (GFR) using the Modification of Diet in Renal Disease (MDRD) study equation. One problem has been the lack of standardization of commercially available creatinine assays resulting in varying estimates of GFR. A revision of the MDRD equation offers traceability to a reference method. This study evaluates the use of isotope dilution mass spectrometry (ID-MS), the compensated Jaffé and enzymatic creatinine methods compared with the Beckman CX3 Jaffé assay used to derive the MDRD equation and investigates their impact on GFR estimation using both the original and ID-MS-traceable MDRD equations.

Methods. Serum creatinine was measured in 277 patients by (i) ID-MS, (ii) a Roche enzymatic assay, (iii) a Roche compensated kinetic Jaffé assay and (iv) a Beckman CX3 kinetic Jaffé assay. Estimated GFR was calculated using the MDRD equations.

Results. The ID-MS (−7.5%), Roche enzymatic (−8.6%) and compensated kinetic Jaffé (−11.9%) assays were all negatively biased (P < 0.0001) compared with the Beckman CX3 assay, causing predictable, clinically significant, overestimation of GFR when the original MDRD equation is used. This positive bias was reduced (ID-MS 6.7 to 0.4%; enzymatic 8.8 to 3.4%; compensated kinetic Jaffé 13.7 to 7.1%) when GFRs were calculated using the ID-MS-traceable MDRD equation.

Conclusions. Compensated assays that account for non-creatinine chromogen interference produce significantly higher estimates of GFR when using the original MDRD equation. Use of the ID-MS-traceable MDRD equation ameliorates this effect. There is good agreement between estimated GFR derived from the original MDRD equation using Beckman Astra CX3 data and estimated GFR derived from the new ID-MS-traceable MDRD equation using a local ID-MS creatinine assay. This suggests that the ID-MS-traceable MDRD equation may be reliably used with both ID-MS and true ID-MS-traceable creatinine assays without the requirement for standardization to the MDRD laboratory.

Keywords: chronic kidney disease; creatinine; glomerular filtration rate; isotope dilution mass spectrometry; Jaffé assay; MDRD equation

Introduction

International recommendations suggest that measurement of serum creatinine should be supplemented with an estimate of the glomerular filtration rate (GFR) and the Modification of Diet in Renal Disease (MDRD) study equation [1,2] is generally thought to be the least biased and most accurate method of achieving this [3,4]. It is well understood that the equation is susceptible to changes in the standardization of creatinine assays, particularly at creatinine concentrations in the normal to high-normal range [5–8]. The development of the MDRD equation was undertaken using a kinetic Jaffé assay on a Beckman Astra CX3 analyser [1]. Recently, a revised isotope dilution mass spectrometry (ID-MS) traceable version of the equation was published, validated for use with methods demonstrating zero or minimal bias compared with ID-MS [9].

In addition to known problems of calibration [10–12], creatinine assays that use the Jaffé reaction are also known to be susceptible to interference from
non-creatinine (‘pseudo’) chromogens, which may account for up to 20% of the apparent creatinine component of normal serum. Enzymatic creatinine assays do not typically suffer from this interference. In an attempt to improve this situation, a major laboratory reagent manufacturer, Roche Diagnostics Ltd, have introduced the so-called ‘compensated’ Jaffe assay for measurement of serum creatinine, in which a fixed component is automatically subtracted from each result to account for reaction due to non-creatinine chromogens.

In the present study, we have compared creatinine results produced by both the Roche compensated Jaffe and enzymatic assays with those produced by the Beckman Astra CX3 assay in the MDRD laboratory, and have evaluated their practical application in estimating GFR using both the original and ID-MS-traceable MDRD equations. Verification of true creatinine concentration was achieved using an ID-MS approach, which has been proposed as the reference method [13].

Subjects and methods

Serum samples were available from 277 patients, including 203 patients enrolled in a study of chronic kidney disease (CKD), the clinical details of whom have been reported elsewhere [14], and 74 residual, de-identified samples from the diagnostic laboratory to provide a representative range of creatinine concentrations.

