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Round 1

Reviewer A

First, English language of this paper was rather poor, which needs professional editing after extensive revisions.

Reply 1: Thank you for your advice on the language of our article and we have professional editing now.

Changes in the text: N/A.

EDITORIAL CERTIFICATE

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Manuscript Title:
A clinical and laboratory-based nomogram for predicting nonalcoholic fatty liver disease in non-diabetic adults

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Second, please indicate non-diabetes in the title.

Reply 2: Indeed, there is a close relationship between insulin resistance(IR) and NAFLD, with high prevalence of NAFLD among patients suffering from diabetes(PMID: 30556145), while there is still unknown when talking about the prediction of NAFLD among adults without diabetes. Furthermore, due to the epidemic proportion of patients with NAFLD, a convenient and economical prediction model is urgently needed. In this study, we developed and validated a personalized and novel model to predict the risk of NAFLD among adults without diabetes.

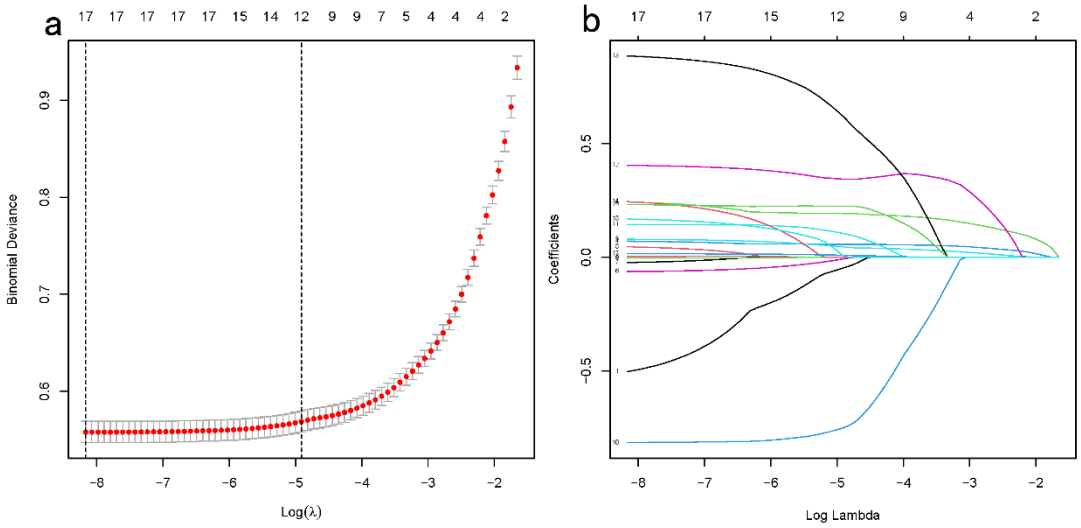
Changes in the text: We have modified our text as advised (see Page 1, line 2-4; Page 4,line 68-76)

Third, the abstract is inadequate. Please indicate why there is a need for the nomogram and why nomogram is suitable for answering the research question. In the part of method, the authors should describe how the training and validation samples were generated and how these variables for predicting were selected. The conclusion should be made with cautions because the study population is a health-check-up population, not the general population.

Reply 3: We can't agree with you more and thank you for your advice on our Abstract, Method and Conclusions. First, in abstract, in view of increasing prevalence, severe comorbidities and financial burdens of nonalcoholic fatty liver disease(NAFLD), the early detection of NAFLD is significant. Although the close relationship between NAFLD and diabetes is clear, there is still a lack of a convenient tool to predict NAFLD among adults without diabetes. So, our nomogram is a quick and economical tool to predict NAFLD. This novel model which took so many associated risk factors into consideration would be an accurate tool to predict the disease and the simple nomogram could help physicians make better decisions. In thus, it's suitable for answering the research question. Second, in methods, we describe that 14,251 NAFLD patients were randomly divided into a training dataset with 10,689 participants and a validation dataset with 3,562 participants at a ratio of 3:1 using R caret package which is an algorithm. And importantly, we have complemented many details about how the six variables for predicting were selected. Variables for predicting were selected by multivariable logistic regression analysis, LASSO method and clinical experience. The least absolute shrinkage and selection operator (LASSO) method is also a suitable tool for the reduction in high-dimensional data. Features with nonzero coefficients in the LASSO regression model were selected(Figure 1A and B). In thus, variables for predicting were selected by multivariable logistic regression analysis, LASSO method and clinical experience. Third, actually the study population is a health-check-up population without diabetes, so we did some changes in our text.

