Paris, 11/4th/2020

Dear Editor,

Thank you for your invitation to submit our manuscript: «Adult human liver slice cultures: Modelling of liver fibrosis and evaluation of new anti-fibrotic drugs" to World Journal of Hepatology.

Please find our point-by-point answers to Reviewer and Editors. We are grateful for their critical remarks, proposed corrections and suggestions. The manuscript with corrections (Manuscript NO.: 59138, Observational Study) is attached.
Since, our work uses human liver samples as a model for testing anti-fibrotic, and anti-viral drugs, and also tissue molecular responses, we suppose that the work is not a classical "observation study", but rather a "basic study. Of course, we will comply with your final choice.

Kind regards,

Sylvie Lagaye PhD, HDR

Point-by-point answers to the reviewers

Dear Editors and Reviewer,

Please find our point-by-point answers to Reviewer and Editors. We would like to thank the Reviewer #1 and the Science editor for their helpful criticisms and suggestions. We believe, and hope the editors / reviewer agree, that the manuscript has now been improved.
The manuscript with corrections (Manuscript N°.: 59138, Observational Study) is attached.

Reviewer #1:

Specific Comments to Authors:

Authors developed 3d human liver tissue slice (HLS) culture for 21 days from patients with F0-1 vs. F2-4 fibrosis, created fibrosis model (FLS) for hepatitis C (HCV)and alcoholic hepatitis (ALD) or HCV and steatohepatitis (NASH), and then evaluated the anti-fibrotic effects of ursodeoksi cholic asid (UDCA) and/or alpha-tocopherol.

My comments: • Since fibrosis was induced by HCV infection in HLS, what is the rational for developing HLS from patients with F0-1 vs. F2-4.

Liver fibrosis is a multifactorial condition induced by multiple causes, including hepatitis C, alcohol and triglyceride accumulation in the liver.

For the first time, we established the non-fibrotic (F0-F1) and fibrotic (F2-F4) liver slice cultures that last for 21 days. The use of F0-F1 HLS cultures enabled effective studies and modelling of the early steps of liver fibrosis related to HCV infection, EtOH or lipids exposure, thus, mimicking human viral, alcoholic, and NASH liver diseases.

Expression of the biomarkers of fibrosis and the progression to steatosis (estimated by triglycerides production) was increased significantly with the addition of HCV and /or EtOH or palmitate, but also in non-infected liver slices treated either with alcohol (figure 3 A, C, E, G, I, K) or palmitate acid (figure 4).

Sure, HCV infection accelerates the profibrogenic response, since we observed a significant increase in the expression of fibrosis markers, which well corresponds to the clinical picture: liver comorbidities increase the severity of fibrosis.

The rationale for developing HLS from patients with F0-F1 vs F2-F4 was to approach conditions in vivo. From clinical studies it is known that fibrotic liver is, the faster is progression to fibrosis. In fact, we saw this effect in our studies. In this regard, we believe that our model reflects rather well the events happen in human liver, as liver fibrosis develops.

Is there any data that shown that HLS obtained from patients with different levels of fibrosis (F0-4) differ in their fibrosis score on HLS cultures?

We did not find any reference concerning this point. It is likely that the time of culture was too short to see any change in the score of fibrosis. We detected a significant increase of collagen deposition in F0-F1 HCV infected liver slice culture treated with 5 mM EtOH on day 6, compared to day 1 (Figure 8M).

• Fibrosis is a response to chronic inflammation, therefore HLS culture for 21 days is not a suitable model for studying fibrosis in chronic hepatitis.

You are right; it is not possible to study transition from fibrogenesis to established fibrosis in chronic hepatitis. The principal restrictions are: duration of chronic hepatitis and access to clinical material, which preferentially should be from the same patient. Nevertheless, we were able to survey the first step of fibrogenesis (increased expression of fibrotic markers) in non-fibrotic (F0-F1) liver slice culture. In addition we

reveal increased expression of fibrosis markers in F2-F4 liver slice culture, where the chronic hepatitis was already established.

So, during 21 days liver slice culture, we observed principal manifestation of fibrotic process, which was earlier described in clinical studies. Together, increase of fibrosis markers expression, a significantly stronger production and deposition of collagen, as well as synergy under the influence of damaging factors (alcohol and HCV, palmitate and HCV) are factors in favor of 3D model feasibility.

Since the hepatic slice model is a 3D model, it contains all the structural components of the organ, including immune cells, and Kupffer cells, which are the most powerful generator of inflammatory process in the liver.

In this manuscript, we did not focus on the markers of the inflammatory process.

• Color label of each group should be same all through the figures.

Use of line charts and bar graphs in an intermingled way is confusing. If line charts were preferred, the figures would be simplified and become much more easy to understand.

As requested by Reviewer, we have harmonized all the graphs. We preferred to use bar graphs, as it seems easier to compare each group on the same day of kinetic studies. As requested, we put the same color label for each group of bar graphs.

