Peer Review File

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Reviewer Comments

Reviewer A

Comment 1: Thank you for allowing me to review this manuscript. I appreciate the author's effort in investigating elevated LDH in various diseases through literature and in developing an algorithm for elevated blood LDH concentrations.

Reply 1: Thank you for your kind input, your time is much appreciated. We have underlined the changes we made to the text.

Comment 2: The manuscript, particularly the introduction, would benefit from a more comprehensive review and reference.

Reply 2: Thank you we will add to the introduction.

Comment 3: Please correct the error in lines 77-80 to: "At the molecular level, LDH is a tetramer..." instead of "tetramers".

Reply 3: done, thanks.

Comment 4: Revise lines 114-118 to conform to the JLPM journal format. Consider a more academic tone, such as, “Elevated levels of LDH arise from multiple etiological factors..."

Reply 4: completely agree, the more colloquial terminology has been addressed.

Comment 5: Clarification on why certain databases were omitted from the literature search for the narrative review would be helpful.

Reply 5: Primarily time and resource was limited and therefore the review is thus limited. Basically within the lab community algorithms are in common usage from publications (books) that are no longer printed (particularly when teaching trainees to approach biochemical problems systematically). The main aim in the two invited series of articles was to address the fact that the algorithms were exclusive (i.e. very few people would have access) and also out-of-date. It is assumed that other groups may also similarly be using out-of-date, hard copies of algorithms. We would agree that therefore this was not approached with the same rigor and resource one would want from a true systematic review but to attempt to provide something at least better than what is in common use. I have added a bit to the end of the introduction and ‘limited’ in the method. Hopefully, this also creates a start for comment and critical review in the literature and, given the increasing use of AI, there does seem to be a dearth of algorithms in the literature as a platform for research into the most optimum design. Of course, one would wish this to be as good as we can make it but we simply struggled with resources (man power primarily).

Comment 6: Kindly detail the inclusion/exclusion criteria for article selection in the narrative review.
Reply 6: thank you I have added an exclusion criteria and the missing table (S1) is actually/hopefully informative, sorry it was indeed missed in the submission.

Comment 7: The mention of spurious LDH in lines 105-111 during the discussion on LDH measurement requires an explanation.
Reply 7: thank you, additional wording was added to make this easier to understand for those less familiar with laboratory interference

Comment 8: While the authors mention that Figure 3 originates from an article in this series, a detailed explanation of the diagnostic algorithm's creation would benefit the reader.
Reply 8: thank you we have added a few words but effectively this is the 13th article in a series of diagnostic algorithms all following the same methodology i.e. limited narrative reviews. The bilirubin is under review and so we cannot reference it yet, we would be most grateful to the editorial staff if they were able to add the reference during processing if possible.

Comment 9: There seems to be a discrepancy in line 158. While reference 26 from 2006 is cited as a "more recent study", reference 24 is from 2020. Additionally, the reviewer is unsure why low LDH levels are discussed in lines 330-335 when the focus is on elevated plasma LDH levels.
Reply 9: Sorry, it looks like in the submitted form something has happened to the Endnote referencing system, we have rectified it. Apologies, we set out to cover what to do with an abnormal LDH, both low and high, and somewhere along the line we lost clarity about that, I have changed the introduction.

Comment 10: The inclusion of Table 1 seems misaligned with the manuscript's objective on elevated LDH levels in plasma.
Reply 10: we aimed to cover abnormal LDH levels – both high and low, but low is so poorly described, partly because it is rarely clinically relevant, that it takes up very little space in our article. I realise we were not clear about that, apologies, so I have clarified that in the introduction.

Comment 11: The manuscript should explain in detail how the algorithm presented in the manuscript was created.
Reply 11: (see 8) I have added a few words into the methods but I must admit I am not sure what to say about this, we did a thorough literature search and then put our best ideas down into a figure and played around with it until it made sense. We have a mix of a physician clinician and a clinical pathologist so hopefully, we have a balance between clinical and laboratory viewpoints in this algorithm.

Comment 12: The authors should elucidate how their algorithm is better than those

previously published and currently utilized in clinical settings for the reader's benefit.
Reply 12: We cannot find any algorithms published in journals that is generalized and laboratory-based and so we are unable to do this. Although most other analytes have algorithms LDH seems to be consistently excluded.

Comment 13: The limitations of the proposed algorithm in comparison with other LDH algorithms would be valuable to the reader.
Reply 13: Again, we are unable to do this as there is nothing we can compare to.

