29 March 2022

Jin-Lei Wang
Editor in Chief
World Journal of Hepatology

Dear Dr Wang,

RE: Prognostic non-invasive biomarkers for all-cause mortality in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis

I am writing to address the suggested revisions to our submitted manuscript titled, " **Prognostic non-invasive biomarkers for all-cause mortality in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis** ". Thank you to reviewers for taking the time to provide a comprehensive review.

We have attempted to address each of the reviewer's comments, as listed below:

Reviewer 1:
Specific Comments to Authors:

Current review sums up data regarding the important question in hepatology. It is well designed and argumentative.

Thank you very much for your interest in this systematic review. We hope the review will attract similar response from the journal's wider audience and readership.

It would be beneficial if the authors will concretize possible difference in the role of the discussed laboratory parameters and scoring systems in patients with different stages of NAFLD steatosis, steatohepatitis, liver fibrosis and cirrhosis associated with fatty liver.

Cohort studies have consistently shown association of fibrosis stage in NAFLD with overall and disease specific. These studies investigated as to whether NAFLD activity Score (NAS) or NASH as a stage were associated with all cause and disease specific mortality and found no such associations. Our aim was to perform a systematic review to identify non-invasive markers that are associated with increased risk of all-cause mortality. Identifying different stages of NAFLD was not our objective and this wasn't within the scope of our systematic review.

Reviewer 2:
Specific Comments to Authors:
The manuscript was reviewed for publication in the journal. The review manuscript was designed to evaluate available evidence on the use of non-invasive test(s) as prognostic factors for mortality in NAFLD. It is the reviewer's opinion that the review is interesting and that the manuscript is easy to follow. However, it appears that there are a couple of concerns in the manuscript.

Thank you very much for your interest in review.

1) The authors evaluated available evidence on the use of non-invasive test(s) as prognostic factors for mortality in NAFLD in the study. Non-invasive test(s) such as NFS, FIB4, BARD,

and APRI appears to be useful to predict liver fibrosis. Therefore, the use of these tests as prognostic factors for mortality in NAFLD may be incomprehensible. The authors should discuss the issue.

Thank you for your comment. We agree with reviewer the Non-Invasive scoring systems such as NFS, FIB4, BARD, and APRI have been used to stage the liver fibrosis. Their use as marker to predict all cause or liver specific mortality is a concept that has not been well explored in literature. Therefore, the aim of current review was to evaluate available evidence on the use of any non-invasive test, including serum biomarkers, non-invasive scoring systems, and imaging modalities, in predicting all-cause mortality, and disease-specific mortality, in NAFLD. We believe by identifying markers which are associated with the risk of mortality will help clinicians risk stratify patients and implement necessary interventions.

"Cohort studies have consistently shown association of fibrosis stage in NAFLD with overall and disease specific mortality. The algorithms that we have identified include parameters such as age, BMI and type 2 diabetes which are well recognised risk factors for cardiovascular and all-cause mortality. Therefore, it is understandable that particular biomarkers are also associated with all-cause mortality." We have now included this section in the discussion (page 12)

2) The authors discussed the prognostic markers for mortality in NAFLD, but not NASH. How about the definition of NAFLD? NASH may be more prognostic state for mortality. The authors should explain the point.

Thank you for your comment. We now have provided definition of NAFLD in methods section.