Serum creatinine concentrations were measured by the following:

(i) ID-MS, a modified liquid chromatographic ID_MS/MS method [15]. Serum (5 μl) was diluted with 250 μl of deionized water containing 500 pmol d3-creatinine and 250 μl acetonitrile with 0.05% formic acid. Following mixing and centrifuging, supernatant (2 μl) was automatically injected using an HTS PAL autosampler (CTC Analytics AG, Switzerland) into a 200 μl/min solvent stream of acetonitrile:water (50:50 v/v) with 0.025% formic acid. Chromatography was performed on a Symmetry-C8, 3.5 μm, 2.1 × 50 mm column (Waters Corporation, UK) and precursor–product ion pairs (m/z 114.2/44.2, 117.2/47.2) were acquired in positive-ion multiple-reaction-monitoring mode using an API4000 (Applied Biosystems, UK). Results were calculated using Analyst version 1.3.1. Stock aqueous creatinine standard, 10 mmol/l, was prepared by dissolving creatinine [BDH (Chemicals) Ltd, UK] in 0.1 M HCl and stored at 4°C. The concentration of the stock standard has been confirmed using National Institute of Standards and Technology (NIST) standard reference material, creatinine 914a (Laboratory of the Government Chemist, UK). Assay calibrators at 50, 250 and 1000 μmol/l were prepared from the stock standard by dilution with deionized water. Between-day coefficients of variation in control sera were <5% at concentrations of 112 and 256 μmol/l.

(ii) A kinetic Jaffe assay on a Beckman Astra CX3 analyser (Beckman-Coulter Ltd, UK). These analyses were provided by the African-American Study of Kidney Disease (AASK) Core Biochemistry laboratory (Cleveland Clinic Foundation), which supported the MDRD study [1]. Between-day coefficients of variation were 10.0 and 3.7% at concentrations of 95 and 360 μmol/l. The method uses two aqueous calibrators at values of 88 and 707 μmol/l. Absorbances are read at 520 and 560 nm, 25.6 s after sample addition. The assay has been reported to have an average 6 μmol/l positive bias compared with a high-performance liquid chromatography (HPLC) procedure [16]. For this assay only, samples were analysed in duplicate and data were analysed using the mean of duplicates.

(iii) A kinetic compensated Jaffe assay on an Integra 800 analyser (cat. no. 20764345, Roche Diagnostics Ltd, UK). This is a rate assay without deproteinization, measuring the increase in absorbance at 512 nm between 55 and 70 s after initiation of the reaction. Absorbance blanking at 583 nm is used. The assay is calibrated with a lyophilized human serum calibrator (C.f.a.s., Roche Diagnostics Ltd) in which the creatinine concentration (normally ~320 μmol/l) is traceable to an ID-MS determination. To compensate for non-creatinine chromogens, values are automatically corrected by subtracting 18 μmol/l. The between-day coefficients of variation were 4.4 and 3.9% at concentrations of 93 and 316 μmol/l, respectively.

(iv) An enzymatic wet chemistry method on an Integra 800 analyser (creatinine plus ver. 2, cat. no. 03263991, Roche Diagnostics Ltd). The assay principle utilizes a creatininase–creatininase–sarcosine oxidase system with detection at 552 nm and absorbance blanking at 659 nm. The assay is calibrated with C.f.a.s. (Roche Diagnostics Ltd) as above. Water is used as the zero standard. The between-day coefficients of variation were <2.5% at concentrations of 109 and 371 μmol/l.

Estimated GFR was calculated using the ‘original’ MDRD equation [2]: GFR (ml/min/1.73 m2) = 186 × [creatinine (μmol/l) × 0.011312]−1.154 × (age)−0.203 × 0.742 (if female) × 1.212 (if black) in addition to the ID-MS-traceable MDRD equation [9] in which the constant 186 is replaced by the constant 175.

Statistical analysis

All analyses were undertaken using Analyse-It™ (Analyse-It Software Ltd, Leeds, UK) and InStat (GraphPad Software Inc., San Diego, CA, USA). To determine whether it is appropriate to use the Roche Jaffe or enzymatic assays in the MDRD equation, and to check the validity of the ID-MS-traceable equation using a local ID-MS creatinine assay, the Beckman Astra CX3 results were used as the reference method against which other methods were compared by studying bias (plots of difference between test method and Beckman method plotted against Beckman results) and agreement (linear regression analysis). The significance of bias compared with the Beckman method was tested using the Wilcoxon’s matched-pairs signed-ranks test, and the relationship between bias and concentration was tested using linear regression analysis. Bias of the three colorimetric methods against ID-MS was also tested. Each of the serum creatinine data sets had a non-parametric distribution, as confirmed using the Shapiro–Wilk W test.
Therefore, log-transformed data were used for bias analyses [17]. To illustrate the effect of assay bias on estimated GFR, the regression equations were used to generate creatinine results for all three assays relative to the Beckman assay at 10 μmol/l intervals covering the range 80–250 μmol/l. These data were then used to generate illustrative estimates of GFR using both the original and ID-MS-traceable MDRD equations assuming the subject to be a 60-year-old Caucasian male. The significance of differences in estimated GFRs (eGFRs) was tested using Student’s t-test. Amongst the subset of 203 chronic kidney disease (CKD) patients, where age, gender and ethnicity were known, eGFRs were calculated using the original and ID-MS-traceable MDRD equations for each creatinine method; data were compared using bias plot analyses.

