Montreal, July 24th 2021

Dear Editor,

Please find enclosed our manuscript entitled "**Non-alcoholic steatohepatitis in liver transplant recipients diagnosed by serum cytokeratin 18 and transient elastography: a prospective study**", that we here resubmit after revision according to the reviewers' comments.

A point by point reply to the reviewers' comments is attached to the submission. We are grateful to the editor and reviewers for the time and energy they dedicated to our work and we hope that the paper will now be suitable for publication in the *World Journal of Hepatology*.

Kindest regards on behalf of all the co-authors and thank you for your consideration.

Dr Alshaima Alhinai

Dr Giada Sebastiani

Non-alcoholic steatohepatitis in liver transplant recipients diagnosed by serum cytokeratin 18 and transient elastography: a prospective study - World Journal of Hepatology Manuscript NO: 69318

Response to Editor and Peer Reviewers

Comments	Author response	Revision to manuscript
Associate Editor:		
We are pleased to inform you that, after preview by the Editorial Office and peer review, as well as CrossCheck and Google plagiarism detection, we believe that the academic quality, language quality, and ethics of your manuscript (Manuscript NO.: 69318, Prospective Study) basically meet the publishing requirements of the World Journal of Hepatology. As such, we have made the preliminary decision that it is acceptable for publication after your appropriate revision. Upon our receipt of your revised manuscript, we will send it for re-review. We will then make a final decision on whether to accept the manuscript or not, based on the reviewers' comments, the quality of the revised manuscript, and the relevant documents.	We are very grateful for the careful review and preliminary acceptance of our article by the editorial board and reviewers. We provide a point-by-point response to reviewer's comment below.	
Reviewer #1:		
GENERAL Scientific Quality: Grade B (Very good) Language Quality: Grade A (Priority publishing) Conclusion: Accept (General priority) This is a good paper, useful for practice!	We thank the reviewer for the careful and thoughtful review.	
1. please add to keywords NAFLD	Thank you. NAFLD has been added to keywords.	Text added.
2. use type 2 diabetes mellitus instead of diabetes patients	Thank you. We have replaced "diabetic/diabetes patients" with "type 2 diabetes mellitus" throughout the manuscript.	Text revised.

3. why did you take 270 dB/m as cut off for CAP. You can try to use the cut off of 290 dB/m as presented in a recent paper by Eddowes (Gastroenterology 2019).	We appreciate the reviewer's question and comment. We selected the cut-off 270 dB/m based on the study by Siddiqui et al published in Clinical Gastroenterology & Hepatology 2020 (https://doi.org/10.1016/j.cgh.2020.03.067). We chose the cut-off from this study specifically because it was performed in post liver transplant patients, which is our study population. Siddiqui et al reported that a cut-off of 270 dB/m to detect steatosis grade 0 vs 1-3 had an AUROC of 0.88 (95% CI, 0.78–0.93) with a sensitivity of 0.74, specificity of 0.87, PPV of 0.78 and NPV of 0.84. The results were very good and relevant to our study population.	Text unchanged.
4. please give more explanation for the low accuracy of TE (57.8%) for fibrosis assessment!	We thank the reviewer for pointing this out. We have now added further explanation in the discussion for the low accuracy of non-invasive fibrosis tests. *"Similar findings have been reported previously in post-LT patients with HCV recurrence. El-Meteini et al. concluded that TE and APRI were not correlated with the degree of fibrosis in liver biopsy done at 3 months post-LT in 31 patients. Other studies reported a poor diagnostic accuracy of APRI and FIB-4 compared to liver biopsy for the presence of advanced fibrosis post-LT. Indeed, some of our patients experienced an important variation in LSM, FIB-4 and APRI particularly during the first 6 months post-LT. This could be due to several reasons. Inflammation due to congestion or cholestasis is common post-LT and could be one reason for the inaccuracy of fibrosis tests. Fluctuations liver enzymes and platelets during the first 6 months may also account for these findings as LT recipients have started receiving and adjusting their immunosuppressive medications. Since a majority of our liver recipients were overweight, this could have interfered with the LSM results. Since our study and the previous studies were performed on small cohorts, a conclusion regarding the accuracy of non-invasive fibrosis tests cannot be made."*	Text added in the discussion section.
Reviewer #2:		
GENERAL Scientific Quality: Grade E (Do not publish)	We thank and appreciate the reviewer for the careful and thoughtful review.	

