Professor Ruo-Yu Ma,

Science Editor, Science Editor Office

World Journal of Hepatology

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Low phospholipid-associated cholelithiasis syndrome: A rare cause of acute pancreatitis that should not be neglected.

Dear editor and reviewers,

Thank you for your e-mail from May 9, 2020 and the interesting comments made by the editor and the reviewers. Please find attached the revised copy of the above-mentioned manuscript, as well as our reply to the questions raised.

Thank you for considering our revised paper for publication in World Journal of Hepatology, in the form of an observational study.

Best regards,

Nicolas GILLE

For and on behalf of all authors

Answer to the Editor

Thank you for considering our paper. We have made all modifications requested in document "54024_List of issues that need to be addressed by authors in conditionally accepted manuscript". We have added the following, written in red: "Received, Revised, Accepted, Published", the citation, the acknowledgements, and the footnotes.

We have also made the formatting modifications requested.

Additionally, as recommended for the title of our manuscript, we have replaced the abbreviation "LPAC" with "Low phospholipid-associated cholelithiasis". We can update accordingly based on your preference.

Reviewer #03271124

Question 1. What is the criteria for diagnosis of the LPAC syndrome in this study? The author should clearly state in the method section. Because of the diagnosis of the LPAC is vary from the previous studies. For example, the patients meet the following two of five criteria from the one previous study (1). Another study reports the diagnosis of the LPAC syndrome seem to be relied on the genetic study (mutation of APCB4) (2).

Reference 1. Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. The spectrum of liver diseases related to ABCB4 gene mutations: pathophysiology and clinical aspects. Semin Liver Dis. 2010;30(2):134-46.

Reference 2. Goubault P, Brunel T, Rode A, Bancel B, Mohkam K, Mabrut JY. Low-Phospholipid Associated Cholelithiasis (LPAC) syndrome: A synthetic review. J Visc Surg. 2019;156(4):319-28.

Authors' reply: Thank you for your comment. In our study, the diagnosis of LPAC syndrome was made by ultrasound examination when the following findings were detected in the intrahepatic bile ducts: hyperechoic foci in the form of comet-tail artifacts, microlithiasis, or stones with acoustic shadows. Ultrasound examinations were performed on patients who had a clinical suspicion of LPAC syndrome, *i.e.* who had at least one of the following features: the onset of biliary pain before the age of

30; biliary pain recurring after a cholecystectomy; a personal history of acute pancreatitis with unknown etiology; a personal history of pregnancy cholestasis; or a family history of gallstones before the age of 30 in first-degree relatives.

We included our criteria for diagnosis in our Patients section, but as you have suggested, we have moved the following sentences into the Methods section: *"LPAC syndrome was suspected when at least one of the following features was present: the onset of biliary pain before the age of 30 years; biliary pain recurring after cholecystectomy; a personal history of acute pancreatitis with unknown etiology; a personal history of pregnancy cholestasis; or a family history of gallstones before the age of 30 years in first-degree relatives"* and *"The diagnosis was made by ultrasound examination when the following findings were detected in the intrahepatic bile ducts: hyperechoic foci in the form of comet-tail artifacts, microlithiasis, or stones with acoustic shadows."*

Question 2. How many patients in this study performed the genetic testing?

Authors' reply: In our study, the genetic testing was performed on seven patients. We added this information within the Results section, with the following: *"Genetic testing was performed on seven patients, none of which had a mutation in the ABCB4/MDR3 gene."*

Question 3. There are ten patients who have associated gallbladder and common bile duct pathology including gallstones, sludge, gallbladder hydrops and common bile duct stones. How can you differentiate the biliary symptom which arise from these conditions or intrahepatic stone?

Authors' reply: Thank you for this question. We believe the biliary symptoms of these ten patients were related to intrahepatic stones because:

- All ten patients also had intrahepatic bile duct lithiasis diagnosed *via* ultrasound examination: comet-tail artifacts, microlithiasis, and stones with acoustic shadows.

- All biliary symptoms were recurrent and occurred in patients with clinical suspicion of LPAC Syndrome (onset of biliary pain before the age of 30; biliary pain

recurring after a cholecystectomy; a personal history of acute pancreatitis with unknown etiology; a personal history of pregnancy cholestasis; or a family history of gallstones before the age of 30 in first-degree relatives).

