Format for ANSWERING REVIEWERS

July 15, 2013

Dear Editor,

Thank you for your kind email letter, regarding about our manuscript entitled "Clinicopathological study of primary biliary cirrhosis with interface hepatitis compared to autoimmune hepatitis" (manuscript No.3843).

We have revised our manuscript taking the Editor's and two Reviewers' kind and thoughtful comments and suggestions into consideration. We are sending a revision package to the Managing Editor, which includes a sheet of answering report with the point-by-point responses to the Editor's and two reviewers' comments, a revised manuscript as a text file and figures. In the following responses, the comments and queries of the editor and reviewers are shown by blue color, and our point-by-point responses are shown by brown color and the revised parts are shown by red color. In the revised manuscript submitted, revised parts were shown by color.

We trust the revisions of the manuscript are in order. We are glad and grateful if this revised paper would be accepted for publication on your journal, World Journal of Gastroenterology.

Thank you very much for your kindness and cooperation in advance.

Please find enclosed the edited manuscript in Word format (file name: 3843-review').

Title: Clinicopathological study of primary biliary cirrhosis with interface hepatitis compared to autoimmune hepatitis

Author: Mio Kobayashi, Yuko Kakuda, Kenichi Harada, Yasunori Sato, Motoko Sasaki, Hiroko Ikeda, Mitsuhiro Terada, Munenori Mukai, Shuichi Kaneko, and Yasuni Nakanuma

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3843

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers.

To Editor:

(1) We attached here a language certificate by a professional English language editing company.

(2) As for zip cord, we added them in the revised manuscript (Page 1).

(3) As for core tip, we added it in the revised manuscript (Page 4).

(4) As to the citation of reference in the manuscript, we marked all of the citation in accordance with the format in the revised manuscript. The following is an example.

In the revised manuscript, Page 5 line 2- 3:

Primary biliary cirrhosis (PBC) is characterized by the progressive destruction of interlobular bile ducts (chronic

nonsuppurative destructive cholangitis) and frequent anti-mitochondrial antibodies (AMAs) in the serum[1-3].

(5) As for the revision in the reference, we added PubMed and DOI citation numbers for the reference list. Please see the reference in the revised manuscript. The following is one example.

1. Poupon R. Primary biliary cirrhosis: a 2010 update. J Hepatol. 2010;52:745-58. doi: 10.1016/j.jhep.2009.11.027. PMID: 20347176

To Reviewer No.01314835:

Major comments

(1)The investigators have not achieved your desired goal.

Our responses

Thank you very much for your critical comments.

Our goal of this study is, of course, to clarify whether the pathogenetic mechanisms of hepatitic activities of PBC with AIH features, particularly interface hepatitis, is different from or identical to those of AIH. So, we examined three issues for this goal, which are shown in the last paragraph of the Introduction section, as follows.

1) To examine similarities and differences in interface hepatitis and the lobular changes in AIH and PBC with interface hepatitis.
2) To compare the subtypes of infiltrating mononuclear cells in interface and lobular hepatitis in AIH and PBC with interface hepatitis.
3) To correlate such histologic and immunohistochemical features of hepatic necroinflammation with the clinical and laboratory features of these two groups.

We think that the above-mentioned aims were carefully examined and conducted clinicopathologically and immunohistochemically in this study, and that the reasonable results were obtained as described in this manuscript. These results were not conclusive whether the hepatitic activities of PBC with interface hepatitis were identical to AIH, or that the former was completely different from the latter. In this context, we could not have achieved our desired goal, as you pointed out. However, this study raised several important points which are discussed in the discussion section. In this context, while we have not definitely achieved our desired goal, this study guarantees the further studies which may more clearly clarify the similarities and differences of the hepatitic phenomena between PBC with interface hepatitis and AIH.

Minor comments

(1) Diagnosis criteria of PBC should be addressed.

Our responses

Thank you for your nice comment. We made a diagnosis of PBC histologically in the patients with compatible laboratory and serologic findings with PBC. Other hepatobiliary diseases were excluded in these cases. We added the following sentence and one reference for a diagnosis of PBC in the revised manuscript, as follows.

Materials and Methods,

Revised manuscript, Page 6 line 1-3 from the bottom-Page 7 line 1:

diagnostic features of PBC[1] and AIH type I [10, 12], respectively. Regarding a diagnosis of PBC, when histological changes compatible with PBC such as chronic cholangitis and/or bile duct loss were found in the patients with elevation

of biliary enzymes and serological data compatible with PBC, such cases were diagnosed as PBC and included in this study. Other hepatobiliary diseases were excluded in these cases. No patient had a ---

(2) Since titers of ANA and AMA are not correlated with disease severity, it should be assessed as positive or negative.

