Peer Review File

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Reviewer 1:
Introduction:

Reviewer Comment:
MMP-7 "MMP7-based diagnostics may facilitate early diagnosis of BA…" cutting edge certainly but not substantiated in the real world. All the published series are Chinese in origin and the Taiwanese one is much less discriminatory with a completely different cut-off and normal range (Wu et al. J Pediatr. 2019 May;208:30-37.e1. doi: 10.1016/j.jpeds.2018.12.006.).

Author reply:
We agree with the reviewers' comment that the patient cohort published in Hepatology. 2018 Dec; 68(6): 2069-2077 (Yang L, *et al*. Diagnostic Accuracy of Serum Matrix Metalloproteinase-7 for Biliary Atresia) are from three major pediatric centers in Wuhan, China. Here, the median concentration for MMP-7 in normal controls MMP-7 was 2.86 ng/mL, 11.47 ng/mL for non-BA, and 121.1 ng for BA ($P < 0.0001$), which is highly discriminatory. This publication from our institution was a follow-up study building upon our earlier discovery of MMP-7 as a critical and potential diagnostic biomarker (Lertudomphonwanit C, *et al*. Large-scale proteomics identifies MMP-7 as a sentinel of epithelial injury and of biliary atresia. Sci Transl Med. 2017 Nov 22;9(417): eaan8462). We have now included this reference in the revised manuscript (Reference #4; PAGE 4). Furthermore, the MMP-7 test is now being routinely used by physicians across the US for patients with suspected diagnosis of biliary atresia. Please see: http://enewsletter.cincinnatichildrens.org/physicians/gastroenterology/2019/06/new-test-expedites-the-diagnosis-of-biliary-atresia. This clinical test successfully utilizes the above mentioned MMP-7 diagnostic ranges for discriminating patients with and without BA.

Reviewer Comment:
"KHPE is mostly successful only if performed within the first 45 days of life with greater benefits of survival with native liver at 2 (~65%) and 15 (~40%) years of age."
Exactly where are these statistics from?

Author reply:
Thank you for the clarification. The data has been updated and references have been added (PAGE 4).

Triggers:

Reviewer Comment:
Viral hypothesis – this is a nice review of the laboratory basis but absolutely no mention of any clinical correlates either for (Zani et al. J Pediatr Surg. 2015; 50: 1739-

45. Xu et al. Clin Pediatr (Phila). 2012; 51:109-13. Parolini et al. J Pediatr Surg 2019; 54: 1941-1945) or against (Rauschenfels Eur J Pediatr 2009;168:469-76.)

Author reply:
Thank you for the comment. The primary goal of our review was to highlight the ability to artificially induce a clinically relevant animal or an in-vitro model. As elucidated in the review, the only available in-vivo model that is reliably used to recapitulate clinical phenotype is the RRV-induced newborn mouse model. However, in our experience and from published reports by other laboratories, this model presents primarily as an inflammation-driven disease phenotype. The chemical model is a rapidly transforming fibrosis model typified by the most commonly diagnosed Type III BA in human infants. While we have highlighted this clinical correlation, the reviewer is accurate in suggesting the need to correlate with clinical findings. As suggested by the reviewer, we have now revised the manuscript (PAGE 9–10) to include the findings of virus signatures in patients with BA.

Reviewer Comment:
VEBDS – it is indeed entirely speculative, and we have already one putative trigger (virus) that still has not been substantiated in 30 years of work. VBDS may have some basis in the post-Kasai infant but none of the papers listed are in any way to do with BA.

Author reply:
Thank you for highlighting the concept of VEBDS. We completely agree with the reviewer's comment about VEBDS being speculative and the literature does not describe this process to be operative in BA. Nonetheless, the findings in the newly developed chemical model of BA may have underlying features of vanishing duct like syndrome described within the liver as a result of DILI or chronic cholestasis. In the EHBDs, this process may be acute and reflect the high susceptibility of extrahepatic cholangiocytes to noxious stimulus. We are also proposing this hypothesis as a foundation for future cellular and molecular studies involving the chemical model that shows complete absence of the common bile duct. Such studies are expected to reveal how similar or divergent the processes are when compared to human proteomics and transcriptomics data. In parallel with this hypothesis, bile duct remnants from the youngest patients show inactive homogeneously bland fibrous cords with very little or no inflammation. This information has been added in Page 14. (Reference #90 in revised manuscript).

Reviewer Comment:
BALF – where are the references to this work?

Author reply:
Thank you for the suggestion. The Biliary Atresia Liver Fibrosis (BALF) was first used to study the role of N-acetylcysteine in preventing liver fibrosis. This work is already cited in the manuscript. However, we have now included this reference at the first instance where BALF is used. The reference is: Luo, Z., et al., *Gene Expression Signatures Associated with Survival Times of Pediatric Patients with Biliary Atresia Identify Potential Therapeutic Agents.* Gastroenterology, 2019. **157**(4): p. 1138-1152 e14.

Reviewer Comment:
[p18] Struecker et al. successfully applied tissue bioengineering principles and performed EHBD….. This is completely different direction from BA. The problem is not the extrahepatic ducts but the intrahepatic and their connections at the portal plate. All this would ever do is replace the Roux loop.

Author reply:
We agree with the reviewer that Struecker et al.'s work constitutes an approach to circumvent portoenterostomy. While the progressive liver damage post Kasai involves intrahepatic biliary epithelial cells, the segment of bowel loop has the propensity to drive single or multiple episodes of recurring ascending cholangitis, secondary biliary cirrhosis, etc. With emerging data implicating intestinal microbiota and microbe-derived metabolites in the pathogenesis of BA, an alternate approach to replacing the Roux loop may hold promise in promoting long-term native liver survival post Kasai. Generation of neo bile ducts either by tissue engineering, hepatic/cholangiocyte organoids or organ-on-a-chip techniques are expected to be future drivers of novel surgical approaches. We have incorporated these suggestions in the article (PAGE 21). Thank you for the suggestion.

Reviewer Comment:
I would have preferred at least a paragraph or two on developmental aspects which are almost completely ignored and are the closest explanation we have for at least the syndromic form of BA.

Author reply:
Thank you for the comment. We would like to emphasize our article is centered around perinatal etiological drivers that constitute greater than 80-90% of acquired form of BA. The review article also discusses our ability to develop animal models that closely recapitulate clinical features of BA seen in human infants. While syndromic or the embryonic form constitutes a minor proportion of the overall infants diagnosed with BA, no relevant models have ever been developed due to a) limited or non-existent disease-modeling studies and b) challenges in identifying the critical drivers of embryonic BA. As suggested by the reviewer, we have included details on this form of BA to benefit the readers (PAGE 6–8).