Question 4. In my opinion, the patients who have associated gallbladder pathology and genetic testing were not performed should be excluded from the study.

Authors' reply: Thank you for this comment. We chose not to exclude these patients for the two following reasons:

1. Several studies showed that genetic mutations are detected in only 50%–65% of patients with LPAC syndrome (References: 1, 2, 3, 4). LPAC Syndrome diagnosis is made *via* ultrasound examination by detecting intrahepatic lithiasis or comet-tail artifacts on patients with recurrent biliary symptoms. Genetic mutations are not required to diagnose the disease.

Reference 1. Rosmorduc O, Poupon R. Low phospholipid associated cholelithiasis: association with mutation in the MDR3/ABCB4 gene. Orphanet J Rare Dis. 2007 Jun 11;2:29.

Reference 2. Poupon R, Rosmorduc O, Boëlle PY, Chrétien Y, Corpechot C, Chazouillères O, et al. Genotype-phenotype relationships in the low-phospholipid-associated cholelithiasis syndrome: a study of 156 consecutive patients. Hepatology. 2013 Sep;58(3):1105–10

Reference 3. Erlinger S. Low phospholipid-associated cholestasis and cholelithiasis. Clin Res Hepatol Gastroenterol. 2012 Sep;36 Suppl 1:S36-40

Reference 4. Erlinger S. [Right upper quadrant abdominal pain and fever. Genetic phospholipid deficiency]. Gastroenterol Clin Biol. 2009 Oct;33(10-11 Suppl):F50-55

2. All patients with associated gallbladder pathology also had intrahepatic bile duct lithiasis diagnosed *via* ultrasound examination, and recurrent biliary symptoms, making the diagnosis of LPAC Syndrome certain.

Nevertheless, the fact that genetic testing was not performed systematically is a limit of our study, as we acknowledged within the Discussion section with the following: *"Genetic testing was not performed systematically, which hinders the analysis of all possible mutations present".*

Question 5. In the follow-up period, the stone from ultrasound examination is one of the crucial points for the evaluation of the treatment outcome. The author should show the data of the ultrasound examination after treatment.

Authors' reply: Thank you for this comment. To evaluate the treatment outcome during our follow-up period, the patients were systematically asked about their symptoms through in-person medical appointments or phone calls. We did not perform ultrasound examination during follow-up. Nevertheless, as 94% of patients described a complete disappearance or a significant decrease of biliary pain intensity or frequency, this result suggests that symptoms are not directly related to stones, but may be due to inflammation of intrahepatic bile ducts or to cholesterol crystals not detected by echography.

We have added the following sentence within the Methods section: *"Follow-up was based on clinical evaluation and not on ultrasound examination."*

Reviewer #00186426

Question. In fact, this is a very interesting study and it was presented in a very didactic form. It would be very important to include this kind of study in children with cholelithiasis or in children with "idiopathic" pancreatitis. I have an important question: how many patients had to undergo a Roux-en-Y procedure due to the intrahepatic lithiasis?

Authors' reply: Thank you for this comment. It is certainly true that there exists a lack of studies that include pediatric patients with cholelithiasis or "idiopathic" pancreatitis.

None of our 24 patients had to undergo a Roux-en-Y procedure. We have added the following within the Results section: *"None of the 24 patients had to undergo a Roux-en-Y procedure"*.

Reviewer #02461932

Question. Major Comment: The manuscript is well documented and worth publishing. Minor Comment: Please explain "*2.8N and 1.7N*" in Method paragraphs of Abstract and Main Text.

Author's reply: Thank you for this point. The sentence *"2.8N and 1.7N"* means that serum aspartate and alanine transaminase activities were 2.8 times higher than normal, and that the alkaline phosphatase level was 1.7 times higher than normal. As you have recommended, we have now incorporated an explanation within Method paragraphs of Abstract and Main Text: "*Cytolysis and cholestasis were expressed compared to the normal values (N) of serum aspartate and alanine transaminase activities, and to the normal value of alkaline phosphatase level, respectively*".