MAFLD identifies patients with significant hepatic fibrosis better than NAFLD

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Abbreviations: MAFLD, metabolic associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; SWE, shear wave elastography; BMI, body mass index; WC, waist circumference; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; TG, triglycerides; HDL-cholesterol, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; APRI, AST to Platelet Ratio Index; kPa, kilopascals; OR, odds ratios; CI, confidence intervals.

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Abstract

Background & Aims: Diagnostic criteria for metabolic associated fatty liver disease (MAFLD) have been proposed, but not validated. We aimed to compare the diagnostic accuracy of the MAFLD definition versus the existing NAFLD criteria to identify patients with significant fibrosis and to characterize the impact of mild alcohol intake.

Methods: We enrolled 765 Japanese patients with fatty liver (median age 54 years). MAFLD and NAFLD were diagnosed in 79.6% and 70.7% of patients, respectively. Significant fibrosis was defined by FIB-4 index ≥1.3 and liver stiffness ≥6.6 kPa using shear wave elastography. Mild alcohol intake was defined as <20 gms/day. Factors associated with significant fibrosis were analyzed by logistic regression and decision-tree analyses.

Results: Liver stiffness was higher in MAFLD compared to NAFLD (7.7 vs. 6.8 kPa, P=0.0010). In logistic regression, MAFLD (OR 4.401; 95%CI 2.144–10.629; P<.0001), alcohol intake (OR 1.761; 95%CI 1.081–2.853; P=0.0234), and NAFLD (OR 1.721; 95%CI 1.009–2.951; P=0.0463) were independently associated with significant fibrosis. By decision-tree analysis, MAFLD, but not NAFLD or alcohol consumption was the initial classifier for significant fibrosis. The sensitivity for detecting significant fibrosis was higher for MAFLD than NAFLD (93.9% vs. 73.0%). In patients with MAFLD, even mild alcohol intake was associated with an increase in the prevalence of significant fibrosis (25.0% vs. 15.5%; P=0.0181).

Conclusions: The MAFLD definition better identifies a group with fatty liver and significant fibrosis evaluated by non-invasive tests. Moreover, in patients with

MAFLD, even mild alcohol consumption is associated with worsening of hepatic fibrosis measures.

Keywords: steatosis, metabolic associated fatty liver disease, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, significant hepatic fibrosis, alcoholic intake

Lay summary

- The MAFLD definition was more suitable than the NAFLD definition to identify patients with significant fibrosis as evaluated by non-invasive tests.
- Overweight/obesity *per se* was associated with a risk for significant liver fibrosis in patients with fatty liver.
- The prevalence of significant fibrosis was greater in lean patients with fatty liver and ≥2 metabolic risk abnormalities.
- Even mild alcohol consumption was associated with worsening of hepatic fibrosis measures in patients with MAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the commonest global liver disease and affects about a quarter of the population.[1] The rise in NAFLD prevalence is fueled by rapid increases in the prevalence of poor metabolic health even in individuals with normal weight, and relates to modern patterns of excess consumption of poor quality foods and reduced physical activity.[1]

An international panel has recently proposed a new definition of fatty liver, metabolic associated fatty liver disease (MAFLD), which is based on a set of positive diagnostic criteria for fatty liver disease associated with metabolic dysfunction.[2] These criteria shift the diagnostic burden from one of exclusion to one of inclusion and are based on evidence of fatty liver in addition to one of 1) overweight/obesity, 2) presence of metabolic dysregulation with at least two risk features, or 3) the presence of type 2 diabetes mellitus (T2DM).[2] Validation of these criteria and their utility in real-world cohorts is imperative.

There is abundant evidence that fibrosis is the major determinant of adverse outcomes in patients with MAFLD.[3, 4] Hence, early and accurate identification of patients with significant fibrosis is essential. Stemming from this, it is pivotal to evaluate whether the new definition identifies patients with significant fibrosis, at least as well as the previous NAFLD criteria.

A second aspect that requires clarification and is important for clinical management is the impact of mild amounts of alcohol intake (<20 gms/day) on the severity of liver disease. An implicit assumption of the NAFLD definition is that alcohol consumption <20 gms/day in women and <30 gms/day in men does not meaningfully impact liver disease progression. On this basis, gender-based

intakes of 20-30 gms of alcohol a day are permissible and consistent with a diagnosis of NAFLD.[5] In contrast, the MAFLD definition is not based on alcohol intake and thus allows for a fresh examination of the impact of mild amounts of alcohol on liver disease.[6-9]

In this work on a prospectively enrolled large (n=765) cohort of patients, we evaluated the 1) diagnostic accuracy of the MAFLD and NAFLD definitions to identify patients with significant fibrosis evaluated by non-invasive tests, and 2) characterized the impact of mild alcohol intake on fibrosis severity.

Patients and Methods

Study design and ethics

This study was designed as a single-center, observational cohort study in Japan. The protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by prior approval from the institutional review board of Kurume University School of Medicine (ID 20092). This research was performed in accordance with relevant guidelines and regulations. An opt-out approach was used to obtain informed consent from patients and personal information was protected during data collection.

Study population and selection of patients for analysis

We enrolled 2,442 consecutive health check examinees who visited the Saga Health and Clinical Examination Center in Japan from May 2017 to December 2019 (Supplementary Figure 1). All patients were of Japanese ancestry and underwent abdominal ultrasonography as part of their clinical review. We excluded 1,174 participants because fatty liver was not evident on sonography; 1,268 participants with fatty liver were thus included. Of these, 320 participants were excluded because of duplicate records (n=143), a lack of data for a diagnosis of MAFLD (n=131), platelet count (n=10), or alcohol consumption (n=15). Patients with hepatitis B virus infection (n=5), hepatitis C virus infection (n=4), and ≥ 60 gms/day alcohol consumption (n=12) were also excluded. In the remaining 948 non-overlapping participants, liver stiffness was evaluated by shear wave elastography (SWE). Of these, 183 were excluded because of unreliable SWE measurements (interquartile range >30%). The

study cohort thus comprised 765 individuals (Supplementary Figure 1).

Data collection

All data were collected prospectively at the time of the medical check-up. The following information was obtained using a self-reported questionnaire: age, sex, exercise habits (<6,000 or ≥6,000 steps/day), sleep disturbance, comorbidity, and medication use. At the clinical review, we obtained the following data: body mass index (BMI), waist circumference (WC), blood pressure, presence/absence of T2DM, hypertension, and dyslipidemia; these were diagnosed according to standard criteria. [2, 10-12] We also obtained the data for current alcohol intake. Alcohol intake habit was defined as intake of 1-59 gms/day alcohol. Mild alcohol intake was defined as alcohol intake less than 20 gms/day (i.e., not more than one drink a day).

Biochemical analysis

Patients fasted overnight before collection of a blood sample for the following tests: full blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, γ-glutamyl transpeptidase (GGT), lactate dehydrogenase, total protein, albumin, total bilirubin, total cholesterol, triglycerides (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, amylase, blood urea nitrogen, creatinine, estimated glomerular filtration rate, C-reactive protein, uric acid, electrolytes, fasting glucose, hemoglobin A1c (HbA1c) and cholinesterase.

Diagnosis of fatty liver

The diagnosis of fatty liver was based on the following at abdominal ultrasonography: increased hepato-renal contrast, increased echogenicity of liver parenchyma, unclear visualization of the intrahepatic vessels, and/or impaired visualization of the diaphragm as previously described.[13]

Diagnosis of NAFLD and MAFLD

A diagnosis of NAFLD was according to the EASL-EASD-EASO and American Association for the Study of Liver Diseases Clinical Practice Guidelines for the Management of NAFLD[5, 14]: (1) fatty liver by abdominal ultrasonography, (2) alcohol consumption no more than 30 gms/day for men and 20 gms/day for women, and (3) no competing etiologies for fatty liver or coexisting causes of chronic liver disease.[5, 14]

MAFLD was diagnosed according to the criteria proposed by an international expert panel.[2] The criteria include evidence of fatty liver (hereby ultrasonography), in addition to one of the following: overweight/obesity, presence of T2DM, or lean/normal weight with evidence of metabolic dysregulation. Overweight was defined as BMI ≥23 kg/m^2 in this Asian cohort and T2DM was defined as HbA1c ≥6.5% or specific drug treatment. Metabolic dysregulation was defined as the presence of at least two metabolic risk abnormalities: 1) WC ≥90/80 cm in men and women, respectively, 2) blood pressure ≥130 mmHg or specific drug treatment, 3) plasma TG ≥150 mg/dL or specific drug treatment, 4) plasma HDL-cholesterol <40 mg/dL for men and <50 mg/dL for women or specific drug treatment, and 5) prediabetes (fasting glucose

levels 100 to 125 mg/dL or HbA1c 5.7%-6.4%).[2] Since all patients were Japanese, BMI and WC were evaluated using cut-off values for Asians.[2] Although homeostasis model assessment-insulin resistance score and plasma high-sensitivity C-reactive protein level are metabolic risk abnormalities,[2] these were not available in our dataset.

