Reply to editor and reviewers.

We are pleased to inform you that, after preview by the Editorial Office and peer review, we believe that the academic quality, language quality, and ethics of your manuscript (Manuscript NO.: 89634, Retrospective Cohort Study) basically meet the publishing requirements of the World Journal of Gastroenterology. As such, we have made the preliminary decision that it is acceptable for publication after your appropriate revision.

Response: We thank the editors and reviewers for their great comments enhancing our manuscript. Please see below for our revised versions and comments based on the editor and reviewers' suggestions. Please let us know if there is anything else we can do to enhance our manuscript.

Reviewer #1:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

This study addresses an important question: Within the range of ALT scores currently considered normal, 0-40 U/mL, does an ALT score of 21-40, with rising ALT score over a 3-year period indicate an increased risk of MAFLD? The study is well designed.

1. The manuscript needs increased clarity, especially in abstract and introduction, addition of the ROC used to determine the cutoff of 18.5, and correction of errors. Also, why was there not a revisitation of the data using a 20 U/mL level as a criteria for entry to the study, and reanalyse the data using the 18.5 U/mL cutoff that was later derived.?

Response: Thank you for your suggestion. First of all, we rewrite the abstract and introduction, especially about ROC used to determine the cutoff of 18.5, such as "Some evidence has suggested that the Youden index, a popular summary statistic for receiver-operating characteristic curves, provides the optimal cut-off point for a biomarker to distinguish diseased and healthy individuals [15]. In a study of adolescents with obesity, the optimal ALT cut-off points for diagnosing NAFLD were 36 U/L for males and 33 U/L for females [16].

Then for using the 18.5 U/mL cutoff instead of 20 U/L, the reasons were: (1) **Fig.**

2(A) (below) shows the distribution of ALT levels in participants with MAFLD, where the ALT level grouping every 20 U/L is artificially set to display the distribution of MAFLD patients , and the aim is to indicate that a significant proportion of MAFLD patients have normal ALT levels (36.36%+46.77%=83.13%).

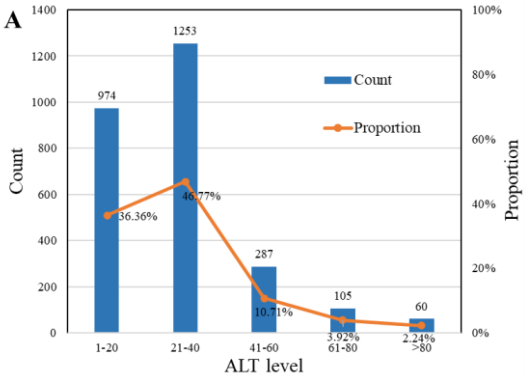


Figure. Distribution of ALT levels in participants with MAFLD.

(2) Our previous study (reference 14, below) indicated that ALT trajectory in normal level have been confirmed to be associated with the risk of new-onset MAFLD, where after adjusting for multiple confounding factors, the risks of MAFLD in ALT medium-stable and the high-stable group were still 1.422 times (95%CI:1.115-1.813) and 1.483 times (95%CI:1.040-2.114) of low-stable ALT group (P<0.05). This study is qualitative and fails to determine the optimal cut-off point value for ALT levels. That is, repeated high-normal ALT levels increase the risk of new-onset MAFLD.

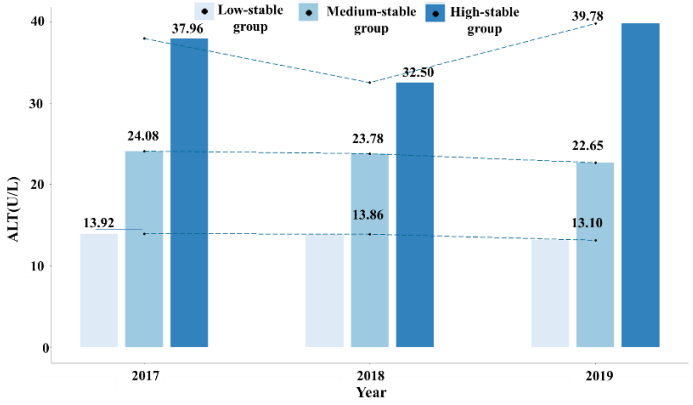


Figure. ALT trajectories of three groups.

