July 20, 2015

Dear Editor,

I am resubmitting to World J Gastroenterology the revised invited editorial article **#18522** titled "*Non-alcoholic fatty liver disease: Disparate predictive ability for cardiometabolic risk and all-cause mortality*". The response to the comments of the Reviewer is presented below.
All changes in the manuscript are highlighted in red.

With kind regards,

AltanOnat, MD, FESC

Manuscript Number **18522**

Manuscript Title *Fatty liver disease: Disparate predictive ability for cardiometabolic risk and all-cause mortality*

Reviewed by 01136482 Jun 4

Epidemiology of NASH in MetS and the association of liver steatosis with (central) fat mass and BMI. This request has been briefly outlined (Lines 240-41).

The close associations of impaired glucose regulation and lipid metabolism with NAFLD are widely recognized (28).(new reference).

The cardiovascular risk profile of Turkish adults is briefly reviewed on lines 105-108 as follows: Cardiovascular risk profiles are characterized by a high prevalence of abdominal obesity in males, overall obesity in females, low HDL-cholesterol, high triglyceride and intermediate total cholesterol levels. Current smoking protects against abdominal obesity (18). One-fifth of non-diabetic Turks exhibit impaired fasting glucose (20).

Reviewed by 02861137 Jun 18

- The diagnosis of NAFLD is broader than presence of liver steatosis. Patients with NAFLD must have > 5% of hepatocytes with liver fatty associated with no alcohol abuse or use of few drugs that lead to steatosis. In addition, other chronic liver diseases must be excluded. According to Bedogni et al (BMC 2006), Fatty Liver Index (FLI) was not developed to diagnosis of NAFLD. It was validated using abdominal ultrasound as the reference to diagnosis fatty liver. Thus, authors should not state in the paper that NAFLD was or not associated with severe outcomes. In my point of view, the better is to evaluate the prognostic value of the FLI instead of NAFLD.

The terms Fatty Liver Index (FLI) or FLD replaced NAFLD.

- Alcohol consumption is crucial point in NAFLD and its evaluation should be better described (lines 137-138). Does the authors used a validated questionnaire, such as CAGE or AUDIT, or estimated alcohol use in g/day ?

On page 7 (top), following information was added. Fourteen % of men and 1% of women were categorized as alcohol users. Six % of males were estimated to use alcohol at a daily mean equivalent to ca. 19 ml ethanol, the remainder much less.

- Population sample (lines 118-130): The original study of the cohort (Turkish Adult Risk Factor Study, Onat Atherosclerosis 2001) had 3687 individuals. Why only half of them were evaluated in the present study (n=1822)? Authors should include a flow-chart of eligible and included patients.

We have clarified that section of the Methods as follows. Serum ɣ-glutamyltransferase (GGT) determinations were made in the survey 2003/'04, during which GGT was measured in all the 1822 participants who attended the survey (60%) out of an eligible number of 3037 participants. By then, 257 participants had died. Follow-up extended to the survey 2012/'13.

- Definitions (lines 155-166): Authors should define CHD. Authors should describe how they have access to death information: National Death Registry? Information with family? Did deaths have a classification according to ICD-10 codes ?

CHD is defined. Death had been described to be ascertained from all the three sources

- Data analysis (lines 175-183): Authors should describe the use of ANOVA (applied in Table 1) and Cox regression (described in the core tip and applied in Tables 3 and 4). Did the p values on Table 1 are corrected by Bonferroni (authors stated in the footnote that in bold denote significant difference between 2 groups)?

"ANOVA was used to detect difference across multiple groups, whereby difference between two groups was determined using Bonferroni corrections" was added to Data analysis.

6. Results:

Authors should provide a K-M graphic for incidence of (or survival without) diabetes and CHD and overall mortality. The Cox analysis (Tables 3 and 4) should be repeated replacing the FLI (NAFLD for the authors) by its parameters (BMI, GGT, triglycerides and waist circumference). These analyses should be provided in a Supplementary Material. Severe outcomes might be related to metabolic factors (BMI, etc) and not with fatty liver estimated by FLI.

- Lines 216-220. Kaplan-Meier plots were constructed for survival and survival free of diabetes/CHD, as seen in Suppl. Fig. 1. These demonstrated significantly lower survival free of diabetes and of incident CHD for participants with FLD at baseline. Subjects categorized as probable FLD also separated from those with no FLD in regard to CHD. However, overall survival curves were similar in the three groups.

- Lines 235-239: Cox with BMI, GGT, triglycerides and waist circumference.

  In order to assess whether some of the components of the FLI, rather than the overall algorithm, were determinants of outcomes, we analyzed the Cox models separately with the 4 components (Suppl. Table 2). These demonstrated a greater impact of triglycerides and GGT levels and –to some extent- of abdominal obesity, but not of overall obesity which interestingly and significantly protected against risk of death.