Calculation of Fatty liver index, APRI, NAFLD fibrosis score, and FIB-4 index

Fatty liver index was calculated using BMI, WC, and serum levels of TG and GGT as previously described.[15] AST to platelet ratio index (APRI) was calculated using serum levels of AST and platelet count as previously described.[16] NAFLD fibrosis score was calculated using age, BMI, the presence of impaired fasting glucose or diabetes, platelet count, and serum levels of AST, ALT, and albumin as previously described.[17] FIB-4 index was calculated using age, serum levels of AST, ALT, and platelet count as previously described.[18]

Shear wave elastography

To measure liver stiffness, 2 dimensional-SWE was employed during the ultrasound examination using a LOGIQ S8 with the R3.1.9 software and the C1-6-D abdominal convex probe (GE Healthcare, Wauwatosa, WI) as previously described.[13] Briefly, liver stiffness measurements were performed by three sonographers blinded to the clinical data. Three valid SWE measurements were performed on each patient and the median value was calculated based on the European Federation of Societies for Ultrasound in Medicine and Biology Guidelines and Recommendations on the Clinical Use of Liver Ultrasound

Elastography.[19] The SWE measurement was expressed in kilopascals (kPa). Invalid results were defined as an interquartile range/median value >30% as recommended by the above society.[19]

Definition of significant hepatic fibrosis

According to the algorithm of a previous study,[20] liver fibrosis was assessed in the following two steps: 1) assessment of FIB-4 index and 2) elastography. Significant fibrosis was defined by FIB-4 index ≥1.3 and liver stiffness ≥6.6 kPa using SWE; this corresponds to ≥F2 fibrosis stage.[13, 20]

Effects of alcoholic consumption on Fatty liver index, APRI, NAFLD fibrosis score, FIB-4 index, and liver stiffness in patients with MAFLD

In the analysis for the evaluation of alcohol consumption on biochemical parameters and hepatic fibrosis measures, participants with MAFLD were classified into MAFLD with no alcohol consumption (0 gm/day) and those with 1-59 gms/day alcohol consumption. In a subset analysis, we examined the effects of mild alcohol consumption (<20 gms/day and within the threshold to define NAFLD) on hepatic fibrosis indices.

Statistical analysis

Continuous variables are expressed as median and range or number. Categorical variables are expressed as frequencies and percentages. The differences between groups were analyzed using the Wilcoxon rank-sum test for continuous variables and the Fisher's exact test for categorical variables. A

logistic regression model was used to identify independent factors associated with significant hepatic fibrosis. Explanatory variables were selected stepwise minimizing the Bayesian information criterion, as previously described.[21] Data were expressed as odds ratios (OR) and 95% confidence intervals (CI). A decision-tree algorithm was constructed to reveal profiles associated with significant hepatic fibrosis, as previously described.[21] The performance of MAFLD and NAFLD diagnosis for detection of significant fibrosis and for the exclusion of non-significant fibrosis were also evaluated by sensitivity, specificity, accuracy, positive predictive value, negative predictive value. $P<0.05$ was considered to indicate statistical significance. Data were analyzed using the JMP Pro14 (SAS Institute Inc., Cary, NC).

Results

Patient characteristics

The participant characteristics are summarized in Supplementary Table 1. The median age was 54 years and women represented 54% of the cohort. The median BMI was 24.1 kg/m^2, while the percentage with large WC was 63.0% of the participants. The percent of participants with T2DM, hypertension, and dyslipidemia were 11.6%, 33.6%, and 39.2% of the participants, respectively. The percent of participants with alcohol intake was 50.7% and mild alcohol (<20 gms/day) intake was seen in 21.4% (Supplementary Table 1). The median Fatty liver index, APRI, and NAFLD fibrosis scores were 29, 0.3, and -2.050, respectively. Significant hepatic fibrosis evaluated by FIB-4 index and liver stiffness was observed in 15.0% of the participants (Supplementary Table 1).

Difference in characteristics and fibrosis indices using the NAFLD and MAFLD definition

NAFLD and MAFLD were present in 70.7% and 79.6% of all participants (n=765), respectively. Patients overlapping NAFLD and MAFLD comprised 55.4% (424/765) of all participants. Non-overlapping NAFLD and MAFLD was observed in 15.3% (117/765) and 24.2% (185/765) of all participants, respectively (Figure 1). Clinical and biochemical characteristics of patients with NAFLD and MAFLD are depicted in Table 1. MAFLD patients were more likely to be male and had higher BMI and WC, and a worse metabolic profile, including significantly higher frequencies of hypertension, and

dyslipidemia as well as higher serum levels of creatinine and uric acid compared to their NAFLD counterparts. Participants with MAFLD had higher serum liver enzymes (AST, ALT, GGT), Fatty liver index, and fibrosis scores including APRI and NAFLD fibrosis score compared to NAFLD patients (Table 1). Similarly, liver stiffness was higher in the MAFLD group (Table 1). Moreover, in the comparison between non-overlapping MAFLD and non-overlapping NAFLD patients, there were significant elevations in Fatty liver index, APRI, NAFLD fibrosis score, and liver stiffness in the non-overlapping MAFLD patients compared to the non-overlapping NAFLD group (Table 2).

Independent factors and profiles associated with significant hepatic fibrosis

To adjust for confounding, in a subsequent analysis, we compared MAFLD and NAFLD definitions for associations with significant hepatic fibrosis evaluated by FIB-4 index and liver stiffness, using multiple logistic regression analysis. In this analysis, the association was significantly stronger for MAFLD (OR 4.401; 95%CI 2.144–10.629; P<.0001) than NAFLD (OR 1.721; 95%CI 1.009–2.951; P=0.0463). Alcohol intake habit (<60 gms/day) was also identified as an independent risk factor for significant fibrosis in this analysis (OR 1.761; 95%CI 1.081–2.853; P=0.0234), though again with lesser association compared to MAFLD (Figure 2A). Moreover, we performed subgroup analysis according to the amount of alcohol intake (<20 gms/day). The presence of MAFLD (OR 4.798; 95%CI 2.078–13.935; P<.0001) remained an independent factor associated with significant fibrosis, and the odds ratio was higher than in those with <20 gms/day of alcohol consumption (OR 1.757; 95%CI 1.077–2.846;

P=0.0242) (Supplementary Figure 2A).

A decision tree classifier approach is a valuable data mining analysis to reveal a series of classification rules by identifying priorities. In addition, this method overcomes the constraints of linear models in handling highly skewed clinical data, and is well suited to analyze data with high degrees of collinearity between variables.[22] Hence, we next compared MAFLD and NAFLD for associations with significant hepatic fibrosis adopting a decision-tree algorithm (Figure 2B). Notably, MAFLD was selected as the most important classifier for significant fibrosis. This was followed by alcohol intake, in order of importance, while NAFLD was not a predictor during pruning (Figure 2B). Thus, MAFLD has significantly higher predictive value for identifying patients with fatty liver disease and significant fibrosis compared to NAFLD, independent of alcohol intake and other confounding factors. In addition, the presence of MAFLD was also the most important classifier associated with significant fibrosis in sub-analysis according to <20 gms/day alcohol consumption (Supplementary Figure 2B).

Performance of MAFLD and NAFLD criteria for the identification of significant fibrosis and for the exclusion of non-significant fibrosis

To further evaluate the MAFLD and NAFLD definitions, we explored their performance for the identification of significant fibrosis evaluated by FIB-4 index and liver stiffness. In this cohort, 115 patients had significant fibrosis. The MAFLD criteria identified 24 (20.87%) additional patients with significant fibrosis (n=108) compared with the NAFLD definition (n=84). The MAFLD criteria had a

higher sensitivity (93.9% vs. 73.0%) and negative predictive value (95.5 vs. 86.2%) than the NAFLD definition (Table 3). Moreover, the sensitivity and negative predictive values of MAFLD were more than 90% in the sub-analyses according to <20 gms/day and 0 gms/day of alcohol consumption (Table 3).

Characteristics of patients with subgroups of MAFLD

Having established the validity of the MAFLD definition and its ability to capture patients with both severe metabolic and liver injury, we focused on the various MAFLD subgroups. MAFLD prevalence among overweight/obesity, lean/normal weight with metabolic dysregulation, T2DM, and overweight/obesity plus T2DM groups were 67.2%, 18.2%, 3.4%, and 11.2%, respectively (Figure 3A). Large WC and prediabetes were identified as major metabolic abnormalities in both the overweight/obesity group and the lean/normal weight with metabolic dysregulation group (Figure 3A).

According to the increase in the number of metabolic abnormalities, there was an increase in the prevalence of significant fibrosis in overweight/obesity MAFLD patients (Figure 3B). However, only 2% of the overweight/obesity MAFLD patients showed no metabolic abnormalities; 98% were accompanied with at least one metabolic comorbidity (Figure 3C).

The prevalence of significant fibrosis was higher in the lean/normal weight patients with ≥2 metabolic abnormalities compared to those with <2 metabolic abnormalities (Figure 3D, p=0.0006). Thus, among patients with MAFLD lean/normal weight with metabolic dysregulation, the risk of significant fibrosis increases stepwise with more risk-factor variables.

Differences in characteristics and fibrosis indices between MAFLD and non-MAFLD with fatty liver

The diagnosis of NAFLD based on exclusion criteria contributes to the well-known heterogeneity of the disease and impacts both management and clinical trial outcomes. MAFLD overcomes this limitation and helps to define a more homogenous group of patients. Hence, we explored the difference in characteristics and fibrosis indices between MAFLD and those not meeting the MAFLD definition (non-MAFLD with fatty liver). Consistently, MAFLD patients were older, more likely to be male, had higher BMI and WC, and worse metabolic profiles, including higher creatinine and uric acid levels compared to those with fatty liver without MAFLD (Supplementary Table 2). In addition, the MAFLD cohort had higher serum levels of AST, ALT, and GGT compared to non-MAFLD with fatty liver (Supplementary Table 2). Similarly, the Fatty liver index, APRI, NAFLD fibrosis score, and liver stiffness measurements were higher in MAFLD than in the non-MAFLD fatty liver group (Supplementary Table 2; $p < 0.05$).

Differences in characteristics and fibrosis indices between MAFLD with no-alcohol consumption and MAFLD with alcohol consumption

The effects of alcohol consumption on hepatic fibrosis remain unclear in real-world populations. Thus, we determined differences in characteristics and fibrosis indices between patients with MAFLD with no alcohol consumption (0 gms/day) and those with MAFLD and alcohol consumption (1-59 gms/day).

In this analysis, there was no difference in age, BMI, blood pressure, HDL-cholesterol, and TG levels between the two groups; however, patients with MAFLD and alcohol consumption (1-59 gms/day) were more likely to be male and to have higher fasting blood glucose, creatinine, and uric acid levels compared to those with MAFLD and no alcohol consumption. The WC and HbA1c were higher in MAFLD with no alcohol consumption compared to MAFLD and alcohol consumption (1-59 gms/day) (Supplementary Table 3). As expected, serum levels of AST and GGT were higher in the context of alcohol consumption compared to those with MAFLD and no alcohol consumption (Supplementary Table 3).