(3) The study applied the receiver operating characteristic (ROC) curve with the maximum value of the Youden index (sensitivity+specificity-1) to determine the optimal ALT cut-off points for the diagnosis of MAFLD using ALT in 7817 participants from 2017 to 2019. The results indicated the optimal ALT cut-off points were 18.5 U/L in 2017, 18.5 U/L in 2018, 17.5 U/L in 2019, and 18.5 U/L in 2017–2019, as shown in

the following figure. Thus, we determined the optimal ALT cut-off point to be 18.5 U/L.

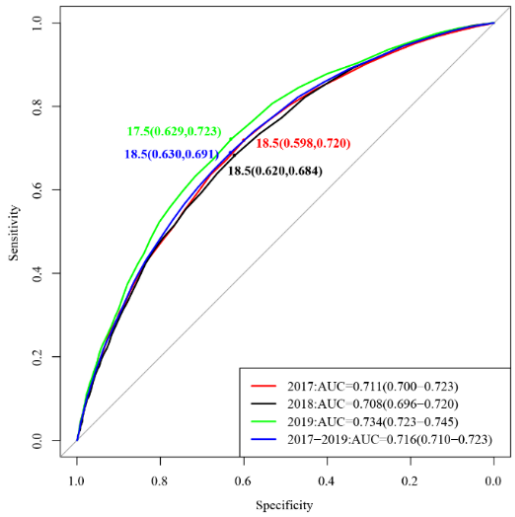


Figure. Optimal ALT cut-off points for diagnosis of MAFLD.

2. The major limitation of the study lacking biopsy is noted in Discussion. Now that biopsy is far less frequent, which is appropriate, it is important that studies now turn to derive diagnostics that are not pinned to biopsy. Biopsy should be minimal in hepatology.

Response: Thank you for your suggestion. We have removed the inappropriate description about "MAFLD was diagnosed using ultrasound instead of the gold standard (i.e., liver biopsy)", and revised it as "Additionally, randomized controlled trials with different lifestyle interventions (including weight loss through diet and physical exercise) will be conducted to explore whether those interventions can improve long-term ALT levels in individuals who are high-normal and ultimately prevent MAFLD.

3. ALT is higher in male than female and the follow-up cohort had 49% male: It would be interesting to know whether the analyses can be applied separately to males and females: Is 18.5 U/mL appropriate for both male and female if male and female were separated into two cohorts? Does the eALT work apply more strongly to males vs females?

Response: Thank you for your suggestion. Considering that ALT is higher in males than females, it is not appropriate to use 18.5 U/L for both males and females if males and females were separated into two cohorts. Thus, we determine the optimal ALT cut-off point in women and men separately, where the female is 15.5 U/L and the

male is 21.5 U/L, as shown in the following figures.