Our responses

Thank you for your nice comment. Accordingly, we re-evaluated the disease severity with AMA or ANA positivivity but not with their titers in the revised paper. We combined AMA and M2 as one item as AMA or M2, because only either AMA or M2, not both, was examined in several hospitals for a serological diagnosis of PBC.

In the revised paper, we evaluated the degree of interface hepatitis and that of infiltration of mononuclear cells positive for CD3, CD38, IgG, or IgM between the positive cases and negative cases for AMA or M2 and ANA. We revised Table 4, and deleted Table 5B, and newly added Table 6 in the revised manuscript. Please see these Tables.

There were no difference in the degree of interface hepatitis and the degree of mononuclear cells infiltration between the patients positive for and those negative for AMA or M2 and ANA in PBC and AIH. This data was shown in Table 6 in the revised manuscript.

There were also no difference in the positivity of AMA or M2 and ANA between IgG+ cell-infiltration dominant and IgM+ cell infiltration-dominant groups in PBC. This was shown in revised Table 4 in the revised manuscript.

We rewrote the relevant parts in the revised paper as below.

Materials and methods,

Revised manuscript, Page 7 line 3-4:

The main clinicopathological and laboratory findings of these cases are shown in Table 1. As for AMA positivity, AMA and/or M2 were evaluated in a combination in individual cases.

Results,

Revised manuscript, Page 11 line 2 from the bottom:

--- which suggests that PBC in group B may share more features with AIH. There were no correlation in the positive ratio of AMA or M2 and that of ANA between these two groups.

Revised manuscript, Page 12, last paragraph:

Correlation between AMA or M2 and ANA positivity and the degree of interface hepatitis and infiltration of mononuclear cells positive for CD3, CD38, IgG, and IgM: There was no difference in the degree of interface hepatitis between AMA or M2 and ANA positive and negative groups in both AIH and PBC (Table 6). There was also no difference in the degree of infiltrated mononuclear cells expressing CD3, CD38, IgG, or IgM at the interface between AMA or M2 positive and negative group in PBC or between the ANA positive and negative group in AIH.

Discussion,

Revised manuscript, Page 15, 2nd paragraph:

More than 90 % of PBC patients are AMA-positive, and more than 90 % of AIH patients are ANA-positive[11, 21, 22]. These antibodies have been considered to be important for the diagnosis of PBC and AIH, though their pathogenic roles in hepatocellular injuries are speculative. This study found no difference in the degree of interface and lobular hepatitis or mononuclear cell infiltration at the interface irrespective of an AMA and ANA positivity in PBC and AIH. Therefore, it appears likely that ANA and AMA have no direct influence on interface and lobular hepatitis. While ANA and AMA

may play a role in hepatocyte or cholangiocyte injury[21, 23], these autoantibodies are not necessarily linked to the severity and pathogenesis of PBC and AIH[22-27], which was supported by the results of this study.

(3) Although you selected activity scores more than 2, score 1 in PBC were included.

Our responses

Portal/periportal activity score 1 in PBC means no interface hepatitis. In this study, we compared PBC with interface hepatitis with AIH. AIH is always characterized by interface hepatitis itself. So, inclusion of PBC with score 1 may simply compare with PBC with AIH. In this context, we did not include PBC cases with score 1 and 0 in this study. To make clear our purpose of this study and avoid misunderstanding, we added the following sentences and rewrote the material and method section as follows.

Materials and methods,

Revised manuscript, Page 6 line 8-17:

Selection of Patients with PBC and AIH and tissue preparation

In this study, we tried to compare PBC with interface hepatitis with AIH, and we selected PBC patients with interface hepatitis, by adopting Batts and Ludwig's scoring system[17] In this system, the activity of periportal (interface) and lobular inflammation was scored semiquantitatively in routinely processed sections; portal/periportal activity score 0 (none or minimal activity), 1 (portal inflammation only), 2 (mild interface hepatitis), 3 (moderate interface hepatitis), and 4 (severe interface hepatitis), and lobular activity score 0 (none), 1 (inflammatory cells, but no hepatocellular death), 2 (focal cell death), 3 (severe focal cell death, confluent necrosis without bridging), and 4 (damage including bridging necrosis). We collected liver specimens PBC with portal/periportal activity scores ≥ 2 (Fig. 1A, B).

