Imperial College London

Liver Unit/ Division of Digestive Diseases,
Department of Metabolism, Digestion
and Reproduction
Imperial College London

St Mary's Hospital Campus
10th Floor, QEQM Wing
South Wharf Road, Paddington
London, W2 1NY, United Kingdom
Tel: +44 (0)2033126454 Fax: +44
(0)2077249369
pinelopi.manousou@nhs.net
p.manousou@imperial.ac.uk
www.imperial.ac.uk/people/p.manousou
Dr Pinelopi Manousou MD PhD FRCP
Consultant Hepatologist

2nd July 2021

Editorial Team
World Journal of Hepatology

Dear Editors,

Re: Manuscript ID: 67327. Liver function tests and NAFLD: changes in upper normal limits, does it really matter?

Thank you very much for considering our paper for publication within the *World Journal of Hepatology*. Moreover, we would like to thank you for your valuable comments which we have embedded in our paper.
You can find attached our response to the reviewers

Yours Faithfully

Dr Pinelopi Manousou
Consultant Hepatologist and Honorary Senior Lecturer

1 Peer-review report

Reviewer #1: Congrats to the authors for this nice study.

We thank the reviewer n.1 for his favourable comments.

Reviewer #2: In this study "Liver function tests and non-alcoholic fatty liver disease (NAFLD): Changes in Upper Normal Limit, Dose it really matter? ", Liver Stiffness Measurement (LSM) and liver biopsy were used to distinguish the severity of NAFDL and NASH, and combined with liver function (especially ALT) to explore whether the LFT classification can be used to judge the prediction and prognosis of NAFLD and NASH. Actually, the ALT value of NAFLD and NASH patients can range from normal to 200~300 U/L, rarely more than 10 times higher than the normal value. However, when NASH progresses to cirrhosis, the values of ALT and AST may return to normal values, but the disease is worsening. Use ALT and AST values as the only basis for disease tracking is not clinically feasible. In response to this research, a few comments are as follows:

1. Other indicators may be more suitable for this study, such as non-alcoholic fatty liver disease fibrosis score (NFS) and Fibrosis-4 (FIB-4). NFS = (−1.675) + 0.037 − age (years) + 0.094 − BMI (kg/m2) + 1.13 × impaired fasting glucose (IFG) / diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet count (× 109 /L) − 0.66 x albumin (g/dL); FIB-4 = age × AST (IU/L) / platelet count (× 109 /L) × √ALT (IU/L). In NFS, advance fibrosis can be reliably excluded (negative predictive value [NPV], 93%) using the low cut-off score (< −1.455) and diagnosed with high accuracy (positive predictive value [PPV], 90%) using the high cut-off score (> 0.676) [1]. In FIB-4 scoring, advanced fibrosis can be reliably excluded (NPV, 90%) using the low cut-off score (< 1.45), and a FIB-4 of >3.25 is 97% specificity, with a PPV of 65% for advanced fibrosis [2]. 1. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a non-invasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45:846-54. 2. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple non-invasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43:1317-25.

We thank the reviewers for the comments. We agree that it would be useful to include the non-invasive markers of fibrosis in the analysis. Here we have shown that FIB-4 and NAFLD fibrosis score perform better than LFTs alone in predicting the presence of fibrosis and NASH in the NAFLD population.

We have made the following changes to the manuscript:

Page 9, line 6–12: "Overall, FIB-4 and NAFLD fibrosis scored performed better than ALT in diagnosing F>F3. Specifically, the AUROC of ALT for diagnosing F≥F3 was 0.45 (95% CI: 0.38-0.53,

p=0.05) compared to 0.71 (95%CI: 0.63-0.79, p=0.0001) for FIB-4 and 0.65 (95%CI: 0.59-0.72, p=0.0001) for NAFLD fibrosis score. However, ALT, FIB-4 and NAFLD fibrosis score performed similarly in diagnosing "definite MASH". In particular, the AUROC of ALT was 0.55 (95% CI: 0.47-0.62, p=0.049), compared to 0.47 (95%CI: 0.39-0.54, p=0.01) for FIB-4 and 0.5 (95%CI: 0.42-0.58, p=0.05) for NAFLD fibrosis score (Figure 3A and Figure 3B)."