Results

Comparison of creatinine methods

The ID-MS (−7.5%), Roche enzymatic (−8.6%) and compensated kinetic Jaffe (−11.9%) methods all produced creatinine results that were negatively biased (P < 0.0001) compared with those obtained from the Beckman Astra CX3 assay (Table 1, Figure 1). The relatively similar negative intercepts of the ID-MS (−16.3 μmol/l), Roche enzymatic (−16.5 μmol/l) and compensated kinetic Jaffe (−17.5 μmol/l) assays compared with the Beckman Astra CX3 assay suggest that a large component of this bias may relate to pseudo-chromogen interference in the Beckman assay, which does not affect the ID-MS or enzymatic assays and has been compensated for in the Roche Jaffe assay. In keeping with this suggestion, there was a constant relationship between bias (test—Beckman Astra CX3) and creatinine concentration for the ID-MS method. For both Roche assays the difference increased with increasing creatinine concentration, suggesting an additional component of calibration bias (Figure 1). In the case of the enzymatic assay, a weak, but significant, concentration-bias effect (R² = 0.06, P < 0.0001; difference = 0.0173 × creatinine concentration − 16.5) was seen. A greater concentration-bias effect was seen with the compensated Jaffe assay (R² = 0.41, P < 0.0001; difference = −0.0434 × creatinine concentration − 17.2).

The Roche compensated kinetic Jaffe assay demonstrated significant negative bias against ID-MS [mean bias = −4.7%, 95% confidence interval (CI) −5.5 to −4.0%, P < 0.0001]. Although the Roche enzymatic assay demonstrated statistically significant negative bias compared with ID-MS, in practice the differences were minimal (mean bias = −1.3%, 95% CI −2.1 to −0.0%, P < 0.0001).

Estimated GFRs using an illustrative dataset

Estimated GFRs derived from the ID-MS, Roche enzymatic and compensated kinetic Jaffe creatinine assays were significantly greater than those derived using the Beckman CX3 assay, irrespective of whether the original or ID-MS-traceable MDRD equation was used (P < 0.005 in all cases) (Table 2). The greatest percentage differences were seen at lower creatinine concentrations: as creatinine concentration increases the percentage overestimation of GFR decreases for all three methods compared with the Beckman Astra CX3 assay. Using the original MDRD equation, all three assays produced GFR estimates with >15% positive bias compared with the Beckman assay in the creatinine range 80–150 μmol/l. The overestimation was ameliorated, though not abolished, when the ID-MS-traceable MDRD equation was used (Table 2).

Effect of ID-MS-traceable MDRD equation on estimated GFR in CKD patients

The ID-MS, Roche enzymatic and compensated kinetic Jaffe assays all produced estimated GFRs, calculated by the original MDRD equation, that were

Table 1. Relationship of three serum creatinine methods (y) to the Beckman Astra CX3 assay (x) employed in the MDRD study. All four serum creatinine data sets demonstrated a non-parametric distribution and difference plot analyses shown were therefore calculated following log transformation

<table>
<thead>
<tr>
<th>Creatinine method</th>
<th>n</th>
<th>Creatinine (μmol/l)</th>
<th>Regression analysis</th>
<th>Difference plot analysis (y−x)</th>
<th>Limits of agreement (%)</th>
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<tr>
<td></td>
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<td>Median (2.5–97.5th centiles)</td>
<td>R² Slope Intercept</td>
<td>Mean bias (%) (95% CI of mean bias)</td>
<td>Limits of agreement (%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Upper</td>
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<tr>
<td>Beckman CX3</td>
<td>277</td>
<td>303</td>
<td>74–920</td>
<td>0.99 0.9982 −16.3b</td>
<td>−7.5 −20.6</td>
</tr>
<tr>
<td>ID-MS</td>
<td>277</td>
<td>289a</td>
<td>57–914</td>
<td>(−6.7 to −8.2) −18.6</td>
<td>(−19.3 to −21.7) 7.9</td>
</tr>
<tr>
<td>Enzymatic (Roche)</td>
<td>277</td>
<td>279a</td>
<td>61–921</td>
<td>0.99 0.9827 −16.5b</td>
<td>(−8.0 to −9.2) 2.8</td>
</tr>
<tr>
<td>Compensated kinetic Jaffe (Roche)</td>
<td>277</td>
<td>269a</td>
<td>57–853</td>
<td>1.00 0.9566 −17.5b</td>
<td>−11.9 −22.9</td>
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<td>(−11.1 to −12.6) (−21.8 to −24.0) −0.7 to 2.3</td>
</tr>
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ID-MS; isotope dilution mass spectrometry.

