The Ability of the Framingham Steatosis Index (FSI) to Predict Non-Alcoholic Fatty Liver Disease (NAFLD): A Cohort Study

Nima Motamed[1], Mehdi Nikkhah[2], Mohammad Hadi Karbalaie Niya[2], Mahmoodreza khoonsari[2], Dhayaneethie Perumal[3], G. Hossein Ashrafi[4], Amir Hossein Faraji[2], Mansooreh Maadi[2], Hossein Ajdarkosh[2], Fahimeh Safarnezhad Tameshkel[2], Maziar Moradi-Lakeh[5], Seyyed Mohammad Miri[6], Shahram Arsang-Jang[7], Farhad Zamani[2]*

1. Department of Social Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
2. Gastrointestinal and Liver Disease Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Faculty of Science, Engineering and Computing, Kingston University, Kingston, United Kingdom
4. Cancer theme SEC faculty Penrhyn road, Kingston university London KT1 2EE
5. Preventive Medicine and Public Health Research Center, Iran University of Medical Sciences, Tehran, Iran
6. Nedmedica, Heerlen, Netherlands
7. Department of Biostatistics and Epidemiology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

***Corresponding Author:** Farhad Zamani, MD, Professor of Medicine, Gastrointestinal and Liver Disease Research Center, Iran University of Medical Sciences, Firoozgar Hospital, Tehran, Iran. Tel/Fax: +98 218894183. Mobil: +98 9121547311. email: Zamani.f@iums.ac.ir, zamani.farhad@gmail.com

Abstract

Background: Utilization of indexes for the diagnosis of Non-Alcoholic Fatty Liver Disease (NAFLD) can be valuable. The present study was conducted to determine the ability of the Framingham Steatosis Index (FSI) to distinguish between individuals with and without NAFLD to predict the risk for NAFLD so as to establish the need for lifestyle modifications in individuals at risk to develop this disease.

Methods: The study was conducted in two time phases: 2009-2010 (phase I) and 2016-2017 (phase II). A total of 4670 people in Northern Iran were included in the present study. NAFLD was diagnosed using ultrasound. The FSI was calculated based on age, sex, hypertension, diabetes mellitus status, liver enzyme levels, and triglyceride levels. Receiver Operating Characteristic (ROC) analysis was conducted to determine the discriminatory and predictive abilities of the FSI. To remove the confounding effects of potential mediators, logistic regression was performed in which NAFLD was considered as the outcome and the FSI as the predictor.

Results: The odds ratios of the FSI, when the outcome was the prevalence of NAFLD in phase I and when the outcome was new cases of NAFLD from 2009-2010 to 2016-2017, were 4.909 (4.243-5.681) and 2.453 (2.024-2.972), respectively ($P<0.001$). Also, the Areas under the Curve (AUCs) for the discriminatory and predictive abilities of the FSI were 0.8421 (95% CI: 0.8314-0.8527) and 0.7093 (95% CI: 0.6863-0.7322), respectively.

Conclusion: The FSI has a strong ability to diagnose NAFLD while it has an acceptable ability to predict the occurrence of new cases of NAFLD.

***Keywords*:** Framingham Steatosis Index (FSI); Non-alcoholic Fatty Liver Disease (NAFLD); Cohort study

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is defined as the accumulation of fat in the liver tissue diagnosed via imaging or histological evaluation after excluding other factors associated with hepatic steatosis [1]. NAFLD has become one of the most common contributors to chronic liver disease as a result of the global obesity epidemic [2]. Although in the previous decades the prevalence of other chronic liver diseases has not significantly changed or has even decreased, the prevalence of NAFLD has increased markedly in parallel with the obesity epidemic [3-6]. Based on the results of a meta-analysis of 86 studies conducted in 22 countries, the global prevalence of NAFLD was reported to be 25.2% [7]. Several procedures can be used to diagnose NAFLD. Although liver biopsy is the gold standard for the diagnosis of NAFLD, its routine application in clinical settings cannot be considered a logical medical diagnosis decision becasue it is both invasive and expensive [8].

Imaging procedures, including ultrasound (US), Computed Tomography, and Magnetic Resonance Imaging, have shown appropriate accuracy for the diagnosis of fatty liver diseases [9, 10]. In addition, some simple models have been developed based on the available demographic, anthropometric, and laboratory data to identify patients with NAFLD. The fatty liver index which was suggested by Bedogni et al in 2006 had an appropriate discriminative ability for NAFLD diagnosis [11, 12]. The Lipid Accumulation Product (LAP) and NAFLD liver fat score are other models suggested for the diagnosis of NAFLD [13-15] with the latter revealed to have promising results in the diagnosis of fatty liver diseases. The Framingham Steatosis Index (FSI) is another tool used to determine NAFLD occurrence according to age, Body Mass Index (BMI), Triglycerides (TGs), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), diabetes history, and hypertension status [16]. It has been reported that ultrasound is an inexpensive and noninvasive procedure for the diagnosis of NAFLD [15], but sometimes clinical data could predict the development of NAFLD without using imaging procedures.

Accordingly, in the present study, we aimed to determine the ability of the FSI to diagnose NAFLD and to identify the new cases of NAFLD in a population-based cohort study during a seven-year follow-up.

Methods

Study population

We began our cohort study in 2009 in Amol, a populated city in Northern Iran. To date, two phases of the study have been conducted: phase I between 2009-2010 and phase II between 2016-2017. Health houses in rural areas and health posts in urban areas were the target sites for sampling. The samples were divided into 16 strata according to sex and age groups, including 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, and 80-89 years. The sample size in each stratum was considered proportionate to the size of the population in the same stratum. After initiation of phase I, we contacted the study participants annually in order to collect data on possible medical complications. All participants in phase I were invited once again to participate in phase II of the study. The comprehensive evaluations during phase II of our cohort study were initiated in 2016 and continued over a year. In phase II, we evaluated the demographic, anthropometric, and laboratory data in addition to assessing the related outcomes. A schematic view of the enrollment of the study population is shown in Figure 1.

Measurements:

NAFLD Diagnosis

Ultrasound was applied to diagnose NAFLD. Thus, NAFLD was defined as the evidence of hepatic steatosis on ultrasound examination, without any history of excess alcohol consumption, drug-related steatosis, or viral or hereditary steatogenic hepatic conditions. An expert radiologist performed all the ultrasounds. This radiologist was blinded to the study protocol and was not directly involved in the cohort study. The different ultrasound views, including sagittal, longitudinal, lateral, and intercostal were obtained using a 3-5 MHz transducer. The associated criteria to confirm the fatty liver disease from the sonographic data were as follows: a marked increase in hepatic echogenicity and an abnormal appearance of hepatic vessels and diaphragm.

