Nomogram for predicting the risk of nonalcoholic fatty liver disease in older adults in Qingdao, China: A cross-sectional study

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ABSTRACT

Background and Objectives: To explore the risk factors for non-alcoholic fatty liver disease (NAFLD) and to establish a non-invasive tool for the screening of NAFLD in an older adult population. **Methods and Study Design:** A total of 131,161 participants were included in this cross-sectional study. Participants were randomly divided into training and validation sets (7:3). The least absolute shrinkage and selection operator method was used to screen risk factors. Multivariate logistic regression was employed to develop a nomogram, which was made available online. Receiver operating characteristic curve analysis, calibration plots, and decision curve analysis were used to validate the discrimination, calibration, and clinical practicability of the nomogram. Sex and age subgroup analyses were conducted to further validate the reliability of the model. **Results:** Nine variables were identified for inclusion in the nomogram (age, sex, waist circumference, body mass index, exercise frequency, systolic blood pressure, fasting plasma glucose, alanine aminotransferase, and low-density lipoprotein cholesterol). The area under the receiver operating characteristic curve values were 0.793 and 0.790 for the training set and the validation set, respectively. The calibration plots and decision curve analyses showed good calibration and clinical utility. Subgroup analyses demonstrated consistent discriminatory ability in different sex and age subgroups. **Conclusions:** This study established and validated a new nomogram model for evaluating the risk of NAFLD among older adults. The nomogram had good discriminatory performance and is a non-invasive and convenient tool for the screening of NAFLD in older adults.

Key Words: non-alcoholic fatty liver disease, aged, nomogram, non-invasive model

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by the excessive accumulation of fat in the liver in the absence of excessive alcohol consumption.[1] NAFLD encompasses various liver pathologies, spanning from simple steatosis (fatty liver) to nonalcoholic steatohepatitis. NAFLD has the potential to progress to liver fibrosis, cirrhosis, and even hepatocellular carcinoma.[2] The global prevalence of NAFLD is 32.4%.[3] In China, the prevalence of NAFLD is 29.2%.[4] NAFLD affects individuals of all ages but is more prevalent among older adults[5] due to the effects of various age-related factors, such as hormonal fluctuations, reduced physical activity, and metabolic alterations. Given the global aging trend, NAFLD has emerged as a major public health concern.[6] The consequences of NAFLD for older adults are severe. Older adults are more susceptible to advanced liver diseases, such

as fibrosis and cirrhosis.[7] Furthermore, NAFLD is frequently associated with other metabolic disorders, including obesity, type 2 diabetes, dyslipidemia, and hypertension,[8] which further augment the elevated risks of cardiovascular diseases and mortality in this population.[9] Given these facts, identifying older adults at risk of progressive NAFLD is imperative.

Liver biopsy is regarded as the gold standard for NAFLD diagnosis despite its invasive nature and potential for complications.[10] Ultrasound and other imaging techniques are non-invasive and effective for NAFLD diagnosis;[11] however, they are costly and unsuitable for use in large-scale screening programs. Given the inherent limitations of liver biopsy and imaging modalities, a new screening approach is required. Early detection is essential for effective interventions in NAFLD.

Several NAFLD prediction models have been developed. The most common variables in NAFLD prediction models are biochemical measures, such as high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), body mass index (BMI), and alanine transferase (ALT).[12-16] However, the majority of NAFLD prediction models incorporate biomarkers that are not typically included in routine health assessments, such as haptoglobin and α2-macroglobulin levels.[17,18] Furthermore, few models include lifestyle characteristics, such as dietary habits and exercise frequency. Pan et al.[19] considered dietary habits but did not consider indicators of physical activity, which are crucial for evaluating the risk of NAFLD and the effects of further interventions.[20] Additionally, few studies have focused on older adults, a population with distinct characteristics and NAFLD-associated challenges. This study is the first to use exercise frequency as an indicator in an NAFLD prediction model.

This study developed and validated a nomogram for predicting the risk of NAFLD in a large sample of older adults from Qingdao, China. Additionally, this study provided a personalized and user-friendly prediction tool that incorporates cost-effectiveness variables. The developed model can serve as an early warning and prediction system, aiding medical practitioners in identifying and selecting high-risk individuals for further diagnostic examinations and nonmedical health interventions at an early stage, thereby delaying or preventing the progression of NAFLD.

MATERIALS AND METHODS

Participants

This cross-sectional study enrolled individuals who underwent annual health examinations from 2020 to 2021 as a part of the Qingdao Diabetes Prevention Programme at community health service institutions in Qingdao, China.

Individuals were included if they were aged ≥60 years and had fatty liver. Individuals were excluded if they had excessive alcohol intake (>140 g/week for men and >70 g/week for women),[21] if they had a history of hepatotoxic drug use or a chronic liver disease (viral hepatitis, autoimmune hepatitis, or another chronic liver disease), or if they did not complete the questionnaire or receive an ultrasound examination. NAFLD was identified using abdominal ultrasonography.[1]

In total, 143,852 individuals completed the survey, among which 131,161 received a hepatic ultrasonography examination and thus were included in this study. The study population consisted of 61,514 men and 69,647 women aged 64–107 years. In total, 42,310 participants had NAFLD, and 88,851 participants did not have NAFLD. The flowchart of study population enrollment is presented in Figure 1.

This study was conducted following the principles in the World Medical Association Declaration of Helsinki and the International Ethical Guidelines for Biomedical Research Involving Subjects (GIOMS, Geneva, 1993) and Chinese clinical research management regulations. The study was approved by the Qingdao Municipal Center for Disease Control and Prevention in 2018, and the ethical approval number was Document No. 2. All participants provided written informed consent.

Data collection

Clinical information

Clinical information, including sex, age, height, weight, blood pressure, waist circumference (WC), history of alcohol consumption, smoking status, and medical history, was collected. Participants were instructed to remove their shoes and wear lightweight clothing for the measurement of height and weight. The digital scales used to measure height and weight had an accuracy of 0.1 cm and 0.1 kg, respectively. Standard mercury sphygmomanometers were employed to measure systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the right arm after participants had rested for 5 minutes. BMI was calculated as weight (kg) divided by height squared (m2). WC was measured using plastic tape at the midpoint between the lowest rib and the superior border of the iliac crest as the subject exhaled normally.

Laboratory tests

Venous blood samples were collected after 12-hour overnight fasts. The following parameters were measured: fasting plasma glucose (FPG), ALT, aspartate aminotransferase (AST), hemoglobin (HGB), white blood cell (WBC), platelet (PLT), triglyceride (TG), TC, blood

urea nitrogen (BUN), serum creatinine (Cr), HDL-C, low-density lipoprotein cholesterol (LDL-C), and total bilirubin (TBil). In total, data on 22 parameters were obtained. The blood samples were transported to a central laboratory in the cold chain state within 24 hours after centrifugation. Serum uric acid samples were assessed using enzymatic methods on an automatic biochemical analyzer (Olympus, Japan).