Finally, we undertook a sub-analysis comparing the differences in fibrosis indices between patients with MAFLD (no alcohol consumption [0 gm/day]) and those with MAFLD and mild alcohol consumption (<20 gms/day) (Figure 4). Consistently, elevations in NAFLD fibrosis score and the FIB-4 index were observed in patients with MAFLD and alcohol consumption (<20 gms/day) compared to those with MAFLD and no alcohol consumption (Figure 4B and 4C).

Discussion

We investigated the application of the MAFLD definition for fatty liver associated with metabolic dysfunction in clinical practice. The principal finding was that MAFLD has better ability (~20% higher) to identify patients with significant fibrosis than the NAFLD definition (Figure 5). In addition, even mild alcohol use was associated with higher fibrosis scores that would not have been evident using the NAFLD definition.

NAFLD is a heterogeneous group of patients under one umbrella because all patients without other liver diseases and who have liver fat are included. In fact, no metabolic dysfunction was seen in 21.6% (117/541) of patients with NAFLD in this study. This heterogeneity has negative implications for both management and for clinical trials.[23, 24] In contrast, we showed that MAFLD identifies a homogeneous group and seems to be efficient in identifying a group of at high-risk patients.

In this study, MAFLD was superior to the NAFLD definition for predicting fibrosis at-risk patients across a range of analyses. Lin et al., previously demonstrated that FIB-4 index and NAFLD fibrosis score were higher in MAFLD compared to NAFLD by univariate analysis using the third National Health and Nutrition Examination Surveys database of the United States (1988-1994).[25] They also suggested that the MAFLD definition is more practical for identifying at high-risk patients.[25] Though our studies are in good agreement, we used a more recent database (2017-2019) from Asia, that included liver stiffness measurement by SWE. We believe that our detailed analyses, including multivariable analysis, data-mining approaches and diagnostic performance in a

different population provides additional robust evidence for the superiority of the MAFLD definition.

Exploring the patients diagnosed using the MAFLD definition, overweight/obesity led to the fulfilment of the diagnostic criteria in 67% of the cohort. Nearly all of them (98%) had at least one metabolic dysfunction feature, with large WC and prediabetes being the commonest. A recent large Korean (n=648) cohort study demonstrated that BMI was positively associated with worsening of hepatic fibrosis regardless of metabolic health status in patients with NAFLD.[26] Taken together, it seems to be reasonable to include overweight/obesity as a sole risk criteria in the MAFLD definition.

MAFLD-Lean/normal weight patients account for approximately 20% of MAFLD, coming as the second commonest subgroup in this Japanese cohort. Notably, no significant differences were observed in the prevalence of significant fibrosis between overweight/obesity and lean/normal weight patients with MAFLD. These findings are in agreement with the previous studies. A recent meta-analysis has suggested that ~40% of the metabolic fatty liver population are not obese, indicating the importance of considering metabolic health status rather than focusing on BMI.[27, 28] Kim et al., further reported that non-obese NAFLD patients with metabolic syndrome have a similar degree of hepatic fibrosis compared to obese NAFLD patients.[29] However the definition of ‘metabolic abnormalities’ was unclear. The consensus panel proposed that the presence of at least two metabolic risk abnormalities is a criterion for MAFLD [2] and our results demonstrated the validity of the definition. Wong et al. have reported that the numbers of metabolic abnormalities are associated with a

higher risk of advanced fibrosis in fatty liver patients.[30] In this regard, a study has demonstrated that patients with T2DM who had five risk-factor variables within the recommended target range have little or no excess risk of death or complications as compared to the general population.[31] Stretching the analogy, the MAFLD definition would provide clinicians with a holistic person- and management-centered view of MAFLD.

The MAFLD definition is not based on alcohol intake and thus allowed us to examine the impact of mild amounts of alcohol on liver disease. In the decision-tree analysis, we identified that even <20 gms/day of alcohol intake was the second classifier for significant fibrosis. In addition, NAFLD fibrosis score and FIB-4 index were significantly higher in the MAFLD group (<20 gms/day alcohol intake) compared to those with MAFLD and no alcohol intake (0 gm/day). The Global Burden of Disease Study 2016 collaborators performed a meta-analysis of 592 studies and 694 data sources of alcohol consumption (28 million individuals and 649,000 registered cases for respective outcomes).[32] They demonstrated that there was no safe limit of alcohol consumption for death and disability-adjusted life-years.[32] Consistently, a recent Korean cohort study demonstrated that 1-9.9 gms/day of alcohol intake is associated with an increased risk of worsening hepatic fibrosis indices in patients with NAFLD.[33] Adding to these data, an interaction between the presence of metabolic dysfunction and even mild alcohol intake on the risk of advanced hepatic fibrosis has been reported.[34, 35]

There are some limitations to this study. First, this study was conducted in a single-center in Japan and further validation studies are required. Second,

we did not perform liver biopsy in this study and our study is based on non-invasive tests. We wish to note that the scores are well validated across various cohorts and ethnicities [13, 20] and are incorporated in clinical guidelines.[5, 14] In addition, our adopted algorithm is well validated.[13, 20] Third, the number of patients with MAFLD and T2DM is small and, therefore, we could not evaluate this subgroup in more detail.

There are advantages to the new MAFLD criteria. The criteria allows for assessment of the relative contributions of different etiologies to outcome (for example by comparing MAFLD with chronic hepatitis C versus hepatitis C alone). Such assessments are not possible using the NAFLD criteria as the latter would have been classified as having hepatitis C. Unfortunately, we could not assess this issue in our study because of the small number of patients with viral hepatitis (who were thus excluded). Further studies should focus on the impact of dual aetiology of viral hepatitis and MAFLD on hepatic fibrosis.

In conclusion, the MAFLD definition outperforms NAFLD in identifying a homogenous group of at-high risk patients with metabolic dysfunction and significant hepatic fibrosis evaluated by non-invasive tests. The association with fibrosis increases with accumulating metabolic risk. As even mild alcohol intake is associated with worsening of hepatic fibrosis measures, patients with MAFLD should be advised to limit alcohol consumption.

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Figure legends

Figure 1. The population of MAFLD, NAFLD, and non-MAFLD/non-NAFLD. The Venn diagram indicates the proportion of patients with NAFLD (gray) and patients with MAFLD (blue).

Figure 2. Independent factors and profiles associated with significant hepatic fibrosis. Significant fibrosis was evaluated by FIB-4 index and liver stiffness. (A) Independent factors for significant hepatic fibrosis analyzed by logistic regression analysis, (B) Profiles for significant hepatic fibrosis analyzed by decision-tree analysis. The pie graphs indicate the proportion of patients with significant hepatic fibrosis (black) and patients with Normal-Mild hepatic fibrosis (white).

Figure 3. Characteristics of patients in subgroups of MAFLD. (A: bar graph) Prevalence of each subgroup of MAFLD and (A: table) comorbid metabolic abnormalities and prevalence of significant hepatic fibrosis in each subgroup of MAFLD. Significant fibrosis was evaluated by FIB-4 index and liver stiffness, (B) The prevalence of significant hepatic fibrosis in overweight/obesity MAFLD patients with 0, 1, or ≥2 metabolic abnormalities. (C) number of metabolic abnormalities in the subgroup of MAFLD overweight/obesity, (D) the difference in significant hepatic fibrosis between the lean/normal weight patients with ≥2 metabolic abnormalities and those with <2 metabolic abnormalities.

Figure 4. Sub-analysis for the differences in fibrosis indices between MAFLD

with no alcohol consumption and MAFLD with mild alcohol consumption (<20 gms/day). (A) APRI, (B) NAFLD fibrosis score, (C) FIB-4 index, (D) liver stiffness, (E) FIB-4 index + liver stiffness. Abbreviations: APRI, aspartate aminotransferase to platelet ratio index; MAFLD, metabolic associated fatty liver disease; N.S., not significant; NAFLD, non-alcoholic fatty liver disease; FIB-4, fibrosis-4.

Figure 5. Graphical summary. Performance of MAFLD and NAFLD definitions for the detection of significant fibrosis and for the exclusion of non-significant fibrosis. Significant fibrosis was evaluated by FIB-4 index and liver stiffness. Abbreviations: MAFLD, metabolic associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; NPV, negative predictive value.

Supplementary Figure 1. Study populations. Abbreviations: MAFLD, metabolic associated fatty liver disease.

Supplementary Figure 2. Sub-analysis for independent factors and profiles associated with significant hepatic fibrosis in subjects with <20 gms/day alcohol consumption. (A) Independent factors for significant hepatic fibrosis analyzed by logistic regression analysis, (B) Profiles for significant hepatic fibrosis analyzed by decision-tree analysis. The pie graphs indicate the proportion of patients with significant hepatic fibrosis (black) and patients with normal-mild hepatic fibrosis (white).