Anthropometric measurement

To measure the weight and height, participants were asked to remove their heavy outer garments, take off their shoes, and empty their pockets. Research assistants measured the participants' weight, using a calibrated scale with a precision of 100 grams. Particiapants' heights were measured using a non-stretchable meter while they were standing with a small gap between the legs and the back of their head, shoulder blades, buttocks, and heels touching the wall.

Blood pressure measurement

Participants were asked to seat and rest in a quiet room for a miminum of five minutes with their legs uncrossed and with their backs and arms supported. Blood pressure was then measured using mercury sphygmomanometers. When the cuff was inflated 20-30 mmHg above the point of the disappearance of the radial pulse, the cuff was deflated at a rate of 2 mmHg per second. The Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were recorded based on the onset and disappearance of the Korotkoff sounds, respectively. SBP and DBP were calculated as the average of two measurements.

Biochemical indexes

Biochemical indexes, including Fasting Blood Sugar (FBS), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), ALT, AST, and TG levels, were calculated using an autoanalyzer BS200 (Mindray, Shenzhen, China) and diagnostic kits from Pars Azmoon Company (Pars Azmoon Co., Tehran, Iran). A total of 10 cc of whole blood was obtained from each participant, and serum was separated after centrifugation at 3000 RPM for 15 minutes. Sera was kept at -20°C before analysis.

Framingham Steatosis Index (FSI), LAP, and VAI calculations

The FSI was calculated based on the following formula: [16]

FSI = - 7.981 + 0.011 × age (years) − 0.146 × sex (female = 1, male = 0) + 0.173 × BMI (kg/m^2) + 0.007 × triglycerides (mg/dl) + 0.593 × hypertension (yes = 1, no = 0) + 0.789 × diabetes (yes = 1, no = 0) + 1.1 × ALT/AST ratio ≥ 1.33 (yes = 1, no = 0)

Lipid accumulation product (LAP) formula:

$$\text{LAP in Men} = [\text{WC(cm)} - 65)] \times \text{TG(mmol/l)}$$

$$\text{LAP in Women} = [\text{WC(cm)} - 58)] \times \text{TG(mmol/l)}$$

Visceral adiposity index (VAI):

$$\text{VAI in Men} = \left\{\frac{\text{WC(cm)}}{39.68 + [1.88 \times \text{BMI(kg/m}^2)]}\right\} \times \left[\frac{\text{TG(mmol/L)}}{1.03}\right] \times \left[\frac{1.31}{\text{HDL(mmol/L}}\right]$$

$$\text{VAI in Women} = \left\{\frac{\text{WC(cm)}}{36.58 + [1.89 \times \text{BMI(kg/m}^2)]}\right\} \times \left[\frac{\text{TG(mmol/L)}}{0.81}\right] \times \left[\frac{1.52}{\text{HDL(mmol/L)}}\right]$$

Blinding:

No outcome data (NAFLD status of participants) was available for raters or readers of the related index test, including FSI and its associated components. Moreover, the radiologist did not have any information about the values of FSI and the associated components of the participants.

Statistical analysis

First, to address the pattern of missing data, we performed chi-square tests for nominal variables and t-tests, for continuous (metric) variables, between the missingness indicator, and other observed variables. No statistically significant data was observed. Thus, it can be argued that the pattern of missinggness in our data was random.

Receiver Operating Characteristic (ROC) analysis was performed in which the reference variable was considered NAFLD and the classification variable included the FSI and other continuous variables, such as age, BMI, TGs, FBS, and the ALT/AST ratio in addition to LAP and VAI. Two ROC analyses were conducted in which the presence of NAFLD in phase I and the occurrence of new cases of NAFLD from phase I to phase II were considered as reference variables. Thus, we assessed the abilities (based on areas under the ROC curves [AUCs]) of the

FSI and the related components to distinguish individuals with NAFLD in phase I as well as the occurrence of new cases of NAFLD from phase I to phase II of the cohort. Based on Hosmer and Lemeshow's guidelines [17], we classified $0.5<AUC<0.7$ as "poor" discriminatory ability, $0.7\leq AUC<0.8$ as "acceptable" ability, $0.8\leq AUC<0.9$ as "excellent" ability, and $0.9\leq AUC<1$ as "outstanding" ability. The packages of ROC regression models and ROC curves after rocreg of STATA software, version 12 (STATA Corp., Texas, USA), were utilized to obtain the AUCs and plots of ROC curves.

Simple and multiple logistic regression analyses were performed in which NAFLD was considered as outcome, and the FSI and its related components were considered as independent variables, separately. Therefore, we performed six simple and six multiple logistic regression analyses in which the presence of NAFLD in phase I was considered as the outcome variable and the FSI, age, sex, BMI, ALT/AST ratio, hypertension and diabetes mellitus were considered as the independent variables. Once again, we conducted the related simple and multiple logistic regression in which the new cases of NAFLD from phase I to phase II were considered as the outcome variables. In multiple logistic regression analysis, Gamma Glutamyl Transferase (GGT) levels, Alkaline Phosphatase (ALP) levels, HDL levels, Homeostatic Model Assessment-Insulin Resistance (HOMA-IR), and smoking status were considered covariates. In addition to binary logistic regression, multinomial regression analyses were performed on grade of fatty liver: grade of NAFLD was considered as outcome (reference: without NADLD). The odds ratio, Confidence Interval (CI), and p-value are reported (Table ????). The significance levels for all analyses were considered as ≤ 0.05. All statistical analyses were conducted using SPSS software version 21 (Chicago statistical software, Inc.) and STATA software, version 12 (StataCorp, Texas, United States).

Results

From among 4670 participants in phase I, 2545 were male (54.5%) and 2125 were female (45.5%). Table 1 shows participants' basic and clinical characteristics. The mean age was 44.02 ±16.12 years, and the mean calculated FSI was -1.24±1.57. Based on our results, 29.1% (%27.2-%31.0) of our participants, who had no history of NAFLD at baseline, developed this condition in the seven years of follow up.

In logistic regression analysis, the related odds ratio of the FSI was 5.596 (5.053-6.197) in the simple analysis and 2.151 (1.822-2.539) in the multiple logistic regression analysis when the outcome was considered the prevalence of NAFLD in phase I. On the other hand, the associated odd ratio of the FSI was 2.696 (2.361-3.078) in the simple analysis and 1.677 (1.325-2.098) in the multiple logistic regression analysis when the outcome was the new cases of NAFLD in phase I, as compared to phase II (all associated p values were less than 0.001). Table 2 shows the results of the simple and multiple logistic regression analyses in more details.

In simple multinomial regression analysis, the odd ratios of grade 1, 2, and 3 (relative to no NAFLD as reference) were 4.647 (95% CI=4.187-5.157, P-value<0.001), 9.239 (95% CI=7.963-10.718, P-value<0.001), and 18.488 (95% CI=11.797-28.973, P-value<0.001) for the discrimination of severity of NAFLD, respectively. In multiple multinomial regression analysis, the odd ratios of grades 1, 2, and 3 (relative to no NAFLD as reference) were 4.441 (95% CI=3.874-5.092, P-value<0.001), 10.131 (95% CI=8.199-12.519, P-value<0.001), and 14.362 (95% CI=11.287-18.275, P-value<0.001), respectively [Table 3].