Changes in the text: We have modified our text as advised (see Page 2, Line 28-30; Page 3, Line 76; Page 4, Line 70-80; Page 7, Line 133-137; Figure 1A and B).

Figure 1 Clinical and laboratory feature selection using the LASSO binary logistic regression model. The partial likelihood deviance (binomial deviance) curve was plotted versus log(lambda). Dotted vertical lines were drawn at the optimal values by using the minimum criteria and the 1 SE of the minimum criteria (the 1-SE criteria)(a) and optimal lambda resulted in features with nonzero coefficients(b).



Notes: Application of LASSO binary logistic regression model for selecting variables. **Abbreviations:** LASSO, least absolute shrinkage and selection operator; SE, standard error.

Fourth, in the introduction part, a brief overview of the accuracy and limitations of available methods for assessment of NAFLD is needed, which could indicate the necessity for the nomogram. The authors need to explain why there is a need for early detection, because this is clinically relevant.

Reply 4: Thank you for your good idea for us to overview the accuracy and limitations of available methods for assessment of NAFLD to indicate the necessity for the nomogram. Exactly, we did the work in our text, and now we will provide you a form to describe it clearly.

	strengths	limitations
liver biopsy	gold standard	invasiveness, poor acceptability, cost and sampling variability
anthropometric indicators	simple, low-cost and non-invasive	It is still unknown which one is the best; Cut off points may differ between racial and ethnic groups.
Imaging-derived proton density fat fraction	make a quantitative assessment of liver fat	mostly used in clinical research because of its high-cost
Blood biochemical indexes	simple, low-cost	lack of convenient predictive model to

		combine these indexes together

To sum up, we need a model to combine these indexes together, and nomogram is suitable for physicians to make a quick decision in clinic. Only when we detect the disease early can we provide early intervening treatment. In thus, progression and exacerbation of NAFLD would be avoided.
Changes in the text: We have modified our text as advised (Page 4, Line 59-75).

Fifth, the methodology part is problematic and not scientifically sound because cross-sectional data can not be used for establishing predictive models. In fact, only longitudinal data can allow such analysis. Based on this, this paper should be rejected. The diagnosis of NAFLD is also not a golden method, an indirect and invalid way.

Reply 5: Exactly, the diagnosis of NAFLD is not a golden method, because liver biopsy is of invasiveness, poor acceptability, cost and sampling variability. In Lancet, only 26 patients, who underwent the liver biopsy, were involved in the trial in 2016(PMID: 26608256). In Hepatology, only 291 patients participated in the research and underwent the liver biopsy in 2016(PMID: 26659452). First, our research is a maximus sample clinical survey which can make up the shortfall above. Second, although ultrasound B-mode imaging has a low performance for the detection of mild steatosis, it is widely used for the screening of liver disease, allows to subjectively estimate fatty infiltration in the liver(PMID: 31686762).
Actually, cross-sectional data and longitudinal data can both be used to establish predictive model, with different purposes such as diagnosis and prognosis, respectively. There are some good predictive models based on cross-sectional data(PMID: 31124729, 34544417, 32764769).
Changes in the text: N/A.

Reviewer B

1. In Discussion section, authors should describe the development of NAFLD and NASH. See: Ref. Kanda T, et al. Apoptosis and non-alcoholic fatty liver diseases. World J Gastroenterol. 2018 Jul 7;24(25):2661-2672. doi: 10.3748/wjg.v24.i25.2661.
Reply 1: Thank you for your advice and recommendation. We have learned the article and made a change in our text.
Changes in the text: We have modified our text as advised (Page 10, Line 195-202).