Ultrasound examination and diagnosis of NAFLD

All participants underwent abdominal ultrasonography. Examinations were performed by trained sonographers who were unaware of the clinical data and who used a GE LOGIQ E9 apparatus equipped with a convex 1.0–5.0-MHz probe. Diffuse fatty liver can be defined as enhanced near-field echo (bright liver), attenuated far-field echo, increased liver and kidney echo contrast, intrahepatic vessel blurring, and deep attenuation. Diagnoses of NAFLD were given after excluding diffuse fatty liver caused by alcohol, viral infection, autoimmunity, drugs, and other factors.[1]

Statistical analysis

Clinical observation data were collected and stored in an Excel spreadsheet. Statistical analyses were performed using R software (version 4.3.0).

Continuous variables, which are reported as mean ± standard deviation (SD), were analyzed using t tests. Categorical variables, which are reported as number (percentage), were analyzed using chi-square tests. Participants were randomly divided into training and validation groups at a 7:3 ratio for the construction of the nomogram and its validation.

The least absolute shrinkage and selection operator (LASSO) regression method, serving as the shrinkage selection method, was employed to screen NAFLD-related predictors. By minimizing prediction errors for quantitative response variables and by imposing constraints on model parameters, the regression coefficients of certain variables can be reduced to zero. Nonzero coefficients in the LASSO regression model were incorporated into multivariate logistic regression analysis to establish a prediction model. The diagnostic accuracy of the developed nomogram was evaluated using the area under the receiver operating characteristic curve (AUC). Calibration curves were utilized to measure concordance between practical results and predicted probabilities. Additionally, decision curve analysis (DCA) was applied to evaluate and compare prediction models and to calculate net benefits against threshold probabilities. Finally, sex-stratified and age-stratified analyses were conducted to validate the reliability of the models.

All p values were two-tailed, and $p < 0.05$ indicated statistical significance. LASSO regression was performed using the glmnet package, and the nomogram was constructed using the rms package.

RESULTS

Participant characteristics

A total of 131,161 participants were enrolled. In total, 42,310 participants (32.26%) had NAFLD, of whom 16,519 (39.04%) were men and 25,791 (60.96%) were women. Participant baseline characteristics are provided in Table 1.

The data of participants were randomly divided at a ratio of 7:3 into the training set (n = 91,812) and a validation set (n = 39,349). In the training set, the mean age was 73.32 ± 6.70 years, the proportion of men was 49.99%, and the prevalence of NAFLD was 32.10% (2,324 participants). In the validation set, the mean age was 73.32 ± 6.71 years, the proportion of men was 46.69%, and the prevalence of NAFLD was 32.63% (12,841). No significant differences in demographic or clinical characteristics were observed between the sets ($p > 0.05$). Furthermore, the prevalence of NAFLD did not significantly differ between the sets ($p = 0.058$). The baseline characteristics of participants in the training and validation sets are presented in Table 2.

Independent predictors in the training set

Considering collinearity among some of the included variables, LASSO regression was applied to screen predictive variables. Nine variables with nonzero coefficients were identified, namely age, sex, WC, BMI, exercise frequency, SBP, FPG, ALT, and LDL-C (Figure 2). The specific coefficients corresponding to these variables are listed in Supplementary Table 1.

Multivariate logistic regression analysis including the aforementioned nine variables was performed to identify the factors that were independently associated with NAFLD. All nine variables were significantly associated with NAFLD risk, and the variables in the training set were independent predictors. Multivariate odds ratios were calculated to construct the nomogram (Figure 3). In the training set, female sex, younger age, lower exercise frequency, and higher values for SBP, WC, BMI, FPG, ALT, and LDL-C were associated with an increased risk of NAFLD (Table 3).

Establishment of the nomogram

On the basis of the results of multivariate logistic regression analysis, a nomogram was established to predict the probability of NAFLD by using age, sex, WC, BMI, exercise frequency, FPG, SBP, ALT, and LDL-C as predictors (Figure 3).

Nomograms are visualization tools of multivariate logistic regression that simplify and present complex regression equations. They integrate data with a model, consider influential variables, and quantify the probability of an event through a simple graphical representation. To determine the risk of NAFLD, scores for each variable are obtained on the basis of their corresponding scales, and the total score is aligned vertically with the diagnostic possibility. This study also developed an online application that uses the model established in this study to predict the risk of NAFLD in older adults (https://nomogramforelderly.shinyapps.io/DynNomapp/, Supplementary Materials).

Validation and subgroup analysis of the nomogram

The AUC values of the training and validation sets were 0.793 (95% confidence interval [CI]: 0.790–0.796) and 0.790 (95% CI: 0.785–0.794), respectively. The optimal cutoff values were 0.301 (95% CI: 0.696–0.769) and 0.314 (95% CI: 0.705–0.737) in the training and validation sets, respectively. The sensitivity values for the training and validation sets were 0.750 and 0.737, respectively, and the specificity values were 0.695 and 0.705, respectively. Notably, the AUC of the training and validation set were similar, indicating good model stability.

Sex and age subgroup analyses were conducted. The AUC values were 0.784 (95% CI: 0.780 – 0.788) for men and 0.789 (95% CI: 0.785 – 0.792) for women. The AUC values were 0.795 (95% CI: 0.791 – 0.798) for participants aged ⩽70 years and 0.781 (95% CI: 0.777 – 0.785) for individuals aged >70 years. The model exhibited good predictive ability for all subgroups. Sensitivity, specificity, positive predictive value, and negative predictive value are shown in Table 4.

Clinical usefulness and calibration of the nomogram

The DCA of the nomogram is illustrated in Figure 5. The decision curve demonstrated that this model provided more net benefits for predicting NAFLD risk compared to the “all” or “none” strategies within a threshold probability range of 0.10–0.78 in both the training and validation sets. As shown in Figure 5, the calibration chart indicated good agreement between

the nomogram's predicted risk and the actual risk of NAFLD, demonstrating that the model was well calibrated.

DISCUSSION

In this cross-sectional study, the prevalence of NAFLD was 32.36% overall, 26.85% among men, and 37.03% among women. A nomogram was developed on the basis of nine optimal predictor variables selected using LASSO regression: age, sex, WC, BMI, exercise frequency, FPG, SBP, ALT, and LDL-C. The coefficients of other less significant predictors were shrunk to zero, so they were not included in the model. Among the nine variables, female sex; younger age; higher values for SBP, WC, BMI, FPG, ALT, and LDL-C; and lower exercise frequency were identified as risk factors for NAFLD.

The AUC of the nomogram indicated its good discrimination for predicting the risk of NAFLD in an older adult population. The DCA and calibration plot indicated that the model had good clinical utility and was well calibrated. According to the results of subgroup analyses, the model demonstrated remarkable sensitivity and accuracy as well as good reliability and discriminatory capability in different populations. Furthermore, monitoring changes in the risk of NAFLD over time is simple because the variables are easily attainable. In addition, a web-based nomogram was developed to improve the model's operational feasibility.