Furthermore, in simple multinomial regression analysis, the odd ratios of grades 1, 2, and 3 (relative to no NAFLD as reference) were 2.612 (95% CI=42.260-3.019, P-value<0.001), 2.677 (95% CI=2.163-3.312, P-value<0.001), and 4.461 (95%CI=(3.059-6.505, P-value<0.001) for the prediction of severity of NAFLD, respectively. In addition, in multiple multinomial regression analysis, the odd ratios of grades 1, 2, and 3 (relative to no NAFLD as reference) were 2.314 (95% CI=1.912-2.799, P-value<0.001), 2.408 (95% CI=1.830-3.168, P-value<0.001), and 3.884 (95% CI=2.457-6.140, P-value<0.001), respectively [Table 3].

The power of regression analyses were 1.0000 for all results of FSI except for the prediction of new cases of NAFLD in multiple logistic regression analysis with a power of 0.9998.

The related discriminatory and predictive abilities of the FSI, based on the AUCs, were 0.842 (95% CI: 0.831-0.853) and 0.709 (95%CI=0.686-0.732), respectively. Moreover, the discriminatory and predictive abilities of the FSI for grade 2 and 3 of NAFLD were 0.842 (95% CI: 0.825-0.857) and 0.717 (95% CI=0.695-0.739), respectively. Finally, for grade 3 of NAFLD, these values were found to be 0.865 (95% CI: 0.831-0.900) and 0.748 (95% CI=0.713-0.784) [Table 4 and Figure 2] .

Discussion

Based on our results, the FSI showed an excellent ability to discriminate individuals with NAFLD from those without NAFLD, while it showed only an acceptable ability for the prediction of the new cases of NAFLD. Our results also showed that the FSI had a better discriminatory ability compared with its components and also LAP and VAI. However, this index had only slight superiority over BMI, when the ability of these indexes was evaluated for the prediction of the occurrence of new cases of NAFLD. FSI also showed a greater ability, compared with its components, and also LAP and VAI for the discrimination and prediction of grades two and three of NAFLD. We also showed that the FSI was independently associated with NAFLD when we removed the confounding effects of GGT, TG, ALP, HDL, HOMA-IR, WC, and smoking status.

Long et al. reported an AUC of 0.845 for the FSI for the identification of individuals with NAFLD [16]. As mentioned above, the FSI is calculated based on age, BMI, TGs, ALT, AST, diabetes history, and hypertension status. All these variables are associated with NAFLD [18-22]. According to our findings, the FSI showed a greater ability, compared with its components including age, BMI, FBS, DBP, SBP, TGs, and the ALT/AST ratio, to identify the NAFLD cases and to predict the new cases. However, BMI showed only a slightly lower ability to predict new cases of NAFLD as compared with the FSI. The obesity epidemic may be considered the most important cause of the higher prevalence and incidence of NAFLD worldwide [3-6]. As a result, we can expect that the obesity indexes would show a strong ability to diagnose NAFLD. Another index is the FLI, which includes GGT and WC in addition to BMI and TGs. Numerous previous studies have also reported an excellent ability of the FLI to diagnose people with NAFLD [11, 23]. However, Kim et al reported that the ability of the FLI was even lower than those of WC and BMI [24]. Additionally, the FLI includes fewer variables than the FSI, but the evaluation of GGT, as a necessary component in the calculation of FLI, is not a routine practice in clinical settings and cohort studies [11, 13]. The Lipid Accumulation Product (LAP) is another index based on only TGs and WC [13]. While Dai H reported a strong ability of LAP, Kim et al revealed its lower ability compared with those of the FLI, WC, and BMI [24, 25]. However, none of the mentioned studies assessed the predictive ability of these indexes for the development of new cases of NAFLD [11, 24, 25]. Considering the FSI components except for BMI, TGs showed better predictive ability, but this ability had an AUC <0.7 for NAFLD diagnosis and new case prediction. Based on the previous studies, dyslipidemia is estimated to

affect 70% of NAFLD patients. These studies also showed that a higher level of TGs might have a stronger relationship with NAFLD compared with cholesterol [6, 26].

Our study did not show an acceptable ability for ALT/AST ratio in discriminating NAFLD and or new case prediction. This is not in agreement with the findings reported by Long et al [16]. Although ALT and AST are considered specific liver enzymes that are elevated during liver impairment or inflammation, they are not expected to be elevated in people with simple steatosis. In fact, they could increase with more advanced involvement of hepatic tissues.

The present study has also some limitations which should be considered prior to any generlization. Our outcomes were evaluated using ultrasound, which is not considered an optimal procedure to diagnose NAFLD in clinical practice. However, other procedures such as liver biopsy are invasive and their application in a population-based studies can be considered unethical. On the other hand, the routine application of computed tomography in a population-based study may not be safe. In addition, the application of transient elastography in a large population-based study would be too expensive. Since we evaluated our participants only twice using ultrasound (first in the beginning of our cohort study between 2009-2010 and second in phase II of the cohort between 2016-2017), we were not able to measure the incidence rate of new cases of NAFLD annually. Thus, we did not have the exact time of the incidence of NAFLD, and consequently, we conducted logistic regression analyses instead of Cox proportional regression analysis when we excluded other mediators. However, the main purpose of the present study was to determine the discriminatory and predictive abilities of the FSI that could be done using ROC analysis. Although we excluded from data of target condition annually self reported NAFLD managed by clinicians, we were not able to address interventions for all other conditions that they could potentially have a common etiology or therapeutic management with NAFLD. Missingness was another limitation of our study; however, the pattern of missing data was at random in our study. On the other hand, a large sample size in our study was an advantage that has an important role to control the missingness bias. Nevertheless, the missingness is an unavoidable limitation of longitudinal studies. Finally, another limitation of this study was self-reporting of alcohol use that could culturally lead to social desirability bias. However, using anonymous questionnaires could reduce this bias.

In conclusion, our results showed that the FSI had an excellent ability for NAFLD discrimination. Although the ability of the FSI to predict new cases of NAFLD was acceptable, this ability was slightly better than that of BMI. We propose that the FSI can be applied to diagnose NAFLD if the relevant data for the calculation of FSI is already available for other purposes. Overall, our findings suggest that the FSI could be beneficial in clinical settings to diagnose NAFLD or predict new cases.

Ethical Approval

This project was approved by the ethics committee of Iran University of Medical Sciences, Tehran, Iran (No IR.IUMS.REC.1397.1086). All participants completed and signed a written informed consent form for inclusion in the study.