Figure 1

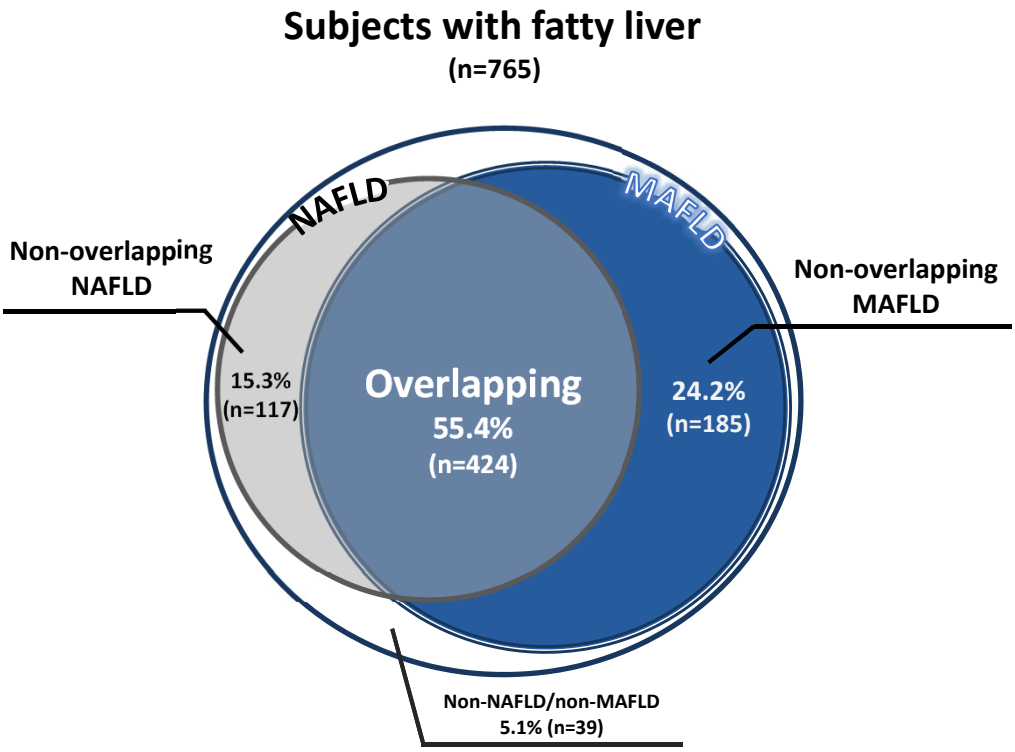
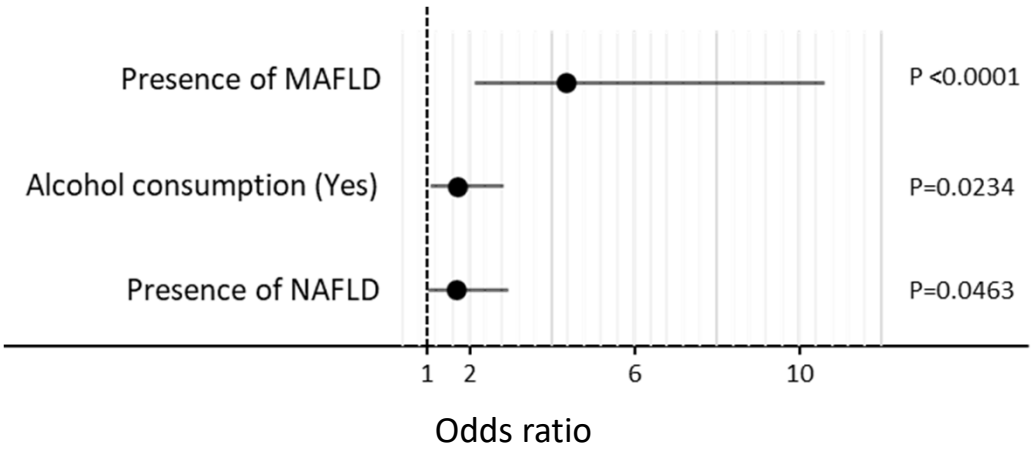

Subjects with fatty liver
(n=765)
NAFLD
MAFLD
Non-overlapping
NAFLD
15.3%
(n=117)
Overlapping
55.4%
(n=424)
24.2%
(n=185)
Non-overlapping
MAFLD
Non-NAFLD/non-MAFLD
5.1% (n=39)

Figure 2

A


Presence of MAFLD
P <0.0001
Alcohol consumption (Yes)
P=0.0234
Presence of NAFLD
P=0.0463
1
2
6
10
Odds ratio

B

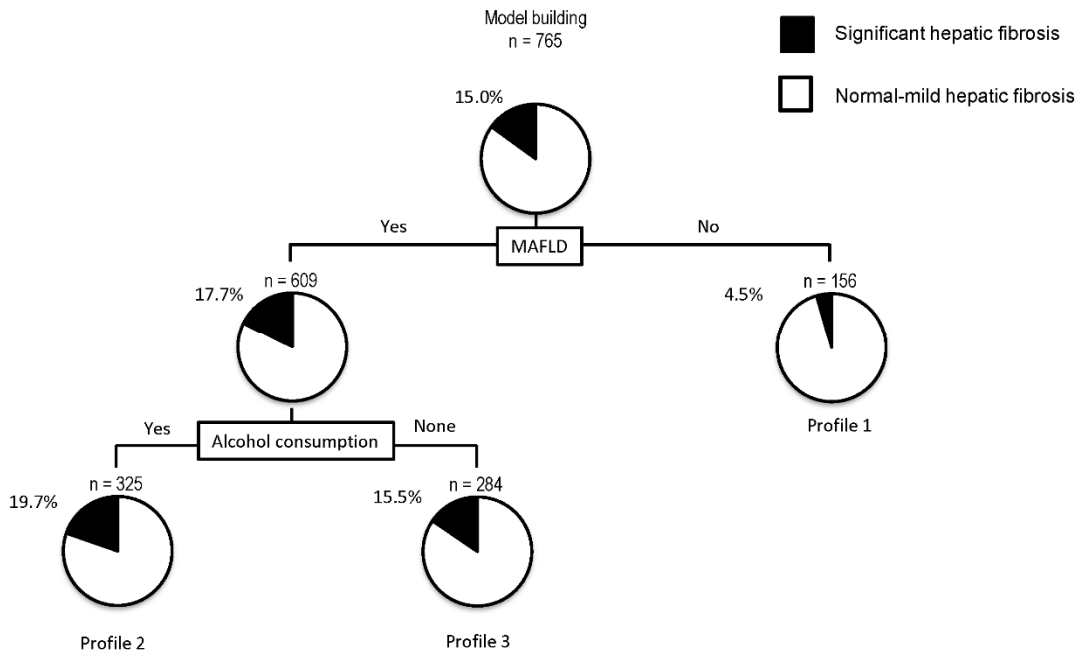
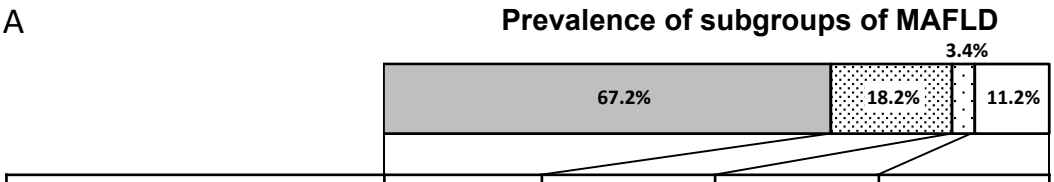

Model building
n = 765
Significant hepatic fibrosis
Normal-mild hepatic fibrosis
15.0%
Yes
MAFLD
No
17.7%
n = 609
4.5%
n = 156
Yes
Alcohol consumption
None
Profile 1
19.7%
n = 325
15.5%
n = 284
Profile 2
Profile 3

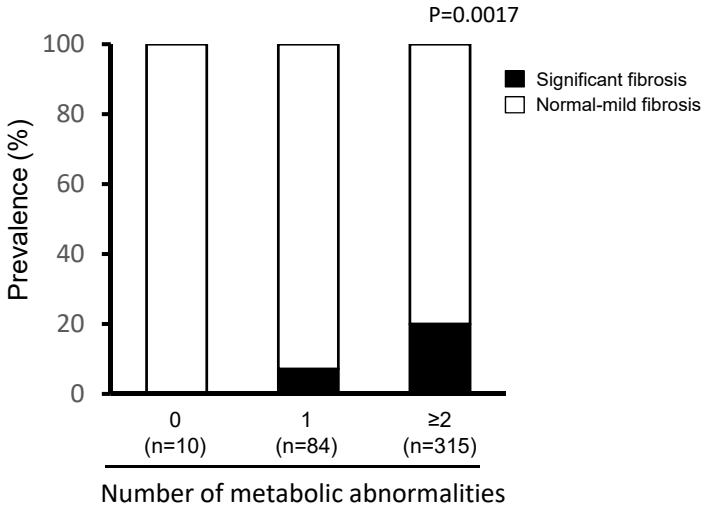
Figure 3

A

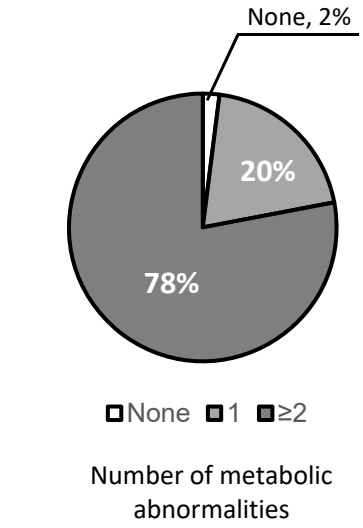


	Overweight/ Obesity (n=409)	Lean/Normal weight (n=111)	T2DM (n=21)	Overweight/ Obesity+T2DM (n=68)
Prevalence of metabolic abnormalities				
Waist circumference	79.0%	61.3%	52.4%	79.1%
Hypertension	35.7%	38.7%	61.9%	73.5%
Hypertriglyceridemia	39.6%	46.8%	33.3%	64.7%
Depressed HDL cholesterol	27.4%	38.7%	38.1%	51.5%
Prediabetes	69.9%	83.8%		
Prevalence of significant fibrosis	16.9%	17.1%	14.3%	25.0%

