Peer Review File

Article Information: https://dx.doi.org/10.21037/tp-23-305

Reviewer A

Study design- The study seems to frame itself on adult studies of VBDS related to DILI. However, bile duct paucity in children can be caused by multiple pathologies. Most commonly, bile duct paucity is seen in the infant age group and is associated with Alagille syndrome or non-syndromic bile duct paucity. These congenital forms of bile duct paucity are quite distinct from VBDS that would present later in life.
The authors therefore need to provide the clinical final diagnosis for all of the VBDS patients
Reply 1: In recent years, Alagille syndrome is diagnosed primarily basing on clinical features and genetic tests, but not liver biopsy in our center. It is reasonable that Alagille syndrome is not diagnosed in this cohort. Per your suggestion, etiologic diagnoses were added in Table 1.
Changes in the text: Etiologic diagnoses were added (See 'Table 1').

2. These patients are deeply cholestatic and typically have GGT values that exceed 1000. Similarly, cholesterol values are also extremely elevated. In grouping children aged 6 months – adolescence, a spectrum of different pathologies is encompassed and the findings are not characteristic of any one pathology. The inclusion of infants with bile duct paucity in this cohort will naturally skew the lab values to the more extreme.
• No mention is made of underlying diagnoses. Was Alagille syndrome included/excluded from the cohort? If DILI cases, what drugs were suspected?
Reply 2: Per your suggestions, etiologic diagnoses were added in Table 1. Alagille syndrome is not diagnosed in this cohort (also see reply 1). DILI was the most common cause, and the implicated drugs included azithromycin (n=3), piperacillin-tazobactam (n=3), Chinese herbal medicine (n=3), etc (See Page 7, line 172-174).
Changes in the text: Etiologic diagnoses and implicated drugs were added (See 'Table 1' and 'Table S1' respectively).

• Time frame from onset of liver injury to biopsy is not commented on and critical to understand the data presented.
Reply 3: Great thanks for your advises.
Changes in the text: Per your suggestion, time frames from the development of jaundice to biopsy were added (See Page 6, line 165-167). They were similar between patients with and without VBDS (See 'Table 2').

• Cohort includes patients who were not cholestatic at the time of biopsy. All of these patients did not have VBDS but their data was included in the analysis. This skews the data for the non-VBDS cohort toward lower lab values.
Reply 4: Sorry for our unclear description. In our center, liver biopsy is performed during the course of acute cholestatic hepatitis. At biopsy, the 16 patients with DB < 17 μmol/L still had elevated transaminase. Their levels of ALT, AST, GGT, and TCH were similar to other 152 patients without VBDS. The main finding of this study is that GGT and TCH can be used as non-invasive biomarkers for identification of VBDS among children with acute cholestatic hepatitis. Therefore, the inclusion of the 16 patients with DB < 17 μmol/L for analysis does not affect the conclusion of this study.
Changes in the text: We revised the sentences (See Page 5, line 105-107, and Page 7, line 174-177).

• Why was CK19 stain used? Is this appropriate for evaluated the bile ducts? Why not CK7?
Reply 5: CK7 and CK19 are biliary markers. Both CK7 stain and CK19 stain are performed for evaluation of bile duct in our center. In this study, we focus in bile duct loss. Bile duct loss was assessed in HE sections, and confirmed in both anti-CK7 and anti-CK19 immunostained sections. Hepatocytes can be CK7-positive due to cholestasis.
Changes in the text: We revised the sentence 'The loss of bile ducts was assessed in hematoxylin-eosin stained sections, and confirmed in both anti-CK7 and anti-CK19 (GeneTech, Shanghai, China) immunostained sections' (See Page 6, line 135-136).

• Included biopsies that had fewer than 10 portal tracts, not ideal for assessing for paucity.
Reply: Liver biopsies with < 10 portal areas are commonly encountered in clinical practice (Bonkovsky HL. *Hepatology*. 2017). In this study, at least 3 portal areas without bile duct were needed for the diagnosis of VBDS if a biopsy had < 10 portal areas. So, the ratio of portal areas without bile ducts is still larger than 25% even if we assume a total of 10 portal areas are detected. It increases the accuracy of the diagnosis of VBDS. However, it is unclear whether these patients belong to partial form of VBDS or classical form of VBDS. We discuss this limitation in the discussion section.
Changes in the text: An additional limitation was added (See Page 8, line 215-216).

6. Time from injury onset to biopsy- This needs to be provided for all cases.
Reply 6: Great thanks for your advises.
Changes in the text: Per your suggestion, time frames from the development of jaundice to biopsy were added (See Page 6, line 165-167, and also 'Table 2').

7. Baseline biomarkers- it would be more clinically relevant and meaningfult to see if the labs at initial presentation/ Injury onset are associated with VBDS at biopsy. The authors used labs closest to time of biopsy- please comment.
Reply 7: The loss of bile ducts is a gradual process, and rarely occurs at initial presentation / injury onset (LiverTox. Vanishing Bile Duct Syndrome. 2019). Values of liver biochemical indices also change as disease evolves. Therefore, it is reasonable to explore non-invasive biomarkers for VBDS by using the values of liver biochemical indices at biopsy.
Changes in the text: Not applicable.