Conflict of interest

There is no potential conflict of interests.

Funding Support

This work was supported by the Gastrointestinal and Liver Diseases Research Center, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran (grant No: 98-1-30-14344).

Acknowledgments

The authors would like to appreciate the helps extended by the GILDRC staff (www.gildrc.ac.ir), the 17-Shahrivar Hospital, Amol, and the health providers.

References

[1] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55(6):2005-23.

[2] Farrell GC, Wong VW-S, Chitturi S. NAFLD in Asia—as common and important as in the West. Nature reviews Gastroenterology & hepatology 2013;10(5):307.

[3] Loomba R, Sanyal AJ. The global NAFLD epidemic. Nature reviews Gastroenterology & hepatology 2013;10(11):686-90.

[4] Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clinical Gastroenterology and Hepatology 2011;9(6):524-30. e1.

[5] Okanoue T, Umemura A, Yasui K, Itoh Y. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in Japan. Journal of gastroenterology and hepatology 2011;26:153-62.

[6] Fan J-G, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. Journal of hepatology 2009;50(1):204-10.

[7] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64(1):73-84.

[8] West J, Card TR. Reduced mortality rates following elective percutaneous liver biopsies. Gastroenterology 2010;139(4):1230-7.

[9] Charatcharoenwitthaya P, Lindor KD. Role of radiologic modalities in the management of non-alcoholic steatohepatitis. Clinics in liver disease 2007;11(1):37-54.

[10] 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. Journal of hepatology 2009;51(3):433-45.

[11] Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC gastroenterology 2006;6(1):33.

[12] Koehler EM, Schouten JN, Hansen BE, Hofman A, Stricker BH, Janssen HL. External validation of the fatty liver index for identifying nonalcoholic fatty liver disease in a population-based study. Clinical Gastroenterology and Hepatology 2013;11(9):1201-4.

[13] Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. BMC gastroenterology 2010;10(1):98.

[14] Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 2009;137(3):865-72.
[15] Macut D, Tziomalos K, Božić-Antić I, Bjekić-Macut J, Katsikis I, Papadakis E, et al. Non-alcoholic fatty liver disease is associated with insulin resistance and lipid accumulation product in women with polycystic ovary syndrome. Human reproduction 2016;31(6):1347-53.
[16] Long MT, Pedley A, Colantonio LD, Massaro JM, Hoffmann U, Muntner P, et al. Development and validation of the Framingham steatosis index to identify persons with hepatic steatosis. Clinical Gastroenterology and Hepatology 2016;14(8):1172-80. e2.
[17] Hosmer Jr DW, Lemeshow S, Sturdivant RX. Applied logistic regression. John Wiley & Sons; 2013.
[18] Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. The journal of clinical endocrinology & metabolism 2015;100(6):2231-8.
[19] Choi S-Y, Kim D, Kim HJ, Kang JH, Chung SJ, Park MJ, et al. The relation between non-alcoholic fatty liver disease and the risk of coronary heart disease in Koreans. American Journal of Gastroenterology 2009;104(8):1953-60.
[20] Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988–1994. American journal of epidemiology 2013;178(1):38-45.
[21] Chang Y, Ryu S, Sung E, Woo H, Cho S, Yoo S, et al. Weight gain within the normal weight range predicts ultrasonographically detected fatty liver in healthy Korean men. Gut 2009;58(10):1419-25.
[22] Friedman L. Liver, Biliary Tract Pancreatic disorders: Non-Alcoholic fatty liver disease. New York: Academic 2015.
[23] Motamed N, Sohrabi M, Ajdarkosh H, Hemmasi G, Maadi M, Sayeedian FS, et al. Fatty liver index vs waist circumference for predicting non-alcoholic fatty liver disease. World journal of gastroenterology 2016;22(10):3023.
[24] Kim JH, Kwon SY, Lee SW, Lee CH. Validation of fatty liver index and lipid accumulation product for predicting fatty liver in Korean population. Liver International 2011;31(10):1600-1.
[25] Dai H, Wang W, Chen R, Chen Z, Lu Y, Yuan H. Lipid accumulation product is a powerful tool to predict non-alcoholic fatty liver disease in Chinese adults. Nutrition & metabolism 2017;14(1):49.
[26] Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. Digestive diseases and sciences 2000;45(10):1929-34.

Table 1: Basic and clinical characteristics of study population at the first phase of the cohort study (n=4670)

Variables	Mean ±SD
Age (years)	44.02±16.12
WC (cm)	91.11±12.86
ALT (IU/mL)	23.33±16.92
AST (IU/mL)	22.27±11.51
GGT (IU/mL)	27.75 ±24.78
ALP (IU/mL)	191.09±96.03
FBS (mg/dL)	100.80±35.36
TG (mg/dL)	143.48±94.77
HDL (mg/dL)	44.72±11.89
LDL (mg/dL)	106.90±31.22
HOMA-IR	2.46±2.23
BMI (kg/m^2)	27.88±5.32
DBP (mmHg)	76.23±12.87
SBP (mmHg)	116.40±16.54
LAP	52.36±48.51
VAI	2.43±1.83
FSI	-1.24±1.57
Distribution of severity of NAFLD based on % (95%CI)	
No NAFLD	70.9 (62.0-72.7)
Grade I	20.6 (18.9-22.3)
Grade II	7.1 (6.0-8.1)
Grade III	1.4 (1.0-1.9)

ALP: denotes Alkaline phosphatase; ALT:Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body Mass Index; CI: confidence interval; DBP: Diastolic blood pressure; GGT: Gamma glutamyl transferase; FBS: Fasting blood sugar; FSI: Framingham Steatosis Index; HDL: High density lipoprotein; HOMA-IR: Homeostasis Model Assessments-insulin resistance; LAP: lipid accumulation product; LDL: Low-density lipoprotein; SBP: Systolic blood pressure; SD: Standard deviation; TG: Triglyceride; VAI: visceral adiposity index; WC: Waist circumference.