Sex and age were nonmodifiable and significant predictors of NAFLD. Epidemiological studies have revealed sex differences in the prevalence of NAFLD, with women generally being more likely to have NAFLD during adulthood than men.[22] However, due to ovarian senescence and estrogen decline, postmenopausal women are as likely or more likely to have NAFLD than men of the same age.[23] In the present study, NAFLD was more prevalent among women than among men, which is consistent with other studies.[24] NAFLD prevalence among older adults varies. Studies have reported an inverted U curve pattern,[22] where the prevalence of NAFLD is highest in late adulthood and then decreases. Therefore, NAFLD is less prevalent among older adults than among younger adults. The lower incidence of NAFLD among older adults has been interpreted as potentially indicating either a specific decline in survival adults with NAFLD[25,26] or a decrease in adipose tissue modifications in advanced non-alcoholic steatohepatitis.[27] Our subgroup analysis revealed that the model well differentiated between age groups (<70 and >70 years) and sex; however, it is only a static prediction model. Long-term longitudinal data are required to verify the reliability of the model. Further research is required to provide a more comprehensive understanding of

whether the observed the lower prevalence of NAFLD among older adults can be attributed to selection bias from earlier mortality among those with NAFLD or to other factors, such as lifestyle disparities among various age groups.

A complex relationship has been reported between NAFLD and metabolic diseases. Metabolic diseases, such as obesity, type 2 diabetes mellitus, and dyslipidemia, are risk factors for NAFLD, but they can also arise as a consequence of NAFLD.[28] Recent calls have been made to rename NAFLD as metabolic dysfunction–associated fatty liver disease(MAFLD) to better reflect the condition's association with metabolic risk factors.[29] Excessively high BMI and visceral obesity are widely recognized and extensively studied risk factors for NAFLD.[30] High BMI scores and WC values are risk factors for NAFLD.

Unlike previous studies,[31-33] our present study identifies LDL-C as a significant predictor of NAFLD. LDL-C is composed of cholesterol ester, which depends on dissociation from the exchangeable apolipoprotein of VLDL by lipoprotein lipase, metabolic abnormal such as elevated liver enzymes can increase the secretion of VLDL particles.[34] A large population-based study involving 60,527 participants reported that increased levels of LDL-C within the normal range may play a role in the development of NAFLD, independent of other confounding factors.[35] Furthermore, LDL-C has been shown to be an important risk factor for cardiovascular disease,[36] suggesting an association between NAFLD and cardiovascular disease.

FPG levels serve as an independent predictor of diabetes mellitus, and FPG levels reflect the secretion and functioning of basal insulin.[37,38] Insulin resistance can lead to increasing lipolysis within white adipose tissue and concomitant increased delivery of free fatty acids to the liver.[39] The present study further expands upon this observation by demonstrating that elevated FPG levels are associated with an increased risk of NAFLD.

SBP is a risk factor for NAFLD and metabolic syndrome.[40] A recent investigation demonstrated that NAFLD was more prevalent among individuals with hypertension than among those without hypertension.[41] This finding implies a potential association between hypertension and the progression of liver disease given the complex interplay between liver regeneration and angiocrine signals.[42]

ALT enzymes serve as a reliable biomarker of liver damage, including hepatic steatosis and steatohepatitis.[43] Several studies have shown that elevated serum ALT levels often accompany the development and progression of NAFLD.[44,45] Consistent with these findings, the present study identified serum ALT concentration as an independent risk factor for NAFLD.

Physical activities such as aerobic and resistance training could reduce intrahepatocellular lipids.[46] A retrospective study in Japan demonstrated that individuals who exercised >250 minutes/week had significantly lower liver fat content than those who exercised <250 minutes/week.[47] The present study found that exercising at least once a week reduced the risk of NAFLD.

Study strengths and limitations

The strengths of this study are as follows. First, the study had a large sample size (131,161 participants), which enhanced the reliability of the study findings and the statistical power of the developed nomogram. Second, the developed nomogram relies on a limited number of easily obtainable variables, making it applicable to diverse populations and ethnicities. Third, the presentation of the nomogram online facilitates convenient assessment of the risk of NAFLD.

The study limitations are as follows. First, this study is cross-sectional, which means that selection bias may be inherent. Second, the study only employed internal validation. Extensive external validation is essential to validating the model's reliability prior to its implementation in clinical practice. Third, liver biopsy, considered the gold standard in NAFLD diagnosis, was not included in this study. Finally, dietary habits, intensity and duration of physical activity, were not considered. Long-term, large-scale, multicenter follow-up studies are required to further validate the study findings on the predictors of NAFLD.

Conclusion

The present study developed a novel nomogram with a relatively good predictive ability for screening NAFLD among Chinese older adults. This study demonstrated that female sex; younger age; higher values for SBP, WC, BMI, FPG, ALT, and LDL-C; and lower exercise frequency were independent risk factors for NAFLD among older adults. Customized treatment plans may be developed for patients on the basis of individual risk. Individuals at high risk should be referred for additional diagnostic tests to confirm NAFLD diagnosis, enabling the early implementation of lifestyle modifications and medical interventions aimed at preventing disease progression.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors have no conflicts of interest to declare.

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REFERENCES

1. Wong VW, Chan WK, Chitturi S, Chawla Y, Dan YY, Duseja A et al. Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017-Part 1: Definition, risk factors and assessment[J]. J Gastroenterol Hepatol, 2018,33(1):70-85. doi:10.1111/jgh.13857.

[2] Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies[J]. Nat Med, 2018,24(7):908-922. doi:10.1038/s41591-018-0104-9.

[3] Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, Swain MG, Congly SE, Kaplan GG, Shaheen AA. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis[J]. Lancet Gastroenterol Hepatol, 2022,7(9):851-861.
doi: 10.1016/S2468-1253(22)00165-0.

[4] Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: a systematic review and meta-analysis[J]. Lancet Gastroenterol Hepatol, 2019,4(5):389-398. doi: 10.1016/S2468-1253(19)30039-1.

[5] Mantovani A, Targher G, Zoppini G. Nonalcoholic Fatty Liver Disease and Implications for Older Adults with Diabetes[J]. Clin Geriatr Med, 2020,36(3):527-547.
doi: 10.1016/j.cger.2020.04.010.

[6] Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention[J]. Nat Rev Gastroenterol Hepatol, 2018,15(1):11-20. doi: 10.1038/nrgastro.2017.109.

[7] Hunt NJ, Kang SWS, Lockwood GP, Le Couteur DG, Cogger VC. Hallmarks of Aging in the Liver[J]. Comput Struct Biotechnol J, 2019,17:1151-1161. doi:10.1016/j.csbj.2019.07.021.

[8] Brady CW. Liver disease in menopause[J]. World J Gastroenterol, 2015,21(25):7613-7620. doi:10.3748/wjg.v21.i25.7613.

[9] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases[J]. Hepatology, 2018,67(1):328-357. doi: 10.1002/hep.29367.