Reviewer B

Wang et al studied to identify non-invasive biomarkers to identify vanishing bile duct syndrome (VBDS) among children with acute cholestatic hepatitis. The study concluded that γ-glutamyl transpeptidase (GGT) and total cholesterol (TCH) can be used as non-invasive biomarkers to identify VBDS.
Overall, the article is well-written. Liver biopsies still remains a gold standard to confirm the diagnosis of VBDS, and GGT and TCH cannot replace them. However, these tests can be used to monitor pediatric patients at high risk for VBDS. Similar results were already published in patients with valporic acid-associated toxicity and Hodgkin lymphoma. The study analyzed pediatric patients with acute cholestatic hepatitis. The results are obtained based on the study of a more generalized population. The article should be published with minor revisions.
My recommendation
1. Please include histologic images: The inclusion criteria for the study is well-defined. Images should be included to reinforce the inclusion criteria.
Reply 8: Great thanks.
Changes in the text: Per your suggestion, histologic images were added (See Fig 2).

2. The conclusion should be more careful. I agree that GGT and TCH could be good monitors but cannot replace liver biopsies. These markers are still potential, and additional tests would be needed to confirm the diagnosis.
Reply 8: Great thanks for your advises.
Changes in the text: We added a sentence 'These markers are still potential, and additional tests are needed to confirm the diagnosis of VBDS' (See Page 9, line 261-262).

3. p8, L238. Duo Typographical error? Due to
Reply 8: Sorry for this typo error.
Changes in the text: We corrected it (See Page 8, line 234).

Reviewer C

The authors tried to evaluate the GGT and TCH as biomarkers for identification of vanishing bile duct syndrome (VBDS) among children with acute cholestatic hepatitis. It is an important topic. However, there are several major concerns about their data.
First, there are many causes of acute cholestatic hepatitis such as infectious etiology, drug toxicity and inherited/metabolic and developmental disorders, some are directly associated with loss of bile duct and increase of GGT and TCH. The paper does not provide any information on it, which limits its clinical application.
Reply 1: Great thanks for your advises.
Changes in the text: Per your suggestion, etiologic diagnoses were added (See 'Table 1').

Second, as the author said, diagnosis of VBDS or loss of bile duct is based on liver biopsy evaluation. It is well known that to reliably determine ductopenia, at least 10 portal tracts must be evaluated. From Table 1, it is obvious that only 12/24 patients had =>10 portal tracts evaluated, which raises the concern of accuracy of their diagnosis.
Reply 2: Biopsies with less than 10 portal areas are often commonly encountered in clinical practice (Bonkovsky HL. *Hepatology*. 2017). In this study, at least 3 portal areas without bile duct were needed for the diagnosis of VBDS if a biopsy had < 10 portal areas. So, the ratio of portal areas without bile ducts is still larger than 25% even if we assume a total of 10 portal areas are detected. It increases the accuracy of the diagnosis of VBDS. However, it is unclear whether these patients belong to partial form of VBDS or classical form of VBDS. We discuss this limitation in discussion.
Changes in the text: A additional limitation was added (See Page 8, line 215-216).

Third, according to their description (page 5, line 139 to 142), only cases with "bile duct paucity" in report were further reviewed by a pathologist who was blind to the patient's clinical information, which is not appropriate.
Reply 3: Histologic reports were giving after liver sections were read jointly by 2 pathologists and at least 1 hepatologist. In this study, a pathologist was needed to confirm the loss of bile ducts, and recounted the numbers of portal areas with or without bile ducts. Blinding to clinical information help avoid bias.
Changes in the text: Not applicable.

Fourth, there is no information about the outcome of cases with VBDS. The quality of data presentation is also concerned.
Reply 4: In this cohort, 1 case died, 1 case had liver transplantation, 2 cases lost to follow-up, and other 20 patients were alive. We also optimized data presentation.
Changes in the text: Per your suggestion, outcome was added (See Table 1).

Reviewer D

Abstract: 303 words. Condense to ensure less than 300 words.
Reply 1: We condensed the abstract.
Changes in the text: The abstract have 265 words now.

Introduction: Very informative introduction. Acute cholestatic hepatitis leading to liver biopsy is only way to confirm VBDS. Very relevant information to many practitioners.
Reply 2: Great thanks.
Changes in the text: Not applicable.

Statistical Analyses: AuROC>0.8 meaning excellent performance.
Reply 3: Thanks for your advise.
Changes in the text: We added a sentence 'AuROC>0.8 meant excellent performance' (See Page 6, line 158).

Discussion: Line 238 typo - "Duo the small sample size" did you mean "Due to the small sample size? I would agree with the limitation that you mentioned, small sample size could have skewed results but I think the results you have are marvelous! Looking at larger studies would only confirm these results but I'm not sure how common VBDS actually is? Would be curious to see this!
Reply 4: Very sorry for this type error. In this cohort, the incidence of VBDS is 12.5% among children with acute cholestatic hepatitis. The ratio may be overestimated (See Page 8, line 217-219). About 5 patient are diagnosed with VBDS per year in our center. More patients with VBDS will be diagnosed in the future.
Changes in the text: Not applicable.

Conclusion: I would agree with the authors conclusion on GGT and TC being a good indicator for VBDS workup in the presence of acute cholestatic hepatitis.
Reply 5: Great thanks.
Changes in the text: Not applicable.

Figures and Tables: Very easy to follow and interpret.
Reply 6: Great thanks.
Changes in the text: Not applicable.