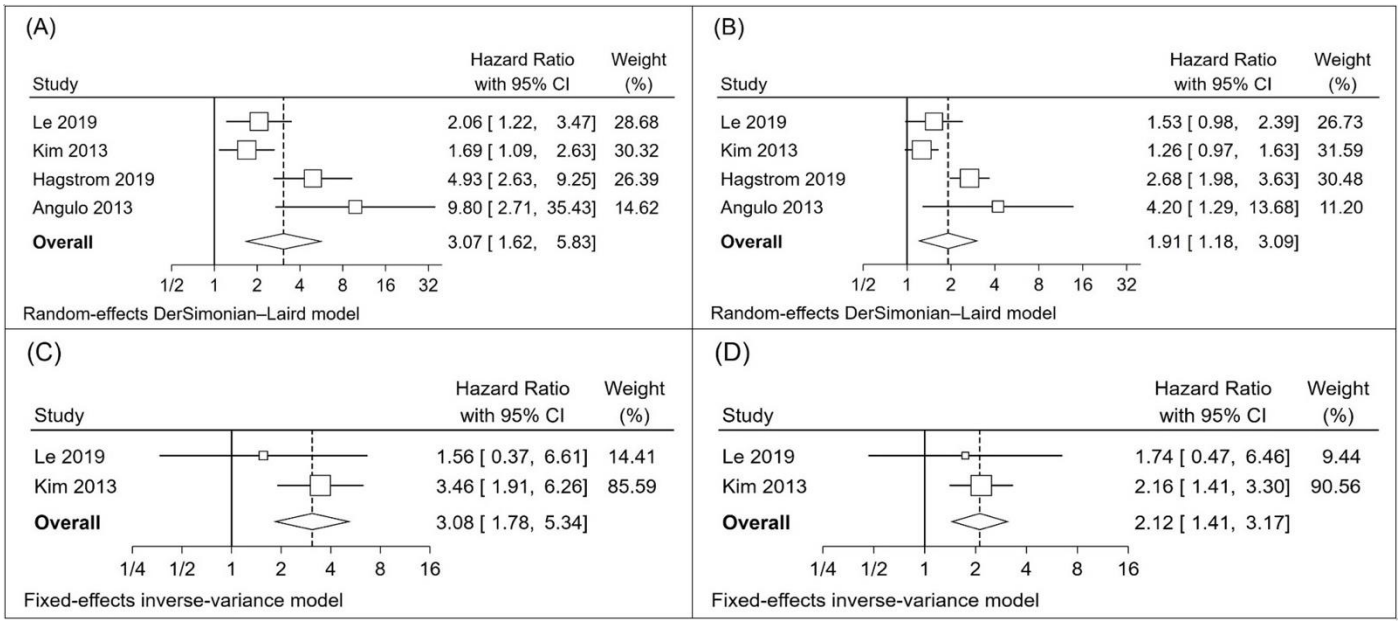
For this review NAFLD was define as " excessive hepatic fat accumulation in liver, as characterised by the presence of steatosis in more than 5% of hepatocytes. The NAFLD encompasses all spectrum of liver disease including non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), various stages of liver fibrosis, and cirrhosis".

"Although our definition of NAFLD included all spectrums of disease, and in the inclusion criteria for the population included in our study we sought to evaluate both NAFL and NASH, however very few studies included subgroups comprising NASH. Indeed, the only studies who did were studies where NAFLD was diagnosed via liver biopsy (Table S1). This is likely to be because currently, international, and national clinical guidelines recommend for NASH to be diagnosed histologically by liver biopsy, so studies where NAFLD was diagnosed by imaging and non-invasive scores would not be able to include NASH as a subgroup. In addition there aren't robust, validated non-invasive markers to identify NASH independent of fibrosis. So, it is unsurprising that in our systematic review we weren't able to identify any relationship between NASH and mortality either liver related or from all causes."

We have added this in discussion section (page 11)

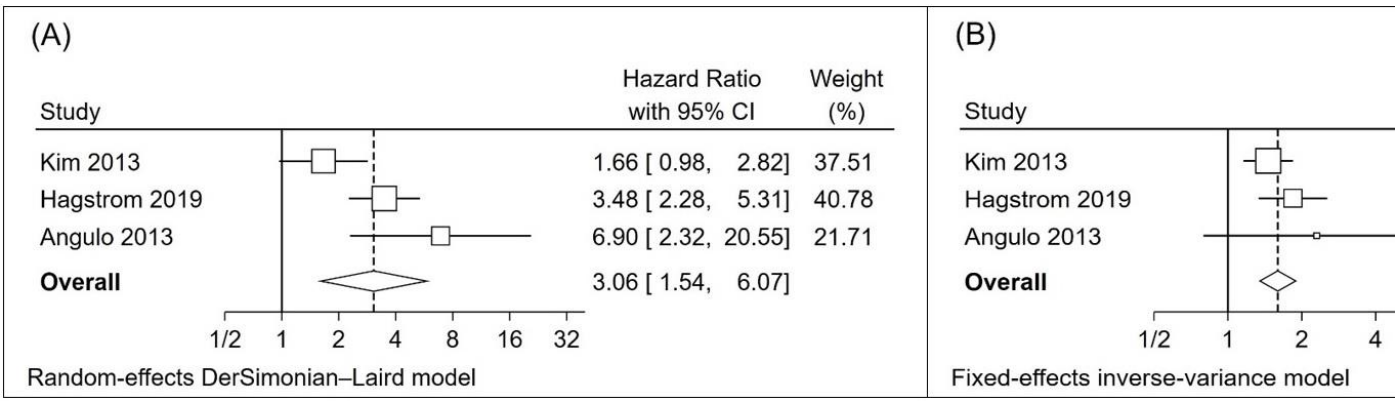
3) The non-invasive scoring system that performed best at predicting all-cause mortality was NFS [pHR 3.07], followed by FIB4 [pHR 3.06]. pHR of NFS and FIB4 appeared to be almost similar. How about forest plots for pHR for FIB4 and all-cause mortality? The authors should explain the point.

