ANSWERING REVIEWERS

May 25 , 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (filename:18162-edited.doc). Thank you very much for your kindly comments on our manuscript (ESPS Manuscript NO: 18162). Those comments are all valuable and very helpful for revising and improving our manuscript as well as the important guiding significance to our researches. Based on your advice and reviewer's comments and suggestions, we have made careful modifications on the original manuscript. All changes made to the text are in yellow color. Point by point responses to the reviewers' comments are listed below this letter.

Title: Thyroid Dysfunction in Chineses HCV patients: Prevalence and Correlation of TPOAb and CXCL10

Author: Renwen Zhang, Cuiping Shao, Na Huo, Minran Li, Hongli Xi, Min Yu, Xiaoyuan Xu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 18162

We would like to express our sincere thanks to the reviewers for the constructive and positive comments. 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:
Replies to Reviewer 02994092
As suggested by the reviewer , corrections (i.e. add words and delete words/space)have been made in the revised version.

(1) Can you please speculate on why the association of TOPAb and CXCL10 was observed with on-treatment TD?

Answer: IFN-α has a well documented side effect profile, including thyroid diseases, which cumulatively could lead to dose reductions in patients and drug discontinuation. Emergence of TD has been proposed as a result of IFN-based therapy, and in some cases interferon induced TD may cause the discontinuation of interferon therapy. Thus, it represents a major clinical problem in hepatitis C patients receiving IFN α therapy. CXCL10 (or IP-10), a member of the CXC chemokine family, is expressed in the liver of CHC patients and selectively recruits activated T cells to the inflammation sites. There was evidence indicating that circulating CXCL10 levels increased in HCV-infected patients who also had autoimmune thyroiditis. Most of thyroid autoimmunity cases have no clinical symptoms. However they may have one or two of the following TAs, including TPOAb, and TGAb. Therefore, we speculate the association of TOPAb and CXCL10 was observed with on-treatment TD.

(2) I don't think you should say "necessary" perhaps" could be helpful to risk stratify,

but this should not exclude subjects from treatment if the majority are reversible.

Answer: I agree with your opinion. As suggested by the reviewer , corrections have been made in the revised version.

(3) Please provide the upper and lower limits of normal for these tests (in Table 2).

Answer: As suggested by the reviewer , corrections have been made in the revised version.

(4) Is this "with" or "without"(For "WTD")?

Answer: After examining the reviewer's comments carefully, we must admit that we have not expressed our meaning correctly in the previous manuscript. Sorry for this confusion. In the revised version, the "WTD" has been corrected as "NTD".

Replies to Reviewer 02995208

Major points

(1) The manuscript must go through a native English correction. Sections like the abstract is hard to be understood as it is, and language errors can be found all over the manuscript.

Answer: Our manuscript had been edited by American Journal Experts (http://www.aje.com) English language editing companies .

(2) The authors stated in discussion/conclusion that: "pretreatment serum CXCL10 levels were associated with PegIFNα-2a/RBV induced TD", however the data presented in table 3 shows higher level of CXCL10 in patients without TD. In the statement all over the manuscript, should be emphasized that is lower levels of pretreatment CXCL10 that are associated with PegIFNα-2a/RBV induced TD.

Answer: After examining the reviewer's comments carefully, we must admit that we have not expressed our meaning correctly in the previous manuscript. Sorry for this confusion. In the revised version, corrections have been made in the revised version. We emphasized that lower levels of pretreatment CXCL10 are associated with PegIFNα-2a/RBV induced TD all over the manuscript.

(3) The authors stated in discussion/conclusion that: "The prevalence of TD was higher in the female patients with TPOAb positivity at baseline". However no direct comparison of proportions of TD in female patients with TPOAb positivity or TPOAb negative is presented. What is presented in table 3 is that in TD there is higher proportion of female and higher proportion of TPOAb positivity when compared with patients without TD. Such statement is not correctly build.

Answer: As suggested by the reviewer , we must admit that we have not expressed our meaning correctly in the previous manuscript. Sorry for this confusion. In our study, female patients had a higher risk for the development of TD than male patients. The baseline TPOAb positivity have been suggested to be a risk factor for TD development secondarily to PegIFNα-2a/RBV treatment. In general, TD prevalence was increased in female patients and patients who were positive for TPOAb at baseline. Corrections have been made in the revised version.

Minor points

(1) In the second paragraph of introduction, the author says: "Preliminary studies have suggested that there are at least two different models by which IFN α may induce thyroid dysfunction". Since this is an important subject along the manuscript, those two models should be better clarified/explained.