B



C



D

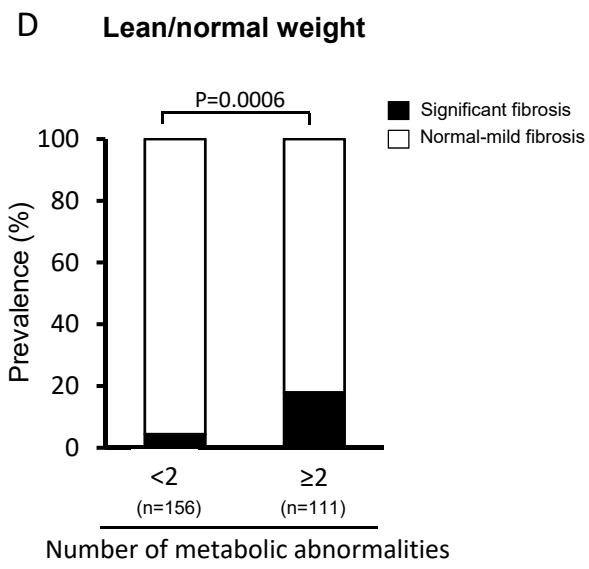


Figure 4

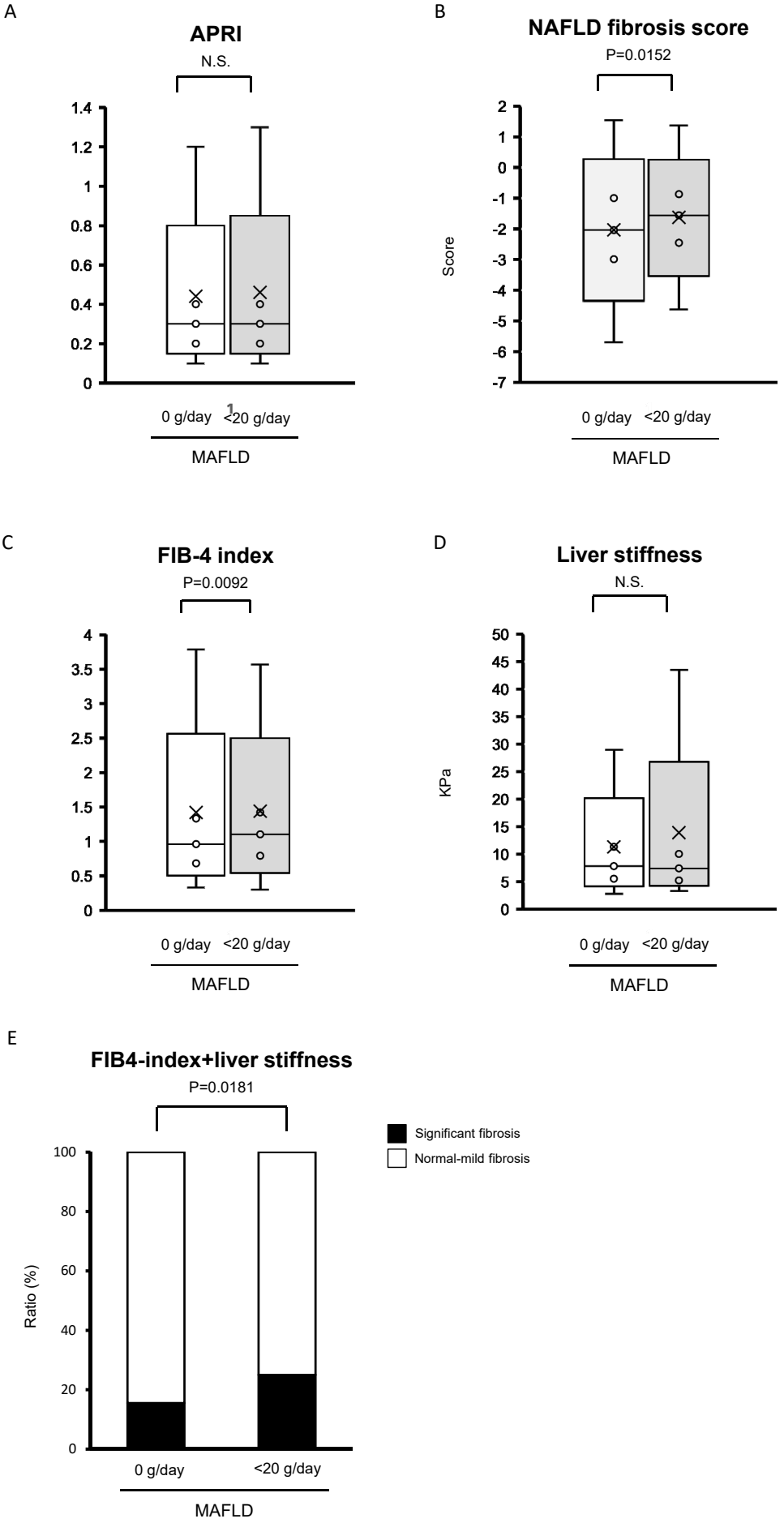

A
APRI
N.S.
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 g/day
<20 g/day
MAFLD
B
NAFLD fibrosis score
P=0.0152
Score
2
1
0
-1
-2
-3
-4
-5
-6
-7
0 g/day
<20 g/day
MAFLD
C
FIB-4 index
P=0.0092
4
3.5
3
2.5
2
1.5
1
0.5
0
0 g/day
<20 g/day
MAFLD
D
Liver stiffness
N.S.
KPa
50
45
40
35
30
25
20
15
10
5
0
0 g/day
<20 g/day
MAFLD
E
FIB4-index+liver stiffness
P=0.0181
Significant fibrosis
Normal-mild fibrosis
Ratio (%)
100
80
60
40
20
0
0 g/day
<20 g/day
MAFLD

Figure 5

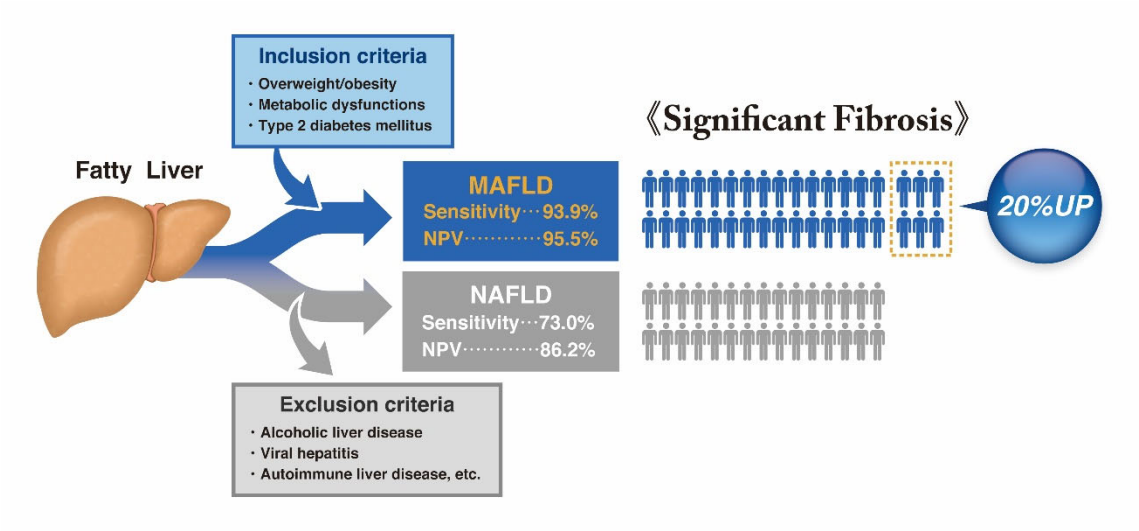

Inclusion criteria
・Overweight/obesity
・Metabolic dysfunctions
・Type 2 diabetes mellitus
Fatty Liver
MAFLD
Sensitivity···93.9%
NPV·····95.5%
NAFLD
Sensitivity···73.0%
NPV·····86.2%
Exclusion criteria
・Alcoholic liver disease
・Viral hepatitis
・Autoimmune liver disease, etc.
《Significant Fibrosis》
20%UP

Table 1. Comparison of patients' characteristic between the NAFLD and MAFLD

	NAFLD		MAFLD		
	Median (IQR)	Range (min–max)	Median (IQR)	Range (min–max)	P
Number	70.7% (541/765)	N/A	79.6% (609/765)	N/A	N/A
Age (years)	55 (47–63)	23–82	56 (49–63)	23–82	0.1225
Sex (female/male)	66.2%/33.8% (358/183)	N/A	49.4%/50.6% (301/308)	N/A	<.0001
Body mass index (kg/m^2)	24.0 (21.7–26.1)	15.9–41.1	25.0 (23.2–26.9)	18.4–42.1	<.0001
Waist circumference (Large/Normal)	65.6%/34.4% (354/186)	N/A	74.8%/25.2% (455/153)	N/A	0.0006
Systolic blood pressure (mmHg)	117 (105–127)	78–207	121 (112–131)	83–207	<.0001

Type 2 Diabetes mellitus (Presence/Absence)	10.9%/89.1% (59/482)	N/A	14.6%/85.4% (89/520)	N/A	0.0609
Hypertension (Presence/Absence)	29.6%/70.4% (160/381)	N/A	41.4%/58.6% (252/357)	N/A	<.0001
Dyslipidemia (Presence/Absence)	38.8%/61.2% (210/331)	N/A	47.5%/52.5% (289/320)	N/A	0.0032
Alcohol intake habit (None/Yes)	69.7%/30.3% (377/164)	N/A	46.6%/53.4% (284/325)	N/A	<.0001
Daily alcohol intake (0gm/<20gms/20-59gms)	69.7%/30.3%/0% (377/164/0)	N/A	46.6%/23.0%/30.4% (284/140/185)	N/A	<.0001
Steps in a day (<6,000/≥6,000 steps)	73.4%/26.6% (397/144)	N/A	74.4%/25.6% (453/156)	N/A	0.6994
Sleep disturbance (Presence/Absence)	61.7%/38.3% (334/207)	N/A	62.7%/37.3% (382/227)	N/A	0.7300