[10] Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis[J]. World J Gastroenterol, 2014,20(2):475-485. doi: 10.3748/wjg.v20.i2.475.

[11]Schwenzer NF, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance[J]. J Hepatol, 2009,51(3):433-445. doi:10.1016/j.jhep.2009.05.023.

[12]Cen C, Wang W, Yu S, Tang X, Liu J, Liu Y, Zhou L, Yu J, Zheng S. Development and validation of a clinical and laboratory-based nomogram to predict nonalcoholic fatty liver disease[J]. Hepatol Int, 2020,14(5):808-816. doi: 10.1007/s12072-020-10065-7.

[13]Zhou B, Gong N, Huang X, Zhu J, Qin C, He Q. Development and validation of a nomogram for predicting metabolic-associated fatty liver disease in the Chinese physical examination population[J]. Lipids Health Dis, 2023,22(1):85. doi:10.1186/s12944-023-01850-y.

[14]Zhao M, Hu Y, Shi C, Wang K, Li J, Song J, Huo C, Xi Y, Bu S, Huang Q. NFI, a clinical scoring tool for predicting non-alcoholic fatty liver in the Chinese population[J]. Public Health, 2022,202:12-17. doi: 10.1016/j.puhe.2021.10.012.

[15]Li D, Zhang M, Wu S, Tan H, Li N. Risk factors and prediction model for nonalcoholic fatty liver disease in northwest China[J]. Sci Rep, 2022,12(1):13877. doi:10.1038/s41598-022-17511-6

[16]Talluri R, Shete S. Using the weighted area under the net benefit curve for decision curve analysis[J]. BMC Med Inform Decis Mak, 2016,16:94. doi: 10.1186/s12911-016-0336-x.

[17]Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers[J]. J Hepatol, 2018,68(2):305-315.
doi: 10.1016/j.jhep.2017.11.013.

[18]Poynard T, Peta V, Munteanu M, Charlotte F, Ngo Y, Ngo A et al. The diagnostic performance of a simplified blood test (SteatoTest-2) for the prediction of liver steatosis[J]. Eur J Gastroenterol Hepatol, 2019,31(3):393-402. doi:10.1097/MEG.0000000000001304.

[19]Pan X, Xie X, Peng H, Cai X, Li H, Hong Q, Wu Y, Lin X, Xu S, Peng XE. Risk Prediction for Non-alcoholic Fatty Liver Disease Based on Biochemical and Dietary Variables in a Chinese Han Population[J]. Front Public Health, 2020,8:220. doi: 10.3389/fpubh.2020.00220.

[20]Rinella ME, Sanyal AJ. Management of NAFLD: a stage-based approach[J]. Nat Rev Gastroenterol Hepatol, 2016,13(4):196-205. doi: 10.1038/nrgastro.2016.3.

[21]Han AL. Association of Cardiovascular Risk Factors and Metabolic Syndrome with non-alcoholic and alcoholic fatty liver disease: a retrospective analysis. BMC Endocr Disord. 2021;21(1):91. doi:10.1186/s12902-021-00758-x.

[22]Eguchi Y, Hyogo H, Ono M, Mizuta T, Ono N, Fujimoto K, Chayama K, Saibara T, JSG-NAFLD. Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study[J]. J Gastroenterol, 2012,47(5):586-595.
doi: 10.1007/s00535-012-0533-z.

[23]Bertolotti M, Lonardo A, Mussi C, Baldelli E, Pellegrini E, Ballestri S, Romagnoli D, Loria P. Nonalcoholic fatty liver disease and aging: epidemiology to management[J]. World J Gastroenterol, 2014,20(39):14185-14204. doi: 10.3748/wjg.v20.i39.14185.

[24]Kavanagh K, Espeland MA, Sutton-Tyrrell K, Barinas-Mitchell E, El Khoudary SR, Wildman RP. Liver fat and SHBG affect insulin resistance in midlife women: the Study of Women's Health Across the Nation (SWAN)[J]. Obesity (Silver Spring), 2013,21(5):1031-1038. doi:10.1002/oby.20077.

[25]Lonardo A, Lombardini S, Scaglioni F, Ballestri S, Verrone AM, Bertolotti M, Carulli L, Ganazzi D, Carulli N, Loria P. Fatty liver, carotid disease and gallstones: a study of age-related associations[J]. World J Gastroenterol, 2006,12(36):5826-5833. doi: 10.3748/wjg.v12.i36.5826.

[26]Koehler EM, Schouten JN, Hansen BE, van Rooij FJ, Hofman A, Stricker BH, Janssen HL. Prevalence and risk factors of non-alcoholic fatty liver disease in the elderly: results from the Rotterdam study[J]. J Hepatol, 2012,57(6):1305-1311. doi:10.1016/j.jhep.2012.07.028.

[27]van der Poorten D, Samer CF, Ramezani-Moghadam M, Coulter S, Kacevska M, Schrijnders D et al. Hepatic fat loss in advanced nonalcoholic steatohepatitis: are alterations in serum adiponectin the cause?[J]. Hepatology, 2013,57(6):2180-2188. doi:10.1002/hep.26072.

[28]Chen TP, Lai M, Lin WY, Huang KC, Yang KC. Metabolic profiles and fibrosis of nonalcoholic fatty liver disease in the elderly: A community-based study[J]. J Gastroenterol Hepatol, 2020,35(9):1636-1643. doi:10.1111/jgh.15073.

[29]Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020;73(1):202-209. doi:10.1016/j.jhep.2020.03.039.

[30]Francque SM, Marchesini G, Kautz A, Walmsley M, Dorner R, Lazarus JV et al. Non-alcoholic fatty liver disease: A patient guideline[J]. JHEP Rep, 2021,3(5):100322. doi:10.1016/j.jhepr.2021.100322.

[31]Zhu W, Shi P, Fu J, Liang A, Zheng T, Wu X, Yuan S. Development and application of a novel model to predict the risk of non-alcoholic fatty liver disease among lean pre-diabetics with normal blood lipid levels. Lipids Health Dis. 2022;21(1):149. doi:10.1186/s12944-022-01752-5.

[32]Peng H, Zhang J, Huang X, Xu M, Huang J, Wu Y, Peng XE. Development and validation of an online dynamic nomogram based on the atherogenic index of plasma to screen nonalcoholic fatty liver disease. Lipids Health Dis. 2023;22(1):44. doi:10.1186/s12944-023-01808-0.

[33]Qian Y, Sun B, Zhang Y, Zhang M, Jiao X, Lai L, Yang W. A clinical and laboratory-based nomogram for predicting nonalcoholic fatty liver disease in non-diabetic adults: a cross-sectional study. Ann Palliat Med. 2022;11(7):2349-2359. doi:10.21037/apm-21-2988.

