Peer Review File

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Reviewer A

Comment 1:

There has been recent interest in transient elastography/LSM at the diagnostic stage in BA. (Wu et al. Transient elastography is useful in diagnosing biliary atresia and predicting prognosis after hepatoportoenterostomy. Hepatology 2018; and Boo et al. Diagnostic performance of TE in BA among infants with cholestasis. Hepatology communications 2021). I wonder if it will be appropriate to include this modality in your article.

Reply 1:

This is an excellent point, there has been recent interest in transient elastography or liver stiffness measurements as a non-invasive tool in the diagnosis and prognosis of BA. Although imaging modalities such as ultrasound, hepatobiliary scintigraphy, magnetic resonance cholangiopancreatography and transient elastography are non-invasive studies that can be very useful in the diagnosis of BA, for the purposes of this review we sought to focus on recent advancements in molecular laboratory studies that are being used in the screening, diagnosis and prognosis of BA. We limited our review to laboratory studies in order to be comprehensive and still stay within the appropriate confines of word limit. An additional review on the imaging modalities in workup and prognostics of BA would be an excellent follow up to this review.

Changes in text:

In the introduction, we state that we will not be reviewing imaging modalities in this review but have now included transient elastography to this list of imaging, to better reflect more up to date techniques. Page 4, Line 77.

Comment 2:

With such superior diagnostic performance as quoted in the paper, do the authors see MMP7 or IL33 eventually become mainstream tests for diagnosis of BA? If not what would be the limitations/obstacles?

Reply 2:

We believe that there is data, as presented in this review, that laboratory studies such as MMP7 and IL33 are emerging tools that will be helpful in guiding management of BA. With increasing utilization and further clinical studies supporting these laboratory modalities, they will likely become more mainstream. However, the limitations on these studies include availability and cost. Currently, in the vast majority of institutions, these lab studies are not done in house and require samples to be sent to specific sites for processing. Not only does this incur cost, but also adds time for results, which can be a hindrance for early diagnosis of BA. As these studies become more mainstream, hopefully availability will increase and in turn the limitation of cost and time to results will improve.

Change in text:

We have included a paragraph on current limitation of these laboratory studies. Page 11, Line 243.

Reviewer B

Comment 1:

Page 4, Line 65: a surgical procedure in which an intestinal roux limb → Roux limb
Page 4, Line 66 – in an attempt to reestablishment biliary drainage. → in an attempt to reestablish biliary drainage.

Reply 1:

Thank you for your attention to detail, we have made these corrections in the text.

Change to text:

We have made the above suggested grammatical correction. Page 4, Line 61 and Line 62

Reviewer C

Comment 1:

The author's review is comprehensive with regard to modern molecular tests for diagnosis of BA. However, although the author thinks that the blood sampling for plasma bile acid or molecular studies are non-invasive, quantitative analysis of sulfated bile acid in the urine is a more non-invasive screening test. It is more effective than stool color screening but has not yet been popular because of the cost. The modern molecular analysis that the author mentions would also cost more expensive. This review would have been more substantial if the author compared the cost among these tests.

Reply 1:

Thank you for this comment, as it is vitally important to consider the limitation of cost in the utilization of emerging molecular testing. Unfortunately, through our review of the literature of these laboratory tests, cost was not included in these studies. Additionally, since cost can vary greatly between institutions, and many of these tests are not done in-house but sent out for processing, accurate cost analysis is not feasible for this review and therefore was not commented on. We have now included a paragraph within the discussion of this review to include limitations of these studies, including cost, availability, and time for processing.

Change in text:

We have included a paragraph on current limitation of these laboratory studies. Page 11, Line 243.

Comment 2:

Traditional tests that include direct bilirubin at the third week of life, followed by ultrasonography of the porta hepatitis and gall bladder would be simple and the most costless modalities when to decide whether the patient should undergo operative cholangiography and liver biopsy for final diagnosis.

Reply 2:

This is a good point, as traditional tests such as direct bilirubin and US of the porta hepatis and gallbladder are still essential in the initial workup of children with cholestasis and potentially BA. In countries with a high incidence of BA, such as Japan and Taiwan, routine labs at three weeks of life, followed by US may be a cost effective and efficient means of screening. However, in the US and Europe screening all infants with blood serum studies and US would likely not be cost effective, due to the significantly lower incidence. In a study by Harpavat et al., and described on line 107 of this review, the use of direct bilirubin as a newborn screening test for BA, was extremely sensitive in identifying infants with cholestasis and BA, unfortunately, due to the very small number of true positive screening results the 95% confidence interval for the sensitivity of direct bilirubin as a screening test for BA was significantly wide and therefore was likely unacceptable for use as a screening test. Additionally, although not directly analyzed in the above study, the authors describe in discussion that the cost-effectiveness was likely also a limitation to the use of direct bilirubin as a newborn screening test for BA.

Furthermore, the use of direct bilirubin and US in differentiating infants with BA versus non-BA cholestasis is poor and lead to invasive operative cholangiogram and liver biopsy for definitive diagnosis. The implementation of more sensitive molecular studies aims to improve management guidelines for those patients at high risk for BA and reduce the number of operative procedures for low risk infants with non-BA cholestasis.

Change in text:

We did not make a change to the text for this comment, however the limitations of direct bilirubin as a screening test are described on page 5-6, lines 94-123.