Language Quality: Grade A (Priority publishing) Conclusion: Rejection Specific Comments: Dr Alshaima Alhinai et al. have performed the current study to evaluate incidence and predictors of NAFLD and NASH by employing noninvasive testing in liver transplant recipients, namely controlled attenuation parameter (CAP) and the serum biomarker cytokeratin 18 (CK-18). They reported that 63.0%, and 48.5% of patients developed NAFLD and NASH during a median follow-up of 16.8 months (interquartile range 15.6-18.0). On multivariable analysis, after adjusting for sex and alanine aminotransferase, body mass index was an independent predictor of development of NAFLD (aHR 1.21, 95% CI: 1.04-1.41; p=0.01) and NASH (aHR 1.26, 95% CI 1.06-1.49; p<0.01). CAP had a 76.5% accuracy to diagnose NAFLD, while the accuracy of CAP plus CK-18 to diagnose NASH was 82.4%. The results were interesting; however, some important concerns are needed to be further clarified.		
1. What's the difference between NAFLD (nonalcoholic fatty liver diseases, line 3) and NAFL (nonalcoholic fatty liver, line 7) in the Introduction section?	We thank the reviewer for this question. The difference between NAFL and NAFLD is that NAFL is simple steatosis while NAFLD is a spectrum of disease ranging from simple steatosis (which is NAFL) to the more serious, inflammation and necrosis of the liver (aka NASH). Therefore, NAFLD encompasses both NAFL and NASH. We have added the sentence "It (NAFLD) ranges from simple steatosis or nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH). Without treatment, NAFL can evolve to NASH…." to the introduction section.	Text added, introduction section.
2. What's the leading reason for liver transplantation? Does NASH rank higher than liver tumor or cirrhosis on the list for transplantation? Pls listed the reason for transplantation in the current study.	We thank the reviewer for his interest and questions. The leading reasons for liver transplantation are mentioned in results section, paragraph 1 *"The most frequent indications for LT were NASH and HCC"*. Cirrhosis due to NASH ranks higher than HCC and higher than cirrhosis due to other etiologies. The numbers are mentioned in Table	Text unchanged

	1-Characteristics of patients at study entry.	
3. When was liver biopsy performed?	We thank the reviewer for his question. The median time between liver biopsies and non-invasive diagnostic testing done in study visits (Scores, CAP, CK-18 and LSM) was 38.6 ± 30 days. This sentence is mentioned in the second paragraph of the results section.	Text unchanged.
4. Why age was not adjusted or included in the final model? As we know, the prevalence of both NAFLD and NASH increased as the age grew older.	We appreciate the reviewer's question. As our sample size was small, therefore we included covariates that were determined a priori to be clinically significant. As per studies, age is considered a risk factor for NAFLD and NASH because it is associated with a the prevalence of metabolic comorbidities, however, when it is taken as an independent risk factor, its role is unclear (Taneja et al translational gastro and hepatology 2020, Collins et al Transplantation 2000). Additionally, studies done on the predictors of NAFLD/NASH post liver transplant did not show significant risks associated with age (Dumortier AJG 2009, meta-analysis by Saeed Transplantation 2019). Accordingly, the univariable analysis in our study showed no effect of age (Table 3) and, as we could not include more variables in the multivariable model for risk of overfitting it, we focused on some variables which showed significance at the univariable analysis ($p<0.10$).	Text added, statistical analysis section.
5. The author described that TE with CAP measurement and plasma to measure CK-18 were also acquired at each study visit, which meant that CAP and CK-18 were repeatedly assessed at month 3, 6, 9, 12 and 18 during the follow up. Further, the author explained that the median time between liver biopsies and non-invasive diagnostic testing was 38.6 ± 30 days (in Result section). Does the author mean liver biopsy were also repeated performed? If so, which one was used as outcome and why the author performed many times of liver biopsy? Similar, because CAP and CK-18 were repeatedly assessed, which one was used as the	We thank the reviewer for their questions and comments. TE with CAP, and CK-18 were acquired at each visit. The outcome was reached for NAFLD and NASH when patients had readings of CAP ≥270 dB/m, CAP ≥ 270 dB/m + CK-18 >130.5 IU/, respectively, for the first time during the follow up visits. At that point the patient was censored (see statistical analysis section). However, it was not the same for liver biopsy. Liver biopsy was not used for the longitudinal analysis component of this study, but only for the diagnostic accuracy analysis (text added for better explanation, thank you). Liver biopsy was performed at the discretion of the treating transplant hepatologist and was only performed in 24 of 40 patients. Most of the 24 patients had one liver biopsy but a few of had repeated liver biopsies as their condition required it (e.g. due to continuous rise of liver enzymes, or due to persistent symptoms/signs of liver injury that was not explained	Text added, methods section.