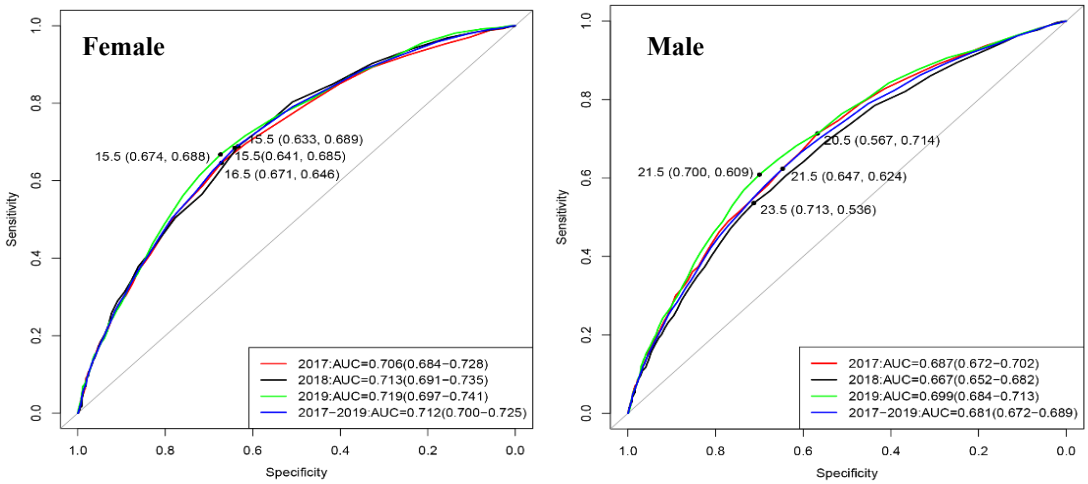


Figure. Optimal ALT cut-off points for diagnosis of MAFLD.

In addition, we separately analyzed the association between the cumulative effects of ehALT and the risk of new-onset MALFD in females and males (**Table 1 and Table 2**). Results indicated that the female was consistent with the total population, while the male was not.

Table 1. Risks of new-onset MAFLD in females stratified by different categories of cumulative effects of ehALT in univariate, WC, SBP, DBP, and BMI–adjusted and multivariate-adjusted regression

Categories	Univariate, *HR* (95% CI)	*P* value	WC, SBP, DBP and BMI–adjusted[a], *HR* (95%CI)	*P* value	Multivariate–adjusted[b]	*P* value
Cumulative occurrences of ehALT (n=3553)						
0 (18/778)	1.000		1.000		1.000	
1 (19/486)	1.764(0.925-3.362)	0.085	1.710(0.896-3.263)	0.104	1.662 (0.867-3.183)	0.126
2 (19/313)	2.786(1.462-5.311)	0.002	2.746(1.436-5.251)	0.002	2.779 (1.442-5.356)	0.002
3 (16/225)	3.162(1.612-6.204)	0.001	2.834(1.439-5.58)	0.003	2.421 (1.217-4.817)	0.012
P for trend[c]	<0.001		0.001		0.004	
Equally weighted cumulative effects of ehALT (n=3553)						
Increase per SD[d]	1.204(1.052-1.379)	0.007	1.16(1.014-1.326)	0.031	1.135(0.968-1.33)	0.119
0 (Reference)	1.000		1.000		1.000	
Q_1 (0.01-2.50 U/L)	2.027(0.993-4.139)	0.052	1.80(0.877-3.695)	0.109	1.713(0.829-3.542)	0.146
Q_2 (2.517.50 U/L)	1.537(0.709-3.33)	0.276	1.773(0.815-3.858)	0.149	1.854(0.846-4.064)	0.123
Q_3 (7.51-17.50 U/L)	3.205(1.652-6.221)	0.001	2.76(1.419-5.367)	0.003	2.681(1.370-5.244)	0.004
Q_4 (≥17.51 U/L)	3.161(1.571-6.361)	0.001	2.965(1.472-5.972)	0.002	2.477(1.210-5.070)	0.013
P for trend[c]	<0.001		0.002		0.012	

Unequally weighted cumulative effects of ehALT (n=3553)						
Increase per SD[d]	1.220(1.059-1.405)	0.006	1.185(1.033-1.358)	0.015	1.138(0.972-1.333)	0.108
0 (Reference)	1.000		1.000		1.000	
Q_1 (0.01-5.00 U/L)	1.711(0.824-3.553)	0.150	1.523(0.729-3.183)	0.263	1.434(0.680-3.024)	0.343
Q_2 (5.01-13.75 U/L)	2.425(1.188-4.95)	0.015	2.564(1.254-5.241)	0.010	2.614(1.275-5.360)	0.009
Q_3 (13.76-34.50 U/L)	2.647(1.349-5.191)	0.005	2.417(1.231-4.745)	0.010	2.356(1.192-4.658)	0.014
Q_4 (≥34.51 U/L)	3.073(1.503-6.28)	0.002	3.081(1.504-6.313)	0.002	2.621(1.261-5.449)	0.010
P for trend[c]	0.003		0.003		0.004	
Single ehALT occurrence (control group, n=925)						
Increase per SD[d]	0.831(0.36-1.921)	0.666	0.866(0.371-2.021)	0.739	0.915(0.397-2.106)	0.834
0 (Reference)	1.000		1.000		1.000	
Q_1 (0.01-1.50 U/L)	1.220(0.282-5.278)	0.790	1.012(0.230-4.458)	0.987	1.194(0.259-5.502)	0.820
Q_2 (1.51-2.50 U/L)	1.543(0.206-11.569)	0.673	1.468(0.193-11.166)	0.710	1.734(0.221-13.622)	0.601
Q_3 (2.51-7.00 U/L)	0(0-.)	0.982	0(0-.)	0.982	0(0-.)	0.983
Q_4 (≥7.01 U/L)	1.115(0.149-8.356)	0.916	1.239(0.164-9.370)	0.835	1.500(0.191-11.781)	0.700
P for trend[c]	0.961		0.920		0.785	