7. Discussion:

The results of the present study are contradictory with the Cremona Study (Calori Hepatology 2011) where FLI had a prognostic value for prediction of overall mortality. The Cremona Study followed more than 2,000 individuals for 15 years and collected mortality data from a National Death Registry. In the present study it was not observed using the same biomarker of liver fat. Thus authors should discuss this

point more deeply than 4 lines (280-283).

Cremona Study may rather be an exception in predicting overall mortality in the general population. In NHANES-III ALT- or US-defined NAFLD did not reveal increased mortality. This issue (possible bidirectional relationship between NAFLD and diabetes with respect to mortality) has been reviewed by P. Loria and associates (which we had cited). We had commented this point also in lines 270 to 273 and 303 to 309. We expanded our comments by adding the following paragraph on lines 315-321.

"Our multivariable analysis with the 4 components of the FLI brought some explanation to the lacking association with risk of death, insofar as BMI emerged (especially in men) as protecting against mortality risk. Moreover, given the information we had reported (37) that disparate independent association existed among sexes between serum GGT and Lp(a) levels, high Lp(a) levels in men, contrasted to low levels in women (reflecting autoimmune activation) may have been pivotal beneath the associations of FLI with the risks of death and incident CHD."

Authors should also discuss the prognostic value of others biomarkers of liver steatosis such as SteatoTest (Perazzo AP&T 2015) and liver fibrosis in NAFLD patients, such as NFS, FIB-4 and APRI (Kim Hepatology 2013).

We expanded the previous sentence on page 9 as follows. A more recent analysis of NHANES-III survey data confirmed that US-defined NAFLD did not increase the risk of mortality (33). However, NAFLD with evidence of advanced fibrosis using non-invasive marker panels (only 1 out of 30 NAFLD cases) was associated with increased mortality, mainly attributable to cardiovascular causes. NAFLD fibrosis score was based on an algorithm using additional data on IFG/diabetes, as well as inflammation-related parameters such as AST/ALT ratio, platelet count and albumin level

Limitations of the study should be discussed such as (i) FLI was not validated to NAFLD diagnosis, (ii) absence of abdominal US to detect liver fat (it is easy, can be done at the bed side and is available worldwide); (iii) co-linearity between FLI and metabolic factors (confounding factors).

"Collinearity between FLI and metabolic factors such as lipoprotein(a) levels or obesity cannot be ruled out" is now added to the Limitations.

Minor Points

- Table 2 and Fig 1 should be placed in Supp Material

o.K. with Table 2. But we think Fig. 1 should be retained in the main text since it shows that the risk of overall mortality among normoglycemic individuals with FLD was lower than with no FLD and that risk increased in prediabetes and tended so in diabetic women, while declining in diabetic men.

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- In Table 1, it is obvious that BMI, tryglicerides, GGT and waist circumference will

be significantly higher in patients with NAFLD, because these parameters are included in the formula of the biomarker (used to define NAFLD by authors).

This is obvious and needs no explanation.

- Line 88 : MetS was defined previously (line 84)

Corrected.

- Line 202: correct the typo steatohepatitis. It might be NAFLD and not NASH

Corrected.

- Rephrase the sentence in lines 297-298. I could not understand what authors means.

The phrase was clarified as follows. “Our observations in men support the view that the development of diabetes from prediabetes attenuates the independent risk of death for FLD (34, 35).”

- HR means risk of, thus authors should replace in the text sentences such as "higher HR" to "higher risk of".

HR means hazard ratio, denoting relative risk per 1 SD increment.

- The manuscript should be reviewed by a native English speaker

Done.

- Number of lines should be re-arranged to a better review of the paper and dropped-out on the references sections (very difficult to identify the number of the reference within the number of lines)

Done.

- Recent reference of Ekstedt (Hepatology 2015) about mortality in NAFLD should be considered

Being a histological-based study on a small sample and very long follow-up, its relevance is relatively little for this manuscript.

Reviewed by 02541357 Jun 24

- The number of subjects was 1822 and was corrected as such.

- On page 7 (top), following information was added. Fourteen % of men and 1% of women were categorized as alcohol users. Six % of males were estimated to use alcohol at a daily mean equivalent to ca. 19 ml ethanol, the remainder much less.

- Liver diseases of specific causes were not reported (page 7).

- Identification of CHD is described on page 6.
- “ANOVA was used to detect difference across multiple groups, whereby difference between two groups was determined using Bonferroni corrections” was added to Data analysis.

- On line 194 we added … including cardiovascular risk factors and HOMA.
- “CHD in 93 and diabetes in 103 subjects were identified at baseline” was added in Results. Continuous values for blood pressure and other MetS components were provided in Table 1.