Peer Review File

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Reviewer Comments

Reviewer A

Comment 1: This is a good narrative review.
-Please, revise the numbers of the references in the section "references", because these are not correct as they do not correspond to the numbers reported in the text.
Reply 1: Thank you ever so much. Yes, we will double-check the numbering of the references, please accept our sincerest apologies about that.

Reviewer B

Comment 1: This is a quite simplistic review of transaminitis, at the same level as a pre-graduate medicine course. I do not see clear improvements over what is already known, and certainly, it is not an Investigative algorithm as claimed: A decision tree is all that I see. And it is partial.
Reply 1: Thank you for your feedback, the article was invited as, and perhaps we may all be surprised, similar articles have been very successful and we have direct feedback of even experienced clinicians utilizing them to help approach patient investigation strategically. The previous articles of similar standard are highly accessed and not directed at experts such as I imagine my esteemed peer reviewers are. The audience includes clinical members of the MDT such as pharmacist, nurses etc who may not have been lucky as most medical students to get focused training in diagnostics (both requesting and interpretation).

This is not a research article per se, therefore there is indeed nothing new here, and others have suggested diagnostic approaches before.

We have used the terminology ‘algorithm’ for the other articles in both series so I hope you don’t mind if we stick with it as so far that has not been raised as an issue and it is in the title of the invited series.

Comment 2: Lines 109-115; not clear to which units the values are referred. Per gram of tissue/ml of serum?
Reply 2: Great question and it turns out very difficult to answer, each paper references another without being explicit re units and we can’t get hold of some of the original papers. We have therefore found a different source and report some different values with units and a reference.

Comment 3: Lines 176-177; time course of liver disease as 176 ALT half-life is longer than AST (18 versus 36 hours), should probably read “36 versus 18 hours”, instead.
Reply 3: Thank you, great spot, corrected.

Comment 4: Lines 354-355: Had a near-perfect sensitivity and sensitivity (100%) for Wilson disease (twice repeated the term)
Reply 4: Good point, we have removed and altered the sentence slightly to make it clearer.
Comment 5: Poor mention of liver autoimmune diseases (autoimmune hepatitis).
Reply 5: We agree but primarily we kept a lot of the discussion limited as we aimed to talk about investigations rather than the pathologies themselves. However, we realize that some conditions have more coverage than others. We have expanded the autoimmune section a little.

Comment 6: Poor mention of iatrogenic hepatitis
Reply 6: This is an interesting point, we agree that besides drugs we haven't mentioned much else as we were likely focusing on results that were unexpected and the source unknown. That of course doesn't rule out iatrogenic causes however we couldn't think of many common other iatrogenic causes that are picked up by laboratory diagnostics.

Comment 7: No mention of the "R" factor to classify hepatocellular vs. cholestatic hepatitis
Reply 7: Yes indeed, this was not mentioned, it has been added to the calculation paragraph.

Reviewer C
Comment 1: Page 4 Line 95-97 and Figure 2. It is confusing what was represented in the pie charts - the total activities or relative activities? None of these were properly reflected in the percentages of both pie charts. Also, as relative activities were referred to ratios of serum activity as 1, there should be no units (U/L).
Reply 1: Thank you, yes we have checked all our figures and units in this. In figure 2 yes, we have set serum activity as 1 U/L for serum so we have removed the unit from the top. I have also attempted to better represent the percentages on the pie chart. It is difficult as percentages for some organs as less than 1% so not clearly visible but I have done a closer approximation and I will make it clear that is is a figurative approximation.

Comment 2: AST is much more non-specific than ALT, therefore, from the lab test utilization perspective, many medical labs have restrictions for AST, with the exception of oncology, GI specialists, and clinical trials. There should be some discussion on this to reflect current lab practice. Also, lab stewardship on AST will substantially reduce AST testing so that much less need for further unnecessary investigations.
Reply 2: Thank you, this is an interesting point that we haven't made and indeed management of demand is a very appropriate consideration. This has been added to the section under calculations.

Comment 3: Table 2 uses the ANA cut-off of 1:40 and 1:80. Due to the high prevalence of ANA in the general population (20-30%), many clinical labs use the ANA cut-off of

1:160 now. How can those low cut-offs be used in current lab/clinical practice? Any role of ANCA IFA for autoimmune hepatitis Dx?
Reply 3: What a brilliant question and we are going to be cowardly in this article and avoid the discussion of the non-specific nature of ANA – it is almost a whole article in its own right. Table 2 is taken from the UK national diagnostic guidelines with their calculated high sensitivity and specifity so we are going to leave it as it is at the moment, but apologies for not tackling this with enthusiasm – perhaps we can write a diagnostic algorithm about what to do with an elevated ANA next time! I have added a comment about how ANCA can be used for the diagnosis of primary sclerosing cholangitis and have increased the discussion on autoimmune hepatitis.

Comment 4: Page 17 Line 392-393. The cut-off for transferrin saturation to reflect HFE genetic testing were too low (40% for male and 30%) for female. The reference cited was old - #63 of 1965. Suggest changing to 45% for both males and females and using some of these newer references:
Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med 2008;358:221–230.
Rossi E, Olynyk JK, Jeffrey GP. Clinical penetrance of C282Y homozygous HFE hemochromatosis. Expert Rev Hematol 2008;1:205–216.
Porto G, Brissot P, Swinkels DW et al. EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis (HH). Eur J Hum Genet 2016;24:479-95.
Waalen J, Felitti VJ, Gelbart T, Beutler E. Screening for hemochromatosis by measuring ferritin levels: a more effective approach. Blood 2008;111:3373-6.
Adams PC, Reboussin DM, Press RD, Barton JC, Acton RT, Moses GC, Leiendecker-Foster C, McLaren GD, Dawkins FW, Gordeuk VR, Lovato L, Eckfeldt JH. Biological variability of transferrin saturation and unsaturated iron-binding capacity. Am J Med 2007;120:999.e1-7.
Reply 4: Thank you ever so much for this and the helpful references. We had actually referenced a 2017 article but we think the references got corrupted a more specific threshold is sensible to quote.

Comment 5: Page 8 Line 177, change to "36 versus 18 hours".
Reply 5: Thank you, great spot, corrected.

Comment 6: Page 13 Line 310-311 and Figure 5: modify the holocaeruloplasmin to conjugate 6 atoms of copper. It showed 8 of them.
Reply 6: Thank you we have altered.

Comment 7: Page 15 Line 354, delete "IU/L".
Reply 7: Thank you, the numbering seems to be different in the version I have, but I think this is the de ritis ratio unit which we completely agree needs to be removed. Thank you.

Comment 8: Page 17 Line 395, add "when screening for autoimmune hepatitis...".
Reply 8: Thank you, added.

Comment 9: Page 17 Line 397, change to "shows".
Reply 9: Thank you, good spot, changed.