[a]Adjusted for WC, SBP, DBP, and BMI.
[b]Adjusted for WC, SBP, DBP, BMI, SUA, TG, HDL-C, LDL-C, FPG, and HbA1c.
[c]For the trend test, Cox proportional hazards regression models were used with group medians in each group instead of grouping variables (e.g., 0, Q_1, Q_2, Q_3, and Q_4). Cumulative occurrences of ehALT were 10 U/L, 13 U/L, 16 U/L, and 21 U/L in four groups, and the equally weighted cumulative effects of ehALT (n=3553) was 0, 1.5 U/L, 5 U/L, 12 U/L, and 29.5 U/L in five groups. The unequally weighted cumulative effects of ehALT were 0, 2.5 U/L, 9 U/L, 21 U/L, and 61.75 U/L in five groups, and single ehALT occurrence (control group) was 0, 0.5 U/L, 2.5 U/L, 4.5 U/L, and 12 U/L in five groups.
[d]SD of equal weight cumulative effect of ehALT was 14.61, SD of unequally weighted cumulative effects of ehALT was 29.80, SD of single ehALT occurrence (control group, only 2019 ALT >15.5 U/L) was 3.85.

Table 2. Risks of new-onset MAFLD in males stratified by different categories of cumulative effect of ehALT in univariate, WC, SBP, DBP, and BMI–adjusted and multivariate-adjusted regression

Categories	Univariate, *HR* (95% CI)	*P* value	WC, SBP, DBP and BMI–adjusted[a], *HR* (95%CI)	*P* value	Multivariate–adjusted[b]	*P* value
Cumulative occurrences of ehALT (n=3553)						
0 (106/875)	1.000		1.000		1.000	
1 (63/434)	1.141(0.834-1.56)	0.409	1.016(0.742-1.392)	0.919	1.018 (0.739-1.403)	0.911
2 (45/250)	1.653(1.166-2.343)	0.005	1.320(0.924-1.886)	0.127	1.281 (0.892-1.841)	0.180
3 (50/182)	2.325(1.660-3.257)	<0.001	1.626(1.152-2.296)	0.006	1.684 (1.189-2.384)	0.003
P for trend[c]	<0.001		0.003		0.002	
Equally weighted cumulative effects of ehALT (n=3553)						
Increase per SD[d]	1.131(1.032-1.24)	0.008	1.069(0.968-1.181)	0.187	1.07(0.969-1.181)	0.182
0 (Reference)	1.000		1.000		1.000	
Q_1 (0.01-2.50 U/L)	1.11(0.727-1.694)	0.628	0.975(0.637-1.493)	0.908	0.963(0.625-1.484)	0.865
Q_2 (2.51-8.50 U/L)	1.824(1.272-2.616)	0.001	1.560(1.078-2.257)	0.018	1.608(1.109-2.331)	0.012