2. Authors should describe the polygenic risk score about NASH. Gao F, Zheng KI, Chen SD, Lee DH, Wu XX, Wang XD, Targher G, Byrne CD, Chen YP, Kim W, Zheng MH. Individualized Polygenic Risk Score Identifies NASH in the Eastern Asia Region: A Derivation and Validation Study. Clin Transl Gastroenterol. 2021 Mar 10;12(3):e00321. doi: 10.14309/ctg.0000000000000321. PMID: 33704100

Reply 2: Thank you for your advice and recommendation, and they are very useful for us. We have learned the article carefully. Exactly, it is a good nomogram which can incorporate both genetic and clinical factors for predicting NASH. However, it is not applied for our article. First, our nomogram is based on the clinical and laboratory indexes which are easily obtained and it is also our purpose to help clinicians to make a quick decision whether non-diabetes adults should undertake imaging examination with the nomogram. Genetic features need Mass ARRAY, Sanger sequencing, or TaqMan assays platform, and it is hard for clinicians to acquire the information about genetic features quickly. Second, it is really a good article worth learning. It's a pity that our research did not include liver biopsy. In thus, it's difficult to distinguish between NAFLD and NASH. After all, polygenic risk maybe a significant factor we should take into consideration, but it is not applied for our research.
Changes in the text: N/A.

Round 2

Reviewer comments

Comment 1: First, please indicate the clinical research of this study in the title.

Reply 1: Thank you for your advice and we have indicated the clinical research of this study in the title.

Changes in the text: A clinical and laboratory-based nomogram for predicting nonalcoholic fatty liver disease in non-diabetic adults: a cross-sectional study (see Page 1, line 2-4).

Comment 2: Second, the abstract needs to report more essential details. In the background, the authors only indicated the knowledge gap but did not describe the clinical importance of NAFLD in non-diabetic patients and needs for the predictive model. Please describe the objectives of this study. The methods part cannot be statistical methods only. Please describe the sampling and inclusion of subjects, assessment of potential predictors and diagnosis of NAFLD, follow up procedures, and the generation of training and validation samples. The conclusion should have comments on the clinical implications of the findings, not the clinical significance of this study.

Reply 2: Thank you for your advice and we have detailed the abstract.

Background: In view of the increasing prevalence, severe comorbidities, and financial burdens of nonalcoholic fatty liver disease (NAFLD), early detection of NAFLD is of clinical significance. Although the close relationship between NAFLD and insulin resistance (IR) has been clarified and there is a five-fold higher prevalence of NAFLD in patients with diabetes compared to that in patients without diabetes, this

is not a reason to focus only on the incidence of NAFLD in people with diabetes because people who are suffering from insulin resistant are not necessarily diagnosed with diabetes, which leads to the overlook of NAFLD in non-diabetic population. As the symptoms are not typical, non-diabetic population may not take the time or initiative to check themselves. Actually, we are obligated to pay more attention to the non-diabetic population for early detection and intervention of NAFLD. To get the accurate diagnosis of NAFLD, detailed present history, imaging tests, blood biochemical indexes, and even liver biopsy are all needed. A personalized and novel model could help physicians make a better decision, whether a non-diabetic person should be asked about a deatiled history of drinking and medicine, to get blood tests for viral hepatitis, immune system disease and liver enzymes. This novel model, which takes many associated risk factors into consideration, provides an accurate tool for predicting the diagnosis of the disease, and the simple nomogram has the potential to save more medical resources and make less missed diagnosis. Objective of this study is to develop and validate a novel clinical nomogram to predict NAFLD among non-diabetic population based on routine biochemical variables and anthropometric parameters.

Methods: Researchers initially enrolled 20,944 patients and then excluded those with history of drinking, known medication usage, viral hepatitis, known liver disease, missing covariant data, age <18 years, and impaired fasting blood glucose, leaving 14,251 adults participating in the baseline analysis, who were randomly divided in a ratio of 3:1 into a training dataset with 10,689 participants and a validation dataset with 3,562 participants, using the classification and regression training (caret) package in R software v. 4.0.3. Variables for prediction were selected by multivariable logistic regression analysis, the LASSO method, and clinical experience, and based on these, we constructed a prediction model. Performance of this model was validated by the area under the receiver operator characteristic curve (AUROC), calibration curve, and decision curve analysis (DCA). Given the cross-sectional study, we didn't describe the follow up procedures.

