Washington University in St. Louis
SCHOOL OF MEDICINE

Department of Pathology & Immunology
Division of Anatomic Pathology

February 6th, 2014

Dear Dr. Garcia-Olmo,

Re: Manuscript 8347

Title: The role of liver biopsy in nonalcoholic fatty liver disease

We would like to thank you and the reviewers for the positive disposition of our above-cited review. Below, please find our answers to the reviewers' queries and suggestions. We feel the suggestions and comments of the reviewers have significantly improved our review.

Thank you for the invitation to write this review for the WJG.

Sincerely,

ILKe Nalbantoglu

Elizabeth M Brunt

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Dear Dr. Garcia-Olmo,

Please find enclosed the edited manuscript in Word format (file name: manuscript number 8347 NAFLD review.doc).

Title: The role of liver biopsy in nonalcoholic fatty liver disease

Authors: lLKe Nalbantoglu, Elizabeth M. Brunt

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 8347

We appreciate the thoughtful and constructive comments of the editors and reviewers. Our responses to comments are in bold. The changes made in the manuscript are highlighted in the resubmission version. We think the manuscript has been improved by the suggestions of reviewers.

1. Per request, the abstract was re-written in an unstructured manner and added to the body of the text along with key words and Core Tips.

2. Revisions have been made according to the suggestions of the reviewers

(1) Reviewer # 02861134
"Starting part of this paper is excellent, especially the abstract. It is concise and organized. The study is a timely research. Objectives are consistent with literature review and analysis. The paper can be recommended to publish with the prior approval of the editorial board. Accept the paper in its current format."
Thank you very much for your comments.

(2) Reviewer # 00069130
"Minor spelling mistakes. Example page 13 last paragraph "toscoring" instead of "to scoring"
The manuscript was carefully re-reviewed and minor spelling/grammar mistakes were corrected.
Page 7, paragraph 2, "who died" was changed to "who died from".
Page 9, paragraph 3 "and and", the extra "and" was deleted from the text.
Page 15, last paragraph "toscoring" was changed to "to scoring".
Page 16, first paragraph, "ederive" was changed to "derive".

"The third paragraph, second line is not clear to me "The intensity and distribution of the inflammation varies within the lobule; in fact, there may be such intense inflammation in zone 3, it may be confused with a portal area with an obscured duct. This can be especially true in the cases when an artery branch is readily appreciated".
This statement was re-phrased. Page 12:

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"The intensity and distribution of the inflammation varies within the lobule. In some cases, the intense inflammation in zone 3 may be confused with a portal area with the duct obscured by inflammation. This may be particularly true in the cases when an artery branch is readily appreciated in zone 3."

"Including some more description about some basic concepts (for example Portal Vs lobular inflammation and their importance) will make the essay more helpful to less specialized readers as well."
Lobular and portal inflammation, their cell types and clinical associations, and differential diagnoses were discussed in specific sections in detail on pages 11 and 12. We have given the detail and literature support that currently exists for these. Thank you for your comment.

"What are periportal progenitor cells? How are they defined structurally or by protein markers?"
Additional descriptions of progenitor cells and their protein markers were added to the text, page 13, paragraph 2:

Hepatic progenitor cells, HPC, reside within the canal of Hering, along the limiting plate. They are rarely visualized by light microscopy unless activated. HPC are characterized by high N:C, round to spindled cytoplasm, ovoid nuclei and positivity for keratin 7 and 19; these characteristics are altered with progressive stages of development towards hepatocellular or biliary epithelium. In certain circumstances of liver injury and repair, stem cell markers, Hedgehog pathway markers and others are also reported in activated HPC.

"Including pictures/additional pictures with explanation of pediatric NAFLD to explain what is mentioned in the text will be very useful for students/an average reader. "
Additional figures were added to display histologic features of pediatric NAFLD. Figures 6A & B.

"NAFLD in octogenarians/ extremely old patients is an area of great curiosity."