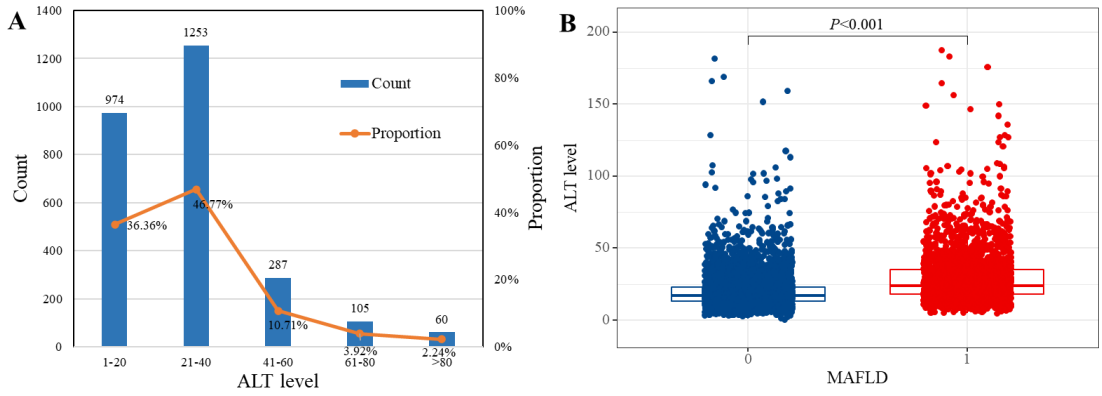
Q_3 (8.51-19.00 U/L)	1.419(0.964-2.088)	0.076	1.167(0.790-1.724)	0.438	1.185(0.801-1.755)	0.396
Q_4 (≥19.01 U/L)	1.693(1.224-2.343)	0.001	1.262(0.905-1.760)	0.170	1.237(0.883-1.733)	0.216
P for trend[c]	0.002		0.213		0.259	
Unequally weighted cumulative effects of ehALT (n=3553)						
Increase per SD[d]	1.145(1.049-1.25)	0.002	1.085(0.986-1.194)	0.093	1.086(0.988-1.193)	0.086
0 (Reference)	1.000		1.000		1.000	
Q_1 (0.01-5.00 U/L)	1.257(0.854-1.851)	0.246	1.018(0.687-1.507)	0.93	1.023(0.686-1.526)	0.912
Q_2 (5.01-15.00 U/L)	1.613(1.086-2.396)	0.018	1.49(1-2.218)	0.050	1.471(0.985-2.196)	0.059
Q_3 (15.01-37.13 U/L)	1.364(0.93-1.998)	0.112	1.113(0.757-1.637)	0.586	1.16(0.787-1.71)	0.453
Q_4 (≥37.14 U/L)	1.824(1.321-2.518)	0	1.375(0.989-1.913)	0.058	1.343(0.961-1.877)	0.084
P for trend[c]	<0.001		0.076		0.004	
Single ehALT occurrence (control group, n=997)						
Increase per SD[d]	1.157(1.035-1.293)	0.01	1.157(1.033-1.295)	0.011	**1.163(1.038-1.303)**	**0.009**
0 (Reference)	1.000		1.000		1.000	
Q_1 (0.01-1.50 U/L)	1.128(0.458-2.777)	0.794	1.204(0.488-2.97)	0.687	0.970(0.377-2.494)	0.950
Q_2 (1.51-4.50 U/L)	1.306(0.481-3.547)	0.601	1.409(0.513-3.867)	0.506	1.252(0.451-3.475)	0.666
Q_3 (4.51-12.75 U/L)	1.386(0.607-3.168)	0.438	1.110(0.484-2.545)	0.806	1.144(0.496-2.641)	0.752
Q_4 (≥12.76 U/L)	1.513(0.616-3.713)	0.366	1.691(0.684-4.176)	0.255	1.667(0.670-4.149)	0.272
P for trend[c]	0.255		0.246		0.251	