exposure, or the average of them was treated as the exposure?	by the first biopsy result). For the accuracy analysis, the results of 35 liver biopsies in 24 patients were compared to the closest corresponding dates of non-invasive tests. Hence, the accuracy values were obtained. The median time between liver biopsies and non-invasive diagnostic testing was 38.6 ± 30 days.	
6. Since only 24 out of 40 performed liver biopsy, how to assess NAFLD and NASH in the remain patients? The ROC was performed in 24 patients?	We appreciate the reviewer's question. Yes, 24 patients had liver biopsy. Some of the patients had more than one biopsy, giving us a total of 35 liver biopsies from 24 patients. The diagnostic accuracy analysis was performed on 35 biopsies. The accuracy was not assessed in the remaining patients who did not have liver biopsy. This is a limitation in our study.	Text unchanged.
7. It seemed that it was a re-identified retrospective cohort study. If so, pls mentioned it in the manuscript.	We thank you the reviewer for the comment. Our study is a prospective longitudinal study that begun recruitment of participants in March 2015 and ended in with the last visit of patients to the clinic in March 2020. Participants details were collected and recorded at each visit post liver transplant. Analysis of data was performed after all data has been collected.	Text unchanged.
8. Did the author try to compare the sensitivity and specificity of CK-18 plus CAP to alanine aminotransferase plus liver image?	We thank the reviewer and appreciate the comment. Comparing the sensitivity and specificity of CK-18 plus CAP to ALT plus liver images is an excellent suggestion. Unfortunately, liver images were not part of our data collection during the study visits. There are literature data suggesting that combining ALT and liver imaging does result in better accuracy than ALT alone (Draijer et al, Eur J Ped 2019). We conducted a diagnostic accuracy analysis for ALT alone to diagnose NASH. We found a diagnostic accuracy of 65%, which is similar to previously reported suboptimal accuracies (Verma et al, Liver International 2013).	Text unchanged.
9. Table 1, as the author described in the material section, LT due to chronic hepatitis C were excluded. However, 8 of 40 were patients with HCV. Another question was the title was non-alcoholic fatty liver, 1 patient with alcoholic fatty liver was included.	We thank the reviewer for the comments and questions. Indeed, as mentioned in Table 1, 8 of 40 patients had LT due to chronic HCV however the HCV genotypes of the patients who met the inclusion criteria were either 1, 2, 4, 5, or 6. Patients with HCV genotype 3 have been excluded from our study as this particular genotype has a different rate of development of steatosis than the other genotypes. We have now	Table 1, text revised.

	added *"(excluding genotype 3)*" next to HCV in the first column in Table 1. As for the second question, our study is looking at de novo/recurrent NAFLD/NASH in liver transplant recipients, thus we are focusing on the incidence and predictors of NAFLD in the post liver transplant setting. Studies have shown that NAFLD/NASH is common after liver transplant regardless of the etiology of liver disease at the time of transplant. In our study, one patient was transplanted due to cirrhosis from alcoholic liver disease however he/she fits the inclusion criteria for the study entry. During the follow up visits, all patients, including this particular one, had negative scores with the AUDIT-C questionnaire. Therefore, if this patient developed NAFLD/NASH post liver transplant, the reason would have been due to metabolic conditions which we discussed in our results and discussion sections.	
10. Table 2, Since only 24 out of 40 performed liver biopsy, the comparison was only possible in 24 patients. Pls clearly explained it.	Thank you. Liver biopsy was performed at the discretion of the treating transplant hepatologist, as part of standard of care during the follow up period. Patients who had signs/symptoms and/or laboratory changes during follow up suspicious of liver injury/disease underwent liver biopsy for confirmation. Patients who had normal examinations and laboratory results were not required to undergo any liver biopsy and our study did not subject liver biopsy due to its limitations (invasive, costly, etc.). Only 24 out of 40 patients required liver biopsy during follow up therefore the comparison was only possible in 24 patients (with total of 35 liver biopsies). Yet, our results suggest the use of non-invasive tests to monitor liver recipients and can be viewed as an opportunity for larger studies to be done on this topic. *"Only 24 out of 40 patients required liver biopsy during follow up therefore the comparison was only possible in these patients, for a total of 35 liver biopsies. Regardless of this, the results obtained from our study provide rationale for the use of non-invasive tests to frequently monitor this patient population, which could not be feasible with liver biopsy, and can be viewed as an opportunity for larger studies to be done on this topic."*	Text added to discussion section. Table 2, text added.