Table 2: Results of simple and multiple logistic regression analysis at the first/second phases of the cohort study

Variable	Simple logistic Regression		Multiple logistic Regression	
	OR (95% CI)	P-value	OR (95%CI)	P-value
Outcome: The prevalence of NAFLD in 2009-2010 (n=4670)				
Gender ratio (men to women)	0.887 (0.794-0.992)	0.036	1.060 (0.879-1.277)	0.543
Type 2 of Diabetes mellitus	3.794 (3.167-4.546)	<0.001	1.339 (1.025-1.750)	0.032
Hypertension	2.630 (2.283-3.030)	<0.001	1.094 (0.878-1.364)	0.425
ALT/AST (Z-score)	1.789 (1.675-1.911)	<0.001	1.171 (1.186-1.425)	<0.001
Age (Z-score)	1.787 (1.683-1.897)	<0.001	1.300 (1.186-1.425)	<0.001
TG (Z-score)	2.117 (1.955-2.293)	<0.001	1.237 (1.113-1.376)	<0.001
BMI (Z-score)	4.616 (4.211-5.060)	<0.001	1.617 (1.366-1.914)	<0.001
LAP (Z-score)	2.126 (1.977-2.288)	<0.001	1.879 (1.676-2.107)	<0.001
VAI (-score	4.729 (4.237-5.277)	<0.001	1.548 (1.426-1.681)	<0.001
FSI (Z-score)	5.596 (5.053-6.197)	<0.001	2.151 (1.822-2.539)	<0.001
Outcome: The new cases of NAFLD from 2009-2010 to 2016-2017 (n=2216)				
Gender ratio (men to women)	0.911 (0.765-1.086)	0.298	0.887 (0.697-1.130)	0.887
Type 2 of Diabetes mellitus	1.370 (0.965-1.945)	0.079	1.090 (0.733-1.621)	0.671
Hypertension	1.562 (1.214-2.010)	0.001	0.915 (0.649-1.288)	0.609
ALT/AST (Z-score)	1.447 (1.310-1.599)	<0.001	1.233 (1.084-1.403)	<0.001
Age (Z-score)	1.078 (0.984-1.181)	0.105	0.898 (0.790-1.021)	0.101
TG (Z-score)	1.674 (1.484-1.888)	<0.001	1.238(1.055-1.453)	0.009
BMI (Z-score)	2.477 (2.196-2.794)	<0.001	1.813 (1.445-2.275)	<0.001
LAP (Z-score)	2.962 (2.503-3.505)	<0.001	1.858 (1.541-2.240)	<0.001
VAI (-score	1.747 (1.559-1.957)	<0.001	1.502 (1.170-1.929)	0.001
FSI (Z-score)	2.696 (2.361-3.078)	<0.001	1.677 (1.325-2.098)	<0.001

ALT: denotes Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body Mass Index; CI: confidence interval; FSI: Framingham Steatosis Index; LAP: lipid accumulation product; NAFLD: non-alcoholic fatty liver disease; OR: odd ratio; TG: Triglyceride; VAI: visceral adiposity index

Table 3: Results of simple and multiple multinomial analysis at the first/second phases of the cohort study

Variables	Grade of NAFLD	Simple Multinomial Regression		Multiple Multinomial Regression	
		Odd ratio (95% CI)	P-value	Odd ratio (95% CI)	P-value
Outcome: The prevalence of different grades of NAFLD in 2009-2010 (n=4670)[reference: without NAFLD]					
Gender ratio (men to women)	Grade 1	1.032 (0.911-1.153)	0.621	1.058 (0.896-1.220)	0.506
	Grade 2	0.822 (0.649-0.995)	0.044	1.062 (0.885-1.1.230)	0.681
	Geade 3	0.810 (0.435-1.185)	0.335	0.887 (0.652-1.112)	0.774
Type 2 of Diabetes mellitus	Grade 1	2.999 (2.457-3.659)	<0.001	1.518 (1.184-1.947)	0.001
	Grade 2	5.572 (4.352-7.134)	<0.001	2.033 (1.431-2.888)	<0.001
	Geade 3	6.841 (4.258-10.991)	<0.001	2.268 (0.959-5.364)	0.062
Hypertension	Grade 1	2.247 (1.920-2.630)	<0.001	1.971 (1.610-2.412)	<0.001
	Grade 2	3.582 (2.892-4.437)	<0.001	3.360 (2.500-4.517)	<0.001
	Geade 3	5.185 (3.342-8.045)	<0.001	6.323 (3.013-13.269)	<0.001
ALT to AST ratio (Z-score)	Grade 1	1.713 (1.595-1.840)	<0.001	1.365 (1.246-1.496)	<0.001
	Grade 2	2.138 (1.948-2.346)	<0.001	1.751 (1.542-1.990)	<0.001
	Geade 3	2.052 (1.727-2.437)	<0.001	1.081 (0.723-1.617)	0.704
Age (Z-score)	Grade 1	1.709 (1.601-1.825)	<0.001	1.689(1.558-1.830)	<0.001
	Grade 2	1.768 (1.601-1.952)	<0.001	1.880 (1.639-2.158)	<0.001
	Geade 3	2.144 (1.720-2.673)	<0.001	2.333 (1.581-3.443)	<0.001
TG (Z-score)	Grade 1	2.002 (1.842-2.177)	<0.001	1.402 (1.268-1.549)	<0.001
	Grade 2	2.268 (2.053-2.505)	<0.001	1.626 (1.418-1.864)	<0.001
	Geade 3	2.477 (2.138-2.870)	<0.001	1.561(1.109-2.198)	0.011
BMI (Z-	Grade 1	3.907 (3.556-4.292)	<0.001	1.411 (1.309-1.513)	<0.001

score)	Grade 2	6.815 (5.958-7.796)	<0.001	3.365 (2.125-4.857)	<0.001
	Geade 3	11.949 (9.380-15.221)	<0.001	10.124 (3.617-19.987)	<0.001
LAP (Z-score)	Grade 1	4.208 (3.761-4.707)	<0.001	1.891 (1.619-2.163)	<0.001
	Grade 2	5.576 (4.904-6.340)	<0.001	3.558 (2.814-4.446)	<0.001
	Geade 3	6.410 (5.468-7.515)	<0.001	4.293 (3.036-5.940)	<0.001
VAI (Z-score)	Grade 1	1.950 (1.805-2.107)	<0.001	1.446 (1.298-1.612)	<0.001
	Grade 2	2.367 (2.148-2.609)	<0.001	1.773 (1.532-2.052)	<0.001
	Geade 3	2.568 (2.158-3.055)	<0.001	1.577 (1.076-2.309)	0.019
FSI (Z-score)	Grade 1	4.647 (4.187-5.157)	<0.001	1.941 (1.774-2.108)	<0.001
	Grade 2	9.239 (7.963-10.718)	<0.001	4.131 (2.199-6.519)	<0.001
	Geade 3	14.362 (11.287-18.275)	<0.001	8.488 (5.388-11.588)	<0.001
Outcome: The new cases of different grades of NAFLD from 2009-2010 to 2016-2017 (n=2216) [reference: without NAFLD]					
Variables	Grade of NAFLD	Odd ratio (95% CI)	P-value	Odd ratio (95% CI)	P-value
Gender ratio (men to women)	Grade 1	0.783 (0.642-0.924)	0.016	0.709 (0.543-0.875)	0.012
	Grade 2	1.333 (0.961-1.705)	0.084	1.730 (1.134-2.326)	0.011
	Geade 3	1.353 (0.684-2.022)	0.385	1.098 (0.485-1.711)	0.824
Type 2 of Diabetes mellitus	Grade 1	1.415 (0.958-2.091)	0.081	0.982 (0.618-1.559)	0.938
	Grade 2	1.199 (0.629-2.285)	0.582	0.712 (0.326-1.556)	0.395
	Geade 3	1.548 (0.467-5.132)	0.475	0.776 (0.211-2.850)	0.703
Hypertension	Grade 1	1.631 (1.232-2.158)	0.001	1.403 (0.979-2.011)	0.065
	Grade 2	1.289 (0.810-2.051)	0.285	1.076 (0.595-1.948)	0.808
	Geade 3	1.948 (0.844-4.498)	0.118	1.428 (0.519-3.928)	0.490
ALT to AST ratio (Z-score)	Grade 1	1.404 (1.260-1.564)	<0.001	1.259 (1.091-1.454)	0.002
	Grade 2	1.530 (1.328-1.764)	<0.001	1.682 (1.383-2.047)	<0.001