Non-invasive tests					
Fatty liver index	25 (12–47)	1–96	37 (21–60)	1–99	<.0001
APRI	0.3 (0.2–0.4)	0.1–1.7	0.3 (0.2–0.4)	0.1–2.1	0.0275
NAFLD fibrosis score	-2.070 (-2.971– -1.129)	-5.694–1.534	-1.783 (-2.801– -0.934)	-6.029–1.534	0.0191
FIB-4 index	0.98 (0.71–1.34)	0.30–3.79	0.99 (0.74–1.37)	0.30–3.79	0.5602
Liver stiffness (kPa)	6.8 (5.0–10.0)	2.8–43.5	7.7 (5.5–11.2)	2.8–43.5	0.0010
Biochemical examinations					
Red blood cell count ($\times 10^4$/μL)	459 (430–491)	326–591	468 (439–500)	326–591	0.0001
Hemoglobin (g/dL)	13.7 (12.8–14.8)	7.5–17.6	14.2 (13.2–15.4)	7.5–18.7	<.0001
Hematocrit (%)	41.1 (38.5–43.7)	25.7–52.7	42.3 (39.7–45.1)	25.7–56.2	<.0001
White blood cell count (/μL)	5,100 (4,300–6,100)	2,300–12,900	5,300 (4,500–6,300)	2,900–15,000	0.0641
Platelet count ($\times 10^4$/μL)	24.8 (21.1–28.7)	9.1–52.8	24.8 (21.1–28.7)	9.1–52.8	0.9967

AST (U/L)	20 (16–24)	7–87	21 (17–26)	7–114	0.0022
ALT (U/L)	19 (14–29)	4–122	22 (16–33)	4–188	0.0001
Lactate dehydrogenase (U/L)	171 (154–191)	65–317	172 (157–191)	65–317	0.2988
ALP (U/L)	207 (173–250)	57–520	207 (174–250)	87–520	0.8655
GGT (U/L)	22 (15–36)	6–408	30 (19–52)	9–408	<.0001
Total protein (g/dL)	7.1 (6.9–7.3)	6.1–8.4	7.1 (6.9–7.3)	6.2–8.8	0.1596
Cholinesterase (U/L)	349 (304–393)	196–671	363 (315–404)	208–671	0.0186
Albumin (g/dL)	4.4 (4.2–4.5)	3.7–5.1	4.4 (4.2–4.5)	3.6–5.1	0.1745
Total bilirubin (mg/dL)	0.7 (0.6–0.9)	0.3–2.9	0.7 (0.6–0.9)	0.3–4.6	0.2631
Total cholesterol (mg/dL)	205 (186–232)	125–351	208 (188–234)	125–351	0.3714
HDL cholesterol (mg/dL)	62 (50–71)	28–111	58 (49–69)	28–111	0.0027
LDL cholesterol (mg/dL)	124 (105–145)	55–254	126 (106–146)	55–254	0.4251
Triglycerides (mg/dL)	98 (71–141)	29–894	111 (82–161)	29–1000	<.0001

Fasting glucose (mg/dL)	97 (92–104)	69–207	100 (94–108)	69–212	<.0001
HbA1c (%)	5.7 (5.5–6.0)	4.9–9.9	5.8 (5.6–6.0)	5.0–9.9	0.0202
Amylase (U/L)	71 (56–85)	25–304	68 (55–82)	25–304	0.0546
BUN (mg/dL)	13.3 (11.2–15.4)	5.8–26.3	13.4 (11.4–15.7)	6.2–26.3	0.4244
Creatinine (mg/dL)	0.64 (0.56–0.77)	0.24–1.52	0.70 (0.59–0.82)	0.24–1.52	<.0001
eGFR (mL/min/1.73 m^2)	80.6 (70.7–90.7)	37.5–227.7	79.3 (70.0–88.5)	37.5–227.7	0.1507
CRP (mg/dL)	0.05 (0.03–0.09)	0.01–2.19	0.06 (0.03–0.11)	0.01–2.19	0.0009
Uric acid (mg/dL)	5.1 (4.2–6.0)	0.6–10.0	5.5 (4.6–6.5)	0.6–11.8	<.0001
Sodium (mmol/L)	142 (141–143)	137–149	142 (141–143)	137–149	0.9762
Potassium (mmol/L)	4.2 (4.0–4.4)	3.4–5.3	4.2 (4.0–4.4)	3.4–5.3	0.2206
Chloride (mmol/L)	106 (104–107)	100–112	106 (104–107)	100–111	0.3404

Note. Data are expressed as median (interquartile range [IQR]), range, or number. Abbreviations: N/A, not applicable; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic associated fatty liver disease; APRI, AST to Platelet Ratio Index; FIB-4, fibrosis-4; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase;

GGT, gamma-glutamyl transpeptidase; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein.

Table 2. Comparisons of the severity of fatty liver and hepatic fibrosis between the Non-overlapping MAFLD and NAFLD groups

	Non-overlapping NAFLD (n=117)	Non-overlapping MAFLD (n=185)	
	Median (IQR)	Median (IQR)	P
Fatty liver index	6 (3–11)	50 (32–71)	<.0001
APRI	0.2 (0.2–0.3)	0.3 (0.2–0.4)	<.0001
NAFLD fibrosis score	-2.582 (-3.366– -1.926)	-1.689 (-2.770– -0.829)	<.0001
Liver stiffness (kPa)	5.2 (4.2–6.3)	7.6 (5.8–11.5)	<.0001

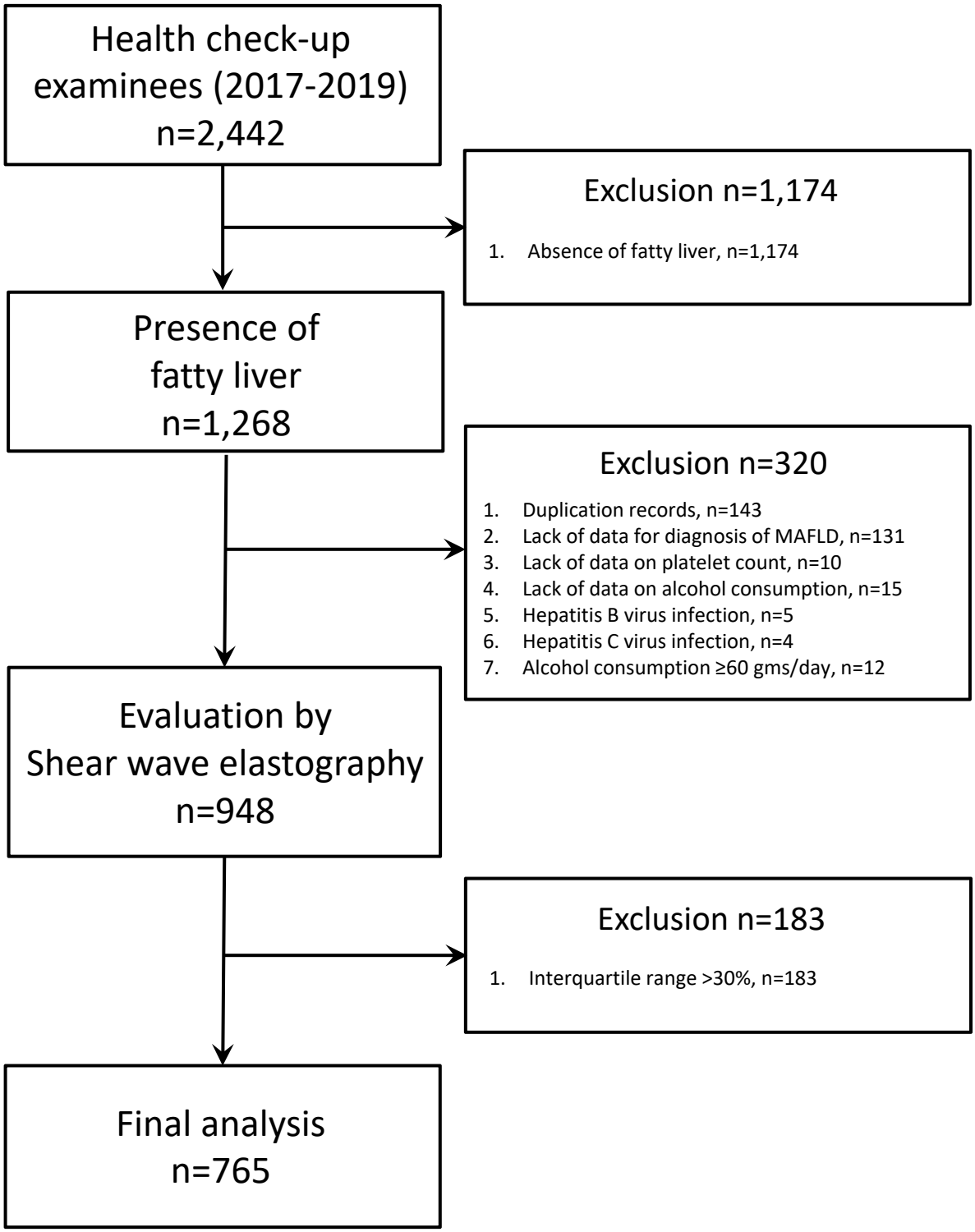
Note. Data are expressed as median (interquartile range [IQR]). Abbreviations: APRI, AST to Platelet Ratio Index; FIB-4, fibrosis-4.

Table 3. Performance of MAFLD and NAFLD criteria for detection of significant fibrosis and for exclusion of non-significant fibrosis

Diagnostic criteria	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	LR+ (95%CI)	LR- (95%CI)
NAFLD	73.0% (64.0%-80.9%)	29.7% (26.2%-33.4%)	15.5% (14.0%-17.2%)	86.2% (81.9%-89.6%)	1.04 (0.92-1.17)	0.91 (0.66-1.25)
MAFLD	93.9% (87.9%-97.5%)	22.9% (19.7%- 26.4%)	17.7% (16.8%-18.6%)	95.5% (91.1%- 97.8%)	1.22 (1.14-1.30)	0.27 (0.13-0.55)
MAFLD (<20 gms/day)	94.1% (86.7%-98.0%)	24.5% (20.6%- 28.7%)	18.6% (17.5%-19.8%)	95.7% (90.4%- 98.2%)	1.25 (1.16-1.34)	0.24 (0.10-0.58)
MAFLD (0 gm/day)	91.7% (80.0%-97.7%)	27.1% (22.3%- 32.2%)	15.5% (14.1%-16.9%)	95.7% (89.6%- 98.3%)	1.26 (1.13-1.40)	0.31 (0.12-0.80)

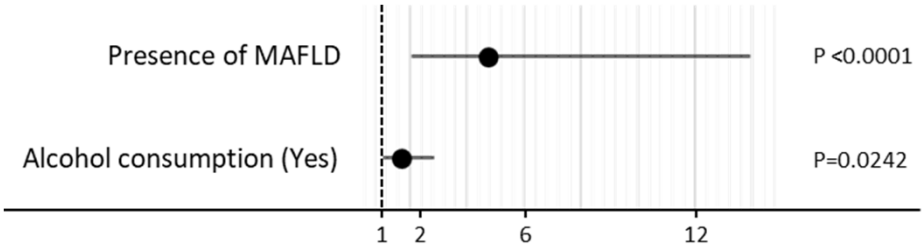
Note. Abbreviations; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio; CI, confidence intervals.

