Format for ANSWERING REVIEWERS

July 18, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name 11627-edited_Rev1_last.doc).

Title: Toll-like receptor-mediated signaling cascade as regulator of the inflammation network during alcoholic liver disease

Author: Sara Ceccarelli, Valerio Nobili, Anna Alisi

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 11627

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

Reviewed by 02445121

Comment: In this review, the author provide an overview of the current evidence of TLR involvement in inflammation during ALD in experimental models and humans. This review is described in detail, which, as a valuable tool, could help the readers that have no first-hand knowledge of this topic to start novel studies. It is suggested that the contents of the paper should be compressed appropriately.

Reply: We would thank the reviewer for his/her suggestions. As recommended, we extensively edited the English language by proofreading English service and we tried to compress when possible some contents of the manuscript.

Reviewed by 00503417

Comment: This is a good review on the subject of TLRs in ALD. The authors, in fact, go beyond the role of TLRs and have reviewed the entire cascade in the evolution of ALD. They may, therefore, be advised to change their title accordingly. The article needs extensive language editing.

Reply: We would thank the reviewer for his/her suggestions. As suggested by Reviewer, we have changed the title with "Toll-like receptor-mediated signaling cascade as regulator of the inflammation network during alcoholic liver disease". Furthermore, as recommended, we extensively edited the English language by proofreading English service (FirstEditing.com).

Reviewed by 00053419

Comment: Ceccarelli et al have performed a very good revision of TLR, their role in alcoholic liver disease and dissect the most relevant signaling pathways involved in TLR-mediated regulation of inflammation. The activity and relevance of TLRs is well summarized proving information from animal models and from patients. In this regard, some discussion about the parallelisms and dissimilarities between findings in animals and patients would be of interest. Some minor concerns are as follows: 1. No reference to table 1 was found in the main text. It should be cited. 2. As mentioned on page 8, progression of ALD-associated liver damage is potentiated by two main factors, LPS and redox imbalance. Oxidative stress is also induced by LPS and participates in the cellular response; this should be mentioned in the text and should be reflected in figure 1. 3. English must be carefully revised.

Reply: We would thank the reviewer for his/her suggestions. We appreciated the advice regarding the parallelisms we could make between animal models and human. We decided to avoid this part preferring to leave it as it is since it would request an additional paragraph. The choose was made to maintain the manuscript as much as possible compressed as the reviewer 00503417 proposed and as it is more suitable for the minireview format.

For minor concerns point by point answers are following.

1. The citations of Table 1 have been inserted in the text
2. We integrated the already present information about the induction of oxidative stress by LPS (see page 12 with ref 33). Furthermore we have already cited in 51 that direct interaction of NADPH oxidase isoenzyme 4 with TLR-4 is involved in LPS-mediated reactive oxygen species production. We also included this in the novel **figure 1**.
3. As recommended, we extensively edited the English language by proofreading English service.

Reviewed by 00003652

Comment: This review covers the current state of knowledge regarding the role of toll-like receptors (TLRs) in the pathobiology of alcoholic liver disease. While it is a comprehensive review overall, the language needs to be carefully revised by a native English speaker. Long, grammatically incorrect sentences exist throughout the text. Specific comments : 1. Given that alcohol-induced intestinal permeability and increase in serum endotoxinaemia is critical to the deleterious effects on the liver, it would be useful to have a detailed discussion about the possible mechanisms by which alcohol increases gut permeability. 2. Does small bowel bacterial overgrowth (SBBO) , increase intestinal permeability? 3. Please define 'toxic alcohol amount" in terms of gms of alcohol per day or cumulative lifetime alcohol consumption. 4. The authors are to be commended on the inclusion of Table 1, which gives a quick overview of all current studies on the subject. 5. Since heavy alcohol consumption is often associated with smoking, it may be useful to include a brief discussion of the possible effects of cigarette on TLRs in the liver.

Reply: We thank the reviewer for his/her comments and interesting suggestions. As recommended, we extensively edited the English language by proofreading English service. Following point by point answers.

Reply to 1: As suggested by the reviewer we added a discussion about mechanisms by which alcohol increases gut permeability. We also inserted two new references in relation to the new part treated in "ALD pathogenesis" paragraph.
Reply to 2: Actually, the bowel bacterial overgrowth (SBBO) is a consequence of increased intestinal permeability.
Reply to 3: We have now reported toxic alcohol amount 40-80 g/day for men and 20-40 g/day for women for 10ys.
Reply to 4: The citations of Table 1 have been inserted in the text.
Reply to 5: As LPS is present in cigarettes, therefore it is plausible that may be one of the factors that tobacco smoke can promote inflammation in smokers. However, we have not found evidence in the liver, thus we believe this mechanism still in embryo to be discussed in this minireview.

Reviewed by 02444949

Comment: Author mentioned that dispensable role of MyD88 adapter in TRL4-mediated liver injury. However, chronic alcohol generally induced MyD88 mRNA expression and its downstream genes, including NF-kB in liver. Did you think about it?

Reply: We really appreciated the specification given by the reviewer. Many studies reported a dispensable role of MyD88 in TLR4-mediated liver injury in ALD. After all, it is known that, during ALD, inflammatory pathways can be driven by NFκB which results in signaling that bypasses MyD88. After all, diverse authors reported an increment in MyD88 levels related to neuro-inflammation study leading to brain damage caused by alcohol. By the way, to date only one very recent study mentioned an increment in MyD88 levels due to alcohol in liver (Kanuri G et al. Effect of acute beer ingestion on the liver: studies in female mice, Eur J Nutr. 2014 Jun 15. Epub ahead of print).

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

Sincerely,
Dr Alisi and CoAuthors.