[34]Sonmez A, Nikolic D, Dogru T, Ercin CN, Genc H, Cesur M et al. Low- and high-density lipoprotein subclasses in subjects with nonalcoholic fatty liver disease. J Clin Lipidol. 2015;9(4):576-582. doi:10.1016/j.jacl.2015.03.010

[35]Sun DQ, Liu WY, Wu SJ, Zhu GQ, Braddock M, Zhang DC, Shi KQ, Song D, Zheng MH. Increased levels of low-density lipoprotein cholesterol within the normal range as a risk factor for nonalcoholic fatty liver disease. Oncotarget. 2016;7(5):5728-5737. doi:10.18632/oncotarget.6799.

[36]Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical

studies. A consensus statement from the European Atherosclerosis Society Consensus Panel[J]. Eur Heart J, 2017,38(32):2459-2472. doi:10.1093/eurheartj/ehx144.

[37]Strandberg AY, Pienimäki T, Pitkälä KH, Tilvis RS, Salomaa VV, Strandberg TE. Comparison of normal fasting and one-hour glucose levels as predictors of future diabetes during a 34-year follow-up[J]. Ann Med, 2013,45(4):336-340. doi:10.3109/07853890.2013.785233.

[38]Lu J, He J, Li M, Tang X, Hu R, Shi L et al. Predictive Value of Fasting Glucose, Postload Glucose, and Hemoglobin A(1c) on Risk of Diabetes and Complications in Chinese Adults[J]. Diabetes Care, 2019,42(8):1539-1548. doi:10.2337/dc18-1390.

[39]Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. J Gastroenterol. 2013;48(4):434-441. doi:10.1007/s00535-013-0758-5.

[40]Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V, Patel A. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis[J]. JAMA, 2015,313(6):603-615. doi:10.1001/jama.2014.18574.

[41]Ciardullo S, Monti T, Sala I, Grassi G, Mancia G, Perseghin G. Nonalcoholic Fatty Liver Disease and Advanced Fibrosis in US Adults Across Blood Pressure Categories. Hypertension. 2020;76(2):562-568. doi:10.1161/HYPERTENSIONAHA.120.15220.

[42]Lorenz L, Axnick J, Buschmann T, Henning C, Urner S, Fang S et al. Mechanosensing by β1 integrin induces angiocrine signals for liver growth and survival[J]. Nature, 2018,562(7725):128-132. doi:10.1038/s41586-018-0522-3.

[43]Sinn DH, Gwak GY, Park HN, Kim JE, Min YW, Kim KM et al. Ultrasonographically detected non-alcoholic fatty liver disease is an independent predictor for identifying patients with insulin resistance in non-obese, non-diabetic middle-aged Asian adults[J]. Am J Gastroenterol, 2012,107(4):561-567. doi:10.1038/ajg.2011.400.

[44]Oh HJ, Kim TH, Sohn YW, Kim YS, Oh YR, Cho EY et al. Association of serum alanine aminotransferase and γ-glutamyltransferase levels within the reference range with metabolic syndrome and nonalcoholic fatty liver disease[J]. Korean J Hepatol, 2011,17(1):27-36. doi:10.3350/kjhep.2011.17.1.27.

[45]Kim CH, Park JY, Lee KU, Kim JH, Kim HK. Association of serum gamma-glutamyltransferase and alanine aminotransferase activities with risk of type 2 diabetes mellitus independent of fatty liver. Diabetes Metab Res Rev. 2009;25(1):64-69. doi:10.1002/dmrr.890.

[46]Bacchi E, Negri C, Targher G, Faccioli N, Lanza M, Zoppini G, Zanolin E, Schena F, Bonora E, Moghetti P. Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 Randomized Trial). Hepatology. 2013;58(4):1287-1295. doi:10.1002/hep.26393.

[47]Oh S, Shida T, Yamagishi K, Tanaka K, So R, Tsujimoto T, Shoda J. Moderate to vigorous physical activity volume is an important factor for managing nonalcoholic fatty liver disease: a retrospective study[J]. Hepatology, 2015,61(4):1205-1215. doi:10.1002/hep.27544.

Table 1. Participant baseline characteristics[†]

Characteristics	Overall (n=131161)	NAFLD (n=42310)	Non-NAFLD (n=88851)	*p*-values
Demographic characteristics				
Age (years)	73.32 ± 6.71	71.97 ± 5.84	73.97 ± 6.99	<0.001
Gender, n (%)				
Male	61514 (46.90)	16519 (39.04)	44995 (50.64)	<0.001
Female	69647 (53.10)	25791 (60.96)	43856 (49.36)	
WC (cm)	86.22 ± 9.32	90.82 ± 9.05	84.02 ± 8.62	<0.001
BMI (kg/m^2)	24.65 ± 3.65	26.80 ± 3.42	23.62 ± 3.29	<0.001
SBP (mmHg)	144.38 ± 22.76	148.36 ± 22.35	142.48 ± 22.71	<0.001
DBP (mmHg)	76.75 ± 12.27	78.58 ± 12.29	75.88 ± 12.17	<0.001
Current smoking, n (%)				
No	109116 (83.19)	36310 (85.82)	72806 (81.94)	<0.001
Yes	22045 (16.81)	6000 (14.18)	16045 (18.06)	
Current alcohol-drinking, n (%)				
No	118081(90.03)	37926 (89.64)	80155 (90.21)	0.001
Yes	13080(9.97)	4384 (10.36)	8696 (9.79)	
Exercise, n (%)				
Never	37532 (28.61)	14984 (35.41)	22548 (25.38)	<0.001
1 time/week	5640 (4.30)	2071 (4.89)	3569 (4.02)	
2-6 times/ week	5924 (4.52)	2239 (5.29)	3685 (4.15)	
Everyday	82065 (62.57)	23016 (54.40)	59049 (66.46)	
Clinical characteristics				
HGB (g/L)	140.93 ± 18.43	142.71 ± 16.98	140.08 ± 19.02	<0.001
WBC (10^9/L)	6.42 ± 6.00	6.58 ± 4.93	6.34 ± 6.45	<0.001
PLT (10^9/L)	222.49 ± 73.65	226.16 ± 63.86	220.75 ± 77.82	<0.001
FPG (mmol/L)	6.16 ± 1.98	6.60 ± 2.23	5.94 ± 1.82	<0.001
ALT (U/L)	20.25 ± 12.07	23.35 ± 13.55	18.77 ± 11.00	<0.001
AST (U/L)	21.24 ± 8.89	21.87 ± 9.88	20.95 ± 8.36	<0.001
TBil (μmol/L)	15.34 ± 17.27	15.17 ± 12.27	15.43 ± 19.19	0.014
Cr (μmol/L)	75.55 ± 21.61	75.56 ± 19.47	75.55 ± 22.56	0.903
BUN (mmol/L)	6.03 ± 13.20	5.85 ± 3.75	6.12 ± 15.83	0.004
TC (mmol/L)	5.48 ± 1.35	5.58 ± 1.27	5.43 ± 1.38	<0.001
TG (mmol/L)	1.46 ± 6.49	1.87 ± 9.99	1.27 ± 3.82	<0.001
LDL-C (mmol/L)	3.14 ± 0.92	3.27 ± 0.93	3.08 ± 0.91	<0.001
HDL-C (mmol/L)	1.52 ± 6.07	1.47 ± 9.34	1.54 ± 3.58	0.064

[†]Data are expressed as mean ± standard deviations or n (%).