Conclusion : Our nomogram provides a convenient and economical model with six meaningful factors for predicting the risk of NAFLD in non-diabetic adults. The value of AUROC, sensitivity and specificity demonstrate the good predictive ability of this model. Calibration curve and decision curve analysis (DCA) show a good performance of this model.

Changes in the text: We have modified our text as advised (see Page 2, line 26-Page 4, line 64).

Comment 3: Third, in the introduction part, please clearly review the clinical importance of NAFLD in non-diabetic patients, comments on limitations and

knowledge gap in relation to predictors and risk factors of NAFLD in non-diabetic patients, and explanations on the clinical needs for the predictive model of NAFLD in non-diabetic patients. All these information is needed to support the necessity of the research topic.

Reply 3: Thank you for your advice and we have retailed the introduction. Actually, it's the same answer to comment 2 (background). In view of the increasing prevalence, severe comorbidities, and financial burdens of nonalcoholic fatty liver disease (NAFLD), early detection of NAFLD is of clinical significance. Although the close relationship between NAFLD and insulin resistance (IR) has been clarified and there is a five-fold higher prevalence of NAFLD in patients with diabetes compared to that in patients without diabetes, this is not a reason to focus only on the incidence of NAFLD in people with diabetes because people who are suffering from insulin resistant are not necessarily diagnosed with diabetes, which leads to the overlook of NAFLD in non-diabetic population. As the symptoms are not typical, non-diabetic population may not take the time or initiative to check themselves. Actually, we are obligated to pay more attention to the non-diabetic population for early detection and intervention of NAFLD. To get the accurate diagnosis of NAFLD, detailed present history, imaging tests, blood biochemical indexes, and even liver biopsy are all needed. A personalized and novel model could help physicians make a better decision, whether a non-diabetic person should be asked about a deatiled history of drinking and medicine, to get blood tests for viral hepatitis, immune system disease and liver enzymes. This novel model, which takes many associated risk factors into consideration, provides an accurate tool for predicting the diagnosis of the disease, and the simple nomogram has the potential to save more medical resources and make less missed diagnosis.

Changes in the text: We have modified our text as advised (see Page 5, line 89-Page 6, line 105; Page 6, line 121-Page 7, line 126, line 132-134).

Comment 4: Fourth, the methodology of this has fatal limitation. A very basic pre-requisite for the development of predictive model is longitudinal data, which uses baseline factors to predict the incident disease, but the current study used cross-sectional data; this is generally not allowed. For example, in this study, if the NAFLD can be directly diagnosed by using vascular blurring, liver brightness, hepatorenal echo contrast, and deep attenuation, it is not necessary to use the proposed predictive model. In statistics, please describe the threshold value of AUC for a good predictive model and indicate the P value of statistical significance.

Reply 4: Thank you for your advice. Actually, cross-sectional data and longitudinal data can both be used to establish predictive model, with different purposes such as diagnosis and prognosis, respectively. There are some good predictive models based on cross-sectional data (PMID: 31124729, 34544417, 32764769), so as ours. To get the

accurate diagnosis of NAFLD, detailed present history, imaging tests, blood biochemical indexes, and even liver biopsy are all needed. A personalized and novel model could help physicians make a better decision, whether a non-diabetic person should be asked about a deatiled history of drinking and medicine, to get blood tests for viral hepatitis, immune system disease and liver enzymes. The simple nomogram has the potential to save more medical resources and make less missed diagnosis. In predictive models, AUC can also be called the C index. The C index ranges from 0.5 to 1.0. C index between 0.5 and 0.7 is considered as low differentiation; 0.7-0.9 was moderate; a value greater than 0.9 is considered high. Tests were two-sided and 0.05 was set as the *P* value for statistical significance.

Changes in the text: We have modified our text as advised (see Page 12, line 251-254).