	Geade 3	1.657 (1.350-2.036)	<0.001	1.614 (1.182-2.205)	0.003
Age (Z-score)	Grade 1	1.128 (1.018-1.250)	0.021	1.044 (0.918-1.186)	0.513
	Grade 2	0.993 (0.841-1.172)	0.932	0.908 (0.739-1.114)	0.354
	Geade 3	0.823 (0.574-1.182)	0.292	0.814 (0.537-1.234)	0.333
TG (Z-score)	Grade 1	1.604 (1.409-1.828)	<0.001	1.402 (1.181-1.663)	<0.001
	Grade 2	1.787 (1.509-2.118)	<0.001	1.431 (1.130-1.813)	0.003
	Geade 3	2.084 (1.623-2.674)	<0.001	1.703 (1.156-2.510)	0.007
BMI (Z-score)	Grade 1	2.478 (2.170-2.829)	<0.001	1.510 (1.402-1.618)	<0.001
	Grade 2	2.316 (1.904-2.816)	<0.001	2.290 (1.787-2.936)	<0.001
	Geade 3	3.423 (2.354-4.978)	<0.001	3.558 (2.281-5.550)	<0.001
LAP (Z-score)	Grade 1	2.818 (2.360-3.364)	<0.001	1.506 (1.411-1.601)	<0.001
	Grade 2	3.210 (2.567-4.015)	<0.001	2.366 (1.760-3.182)	<0.001
	Geade 3	3.990 (2.938-5.419)	<0.001	3.232 (2.202-4.742)	<0.001
VAI (Z-score)	Grade 1	1.718 (1.519-1.944)	<0.001	1.473 (1.225-1.772)	<0.001
	Grade 2	1.752 (1.470-2.087)	<0.001	1.387 (1.056-1.820)	0.019
	Geade 3	2.118 (1.590-2.823)	<0.001	1.566 (1.017-2.410)	0.042
FSI (Z-score)	Grade 1	2.612 (2.260-3.019)	<0.001	1.514 (1.412-1.616)	<0.001
	Grade 2	2.677 (2.163-3.312)	<0.001	2.408 (1.830-3.168)	<0.001
	Geade 3	4.461 (3.059-6.505)	<0.001	3.884 (2.457-6.140)	<0.001

ALT: denotes Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body Mass Index; CI: confidence interval; FSI: Framingham Steatosis Index; LAP: lipid accumulation product; NAFLD: non-alcoholic fatty liver disease; OR: odd ratio; TG: Triglyceride; VAI: visceral adiposity index

Table 4: AUCs of FSI and its components in the discrimination of people with NAFLD and its grades from those without NAFLD and the prediction of the occurrence of new cases of NAFLD and its grades at the first/second step of cohort

Variables	AUC (95%CI)	
	Discriminatory ability of FSI for diagnosis of NAFL (n=4670)	Predictive ability of FSI (in phase 1) for new cases of NAFLD in phase 2 (n=2216)
Age (year)	0.651 (0.636-0.666)	0.520 (0.496-0.545)
BMI (kg/m^2)	0.814 (0.802-0.826)	0.703 (0.681-0.725)
DBP (mmHg)	0.631 (0.615-0.647)	0.519 (0.494-0.545)
SBP (mmHg)	0.638 (0.623-.654)	0.529 (0.503-0.555)
FBS (mg/dl)	0.644 (0.628-0.660)	0.526 (0.500-0.552)
TG (mg/dl)	0.694 (0.679-0.709)	0.625 (0.601-0.650)
ALT/AST	0.6612 (0.6457-0.6767)	0.6122 (0.5866-0.6378)
VAI	0.706 (0.691-0.721)	0.642 (0.617-0.668)
LAP	0.809 (0.796-0.821)	0.710 (0.686-0.734)
FSI	0.842 (0.831-0.853)	0.709 (0.686-0.732)
Variables	Discriminatory ability of FSI for diagnosis of grades 2 and 3 of NAFLD (n=4670)	Predictive ability of FSI (in phase 1) for new cases of grades 2 and 3 of NAFLD in phase 2 (n=2216)
Age (year)	0.607 (0.586-0.628)	0.508 (0.485-0.531)
BMI (kg/m^2)	0.801 (0.788-0.823)	0.691 (0.668-0.714)
DBP (mmHg)	0.636 (0.611-0.660)	0.551 (0.526-0.577)
SBP (mmHg)	0.630 (0.605-.655)	0.571 (0.546-0.596)
FBS (mg/dl)	0.639 (0.613-0.665)	0.572 (0.546-0.597)
TG (mg/dl)	0.685 (0.662-0.707)	0.644 (0.619-0.669)
ALT/AST	0.665(0.641-0.688)	0.645 (0.621-0.669)
VAI	0.693 (0.671-0.715)	0.645 (0.620-0.669)
LAP	0.787 (0.769-0.806)	0.709 (0.686-0.731)
FSI	0.841 (0.825-0.857)	0.717 (0.695-0.739)
Variables	Discriminatory ability of FSI for diagnosis of grade 3 of NAFLD (n=4670)	Predictive ability of FSI (in phase 1) for new cases of grade 3 of NAFLD in phase 2 (n=2216)
Age (year)	0.646 (0.602-0.690)	0.433 (0.388-0.479)
BMI (kg/m^2)	0.831 (0.792-0.870)	0.717 (0.681-0.752)
DBP (mmHg)	0.676 (0.622-0.730)	0.557 (0.516-0.598)
SBP (mmHg)	0.655 (0.596-0.713)	0.601 (0.561-0.641)
FBS (mg/dl)	0.675 (0.613-0.736)	0.562 (0.520-0.604)
TG (mg/dl)	0.718 (0.666-0.771)	0.658 (0.614-0.702)
ALT/AST	0.619 (0.557-0.682)	0.686 (0.644-0.729)
VAI	0.715 (0.664-0.765)	0.647 (0.603-0.691)
LAP	0.818 (0.774-0.861)	0.719 (0.680-0.758)
FSI	0.865 (0.831-0.900)	0.748 (0.713-0.784)