Thank you for your comment. We have now added all the forest plots to the manuscript, as suggested by the reviewer. For reviewer convenience we have also provided the forest plots for pHR for FIB4 and all-cause mortality at end of this letter (**Figure 1: Forest Plots for pooled Hazard Ratios for NFS and all-cause mortality and cardiovascular-related mortality.**



(A) NFS High vs. Low and All-cause Mortality; (B) NFS Intermediate vs. Low and All-cause mortality; (C) NFS High vs. Low and Cardiovascular-related Mortality; (D) NFS Intermediate vs. Low and Cardiovascular-related Mortality;

Figure 2: Forest Plots for pooled Hazard Ratios for FIB-4 and all-cause mortality



(A) FIB-4 High vs. Low and All-cause Mortality; (B) NFS Intermediate vs. Low and All-cause mortality;

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"The forest plots for NFS are shown in Figure 2 and for FIB-4 in Figure 3. The forest plots for the remaining analyses can be found in Figure S1." (page 10)

We have also further supplemented the discussion as below:

"Another non-invasive marker that had very similar performance in predicting all-cause mortality was FIB-4. The pHR, confidence intervals, and heterogeneity levels of FIB-4 and NFS and all-cause mortality were indeed very similar. This can likely be attributed to the fact that all 4 of the individual components of the FIB-4 test (age, AST, platelets, and ALT) are part of the NFS (which in addition to these contains BMI, impaired fasting glucose or diabetes, and albumin). It is encouraging that a scoring system with fewer components seems to have a similar performance, as it may be easier to use in clinical practice. However, our study found only 3 studies, with a total of 5,045 NAFLD patients, that evaluated the prognostic performance of FIB-4. This is significantly less than the 9,725 NAFLD patients included in the analysis of NFS and all-cause mortality. Further epidemiological studies are warranted to enable a head-to-head comparison of NFS and FIB-4 performance, to guide clinical guidelines on the best non-invasive scoring system to use in clinical practice." (page 11)

4) There are a couple of mistakes. Key points: in second sentence, NAFD FIB4 in abstract vs FIB-4 in manuscript? Abstract: in section of Background and Aims, the use of-non-invasive test

Thank you for pointing these out. We have made relevant corrections as suggested by the reviewer.

Science editor:

Specific Comments to Authors:
This manuscript aims to assess the available evidence on the use of non-invasive tests as prognostic factors for NAFLD mortality. Further elaboration on possible differences in the roles of the discussed laboratory parameters and scoring systems in patients with different stages of NAFLD steatosis, steatohepatitis, liver fibrosis, and fatty liver-related cirrhosis is recommended; supplement the discussion on NASH.

Thank you very much for your comment. We now have supplemented the discussion on NASH in the discussion as suggested by the scientific editor.

"Our definition of NAFLD included all spectrums of disease, and in the inclusion criteria for the population included in our study we sought to evaluate both NAFL and NASH, however very few studies included subgroups comprising NASH. Indeed, the only studies who did were studies where NAFLD was diagnosed via liver biopsy (Table S1). This is likely to be because currently, international, and national clinical guidelines recommend for NASH to be diagnosed histologically by liver biopsy, so studies where NAFLD was diagnosed by imaging and non-invasive scores would not be able to include NASH as a subgroup. In addition there aren't robust, validated non-invasive markers to identify NASH independent of fibrosis. So, it is unsurprising that in our systematic review we weren't able to identify any relationship between NASH and mortality either liver related or from all causes." (page 11)