This sentence has been added in the Introduction:

It is currently recognized that mortality in the majority of individuals with NAFLD is more likely from cardiovascular diseases than from liver disease [1], thus, even though recent studies have documented similar epidemiologic and histologic features of NAFLD and NASH in the geriatric population[2, 3], this age group will not be further discussed in this review.

For the referees interest: The largest one is "Rotterdam Study"[2], which includes 2811 patients (mean age 76.4 ± 6.0 years). The diagnosis of fatty liver was made by ultrasound, not confirmed by liver biopsy due to ethical reasons. In this cohort, 35.1 % of participants showed "fatty liver". While the prevalence decreased to 21.1% as the age increased, and the authors commented on "selective mortality" in this population, mainly due to cardiovascular disease.
Another study from Greece was performed on 498 forensic autopsy cases (ages 64.5 ± 17.78 years)[3]. In their cohort, 31.3 % of the cases showed steatosis and 39.8% steatohepatitis which was confirmed by histology. Again the most common cause of death in this cohort was cardiovascular disease. The clinical and epidemiologic features of the participants were similar to other adults in both cohorts.
Since cardiovascular disease is the most common cause of death documented in NAFLD patients[1], it is apparent that patient mortality from CVD occurs prior to liver disease progression to end stage. NAFLD in octogenerians shows similar histologic, clinical and epidemiologic features to adult NAFLD.

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(3) Reviewer # 02541391

"This is a very good manuscript on a heavily debated topic. The structure is excellent and concise, containing information of the highest quality. The article can be useful both to clinicians and to other healthcare professionals that encounter the pathology in their everyday practice, and can serve as a concise reference guide.

Thank you for these supportive comments.

It may be of interest for some readers the addition of some conclusive thoughts on the role of liver biopsy in pediatric and adult NAFLD."

The article discusses the role of liver biopsy including its indications, clinical relevance and advantages, use in clinical trials as well as drawbacks and possible complications, citing several studies in the field. A concluding remark was clarified:

In summary an adequate liver biopsy, with appropriate clinical history, interpreted by a trained liver pathologist, is not only pivotal for an accurate and complete diagnosis (or exclusion) of NAFLD (or NASH), but also is optimal for obtaining detailed information regarding disease pattern, severity and fibrosis. It provides important information with respect to subtypes, potential future risks, possible etiology, and natural history of disease, and sets the ground work for future molecular studies and clinical trials, assisting clinical colleagues and patients with treatments and follow-up.", page 10, last paragraph.

(4) Administrator

The abstract is re-written. The abstract, core tip, and key words are added to the body of the article. The tables are in the format that allows editing. The tables were updated and changed. The website does not allow excel files to be uploaded, therefore the files were converted to PDF and uploaded. The excel files are available upon request.

An additional statement in parenthesis was added to page 8, first paragraph to clarify that NAS score was discussed later.

2. Typesetting and references was corrected

Figure lettering was changed to capital letters to match the actual figures.

The format for reference 90 was updated.

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

Sincerely yours,

ILKe Nalbantoglu
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1 **Rafiq N**, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009; 7(2): 234-238 [PMID: 19049831 DOI: 10.1016/j.cgh.2008.11.005.]

2 **Koehler EM**, Schouten JN, Hansen BE, van Rooij FJ, Hofman A, Stricker BH, Janssen HL. Prevalence and risk factors of non-alcoholic fatty liver disease in the elderly: results from the Rotterdam study. *J Hepatol* 2012; 57(6): 1305-1311 [PMID: 22871499 DOI: doi: 10.1016/j.jhep.2012.07.028. Epub 2012 Aug 4.]

3 **Zois CD**, Baltayiannis GH, Bekiari A, Goussia A, Karayiannis P, Doukas M, Demopoulos D, Mitsellou A, Vougiouklakis T, Mitsi V, Tsianos EV. Steatosis and steatohepatitis in postmortem material from Northwestern Greece. *World J Gastroenterol* 2010; 16(31): 3944-3949 [PMCID: 20712056]

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