Peer Review File
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Reviewer Comments

Reply to reviewers' comments on manuscript ID: JLPM-CALM-01(JLPM-22-16):
"Can ionized calcium-estimating equations replace albumin-corrected calcium? A narrative review"

We are grateful to the Reviewers for their thoughtful and constructive comments, which we believe have led to a greatly improved manuscript. Our specific replies are listed below:

Reviewer A

Comment 1: A discussion of confounding preanalytical variables such as length of time a specimen tube is left open should be included in this review.

Reply 1: The impact of air exposure of serum samples on the performance of I_{Ca}-estimating models is now addressed, with a reference, in the "Applications and limitations of new I_{Ca}-estimating equations" section of the Discussion.

Comment 2: I recommend a discussion and examples case reports of how disease/different populations potentially affect these methods. This section should be moved from the conclusions out into the discussion (some populations include citrate, exposure, monoclonal gammopathy and parathyroid disclosures. Including a section of examples/discussion of the effects of these patient population on the methods discussed will greatly improve the clinical utility of this review.

Reply 2: The "Applications and limitations of new ICa-estimating equations" section now includes both: (a) our suggestion that the such models be applied mainly as screening tools before ordering an I_{Ca} measurement; and (b) a detailed example from outpatient dialysis, a setting in which I_{Ca} measurements are infrequently done, where the output of an I_{Ca} model itself might, in the future, help guide a treatment decision, an example that we expect readers from the other domains, e.g., oncology and endocrinology, would be able to extrapolate to their own similar needs. The discussion of the generalizing these models from relatively ill patients to less seriously ill patients and to patients on citrate has been expanded and shifted to the same section of the discussion.

Reviewer B

Comment 1: The authors build a compelling argument against using albumin-adjusted calcium, which has already been proven, and they nicely outline the three major causes for its poor performance: biochemical, statistics and epidemiology. However, when the time came to discuss other estimating equations, the discussion was a bit underwhelming and I left with no great sense of which is the best equation to use instead. I'm also surprised that the authors did not mention the ROC of their logistic Ion model estimating equation (from reference 20), which was 0.92 and better than the anion gap (ROC 0.89), albumin-corrected (0.82) or Total calcium measurement (0.81) in that critical care population. All that is to say, I think it would be really helpful to the readers if the authors can make a concrete recommendation on which exact equation they recommend today based on their review of the literature and their own studies. They mention correcting for anion gap or phosphate in the conclusion but don't offer the best equation based on today's assessment. If such a recommendation cannot be made and more studies are needed, then that should be clearly stated. Otherwise, this is a bit vague.

Reply 1: The reviewer identified an important gap in the level of detail we provided about the strengths and weaknesses of the various considered I_{Ca} models including our own! We have expanded the Discussion greatly in respect of this important criticism, with detail provided about the strengths and weaknesses of their validation studies (e.g., study-size, ICa-diagnoses examined, variety of analytic platforms) and identify which models have the strongest support at present.

Comment 2: To include in discussion: A major limitation of ionized calcium and the new estimating equations and why clinicians tend to prefer the albumin-corrected calcium, is the need to bring the patient back to collect another sample to do any of them. With albumin, they can just add-on that test to the existing sample and immediately get an answer. While we in the laboratory medicine community recognize the limitations of albumin-correction, it is important that any solution we develop can be as practical and easily added on or reflexed on the same sample were total calcium was measured. Otherwise, it would be hard to implement and convince clinicians to use it. This argument needs to be included in the limitations of upcoming equations, because many of them depend on the measurement of bicarbonate (which cannot be added on after one hour from opening tube due to stability concerns) and also explains why ionized calcium isn't as routinely used (requires recollection of specific tube under specific conditions). We need a practical solution for this, otherwise our clinicians will continue to ask for albumin-corrected calcium, which they like because it is easy to obtain and calculate.

Reply 2: As noted above, the impact of sample air exposure on the performance of I_{Ca}-estimating models that rely on total carbon dioxide concentration is now addressed. Also, the Introduction now reminds the reader that the measurement of I_{Ca} entails demanding-sampling requirements, with a reference.

Comment 3: I recommend creating a table that includes the most popular formulations of the equations that you can reference throughout the discussion. Visually, it would be very appealing to have that and would also be helfpul in case any of the interested readers want to implement an equation, they can look at it in the table and can see what they need to calculate to have it.

Reply 3: We have added a table of the equations, as recommended (a second version of the table, in conventional units, is provided as a Supplement), and agree that it greatly enhances the accessibility of the equations and the readability of the review.

Minor comment 1:

-Line 111: Insert reference interval in brackets after listing "Tca measurement of 2.45 mmol/L", otherwise the following value on line 114 showing a change to 2.57 mmol/L and how significantly different that is would be lost to anyone not using SI units or for labs with different reference intervals.

Reply: We have added the reference interval for TCa, and also added the concentrations in conventional units in parentheses.

Minor comment:
Line 120: Replace "incertitude" with "uncertainty"

Reply 1: We made the requested change.

Minor comment 2:

-Line 126: include reference that supports that statement. Example: Steen O, Clase C, Don-Wauchope A. Corrected calcium formula in routine clinical use does not accurately reflect ionized calcium in hospital patients. Canad J Gen Int Med. 2016;11(3):14-21. DOI: 10.22374/cjgim.v11i3.150.

Reply 2: To support the statement on the difficulty of generalizing a model to patients who are dissimilar to the model's derivation cohort, we now cite two articles, one that describes this issue in general terms (Ramspek et al), and the other (Steen et al) which nicely summarizes the issue in the specific case of albumin-corrected calcium models.

Minor comment 3:

-Line 133: Were the ROCs 0.82 and 0.81 statistically different? I find that hard to believe and would prefer stating that they were similar.

Reply 3: We regret the lack of clarity. It has been revised in the manner suggested by the reviewer.