* significantly (P < 0.0001) different from Beckman CX3.

b significantly (P < 0.0001) different from zero.
positively biased compared with eGFRs produced by the Beckman Astra CX3 assay (Table 3). This positive bias was reduced (ID-MS 6.7 to 0.4%; enzymatic 8.8 to 3.4%; compensated kinetic Jaffe 13.7 to 7.1%) when GFRs were calculated using the ID-MS-traceable MDRD equation, and there was very good agreement between GFRs calculated from our local ID-MS method using the new MDRD equation and GFRs calculated from the MDRD laboratory method using the original MDRD equation.

Discussion

Increasing use of eGFR has re-focused the attention of the scientific community on the shortcomings of creatinine methodology. In line with the National Kidney Diseases Education Programme (NKDEP), the Australasian Creatinine Consensus Working Group [18] recommend that serum creatinine assays should be considered acceptable with respect to bias if their results lie within ±15% of values calculated by ID-MS. Over a broad range of creatinine concentrations the Beckman CX3 assay fulfils this accuracy criterion, as do the majority of commercial kinetic Jaffe assays. At lower concentrations few assays do, largely due to the pseudo-chromogen effect [12]. The NKDEP has set a goal of an overall accuracy of ±30% for the estimation of GFR so as to encompass components of analytical inaccuracy and inaccuracies inherent in the equation. Although there was no ‘gold standard’ estimate of GFR in the present study, it is clear that analytical differences alone can generate GFR estimates in excess of this goal at lower creatinine concentrations; for the assays we studied, the problem was ameliorated by using the more appropriate, ID-MS-traceable MDRD equation.

We have used a direct approach to estimate the errors in estimated GFR associated with use of a compensated kinetic Jaffe assay, when compared with the kinetic Jaffe assay originally used to derive the MDRD equation. Although there are significant concerns relating to the precision of GFR estimates, we concentrated, in this study, on issues of relative accuracy. This is currently compromised by the lack of standardization between creatinine assays and non-consistent correction for pseudo-chromogens. The main clinical consequence of this will be differential recognition of CKD stage 3 (GFR 30–59 ml/min/1.73 m²), where classification is based upon GFR alone, as illustrated in Table 2. At a creatinine concentration of 150 μmol/l obtained using the Beckman CX3 assay, and assuming the subject was a 60-year-old Caucasian male, all methods generated a GFR that placed the patient in CKD stage 3. When the creatinine concentration, obtained from the Beckman CX3 assay, fell to 120 μmol/l, for this individual the ID-MS, Roche enzymatic and compensated kinetic Jaffe assays overestimated GFR and failed to identify CKD stage 3. This was the case irrespective of...
Table 2. Effect on estimated GFR (eGFR) based upon the relative regression equations comparing three creatinine methods with the Beckman Astra CX3 assay. eGFR was calculated using the original MDRD equation (constant = 186) and using the isotope-dilution mass spectrometry (ID-MS)-traceable MDRD equation (constant = 175) assuming the subject was a 60-year-old Caucasian male.

Table 3. Relationship of estimated GFR (eGFR) obtained from three serum creatinine methods (y) to the Beckman Astra CX3 assay (x) calculated using the original MDRD equation (constant = 186) and using the ID-MS-traceable MDRD equation (constant = 175). This table shows difference plot analyses calculated using log-transformed data.

Whether the original or ID-MS-traceable MDRD equation was used.