Answer: As suggested by the reviewer, several sentences have been added in the revised version to address this issue.

(2) The title says "the Role of TPOAb and CXCL10 in TD in HCV", however no specific role is presented. What is presented is the prevalence and correlation of such biological markers and TD in HCV. The title should be adjusted to better summarize what is being presented in the manuscript.

Answer: As suggested by the reviewer, the title have be changed "the prevalence and correlation of TPOAb and CXCL10 in TD in HCV".

Replies to Reviewer 03261346

Major points

(1) A thoroughly revision of the English language would be needed.

Answer: Our manuscript had been edited by American Journal Experts (http://www.aje.com) English language editing companies .

(2) The largest part of the results section enumerates the number of patients with different clinical or subclinical categories of TD. This is also presented in table 2. A more detailed presentation of the TPOAb and CXCL10 results is required, and the use of figures would increase the value of the content.

Answer: Corrections have been made in the revised version. These results were shown in Figure 1A、1B .

(3) The value of TPOAb positivity as a screening marker for treatment induced TD in HCV infected patients is suggested by several studies, including one published by the same authors (reference 15). However, the data presented in the manuscript do not sustain the statement that "screening for … CXCL10 before combination therapy is necessary to identify the high-risk patients who may develop PegIFNα-2a/RBV associated thyroid dysfunction". There is an important variation of the CXCL10 levels in HCV patients with or without TD, demonstrated by the high standard deviation observed for both categories of patients, as presented in table 3. Therefore individual CXCL10 values might be unhelpful to identify patients at risk to develop treatment induced TD, although at a population level the values of CXCL10 are significantly lower in patients who developed TD, at least in the studied population. The conclusions might be reformulated accordingly.

Answer: For TPOAb/TGAb, nine of twenty-four patients with TPOAb/TGAb-positive at baseline developed TD. By constrast, three (one male and two females) of one hundred and fifteen patients without TPOAb/TGAb at baseline developed TD at the end of the treatment (37.5% versus 2.6%, P=0.000). Therefore, the prevalence of TD was increased in patients who were TPOAb-positive at baseline.

Among chemokines of the CXC family, CXC chemokine ligand 10 (CXCL10) plays an important role in several human autoimmune and non-autoimmune diseases. It has been recently reported that HCV patients developing thyroid dysfunction during IFN-α treatment displayed circulating markers of a Th1 immune reaction, as assessed by IFN-γ expression by peripheral blood lymphocytes. Evidence also indicates that circulating CXCL10 levels increase in HCV-infected patients with autoimmune thyroiditis, potentially because CXCL10 recruits T-helper (Th) 1 lymphocytes. These cells, secrete IFN γ and tumor necrosis factor (TNF), promoting further CXCL10 secretion and perpetuating the autoimmune process. Indeed, it is reasonable to hypothesise that the changes in serum CXCL10 might be more evident in patients developing overt thyroid dysfunction, who also show a microenvironment more enriched in T helper type 1 (Th1) molecules. In our study, although high standard deviation are observed for both categories of patients, there is an important variation of the CXCL10 levels in HCV patients with or without TD. At least in the studied population, the values of CXCL10 are

significantly lower in patients who developed TD. Maybe the reason is that the number of overt thyroid dysfunction was too small(only two) in our studied populations. Therefore, our results should be confirmed by much larger sample size. Maybe the reason is that the number of overt thyroid dysfunction was too small(only two) in our studied populations. Therefore our results should be confirmed by much larger sample size. Previous studies and our results both revealed that individual CXCL10 values might be helpful to identify patients at risk to develop treatment induced TD.
As suggested by the reviewer , corrections have been made in the revised version.

Minor points

(1) The title should be reformulated, since the study describes only the prevalence of PegIFN/RBV induced thyroid dysfunction (TD) and its association with TPOAb and CXCL10 levels.

Answer: As suggested by the reviewer, the title have be changed "the prevalence and correlation of TPOAb and CXCL10 in TD in HCV".

(2) The first section might be more focused on the topic, i.e. biological markers associated with thyroid dysfunction in HCV infected patients.

Answer: Several sentences have been added in the Introduction in the revised version to address this issue.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Xiaoyuan Xu

Professor, Corresponding author

Departments of Infectious Diseases,

Peking University First Hospital

NO.8, Xishiku Street, Xicheng District,

Tel: +86-010-83575787

E-mail: xiaoyuanxu6@163.com