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HGB, Hemoglobin; WBC, White blood cell; PLT, platelet count; FPG, fasting plasma glucose; ALT, alanine transferase; AST, aspartate aminotransferase; TBil, total bilirubin; Cr, serum creatinine; BUN, blood urea nitrogen; TC, total cholesterol; TG, total triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 2. Baseline characteristics in training and validation sets

Characteristics	Training set (n=91812)	Validation set (n=39349)	*p*-values
Age (years)	73.32±6.70	73.32±6.71	0.962
Gender, n (%)			
Male	43141 (46.99)	18373 (46.69)	0.328
Female	48671 (53.01)	20976 (53.31)	
WC (cm)	86.22 ± 9.32	86.21 ± 9.29	0.824
BMI (kg/m^2)	24.65 ± 3.65	24.650 ± 3.66	0.878
SBP (mmHg)	144.38 ± 22.79	144.382 ± 22.69	0.985
DBP (mmHg)	76.74 ± 12.29	76.773 ± 12.24	0.665
Current smoking, n (%)			
No	76375 (83.19)	32741 (83.21)	0.934
Yes	15437 (16.81)	6608 (16.79)	
Current alcohol-drinking, n (%)			
No	35449 (90.09)	82632 (90.00)	0.636
Yes	3900 (9.91)	9180 (10.00)	
Exercise, n (%)			
Never	26152 (28.48)	11380 (28.92)	0.344
1 time/week	3972 (4.33)	1668 (4.24)	
2-6 times/ week	4129 (4.50)	1795 (4.56)	
Everyday	57559 (62.69)	24506 (62.28)	
NAFLD, n (%)			
Non-NAFLD	62343 (67.90)	26508 (67.37)	0.058
NAFLD	29469 (32.10)	12841 (32.63)	
HGB (g/L)	140.88 ± 18.42	141.05 ± 18.43	0.117
WBC (10^9/L)	6.43 ± 6.49	6.39 ± 4.65	0.431
PLT (10^9/L)	222.68 ± 69.43	222.06 ± 82.66	0.160
FPG (mmol/L)	6.16 ± 1.99	6.15 ± 1.96	0.819
ALT (U/L)	20.23 ± 12.08	20.29 ± 12.05	0.429
AST (U/L)	21.23 ± 8.81	21.27 ± 9.08	0.410
TBil (μmol/L)	15.32 ± 12.06	15.40 ± 25.58	0.444
Cr (μmol/L)	75.52 ± 21.39	75.63 ± 22.10	0.366
BUN (mmol/L)	5.99 ± 3.99	6.14 ± 23.32	0.052
TC (mmol/L)	5.48 ± 1.33	5.48 ± 1.39	0.792
TG (mmol/L)	1.47 ± 7.71	1.44 ± 1.31	0.433
LDL-C (mmol/L)	3.14 ± 0.93	3.14 ± 0.91	0.512
HDL-C (mmol/L)	1.53 ± 7.25	1.49 ± 0.37	0.272

Data are expressed as mean ± SD or n (%)

Table 3. Multivariate logistic regression in training set

	β	SE	Wald	OR (95% CI)	*p* values
Female	0.270	0.016	16.129	1.310 (1.268-1.354)	<0.001
Age	-0.030	0.001	-22.777	0.971 (0.968-0.973)	<0.001
SBP	0.006	<0.001	17.626	1.006 (1.005-1.007)	<0.001
FPG	0.126	0.004	31.960	1.134 (1.125-1.143)	<0.001
BMI	0.194	0.003	63.144	1.214 (1.207-1.222)	<0.001
WC	0.039	0.001	34.781	1.040 (1.038-1.042)	<0.001
ALT	0.022	0.001	31.027	1.018 (1.005-1.031)	<0.001
LDL-C	0.149	0.009	16.968	1.522 (1.287-1.813)	<0.001
Exercise					
Never				1.00	
1 time/week	-0.167	0.040	-4.164	0.846 (0.782-0.915)	<0.001
2-6 times/week	-0.063	0.039	-1.612	0.939 (0.869-1.013)	0.107
Everyday	-0.343	0.017	-19.188	0.709 (0.685-0.735)	<0.001

SBP, systolic blood pressure, FPG, fasting plasma glucose; BMI body mass index; WC, waist circumference; ALT, alanine transferase; LDL-C, low-density lipoprotein

Table 4. Diagnostic performance of nomogram by sex and age

	AUC (95%CI)	*p* values	Youden	Accuracy	Sensitivity	Specificity	PPV	NPV
Training set	0.793 (0.790-0.796)	<0.001	0.445	0.712	0.750	0.695	0.537	0.854
Validation set	0.790 (0.785-0.794)	<0.001	0.442	0.715	0.737	0.705	0.548	0.847
Gender								
Male	0.784 (0.780-0.788)	<0.001	0.429	0.709	0.727	0.702	0.472	0.875
Female	0.789 (0.785-0.792)	<0.001	0.438	0.714	0.737	0.701	0.591	0.819
Age (years)								
≤ 70	0.795 (0.791-0.798)	<0.001	0.446	0.714	0.742	0.704	0.493	0.876
> 70	0.781 (0.777-0.785)	<0.001	0.427	0.713	0.716	0.711	0.599	0.807

For subgroup analyses stratified by sex, sex was not adjusted; stratified by age, age was not adjusted. PPV, positive predictive value; NPV, negative predictive value