• This manuscript has too much data from several studies; data for development of HLS cultures for 21 days from patients with F0-1 vs. F2-4 fibrosis; creating a fibrosis

on HLS model with HCV, ethanol (ALD) and palmitic asid (NASH), evaluation of antı-fibrotic effects of UDCA and alpha-tocopherol on HCV fibrosis model of HLS culture.

• Why were not ethanol and palmitic asid used alone as a fibrosis model of HLS culture, instead of combining with HCV infection?

In our manuscript, we have presented:

1- results with ethanol and palmitate used alone as a fibrosis model of HLS culture on the following figures:

- Ethanol alone: Figure 7A, C, E, G, I, K; Figure 9A, C, Figure 10A, C, E, G, I, K;E.
- Palmitate alone: Figure 11A-G.
- 2- results with Ethanol combined with HCV infection of HLS culture on the following figures: Figure 7B, D, F, H, J, L; Figure 9B, D, F; Figure 10B, D, F, H, J, L;
- Palmitate combined with HCV infection: Figure 11A-G; Figure 12B.

• Table 3 and 4 should be omitted since the data was given Figure 8-11.

Table 3 and 4 were withdrawn.

• If there was any data supporting the notion that precision medicine could be based on HLS culture for evaluating effectiveness of different drugs, it must be emphasized within the result and discussion sections.

To our knowledge, there were related experiments performed on rats and mouse livers, but follow-up studies were rather short. Citations of these works are given in the Introduction:

Westra M, Oosterhuis D, Groothuis GMM, Olinga P. The Effect of Antifibrotic Drugs in Rat Precision-Cut Fibrotic Liver Slices. PLoS ONE 2014; **9** (4): e95462. [PMCID: PMC3995767 DOI: 10.1371/journal.pone.0095462. eCollection 2014]
Olinga P, Schuppan D. Precision-cut liver slices: a tool to model the liver ex vivo. J Hepatol 2013; **58**: 1252–1253. [PMID: **23336979** DOI: 10.1016/j.jhep.2013.01.009]

Up to now, there is few works on human liver slice culture. The following paper is cited in the Introduction: "Wu X, Roberto JB, Knupp A, Kenerson HL, Truong CD, Yuen SY, Brempelis KJ, Tuefferd M, Chen A, Horton H, Yeung RS, Crispe IN. Precision-cut human liver slice cultures as an immunological platform. J Immunol Methods 2018; 455: 71–79. [PMCID: PMC6689534 DOI: 10.1016/j.jim.2018.01.012]",
The paper indicated that human liver slices collected from resected livers could be maintained in ex vivo culture over a two-week period.
We added this in the discussion.

Science editor: 1

Scientific quality: The manuscript describes an observational study of the adult human liver slice cultures. The topic is within the scope of the WJCC.

(1) Classification: Grade C;

(2) Summary of the Peer-Review Report: The authors developed 3d human liver tissue slice culture for 21 days from patients with F0-1 vs. F2-4 fibrosis, created fibrosis model for hepatitis C (HCV) and alcoholic hepatitis or HCV and steatohepatitis, and then evaluated the anti-fibrotic effects of ursodeoksi cholic asid and/or alpha-tocopherol.

However, some questions raised by the reviewer should be answered; and (3)

Format: There are 5 tables and 13 figures.

A total of 38 references are cited, including 4 references published in the last 3 years.

There are no self-citations.

2 Language evaluation: Classification: Grade B.

A language editing certificate issued by AJE was provided.

3 Academic norms and rules: The authors provided the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, and the Institutional Review Board Approval Form.

The Biostatistics Review Certificate, the STROBE Statement and the written informed consent are not qualified.

No academic misconduct was found in the CrossCheck detection and Bing search.

4 Supplementary comments: This is an invited manuscript.

The study was supported by Assistance Publique-Hôpitaux de Paris (AP-HP, France), by the Institut National de la Santé et de la Recherche Médicale (INSERM, France) and by Institut Pasteur (Paris, France).

The topic has not previously been published in the WJH.

5 Issues raised: (1) The authors did not provide the approved grant application form(s).

Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

It is not appropriated.

The work was supported by regular funds from INSERM (n° U1223-P-A1L4-SE-U1223SE19DA-U122317D/18D/19D/20D) and Pasteur Institute (n°024411E/601010001).

Dr Daria Kartasheva-Ebertz received a PhD fellowship from AP-HP (contract n°AN0212017100337).

(2) The authors did not provide original pictures. Please provide the original figure documents.

Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Now, we have provided decomposable original Figures (whose parts are all movable and editable), organized them into a single PowerPoint file and submitted as "**59138-Figures.ppt**". The figures have been uploaded to the file destination - "Image File".

Now, we have provided decomposable original Tables (whose parts are all movable and editable), organized them into a single Word file and submitted as "**59138-Tables.docx**".

The tables have been uploaded to the file destination - "Table File".

(3) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

The "Article Highlights" section has been added at the end of the main text.

6 Re-Review: Not required.

7 Recommendation: Conditional acceptance.