Supplementary Figure 1

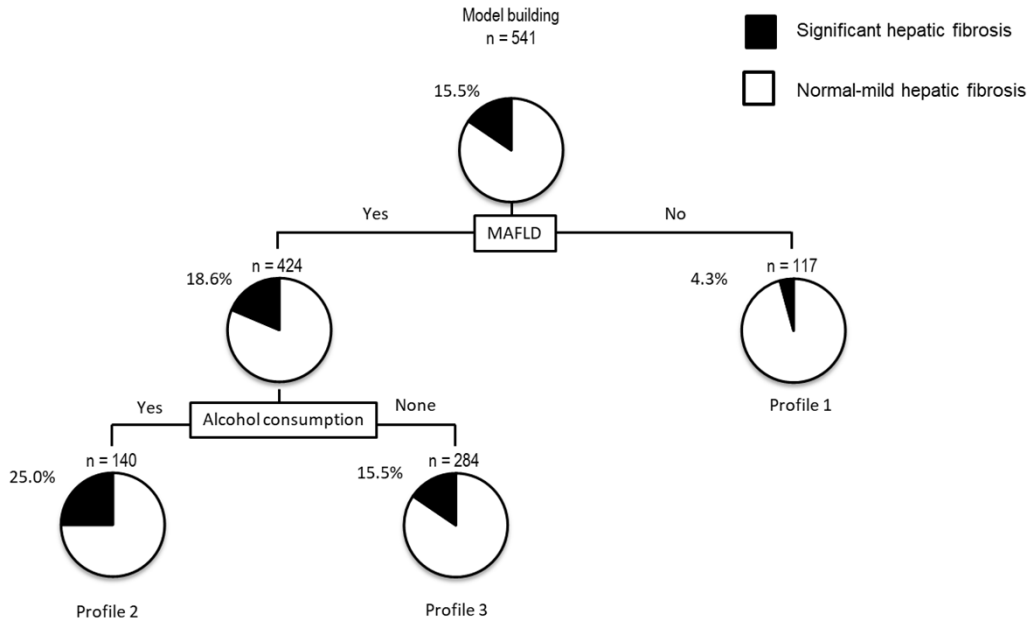


Supplementary Figure 2

A



B



Supplementary Table 1. Patients' characteristics

	Reference Value	Median (IQR)	Range (min–max)
Number	N/A	765	N/A
Age (years)	N/A	54 (47–62)	23–82
Sex (female/male)	N/A	53.9%/46.1% (412/353)	N/A
Body mass index (kg/m^2)	18.5–22.9	24.1 (21.9–26.3)	15.9–42.1
Waist circumference (Large/Normal)	Male <90 Female <80	63.0%/37.0% (481/283)	N/A
Systolic blood pressure (mmHg)	100–129	118 (108–129)	78–207
Type 2 Diabetes mellitus (Presence/Absence)	N/A	11.6%/88.4% (89/676)	N/A
Hypertension (Presence/Absence)	N/A	33.6%/66.4% (257/508)	N/A
Dyslipidemia (Presence/Absence)	N/A	39.2%/60.8% (300/465)	N/A

Alcohol intake habit (None/Yes)	N/A	49.3%/50.7% (377/388)	N/A
Daily alcohol intake (0gm/<20gms/20-59gms)	N/A	49.3%/21.4%/29.3% (377/164/224)	N/A
Steps in a day (<6,000/≥6,000 steps)	N/A	75.0%/25.0% (574/191)	N/A
Sleep disturbance (Presence/Absence)	N/A	63.0%/37.0% (482/283)	N/A
Biochemical examinations			
Red blood cell count (×10^4/μL)	410–530	464 (433–495)	326–591
Hemoglobin (g/dL)	13.1–17.9	14.1 (13.0–15.2)	7.5–18.7
Hematocrit (%)	36.0–45.9	41.8 (38.9–44.4)	25.7–56.2
White blood cell count (/μL)	3,200–8,900	5,100 (4,300–6,100)	2,300–15,000
Platelet count (×10^4/μL)	15.2–36.1	24.7 (21.0–28.6)	9.1–52.8

AST (U/L)	10–30	20 (17–25)	7–114
ALT (U/L)	5–30	21 (14–31)	4–188
Lactate dehydrogenase (U/L)	120–230	169 (153–190)	65－317
ALP (U/L)	119‐303	204 (170–244)	57–520
GGT (U/L)	10–50	26 (17–47)	6–408
Total protein (g/dL)	6.5–7.9	7.1 (6.9–7.3)	6.1–8.8
Cholinesterase (U/L)	240–486	351 (305–395)	196–671
Albumin (g/dL)	4.1–5.1	4.4 (4.2–4.5)	3.6–5.1
Total bilirubin (mg/dL)	0.4–1.6	0.7 (0.6–0.9)	0.3–4.6
Total cholesterol (mg/dL)	140–199	206 (187–233)	125–351
HDL cholesterol (mg/dL)	40–95	61 (50–71)	28–123
LDL cholesterol (mg/dL)	61–119	124 (105–145)	55–254
Triglycerides (mg/dL)	30–149	102 (75–148)	29–1,000
Fasting glucose (mg/dL)	70–99	98 (92–105)	69–212

HbA1c (%)	4.3–5.8	5.7 (5.5–6.0)	4.8–9.9
Amylase (U/L)	44–132	69 (55–83)	25–304
BUN (mg/dL)	8.0–20.0	13.2 (11.2–15.4)	5.8–26.3
Creatinine (mg/dL)	0.60–1.00	0.68 (0.58–0.81)	0.24–1.52
eGFR (mL/min/1.73 m^2)	>60.0	80.2 (71.0–90.1)	37.5–227.7
CRP (mg/dL)	<0.04	0.05 (0.03–0.10)	0.01–2.19
Uric acid (mg/dL)	2.1–7.0	5.3 (4.4–6.4)	0.6–11.8
Sodium (mmol/L)	138–146	142 (141–143)	137–149
Potassium (mmol/L)	3.6–4.9	4.2 (4.0–4.4)	3.4–5.3
Chloride (mmol/L)	99–109	106 (104–107)	100–112
Fatty liver index	<30	29 (14–55)	1–99
APRI	N/A	0.3 (0.2–0.4)	0.1–2.1
NAFLD fibrosis score	N/A	-2.050 (-2.935– -1.052)	-6.029–1.534
FIB-4 index	<1.30	0.98 (0.72–1.33)	0.30–3.79

FIB-4 index (<1.30/ ≥1.30)	N/A	72.7%/27.3% (556/209)	N/A
Liver stiffness (kPa)	< 6.6	6.9 (5.0–10.3)	2.6–43.5
Hepatic fibrosis (Normal-mild /Significant fibrosis)	N/A	85.0%/15.0% (650/115)	N/A

Note. Data are expressed as median (interquartile range [IQR]), range, or number. Abbreviations: N/A, not applicable; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; APRI, AST to Platelet Ratio Index; FIB-4, fibrosis-4.

Supplementary Table 2. Comparison of patients' characteristic between the MAFLD and Non-MAFLD with fatty liver

	Non-MAFLD with fatty liver (n=156)		MAFLD (n=609)		
	Median (IQR)	Range (min–max)	Median (IQR)	Range (min–max)	P
Factors associated with diagnosis of MAFLD					
Body mass index (kg/m^2)	21.0 (19.3-21.9)	15.9–22.9	25.0 (23.2–26.9)	18.4–42.1	<.0001
Waist circumference (Large/Normal)	16.7%/83.3% (26/130)	N/A	74.8%/25.2% (455/153)	N/A	<.0001
Systolic blood pressure (mmHg)	109 (99–116)	78–138	121 (112–131)	83–207	<.0001
Triglycerides (mg/dL)	74 (55–98)	30–495	111 (82–161)	29–1000	<.0001
HDL cholesterol (mg/dL)	68 (61–80)	31–123	58 (49–69)	28–111	<.0001

Fasting glucose (mg/dL)	92 (88–97)	76–115	100 (94–108)	69–212	<.0001
HbA1c (%)	5.5 (5.4–5.6)	4.8–6.2	5.8 (5.6–6.0)	5.0–9.9	<.0001
Patients' background					
Age (years)	48 (43–55)	27–81	56 (49–63)	23–82	<.0001
Sex (female/male)	71.1%/28.9% (111/45)	N/A	49.4%/50.6% (301/308)	N/A	<.0001
Alcohol intake habit (None/Yes)	59.6%/40.4% (93/63)	N/A	46.6%/53.4% (284/325)	N/A	0.0038
Daily alcohol intake (0gm/<20gms/20-59gms)	59.6%/15.4%/25.0% (93/24/39)	N/A	46.6%/23.0%/30.4% (284/140/185)	N/A	0.0121
Steps in a day (<6,000/≥6,000 steps)	77.6%/22.4% (121/35)	N/A	74.4%/25.6% (453/156)	N/A	0.4129
Sleep disturbance (Presence/Absence)	64.1%/35.9% (100/56)	N/A	62.7%/37.3% (382/227)	N/A	0.7506
Biochemical examinations					