ALT: denotes Alanine aminotransferase; AST: Aspartate aminotransferase; AUC: Area under the curve; BMI: Body Mass Index; CI: confidence interval; DBP: Diastolic blood pressure; FBS: Fasting blood sugar; FSI: Framingham Steatosis Index; LAP: lipid accumulation product

product; ROC: receiver operating characteristic; SBP: Systolic blood pressure; TG: Triglyceride; VAI: visceral adiposity index

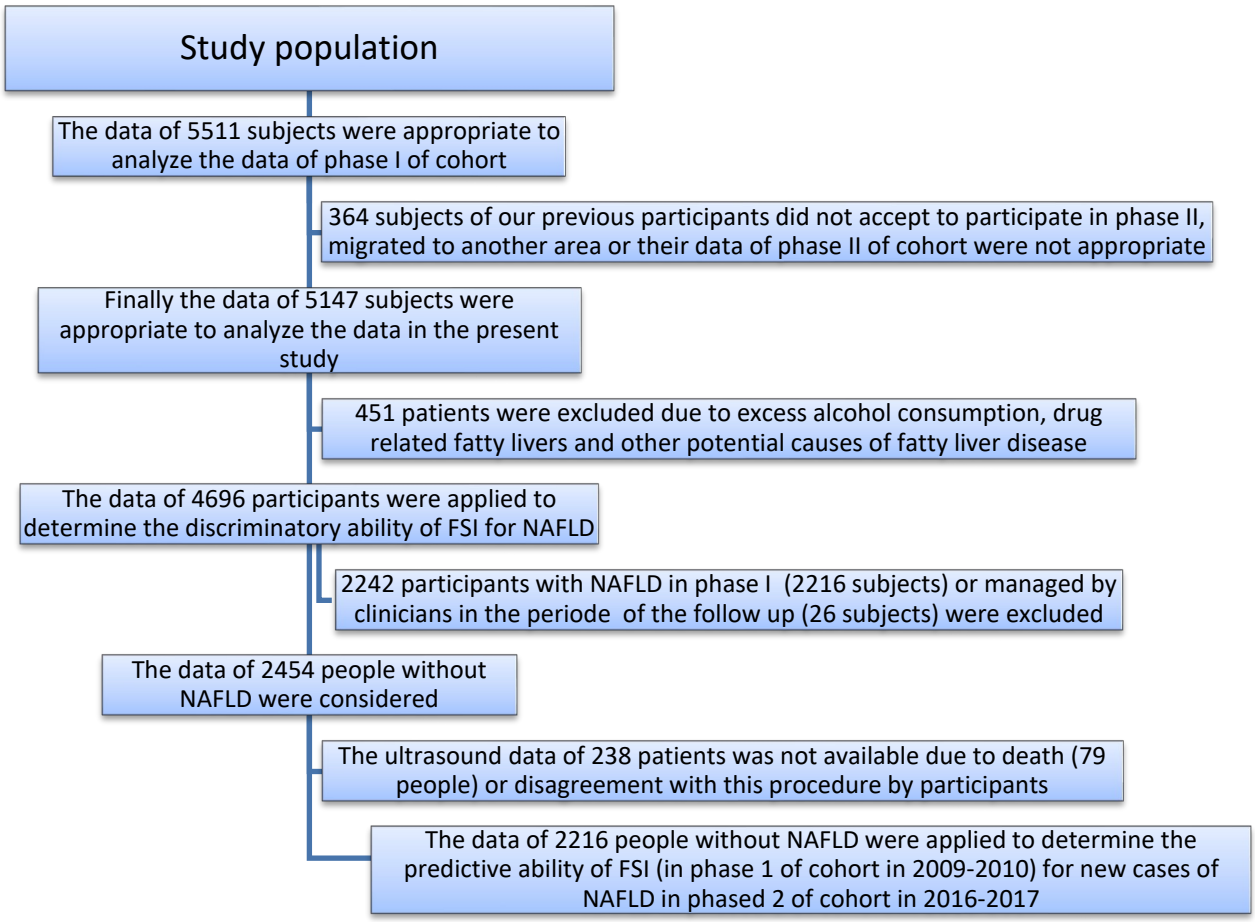
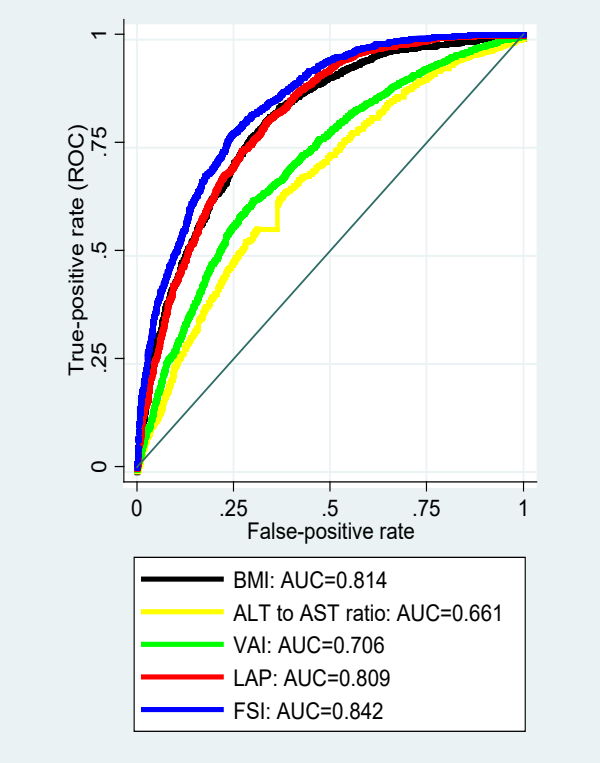
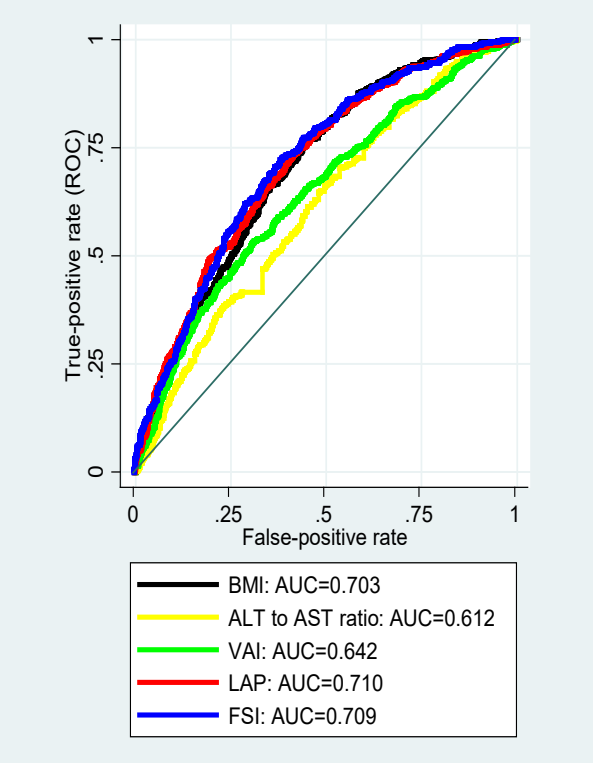


