Author's response to reviews

Title: Derivation and internal validation of an equation for albumin-adjusted calcium

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Author's response to reviews: see over
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BioMed Central Editorial

Dear Dr. Cassady-Cain

Re: MS: 4993710871760052: Derivation and internal validation of an equation for albumin-adjusted calcium

Thank you for providing us the opportunity to resubmit this manuscript to BMC Clinical Pathology. We feel that we have satisfactorily addressed the reviewer’s further comments. We have provided a summary of these changes to the paper below, with the reviewer’s comments provided in bold, followed by our responses. These changes in the manuscript are included in blue font while the earlier revisions in the manuscript remain in red.

Referee 1: Roland N. Dickerson

My concerns have been addressed by the authors.

Referee 2: Patrick Twomey

1. Page 7/8: Difference plots (absolute or relative) are typically used in Clinical Chemistry as opposed to Kappa statistics to assess agreement. I suggest that the authors also use a difference plot (albumin on the x-axis and difference, absolute or relative, on the y-axis) to assess the differences due to the equations. In addition, the authors should construct a table of paired frequencies using either the McNemar tests or the exact binomial probability if the sum of the discordant pairs at not 10 with the former.

Measuring the difference in calcium concentration between the two equations for each subject plotted against their means is a better way of assessing the agreement, and can be used to produce a difference plot, also known as a Bland Altman plot (D.G. Altman and J.M. Bland, “Measurement in Medicine: The Analysis of Method Comparison Studies”, Statistician 32, 307–317(1983)). This plot shows the pattern of the individual differences for the patients with hypoalbuminemia as well as the mean agreement with 95% limits of agreement (see Figure 2 below).
**Figure 2.** Bland-Altman plot showing 95% limits of agreement between corrected calcium by the previously published equation and corrected calcium by the locally derived equation (Note: mean difference=0.09, 95% limits of agreement: 0.043 to 0.136).

The two by two table below shows the classification of calcium status as within or outside the laboratory reference range, with a statistically significant p value of p<0.0001 from the McNemar test for the difference between the two equations.

<table>
<thead>
<tr>
<th></th>
<th>Local Equation*¹</th>
<th>Published Equation*²</th>
<th>Normo-calcemia (Ca 2.10 – 2.55 mmol/L)</th>
<th>Abnormo-calcemia (Ca &lt; 2.10 or Ca &gt; 2.55 mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normo-calcemia</strong></td>
<td></td>
<td></td>
<td>272</td>
<td>5</td>
</tr>
<tr>
<td>(Ca 2.10 – 2.55 mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Abnormo-calcemia</strong></td>
<td></td>
<td></td>
<td>42</td>
<td>24</td>
</tr>
<tr>
<td>(Ca &lt; 2.10 or Ca &gt; 2.55 mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We have added the results from the McNemar test from the above two by two table to the manuscript. Although the Bland Altman plot illustrates the systematic differences in agreement between the two equations, we feel that it does not add further information to the paper, and that the tables of agreement, McNemar’s test, and kappa statistic best communicate to the reader the implications of a local formula versus a previously published form. However we would be willing to comply with the decision of the editor regarding its inclusion in the paper.
2. Tests of Normality should be used for the results on page 7/8 and if non-Gaussian, then non-parametric methods should be used.

We assessed normality of the distribution of calcium levels, confirming a Gaussian distribution for this result. We have clarified this in the methods section as below:

“The distribution of serum calcium concentration was assessed graphically and found to follow a normal distribution.”

3. Page 9, please provide a reference for the statement beginning 'The clinical significance ....'

We have provided the following reference for the statement:


Referee 3: Andries Bakker

1. This paper improved considerably and illustrates the need of revalidation of formula's when analytical methods are altered. However, there are still some issues that I would raise: The word "validation" in the title, in my opinion is not correct, although I understand where it's coming from. Derivation of the equation was performed with a cohort of 4613 samples and "validated" (would "verified" not be a better term?) in a separate cohort of 1500 samples. However, I think that validation means that the correctness of the outcome of the results of equation must be validated in some way, which means that the outcome is compared to e.g. ionised calcium. Since such measurements were not performed, validation is out of the question. The authors just have verified the correctness of the equation.

We have changed the title to “Derivation and internal validation of an equation for albumin-adjusted calcium” to address the above concern and clarify that the relationship between albumin and calcium concentrations was validated in a subsample from the same study population. Within the discussion of the manuscript we have clearly emphasized the limitation that the equation was not validated against a separate gold standard (ionized calcium).