Page 10, line 7-10: "Overall, FIB-4 and NAFLD fibrosis scored performed better than AST in diagnosing F>F3, while the three performed similarly in diagnosing "definite MASH". Specifically, the AUROC of AST for diagnosing F≥F3 was 0.56 (95% CI: 0.49-0.64, p=0.05) and 0.59 (95% CI: 0.52-0.67, p=0.049) for diagnosing "definite MASH" (Figure 3A and 3B)."

Page 11 Line 8-10: "Our results confirm that non-invasive markers based on blood tests (i.e. FIB-4 and NAFLD fibrosis score) perform better than LFTs alone in assessing the severity of liver disease from NAFLD".

2. According to the level of LSM and liver biopsy, authors can try to find the cut-off value of NFS or FIB-4, which can be used to predict the severity of NAFLD or NASH.

We thank the reviewer for this comment. We agree with the reviewer that optimising the cut-off for FIB-4 and NAFLD fibrosis score is a pressing need in clinical practice. However, validating a new cut-off for non-invasive markers of fibrosis vs histology was not within the scope of this work, as we were focusing on changing the upper normal limits in liver function tests. We have, however, emphasised the better diagnostic performance of FIB-4 and NAFLD fibrosis score compared to LFTs alone in tertiary care.

3. Previous papers mentioned that gender and age are the major factors associating the NAFLD and NASH, authors may be can try to subgroup participants.

We thank the reviewer for this comment. In this population, we found that neither age nor gender influenced LFTs level. We have made the following changes to the manuscript:

Page 8, line 15–18: "In the whole population, there was no linear association between ALT and age, as Pearson's correlation was not significant (Rho=-0.86, p=0.07). Moreover, the distribution of ALT across age groups was similar when patients were further stratified per gender (Kruskal Wallis)."

Page 9, line 21–24: "In the whole population, there was no linear association between AST and age, as Pearson's correlation was not significant (Rho=0.01, p=0.99). Moreover, the distribution of AST across age groups was similar when patients were further stratified per gender (Kruskal Wallis)."

2 Editorial Office's comments

1) Science Editor: 1 Scientific quality: The manuscript describes a retrospective study of the effect of lowering the upper limit of normal ALT on the analytical characteristics of this biomarker for the diagnosis of MASH.The topic is within the scope of the WJG.

(1) Classification: Grade C and Grade D;

(2) Summary of the Peer-Review Report: Reviewer 05304884 think that authors need to change the indicators for evaluate the NAFLD and NASH.

(3) Format: There are 3 tables and 7 figures;

(4) References: A total of 23 references are cited, including 4 references published in the last 3 years;

(5) Self-cited references: There is no self-cited reference.

2 Language evaluation: Classification: Grade A and Grade B. A language editing certificate was not provided because the authors are most likely native speakers of English.

3 Academic norms and rules: The authors provided the Biostatistics Review Certificate and the Institutional Review Board Approval Form. The signed Conflict-of-Interest Disclosure Form and Copyright License Agreement were not provided. Please, provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement. No academic misconduct was found by the Google search. An abstract of this study was presented at the International Liver Congress 2019 and published in the proceedings of that Congress. – We have provided the signed conflict-of-interest disclosure form and Copyright license agreement.

4 Supplementary comments: This is an invited manuscript. The study was supported by European association for the study of the liver and National Institute of Health Research (NIHR) Biomedical Research Centre. The topic has not previously been published in the WJG.

5 Issues raised: (1) Please, replace terms with modern terminology (metabolic-associated versus non-alcoholic) – We have replaced NAFLD with modern terminology throughout the manuscript.

(2) some data are duplicated in tables and figures. Please remove tables / figures with duplicate data; - we have removed table n.2

(3) the manuscript is not formatted according to the guidelines of WJG. Please, format the manuscript in accordance with the instructions for authors. – we have modified the manuscript according to the instruction for authors

(4) The "Author Contributions" section is missing. Please provide the author contributions – we have added the author contributions section

(5) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy(ies) of any approval document(s); - we have submitted all necessary documents

(6) The authors did not provide original pictures. Please provide the original figure files. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; - we have provided the Power point version of the images

(7) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout; - we have modified the reference style

(8) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text (and directly before the References); - we have added the article highlights

(9) Please, add keywords "MASH" and "ALT" 6 Re-Review: Required. 7 Recommendation: Conditional acceptance. – we have added MASH and ALT to the keywords

2) Editorial Office Director:

3) Company Editor-in-Chief: I recommend the manuscript to be published in the World Journal of Hepatology.