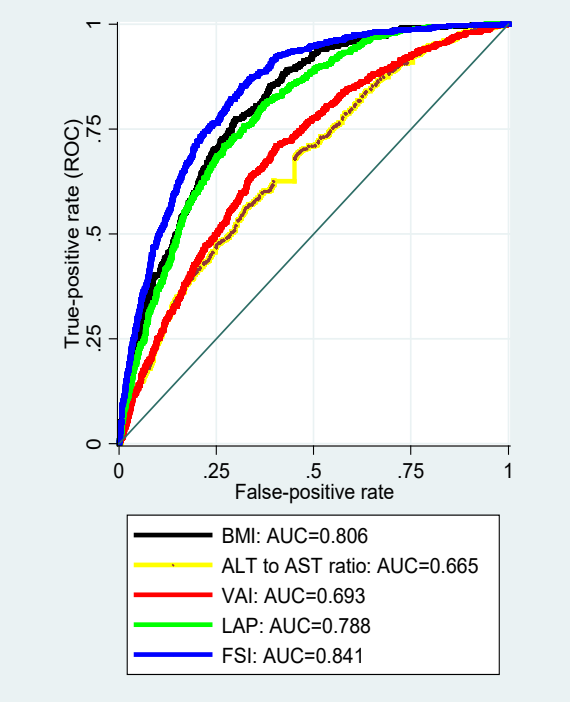
Figure 1: A schematic diagram of the study participants and exclusions



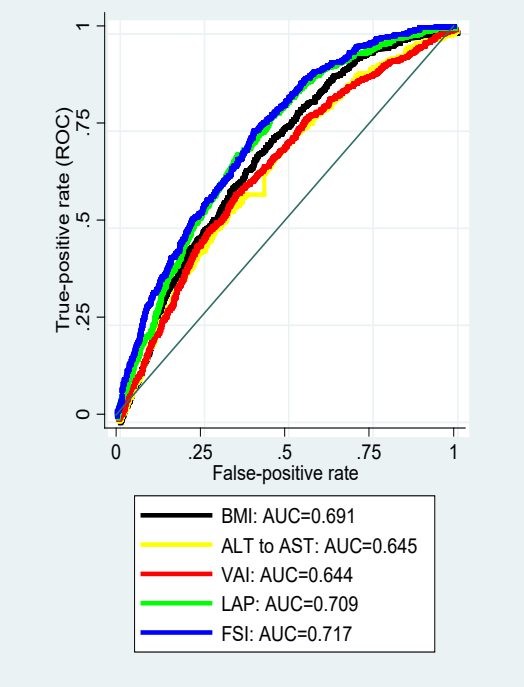
Discriminatory ability of FSI for diagnosis of grade 2 and 3 of NAFLD (n=4670)



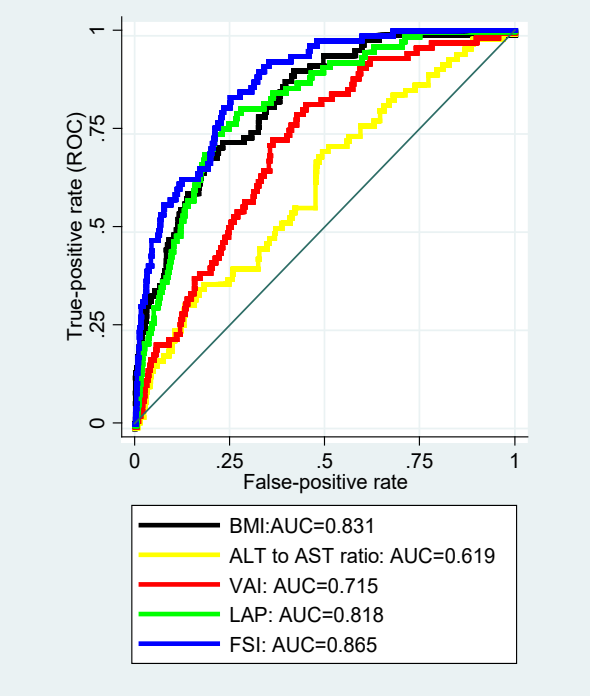
Predictive ability of FSI (in phase 1) for new cases of NAFLD in phase 2 (n=2216)



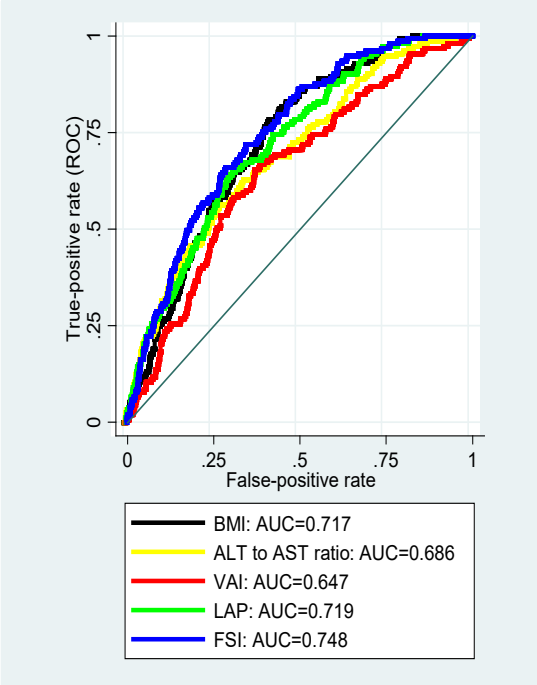
Discriminatory ability of FSI for diagnosis of of grade 2 and 3 of NAFLD (n=4670)



Predictive ability of FSI (in phase 1) for new cases of grade 2 and 3 of NAFLD in phase 2 (n=2216)



Discriminatory ability of FSI for diagnosis of of grade 3 of NAFLD (n n=4670)



Predictive ability of FSI (in phase 1) for new cases of grade 3 of NAFLD in phase 2 (n=2216)

Figure 2: **Related ROC cirves for FSI, LAP, VAI, BMI and ALT to AST ratios for the discrimination and the prediction of the occurrence of new cases of NAFLD and its' grades at the first/second step of cohort**

ALT: denotes Alanine aminotransferase; AST: Aspartate aminotransferase; AUC: Area under the curve; BMI: Body Mass Index; FSI: Framingham Steatosis Index; LAP: lipid accumulation product; ROC: receiver operating characteristic; VAI: visceral adiposity index