Red blood cell count (×10^4/μL)	438 (416–467)	348–566	468 (439–500)	326–591	<.0001
Hemoglobin (g/dL)	13.2 (12.2–14.3)	9.0–17.2	14.2 (13.2–15.4)	7.5–18.7	<.0001
Hematocrit (%)	39.3 (37.0–42.4)	31.1–50.6	42.3 (39.7–45.1)	25.7–56.2	<.0001
White blood cell count (/μL)	4,600 (3,900–5,600)	2,300–12,900	5,300 (4,500–6,300)	2,900–15,000	<.0001
Platelet count (×10^4/μL)	24.3 (21.0–28.5)	10.3–40.8	24.8 (21.1–28.7)	9.1–52.8	0.4688
AST (U/L)	18 (15–22)	11–64	21 (17–26)	7–114	<.0001
ALT (U/L)	15 (10–21)	6–86	22 (16–33)	4–188	<.0001
Lactate dehydrogenase (U/L)	155 (143–182)	113–292	172 (157–191)	65–317	<.0001
ALP (U/L)	193 (152–232)	57–366	207 (174–250)	87–520	0.0015
GGT (U/L)	17 (13–27)	6–320	30 (19–52)	9–408	<.0001
Total protein (g/dL)	6.9 (6.7–7.2)	6.1–8.3	7.1 (6.9–7.3)	6.2–8.8	0.0001
Cholinesterase (U/L)	308 (273–353)	196–625	363 (315–404)	208–671	<.0001

Albumin (g/dL)	4.4 (4.1–4.5)	3.7–5.1	4.4 (4.2–4.5)	3.6–5.1	0.2459
Total bilirubin (mg/dL)	0.7 (0.6–0.9)	0.3–1.8	0.7 (0.6–0.9)	0.3–4.6	0.2043
Total cholesterol (mg/dL)	202 (182–226)	138–315	208 (188–234)	125–351	0.0319
LDL cholesterol (mg/dL)	120 (100–141)	66–216	126 (106–146)	55–254	0.0053
Amylase (U/L)	74 (60–89)	30–159	68 (55–82)	25–304	0.0100
BUN (mg/dL)	12.5 (10.6–14.6)	5.8–20.3	13.4 (11.4–15.7)	6.2–26.3	0.0030
Creatinine (mg/dL)	0.62 (0.55–0.72)	0.42–1.09	0.70 (0.59–0.82)	0.24–1.52	<.0001
eGFR (mL/min/1.73 m^2)	84.7 (74.9–94.6)	52.9–131.4	79.3 (70.0–88.5)	37.5–227.7	<.0001
CRP (mg/dL)	0.03 (0.01–0.04)	0.01–1.45	0.06 (0.03–0.11)	0.01–2.19	<.0001
Uric acid (mg/dL)	4.5 (3.8–5.4)	1.5–7.8	5.5 (4.6–6.5)	0.6–11.8	<.0001
Sodium (mmol/L)	142 (141–143)	139–146	142 (141–143)	137–149	0.8159
Potassium (mmol/L)	4.1 (3.9–4.4)	3.6–5.2	4.2 (4.0–4.4)	3.4–5.3	0.0263
Chloride (mmol/L)	106 (105–107)	100–112	106 (104–107)	100–111	0.4109

Non-invasive tests					
Fatty liver index	7 (4–13)	1–78	37 (21–60)	1–99	<.0001
APRI	0.2 (0.2–0.3)	0.1–1.7	0.3 (0.2–0.4)	0.1–2.1	<.0001
NAFLD fibrosis score	-2.582 (-3.277– -1.974)	-4.800–0.020	-1.783 (-2.801– -0.934)	-6.029–1.534	<.0001
Liver stiffness (kPa)	5.1 (4.0–6.5)	2.6–17.4	7.7 (5.5–11.2)	2.8–43.5	<.0001

Note. Data are expressed as median (interquartile range [IQR]), range, or number. Abbreviations: N/A, not applicable; MAFLD, metabolic associated fatty liver disease; HDL cholesterol, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; LDL cholesterol, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; APRI, AST to Platelet Ratio Index; FIB-4, fibrosis-4.

Supplementary Table 3. Comparison of patients' characteristic between the MAFLD with no alcohol consumption and MAFLD with alcohol consumption (1-59 gms/day) groups

	MAFLD with no alcohol consumption (n=284)		MAFLD with alcohol consumption (1-59 gms/day) (n=325)		
	Median (IQR)	Range (min–max)	Median (IQR)	Range (min–max)	P
Daily alcohol intake (0 gm/<20 gms/20-59 gms)	100%/0%/0% (284/0/0)	N/A	0%/43.1%/56.9% (0/140/185)	N/A	<.0001
Factors associated with diagnosis of MAFLD					
Body mass index (kg/m^2)	25.1 (23.2–27.3)	18.4–41.1	24.8 (23.2–26.7)	19.6–42.1	0.2581
Waist circumference (Large/Normal)	82.0%/18.0% (233/51)	N/A	68.5%/31.5% (222/102)	N/A	0.0001
Systolic blood pressure (mmHg)	119 (109–130)	83–207	123 (113–133)	87–168	0.1050

Triglycerides (mg/dL)	110 (79–158)	29–894	114 (84–167)	35–1000	0.0535
HDL cholesterol (mg/dL)	58 (49–69)	28–111	58 (49–69)	29–109	0.9657
Fasting glucose (mg/dL)	97 (92–106)	71–207	102 (96–110)	69–212	<.0001
HbA1c (%)	5.8 (5.6–6.1)	5.2–9.9	5.7 (5.5–6.0)	5.0–9.2	0.0069
Patients' background					
Age (years)	56 (48–64)	23–79	56 (49–63)	31–82	0.4625
Sex (female/male)	65.9%/34.1% (187/97)	N/A	35.1%/64.9% (114/211)	N/A	<.0001
Steps in a day (<6,000/≥6,000 steps)	71.5%/28.5% (203/81)	N/A	76.9%/23.1% (250/75)	N/A	0.1247
Sleep disturbance (Presence/Absence)	63.4%/36.6% (180/104)	N/A	62.2%/37.8% (202/123)	N/A	0.7548
Biochemical examinations					
Red blood cell count ($\times 10^4/\mu L$)	468 (438–499)	326–591	470 (440–501)	358–587	0.5091

Hemoglobin (g/dL)	13.9 (12.9–14.9)	7.5–17.5	14.6 (13.5–15.6)	7.5–18.7	<.0001
Hematocrit (%)	41.8 (39.2–44.1)	25.7–52.7	42.7 (40.5–45.6)	26.9–56.2	0.0002
White blood cell count (/μL)	5,250 (4,400–6,400)	2,900–12,600	5,300 (4,500–6,200)	2,900–15,000	0.7294
Platelet count (×10^4/μL)	25.6 (21.4–29.7)	9.1–52.8	24.5 (20.7–28.2)	11.8–45.8	0.0663
AST (U/L)	20 (17–25)	10–87	21 (18–26)	7–114	0.0104
ALT (U/L)	22 (15–31)	4–122	23 (16–35)	6–188	0.2544
Lactate dehydrogenase (U/L)	176 (160–192)	65–317	169 (153–189)	127–287	0.0334
ALP (U/L)	209 (179–256)	87–520	205 (171–243)	98–445	0.1972
GGT (U/L)	23 (16–38)	9–408	36 (23–64)	10–381	<.0001
Total protein (g/dL)	7.1 (6.9–7.3)	6.3–8.4	7.1 (6.9–7.3)	6.2–8.8	0.7695
Cholinesterase (U/L)	365 (316–404)	217–671	362 (314–403)	208–573	0.8050
Albumin (g/dL)	4.4 (4.2–4.5)	3.7–5.0	4.4 (4.2–4.5)	3.6–5.1	0.3102
Total bilirubin (mg/dL)	0.7 (0.6–0.9)	0.3–2.9	0.7 (0.6–1.0)	0.3–4.6	0.1969

Total cholesterol (mg/dL)	208 (189–233)	125–351	209 (188–235)	140–339	0.9531
LDL cholesterol (mg/dL)	127 (109–150)	55–254	126 (105–144)	56–240	0.3614
Amylase (U/L)	70 (56–85)	25–192	66 (52–79)	29–304	0.0705
BUN (mg/dL)	13.2 (11.3–15.8)	7.0–26.3	13.6 (11.6–15.6)	6.2–26.0	0.2815
Creatinine (mg/dL)	0.66 (0.56–0.78)	0.24–1.52	0.75 (0.62–0.85)	0.37–1.19	<.0001
eGFR (mL/min/1.73 m^2)	80.7 (69.3–91.0)	37.5–227.7	78.2 (70.6–86.5)	40.1–129.5	0.2222
CRP (mg/dL)	0.06 (0.03–0.11)	0.01–1.81	0.06 (0.03–0.12)	0.01–2.19	0.6533
Uric acid (mg/dL)	5.2 (4.4–6.1)	0.6–10.0	5.8 (5.1–6.7)	1.6–11.8	<.0001
Sodium (mmol/L)	142 (141–143)	137–149	142 (141–143)	138–146	0.4044
Potassium (mmol/L)	4.2 (4.0–4.3)	3.4–5.0	4.2 (4.0–4.4)	3.6–5.3	0.0295
Chloride (mmol/L)	106 (104–107)	100–111	106 (104–107)	100–111	0.7286
Non-invasive tests					
Fatty liver index	34 (17–56)	4–96	41 (25–65)	1–99	0.0003

APRI	0.3 (0.2–0.4)	0.1–1.2	0.3 (0.2–0.4)	0.1–2.1	0.0349
NAFLD fibrosis score	-2.047 (-2.997– -1.003)	-5.694–1.534	-1.618 (-2.655– -0.869)	-6.029–1.369	0.0157
FIB-4 index	0.96 (0.68–1.33)	0.33–3.79	1.05 (0.78–1.40)	0.30–3.57	0.0215
Liver stiffness (kPa)	7.8 (5.5–11.3)	2.8–29.0	7.5 (5.6–11.0)	2.9–43.5	0.5932

Note. Data are expressed as median (interquartile range [IQR]), range, or number. Abbreviations: N/A, not applicable; MAFLD, metabolic associated fatty liver disease; HDL cholesterol, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; LDL cholesterol, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; APRI, AST to Platelet Ratio Index; FIB-4, fibrosis-4.