Point to point responses to reviewers

Dear Editor,
Please, find bellow the answers to all the points raised by the reviewers

COMMENTS TO AUTHORS
Reviewer report Manuscript title: '' High Prevalence of Cholestasis, With Increased Conjugated Bile Acids, in IBD Patients". This retrospective study aimed to investigate the prevalence of cholestasis in a cohort of patients with inflammatory bowel disease. A high prevalence of cholestasis (high total bile acid level in serum) of about 7% was found. The study identified smoking as a factor that reduces the risk of cholestasis in these patients. In this study new data has been provided as regards cholestasis in patients with inflammatory bowel disease and suggest an intrahepatic origin for this. Comments The conclusions stated at the end of the discussion are better to be also added to the abstract.

The conclusion of the abstract has been modified in order to include the conclusion stated at the end of the discussion as suggested by the reviewer while respecting the limit of 26 words…

COMMENTS TO AUTHORS
The findings in this study are interesting because little is known about cholestasis and IBD. However, there are some major points to be mentioned as follows.

Major 1, Since CD and UC are different disease, they should be addressed separately.

I fully agree with this comment on the fact that Crohn's and UC are different disease. One important point is that in the univariate analysis, the type of disease does not emerge as a significant factor. On the other hand, to go in the sense of the comment of the reviewer, we totally took into account that UC and Crohn's are different in the bile acid subtypes analysis. We decided to compare ileal versus colonic disease thinking that ileal Crohn is much more different than Colonic Crohn and UC. It appeared that indeed the characteristics of cholestasis was different. It has been recently shown (from a genetic point of view at least) that there is maybe a continuum between ileal Crohn to colonic Crohn to UC more than a clear dichotomy (Isabelle Cleynen et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. Lancet 2016; 387: 156–67).

2, If you exclude patients with a cause of cholestasis, all PSC patient, ursodesoxycholic acid users and cholestyramine user should be excluded.

Yes, and actually it is what we did as written in the result section on page 10: *"We therefore removed from the final analysis patients with PSC (n=5), patients who underwent organ transplantation (n=3), patients treated with either cyclosporine (n=12) or tacrolimus (n=11), all drugs known to cause cholestasis and lastly patients who received respectively ursodesoxycholic acid (n=16) and cholestyramine (n=25) drugs that can substantially modify the bile acid metabolism."*

3, Other liver function related parameters, such as AST, ALT, gamma GTP or bilirubin needs to be analyzed.

I also agree with this comment, but at the time of the study, only alkaline phosphatase level was collected in the Swiss IBD Cohort, therefore I couldn't include other liver test.

Minor 1, Abstract: In result paragraph, "80>8μmol/l" may be a mistake of "80 had a level >8μmol/l".

"with total bile acid" has been added

2, Materials and Methods: The description about Table 4 need to be move to "Result" section.

Thank you for this remark. This paragraph has been moved to the result section.

3, The number of patients in Supplementary table 1 seems different. According to table 1, the total number of treatment with ursodesoxycholic acid is 17, treatment with cholestyramine is 26 and PSC is 15.

Yes it is maybe a little bit confusing. Table 1 shows all the data for all patients. The supplementary table 1 shows the number of patient excluded. Among those patients there are 5 with PSC but the 10 other patient with PSC are included in those with ursodesoxycholic acid or cholestyramine treatment, because some of them had 2 or 3 "reasons" to be excluded.

COMMENTS TO AUTHORS

The manuscript titled High Prevalence of Cholestasis, With Increased Conjugated Bile Acids, in IBD Patients carried out a retrospective cohort study of the prevalence as well as causes of cholestasis in patients with inflammatory bowel diseases. They found that calcium supplementation was significantly associated with cholestasis, whereas current smoking significantly reduced the risk of cholestasis. Those patients with ileal disease had higher levels of primary, secondary, and tertiary bile acids whereas patients with colonic disease had higher levels of conjugated bile acids. The author concluded that conjugated bile acids are higher in cholestasis indicating a possible role for the liver in pathogenesis. The author also found that smoking appears to reduce cholestasis. As the author mentioned, there is no available data regarding the potential impact of cholestasis on IBD itself. But in cholestasis, the pathways of liposoluble vitamins absorption and bile acids metabolism could be influenced by IBD. Since the exact prevalence of cholestasis in IBD remains unknown and because cholestasis may have a profound impact on the disease course, it is important to deeply identify its aetiologies and its clinical relevance.

Questions and suggestions: • This is a retrospective cohort study, how to evaluate influence of the length of disease time to the changes of TBA and severity of the

disease.

The main issue with retrospective study is the fact that you look back and it is therefore impossible to study a prospective effect of a variable. Nevertheless the suggestion about disease duration is absolutely correct and this data has been added to the Table 1. Disease duration was not different between cholestasis and control group, neither was the severity of the disease attested by the CDAI and MTWI index.

• It seems the patients are from different hospitals or clinics all over Switzerland, and the healthcare physicians are different, how the author can be sure the diagnosis is based on the standard.

Indeed, the patients were included all over Switzerland in different hospitals and clinics. In a large cohort, this is usually the case. But to enter in the study, as inclusion criteria, the diagnosis has to be done following international standard (ECCO guidelines), based on endoscopy, radiology as well as histology. Cases are reviewed at entry by responsible doctors of the cohort.

• Is the bile acids measured in different centers? Do they use the same methods to evaluate the TBA?

No the total bile acid level as well as the subtypes have been done in one lab as explained in the methods section on page 7. *"a total bile acid assay was performed on all serum specimens using a highly sensitive and specific ELISA test (Labor Eberhard, Dortmund, Germany)"* and *"Final analysis was performed on a QUATTRO Micro tandem mass spectrometer (Screening-Labor, Hannover, Germany)"*

• The author concluded that current smoking status seems to be a protective factor against cholestasis. Which may mislead the risk influence of smoking to human health. As the author mentioned in the discussion, the real reason is that the non-smokers probably have better function in absorbing bile acid from bile duct system and transfer into the circulation.

Thank you for this comment. For sure the goal of the statement on smoking is not to encourage patients to smoke! The conclusion has been modified to minimize misunderstanding.