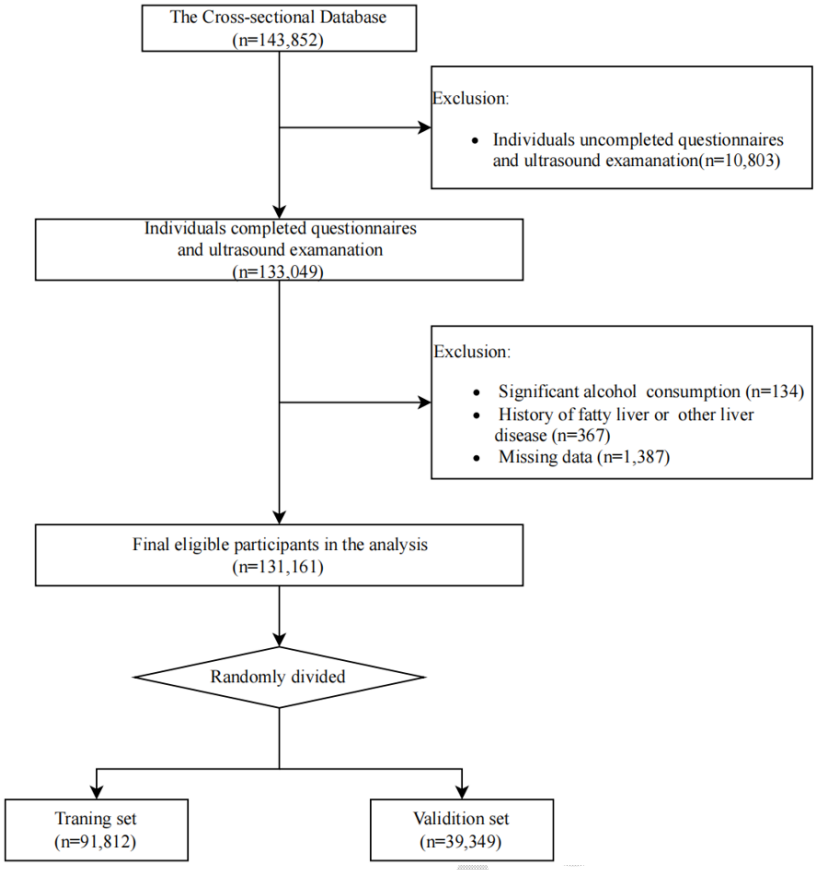

Figure 1. Participant selection process

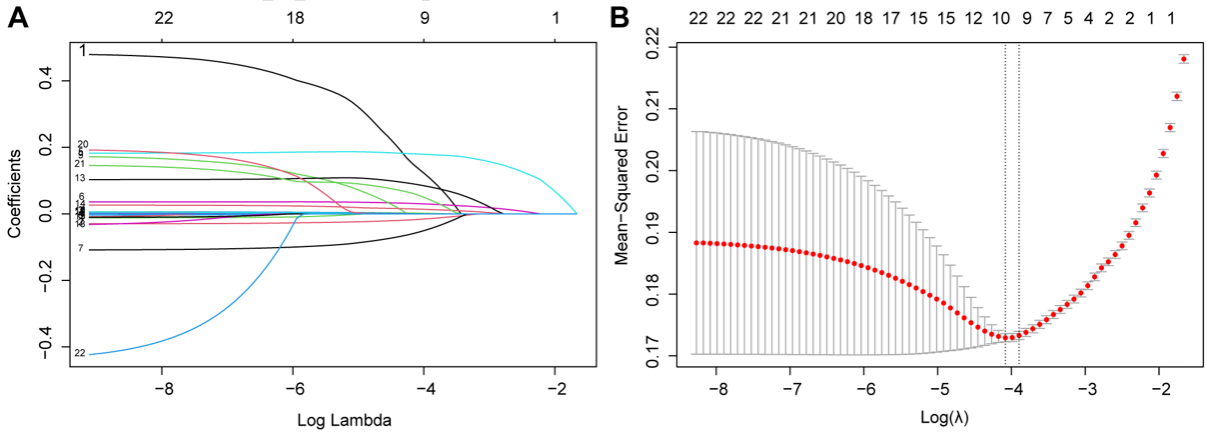

Figure 2. LASSO binary logistic regression model variable selection. (A) Optimization parameters (lambda) of LASSO model were selected using tenfold cross-validation. Mean squared error was plotted versus log (lambda). (B) LASSO coefficient profiles of 22 variables

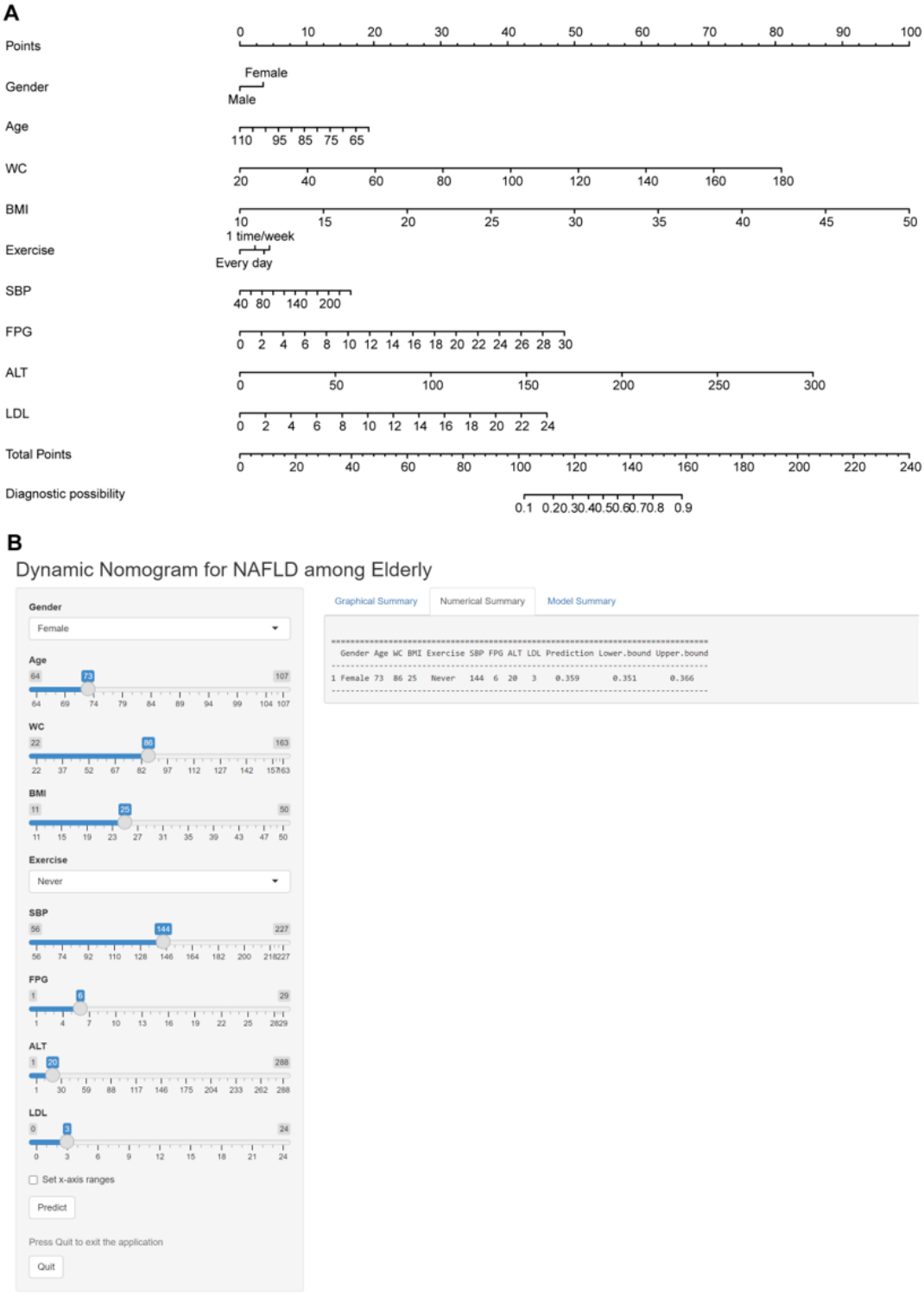

Figure 3. Nomogram prediction model for NAFLD. (A) Nomogram developed in training set for predicting risk of NAFLD. (B) Online dynamic nomogram accessible at https://nomogramforelderly.shinyapps.io/DynNomapp/ depicting example for predicting risk of NAFLD for a 73-year-old woman with WC = 86 cm, BMI = 25 kg/m2, physical activity frequency = never, SBP = 144 mmHg, FPG = 6 mmol/L, ALT = 20 U/L, and LDL-C = 3 mmol/L. Abbreviations: WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; FPG, fasting plasma glucose; ALT, alanine transferase; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease

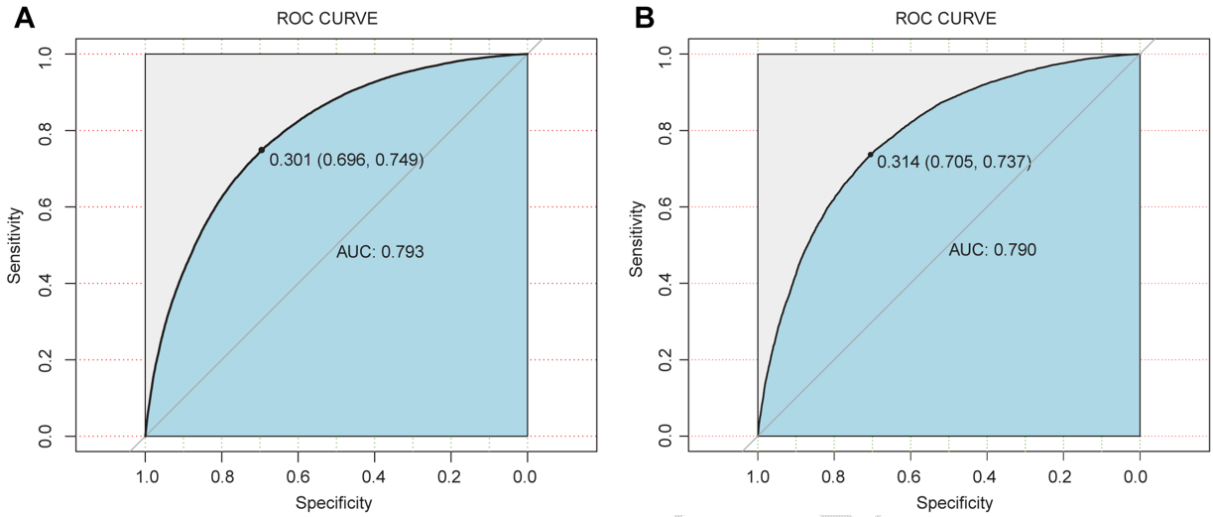

Figure 4. Receiver operating characteristic curves of nomogram in (A) training set and (B) validation set. X-axis is specificity; y-axis is sensitivity

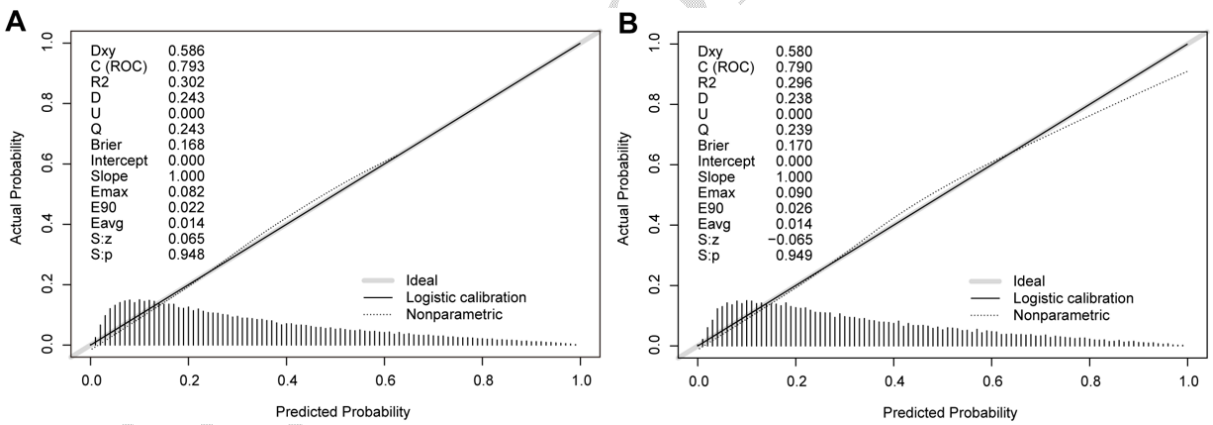

Figure 5. Calibration curves in (A) training set and (B) validation set. X-axis is nomogram predicted risk of NAFLD; y-axis is actual probability.

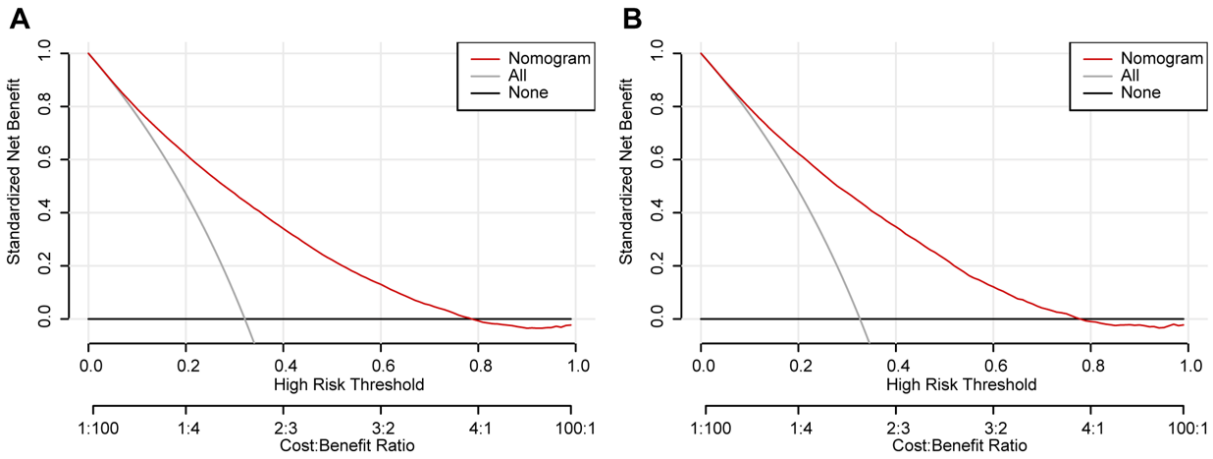

Figure 6. Decision curve analysis in (A) training set and (B) validation set. X-axis measures threshold probability. Y-axis represents net benefits, calculated by subtracting relative harms (false positives) from benefits (true positives)

Supplementary Table 1. LASSO regression analysis in training set

variables	Coefficients	Lambda value
Gender	0.022	0.020
Age	-0.002	
WC	0.006	
BMI	0.034	
Exercise	-0.008	
SBP	0.001	
FPG	0.016	
ALT	0.003	
LDL-C	0.006	