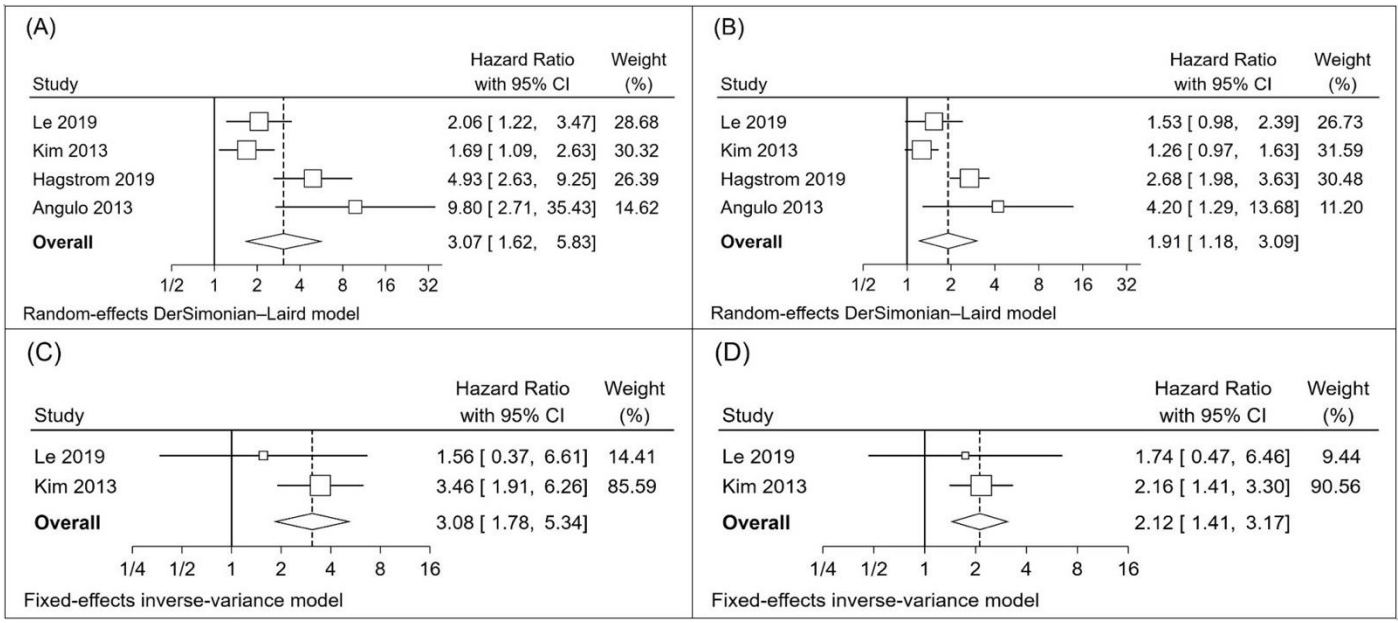
In addition, please indicate the number of the reference cited in the table.

Thank you very much for comment. The number of references (studies) in table 1 were four, and in table 2 were four.

Thank you again for your time and consideration.

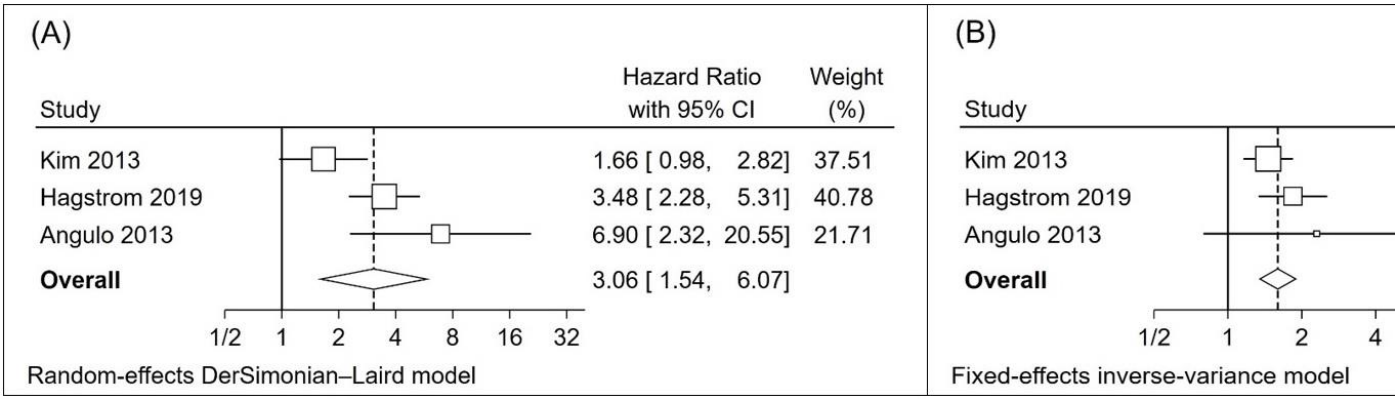
Yours Sincerely,
Dr Nicole Cianci

Figure 1: Forest Plots for pooled Hazard Ratios for NFS and all-cause mortality and cardiovascular-related mortality.



(A) NFS High vs. Low and All-cause Mortality; (B) NFS Intermediate vs. Low and All-cause mortality; (C) NFS High vs. Low and Cardiovascular-related Mortality; (D) NFS Intermediate vs. Low and Cardiovascular-related Mortality;

Figure 2: Forest Plots for pooled Hazard Ratios for FIB-4 and all-cause mortality



(A) FIB-4 High vs. Low and All-cause Mortality; (B) NFS Intermediate vs. Low and All-cause mortality;