[a]Adjusted for WC, SBP, DBP, and BMI.
[b]Adjusted for WC, SBP, DBP, BMI, SUA, TG, HDL-C, LDL-C, FPG, and HbA1c.
[c]For the trend test, Cox proportional hazards regression models were used with group medians in each group instead of grouping variables (e.g., 0, Q_1, Q_2, Q_3, and Q_4). Cumulative occurrences of ehALT were 4 U/L, 18 U/L, 23 U/L, and 29 U/L in four groups, and the equally weighted cumulative effects of ehALT (n=3553) was 0, 1.5 U/L, 5.5 U/L, 13.5 U/L, and 31.75 U/L in five groups. The unequally weighted cumulative effects of ehALT (n=3553) were 0, 2.5 U/L, 9.5 U/L, 24.5 U/L, and 64.5 U/L in five groups, and single ehALT occurrence (control group) was 0, 0.5 U/L, 3.5 U/L, 7.5 U/L, and 20.5 U/L in five groups.
[d]SD of equal weight cumulative effect of ehALT was 15.80, SD of unequally weighted cumulative effects of ehALT was 32.87, SD of single ehALT occurrence (control group, only 2019 ALT >21.5 U/L) was 4.86.

4. Details:

(1) page 8: section 3.1: Error: 83.13% is % with MAFLD who had eALT, 21-40. It is not % who had normal ALT. This error is repeated in 1st para Discussion, page 10.

Response: Thank you for your suggestion. Given that the lines in Figure 2(A) represent cumulative proportions, it is easy to raise doubts. In the revised manuscript, we have made changes to the Figure and description (as shown in the figure below), with 36.36% of participants with MAFLD for ALT levels below 20 and 41% for levels 21 to 40. Thus, we rewrite it as "83.13% (36.36%+46.77%) of participants with MAFLD had normal ALT levels (⩽40 U/L)."



(2) page 8: section 3.1: Error: the ALT levels are written in reverse order of the correct order [correct it to: "with median (IQR) ALT levels 24 (18–35) U/L and 17 (13–23) U/L, respectively (Fig. 2B)".

Response: We revised it as "with median (IQR) ALT levels of 24 (18, 35) U/L and 17 (13, 23) U/L (Fig. 2B)".

(3) Page 8, section 3.2: Show the ROC and derivation of hALT as >18.5 U/mL.

Response: We defined the hALT group as "the optimal ALT cut-off point < ALT ⩽ 40" (U/L), and aALT group as "ALT> 40 (U/L)". In Section 3.2, we added the ROC curve in **Fig.3** and determined the optimal ALT cut-off point as 18.5 U/L, thus the ALT level of hALT group was 18.5-40 U/L. Then, we revised it as "Therefore, the optimal ALT cut-off point was 18.5 U/L based on ROC curve and Youden index, and the derivation of hALT was18.6–40 U/L."

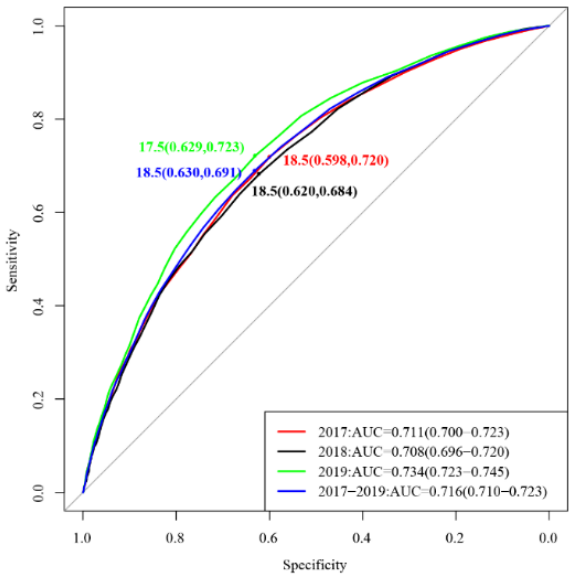


Figure. Optimal ALT cut-off points for diagnosis of MAFLD.

(4) There are several terms unique to this ms: aALT, hALT, ehALT, eALT etc; definitions of all in one place would help with clarity; in a table or fig. 5.