Patient collection: A total of 84 liver needle biopsies were used in this study. They consisted of 41 patients with

(4) Nonparametric analysis should be performed.

First of all, we performed actually nonparametric analysis in this study. Probably because of our insufficient description and presentation, we are sorry that you got a wrong interpretation. We used Mann-Whitney umpaired test to determine the difference between two groups. Accordingly, we added the median of datas in Table 3, 4, 6 in the revised manuscript. We rewrote Figure 2 to show the median of each scores. We performed Fisher's exact probability test to compare the difference of positivity rate of serum autoantibodies. Please see revised and newly added Table 3, 4, 6 and Figure 2 .

Materials and Methods,

Revised manuscript, Page 9, last paragraph:

Statistical Analysis

All statistical analyses were performed using JMP software (version 8.0, SAS Institute Japan, Tokyo, Japan). Values were expressed graphically as the mean ± standard deviation (SD) and median. Statistical differences between groups were determined using a two tailed Mann–Whitney unpaired test and Fisher's exact probability test with 95% confidence interval (CI). The correlation coefficient of 2 factors was evaluated using Spearman's rank correlation test. p values of <0.05 were considered significant.

Figure Legends,

Revised manuscript, Page 20, 2nd paragraph:

Figure 2. Comparison of necroinflammation in autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC) with interface hepatitis.

A: The scores of interface hepatitis (IFH) were not significantly different between PBC (2.49 ± 0.64) and AIH (2.74 ±

0.66) ($p = 0.0599$) because PBC cases with IFH were chosen for this comparative study with AIH; B: The scores of lobular hepatitis (LH) were higher in AIH (2.58 ± 0.70) than in PBC (2.22 ± 0.61) ($p = 0.0003$); C: The scores of hepatitic rosette formation were higher in AIH (0.55 ± 0.83) than in PBC (0.17 ± 0.44) ($p = 0.0134$); D: The scores of emperipolesis were higher in AIH (1.00 ± 0.96) than in PBC (0.32 ± 0.52) ($p = 0.0003$). (Horizontal bars of the graph show the median scores; *$p < 0.05$, ***$p < 0.001$ in the Mann–Whitney test)

(5) Conclusion are too long and should be condensed.

Our responses

According to your suggestion, we rewrote the conclusion briefly as below.

Discussion,

Revised manuscript, Page 16 line 1- :

In conclusion, ~~lobular hepatitis, emperipolesis, and hepatitic rosette formation were milder in PBC with interface hepatitis than in AIH. While the infiltration of CD3+, CD4+, CD8+, and CD38+ mononuclear cells were similarly found in lobular hepatitis and interface hepatitis in AIH and PBC with interface hepatitis, the infiltration scores were higher in AIH than in PBC. The degree of infiltration of these cells correlated with the degree of hepatic necroinflammation and was associated more with the elevation in AST in PBC; however, these features were not evident in AIH.~~ while the immunophenotypes of infiltrating cells at the interface and lobular hepatitis in AIH and PBC with interface hepatitis appeared to be similar, the precise mechanisms of hepatocellular injuries may not be identical. More studies on the precise mechanisms of hepatocellular injuries in PBC are necessary to distinguish PBC with interface hepatitis from AIH.

(6) There are a small number of typo.

Our responses

We corrected some words in the revised manuscript. Thank you very much for your notion.

(7) Language evaluation: Grade B (minor language polishing)

Our responses

This revised manuscript was proofread by a professional English language editing company. .

To reviewer No.02447091

Major comments The authors discuss clinicopathological difference between interface and lobular hepatitis seen in PBC with interface hepatitis and AIH. They conclude that the immunological mechanism in both disease entities is similar but that of hepatocyte injuries may be different, suggesting that autoimmune features observed in PBC with interface hepatitis might not be derived from the overlap of AIH.

Minor comments

(1) Page 1, line 4. Running title should be "AIH and PBC with interface hepatitis", instead of "interface hepatitis".
(2) Page 19, line 5. "J Hepatol. 199: 30: 394-401.". should be "J Hepatol 1999; 30: 394-401.
(3) Page 22, line 16. There are no IgG predominant …… should be, There are no IgM predominant…..

Our responses:

Thank you very much for your kind advices and suggestions. We have corrected words as you pointed out and please see the revised manuscript.

Again, thank you very much for your kind and thoughtful comments.

Sincerely yours,

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