Response to Editor and Peer Re-Reviewer

Comments	Author response	Revision to manuscript
Associate Editor:		
Thank you for submitting your manuscript to World Journal of Hepatology, a peer-reviewed, online, and open access journal. We are pleased to inform you that one of the peer reviewers has completed his/her re-review of your manuscript.	We are very grateful for the careful re-review of our article by the editorial board and reviewers. We provide a point-by-point response to re-reviewer's comment below.	
Reviewer #1:		
GENERAL Dear authors, Thank you for let me review this manuscript. As a disclosure, I do not believe I performed your initial peer-review, but I have read the peer-review report and revised manuscripts. The authors have addressed comments by other peer-reviewers appropriately. Scientific quality [ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish Language quality [Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection Conclusion [ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection Peer-reviewer statements Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No	We thank the reviewer for the careful and thoughtful review.	

I have a few additional comments		
1. CK-18 is not a routine test we order when taking care of liver transplant recipients. Therefore, it may be challenging to apply this result to clinical practice.	Thank you for your comment. CK-18 is currently not a routine test done for LT patients because there are very few to no studies done to determine its accuracy in the LT population. To our knowledge, our study is the first study conducted to determine the efficacy of CK-18 in liver transplant populations. However, CK-18 has the potential to become a routine test in the care of LT patients. Our results showed an accuracy of 82% in diagnosing NASH in this population compared to liver biopsy. Additionally, CK-18 is a non-invasive, readily available, and low-cost test compared to other tests such as imaging tests and liver biopsy. Taking these points into consideration, we believe CK-18 can be considered, at the very least, a preliminary test to risk-stratify patients in the post liver transplant setting for further possible invasive investigations.	We added the following sentence: "Another limitation of our study is that CK-18 is not currently a routine test, as such its application to clinical practice should be further explored" to the discussion section on page 13.
2. The main concern for LFT elevation after a liver transplant is rejection which will require a liver biopsy. It will be hard to attribute LFT elevation to NASH without doing a liver biopsy.	We thank the reviewer for their feedback and comment. Our results reported an 82% accuracy of CAP + CK-18 to diagnose NASH compared to liver biopsy. We also reported a 96% NPV which is excellent. At the current practice, NITs have not replaced liver biopsy and liver biopsy remains the gold-standard to diagnose liver diseases, however our results show that NITs can be used as a risk-stratifying test before undergoing further invasive tests such as liver biopsy. We believe that our results should be replicated in a larger sample for a better understanding of the accuracy of NITs.	Text unchanged.
3. These results may be helpful for a longer duration. It is also valuable to monitor CAP scores to see if they are having a re-occurrence of steatosis.	We appreciate the reviewer's comments. Studies have shown that NAFLD is a common occurrence within 6 months of LT, whereas the onset of NASH occurs in a period of 6 months to 1 year in several studies. Therefore, we aimed to determine the incidences and risk factors of NAFLD/NASH/Fibrosis and accuracy of non-invasive tests specifically in the first year and	We added the following sentence: "The median study length was 16.8 months, so in the future we plan to continue following these patients

	a half following LT. Monitoring CAP scores to check for re-occurrence of steatosis in the long term is indeed a valuable observation, however it is out of the scope of our study. We agree that it is very important to determine our aims in the long term. We have plans to perform a similar study with a longer duration.	for a longer duration by monitoring CAP scores and re-occurrence of steatosis" to the discussion section on page 13.
4. I do not see a table for some reason, so I could not assess this aspect of the manuscript.	We thank the reviewer for pointing this out. We hope that you were able to review the tables by this time. Please share your comments regarding the tables with us anytime. We will gladly respond to them.	Text unchanged.