Comment 14: The authors should provide a 'real-world' validation of the LDH algorithm.
Reply 14: We have been doing a long list of these articles, invited by the editorial team, and so far we have not done this for the previously published 12 articles so for the sake of keeping them all the same we will not do the same here. However, it is a thought for the future and a separate study. We hope that publication of suggested algorithms such as these will allow such studies to be performed and agree validation is very important. We have added that one aim is to provide a framework to be validated.

Comment 15: The conclusion would benefit from highlighting the main insights from the review rather than just restating the challenges of LDH-based diagnosis.
Reply 15: Thank you, great point, we have included more information.

Comment 16: The authors should ensure that references align with the journal's formatting guidelines.
Reply 16: Thank you, we will make every effort to.

Reviewer B
Comment 1: In the topic “diseases” (line 310), considering the health situation (COVID-19) that we are currently experiencing, I expected the authors to address a little more about the lactate dehydrogenase in COVID-19 (including long COVID). I strongly recommend that you create a topic covering lactate dehydrogenase and COVID-19. In this topic I suggest that the authors discuss these three articles that:
Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M, Lippi G. Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. Am J Emerg Med. 2020 Sep;38(9):1722-1726. doi: 10.1016/j.ajem.2020.05.073. Epub 2020 May 27. PMID: 32738466; PMCID: PMC7251362.
Fialek B, Pruc M, Smereka J, Jas R, Rahnama-Hezavah M, Denegri A, Szarpak A, Jaguszewski MJ, Peacock FW, Szarpak L. Diagnostic value of lactate dehydrogenase in COVID-19: A systematic review and meta-analysis. Cardiol J. 2022;29(5):751-758. doi: 10.5603/CJ.a2022.0056. Epub 2022 Jun 28. PMID: 35762075; PMCID: PMC9550334.
Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M, Lippi G. Lactate

dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. Am J Emerg Med. 2020 Sep;38(9):1722-1726. doi: 10.1016/j.ajem.2020.05.073. Epub 2020 May 27. PMID: 32738466; PMCID: PMC7251362.

Reply 1: I have not added additional material about COVID as when considering all infections in the world and how much more common they are, we did not want to spend much time on one recent pandemic as it might skew the reader to discount all other infections as possible causes, which they are. In particular the Henry et al paper is questionable as the heterogeneity is zero, which is concerning suggesting that all papers were done on identical data sets (so either all the Chinese papers were from one patient group or something went wrong with the statistics) therefore we will not be including this paper. Fialek et al is again similar but opposite, with heterogenity this time of 99% and so again calls in doubt the validity of any conclusions, or rather means that no conclusions can be drawn if we follow the correct interpretation of the statistics they presented. The 3rd paper you mention is duplicate of the first, i.e. Henry et al.

Comment 2: Figures 1, 2, and 3 are not self-explanatory. The captions are poor. It is important that authors add more details in the captions to help the reader understand the figures.

Reply 2: Thank you, we have added legends, apologies for missing this.

Comment 3: Table abbreviations must include their respective meanings. Authors must add a footnote.

Reply 3: Thank you, I have looked through the tables and increased the detail so that they are understandable alone and without needing abbreviation definitions from the prose etc.

Comment 4: Considering that the authors studied lactate dehydrogenase, a well-established biomarker in the clinic and with many scientific publications, why did the authors not choose to carry out a systematic review or a scoping review? The problem of narrative review presents many limitations, such as high subjectivity, lack of methodological rigor, limited reproducibility, and potential biases. Another point is the fact that the narrative review almost always has incomplete coverage, that is, there might be a risk of overlooking relevant studies or excluding contradictory evidence, leading to an incomplete or biased understanding of the topic. On the other hand, in a systematic review (or scoping review) we have a rigorous methodology, reproducibility and transparency, comprehensive coverage, objective evaluation, and strength in evidence synthesis. I would like authors to reflect on this.

Reply 4: Thank you for your comments. These articles were commissioned and we were instructed to do the articles this way by the editors. The problem with a systematic review is that it is for answering one question and is indeed the most rigorous way of answering the research question. Here we were set with the task of not answering a research question, but instead creating a novel diagnostic algorithm or flow chart, which is something a systematic review would not have helped us with. We do however

acknowledge that there is a risk papers have been missed but we hope that by publishing this suggested algorithm it provides readers with the opportunity to challenge, validate and research the approach and hopefully result in rigorous discussion on the topic to further knowledge and improve investigative approaches to biochemical abnormalities.