Others [12,19–22] have illustrated the impact of analytical differences on GFR estimations. Some authors have indirectly calibrated their assays to reflect those of the AASK laboratory [23], and some major healthcare providers have also adopted this approach [24]. This is not ideal as the Beckman Astra CX3 method clearly demonstrates significant positive bias compared with the ID-MS reference methodology [12]. One practical solution could be to adjust the equation for assay differences. For example, using the data generated in this study, ‘MDRD-consistent’ GFR estimates can be derived using creatinine concentrations obtained by the Roche compensated Jaffe method by substituting the regression coefficients (Table 1) into the MDRD equation, i.e. GFR (ml/min/1.73 m²) = 186 \times (\text{serum creatinine} \times 1.154 \times \text{age}^{-0.203} \times 1.212 \text{if black}) \times 0.742 \text{if female}, thus effectively adding back in the ‘compensation factor’ and adjusting for calibration differences. The more logical extension to this solution would be to use the ID-MS-traceable MDRD equation and calibrate and/or compensate creatinine assays against this ID-MS ‘gold standard’ method.
Two initiatives of NKDEP may improve this situation. Work is in progress towards the development of high-level commutable reference preparations for serum creatinine calibration [13]. Secondly, the ID-MS-traceable MDRD equation has been presented [9] and is now recommended for use with methods that can demonstrate ID-MS-traceable calibration [4].

We have assessed the impact of the ID-MS-traceable MDRD equation on eGFR using three creatinine methods for which it should be suitable. Estimated GFRs calculated using creatinine data generated using our own ID-MS method gave excellent agreement with those derived from the Beckman Astra CX3 assay using the original MDRD equation. This is reassuring and validates the use of the ID-MS-traceable equation in an independent cohort of patients. The Roche enzymatic assay has been calibrated against an ID-MS reference method and also gave an acceptable agreement with the eGFRs derived from the original MDRD equation. In both cases, agreement was markedly improved by using the ID-MS-traceable equation compared with the original MDRD equation, which tended to cause an overestimation of GFR when calculated with these methods.

In the Roche Integra compensated Jaffe assay, 18 μmol/l is automatically subtracted from each result to account for reaction due to non-creatinine chromogens. The manufacturers have also adjusted the calibration of the assay (‘slope correction’) such that although the compensated assay produces lower concentrations in the normal range compared with their uncompensated assay (e.g. 37 μmol/l compared with 50 μmol/l), at higher concentrations it produces higher results (e.g. 758 μmol/l compared with 700 μmol/l) (manufacturer’s data). The point of equivalence would appear to be ~185 μmol/l [8]. A similar adjustment (−27 μmol/l) has been made to creatinine assays on the manufacturer’s Hitachi platforms. Roche Integra and Hitachi platforms are widely available; nearly 30% of laboratories registered in the United Kingdom National External Quality Assessment Scheme (UKNEQAS) currently use these methods. The Roche compensated Jaffe assay is reported by the manufacturers to be closely aligned to their enzymatic assay and to have been standardized against ID-MS and Standard Reference Material 914. On this basis, the method should be applicable for use with the ID-MS-traceable MDRD equation. Our data demonstrate that the method still produces GFR estimates that are positively biased compared with those generated by the Beckman Astra CX3 assay using the original MDRD equation, although this overestimation is almost halved when the ID-MS-traceable equation is used.

Lack of agreement between creatinine methods leads to differences in estimated GFRs calculated using the MDRD equation that may be of significance at both an individual clinical and healthcare planning strategy level [25]. Use of the ID-MS-traceable MDRD equation improves agreement in GFR estimation for methods that are so aligned, although we have demonstrated that, for the Roche compensated kinetic Jaffe assay at least, significant bias may persist. The majority of creatinine assay results generated by clinical laboratories are not zero-biased compared with ID-MS and demonstrate differing degrees of agreement with the Beckman Astra CX3 method [12]. Some [20] have argued that errors of this magnitude at this level of GFR are unlikely to be clinically significant and that universal alignment is not a practical solution. We feel that laboratories should be able to do better. The application of the National Glycohemoglobin Standardization Programme to glycated haemoglobin measurement is a good example of international alignment that has helped to deliver improvements in patient care. It is hoped that manufacturers will increasingly move towards closer alignment of their creatinine assays to ID-MS methods, as has been urged [13]. In the interim, knowledge of method comparability against either the Beckman Astra CX3 method or ID-MS creatinine methods could be used to facilitate better uniformity in the reporting of eGFR. A feasible approach could be for national external quality assessment organizations to co-ordinate the collection of such method group comparison data.

Acknowledgements. We are grateful to UKNEQAS for permission to use their data and to the WellChild Trust for supporting the ID-MS determinations.

Conflict of interest statement. None declared.

References


Received for publication: 12.1.06
Accepted in revised form: 11.4.06