2. A second issue, I would raise is the procedure of linear regression that was used to estimate the formula: Since two measured analytes were compared to estimate the formula, linear regression should not be used but orthogonal regression or Passing-Bablok regression. These two regression methods are insensitive to outliers and lead to a more reliable estimate of the formula in comparison to least squares linear regression.

There is debate within the literature as to the optimal linear regression method to employ in quantitative method comparison studies (Twomey P, Kroll M: How to use linear regression and correlation in quantitative method comparison studies. Int J Clin Pract 2008, 62: 529-538). Many evaluators prefer Deming regression (DR) over ordinary least squares (OLS); however, some employ OLS depending on the Pearson coefficient despite the lack of supporting evidence. As DR is also a parametric regression method, it is sensitive to outliers in a similar fashion to OLS. Passing and Bablok regression (PBR) also has its advocates over DR (Payne RB. Method comparison: evaluation of least squares, Deming and Passing/Bablok regression procedures for method comparison studies. Ann Clin Biochem 1997; 34: 319–20) whereas others say that it is an alternative to OLS (Linnet K. Evaluation of regression procedures for methods comparison studies. Clin Chem 1993; 39: 424–32). PBR may treat too many data points as outliers. One disadvantage of PBR is that it has no standard deviation of the residuals.
(S_{yx}). In addition to not having $S_{yx}$, Linnet (Linnet K. Evaluation of regression procedures for methods comparison studies. Clin Chem 1993; 39:424–32) has shown that PBR has inadequacies in simulation tests. When OLS and correlation analysis provide poor estimates, Stockl et al. (Stockl D, Dewitte K, Thienpont LM. Validity of linear regression in method comparison studies: is it limited by the statistical model or the quality of the analytical input data? Clin Chem 1998; 44: 2340–6) recommend the investigation of the analytical reason for the poor performance instead of assuming that other linear regression procedures add substantial value to the interpretation of the study. This also applies when there are significant differences between the different regression methods. Stockl et al show the usefulness of OLS, in particular, because it gives a better estimate of $S_{yx}$ than the other procedures.

Given these uncertainties, we explored the effect of these different regression methods on the results of our adjustment formula. We obtained similar results using OLS with bootstrapping, Robust regression, Deming regression, and Passing-Bablok regression. The table below illustrates that the relationship remained robust despite different methods of regression, thus we chose to report in the manuscript only the equation using ordinal least squares regression because it would be most familiar to the majority of readers, and provided for a method with which to assess internal validity using the amount of shrinkage in the adjusted r-square.

**Table 2.** Comparison of regression estimates using OLS, Deming, and Passing-Bablok approaches for our lab data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OLS</th>
<th>Deming</th>
<th>Passing and Bablok</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>S.E.</td>
<td>Estimate</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.87</td>
<td>0.017</td>
<td>1.82</td>
</tr>
<tr>
<td>Slope</td>
<td>0.012</td>
<td>0.00042</td>
<td>0.013</td>
</tr>
<tr>
<td>Residual S.E.</td>
<td>0.106</td>
<td>0.104</td>
<td></td>
</tr>
</tbody>
</table>

3. The third issue is the one on the exclusion criterium on the basis of creatinine level or the estimated GFR. It is the author’s intention to exclude patients with advanced end-stage renal disease. Since age and sex are major determinants for the kidney function, using a creatinine level of 200 µmol/l, some older female patients having advanced end-stage renal disease (eGFR <30 or even <15) might be overlooked. That’s why I suggested to use the eGFR as an exclusion criterion instead of the creatinine level.

We understand the reviewer’s statement that eGFR would provide a more accurate assessment of stage of kidney disease by accounting for age, gender, and race. However, we maintain that the use of serum creatinine concentration is adequate to meet our intention of excluding patients with ESRD. For example, even a 100 year old non-black female with a creatinine of 200 would have an eGFR of 20 ml/min/1.73m$^2$ by the MDRD equation and have a low probability of abnormal serum calcium concentration.

Some typing errors:
In the Background section there is the frequently used formula: ...+ 0.02(40-[albumin][0](g/L)).. which probably should be ...+ 0.02(40-[albumin](g/L))..
In the method section, 7 rows before the result section states.....a published equation[1], which should be :.....a published equation[1]

Thank you for pointing these out. These have been corrected in the manuscript.

Yours truly,

Matthew James
James Zhang

Brenda Hemmelgarn