Response: Thank you for your suggestion. We created a table to define some new terms in the revision:

Table. Definition of some specific terms

Term	Definition
lALT group	ALT ≤ optimal ALT cut-off points (U/L)
hALT group	Optimal ALT cut-off point < ALT ≤ 40 (U/L)
aALT group	ALT> 40 (U/L)
ehALT	ALT−optimal ALT cut-off point, if ehALT<0, redefine ehALT=0
Cumulative occurrences of ehALT	Sum of times that ehALT >0 in 2017–2019, time= {0, 1, 2, 3}
Equally weighted cumulative effects of ehALT	Sum of ehALT levels with a weight of 1 in 2017–2019, i.e., $ehALT_{2017}$ + $ehALT_{2018}$ + $ehALT_{2019}$
Unequally weighted cumulative effects of ehALT	Sum of ehALT levels with an increasing weight in 2017–2019, i.e., 1×$ehALT_{2017}$ + 2×$ehALT_{2018}$ + 3×$ehALT_{2019}$
Single ehALT occurrence	$ehALT_{2019}$ along with $ehALT_{2017}$=0 and $ehALT_{2018}$=0

(5) I have some suggested edits at the end of this comments.

List 6. Page 5: regarding ref 10: how about change to : Liver damage can occur in the presence of normal ALT levels [10]

Response: Thank you for your suggestion. We revised it as “Many studies have suggested that liver damage can occur in the presence of normal ALT levels [11]” in the Introduction.

(6) Page 5: again, as in a number of places, there are statement that normal ALT is associated with MAFLD. This is not correct. Mafld can occur in the presence of normal ALT, but it is not an association with normal ALT.

Response: Thank you for your suggestion. We removed this incorrect description in the revision.

(7) Top of page 11: “this”: what is this? Unclear.

Response: Thank you for your suggestion. We deleted this incorrect description ,

and revised it as “According to the Liver-Bible-2020 cohort study, the best ALT cut-off for steatosis detection was 35 U/L in males and 22 U/L in females, and the best cut-off for fibrosis detection was 27 U/L in males [20]. ”

(8) Abstract needs a rewrite. Re-write of abstract and Key points:

Response: Thank you so much for the compliment and for the valuable comments. We have re-written the abstracts and key points in the revision, and have also consulted a language expert to polish them.

Abstract

***Background*:** Within the normal range, elevated alanine aminotransferase (ALT) levels are associated with an increased risk of metabolic-associated fatty liver disease (MAFLD).

***Aim*:** The associations between repeated high-normal ALT measurements and the risk of new-onset MAFLD were investigated prospectively.

***Methods*:** A cohort of 3553 participants followed for four consecutive health examinations over 4 years was selected. The incidence rate, cumulative times, and equally and unequally weighted cumulative effects of excess high-normal ALT levels (ehALT) were measured. Cox proportional hazards regression was used to analyse the association between the cumulative effects of ehALT and the risk of new-onset MAFLD.

***Results*:** A total of 83.13% of participants with MAFLD had normal ALT levels. The incidence rate of MAFLD showed a linear increasing trend in the cumulative ehALT group. Compared with those in the low-normal ALT group, the multivariate adjusted hazard ratios (HRs) of the equally and unequally weighted cumulative effects of ehALT were 1.651 (95% CI 1.199–2.273) and 1.535 (95% CI 1.119–2.106) in the third quartile and 1.616 (95% CI 1.162–2.246) and 1.580 (95% CI 1.155-2.162) in the fourth quartile, respectively.

Conclusion: Most participants with MAFLD had normal ALT levels. Long-term high-normal ALT levels were associated with a cumulative increased risk of new-onset MAFLD.

Core Tip:

Limited evidence exists regarding the association between persistently elevated high-normal alanine transaminase (ALT) levels and the risk of new-onset metabolic dysfunction-associated fatty liver disease (MAFLD). This cohort study analysed 3553 participants followed for four consecutive health examinations between 2017 and 2020 and measured the cumulative effects of excess high-normal ALT (ehALT). Among the participants, the incidence rate of MAFLD showed a linear increasing trend for the cumulative ehALT group. The hazard ratios of new-onset MAFLD were significantly increased in the third and fourth quartiles of the equally and unequally weighted cumulative effects of ehALT. Among Chinese adults, long-term high-normal ALT levels were related to a cumulative increased risk of new-onset MAFLD.

Thanks again!