Comparative associations of NAFLD and MAFLD with coronary artery calcification: a cross-sectional and longitudinal cohort study

Short title: MAFLD and coronary artery calcification

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Abstract

Background

In cross-sectional and retrospective cohort studies we examined comparative associations between non-alcoholic fatty liver disease (NAFLD) and metabolic dysfunction-associated fatty liver disease (MAFLD) and risk of having or developing coronary artery calcification (CAC).

Methods

Participants who had health examinations between 2010-2019 were analyzed. Liver ultrasonography and coronary artery computed tomography were used to diagnose fatty liver and CAC. Participants were divided into MAFLD and no-MAFLD group, then NAFLD and no-NAFLD group. Participants were further divided into no fatty liver disease (reference), NAFLD-only, MAFLD-only, and both NAFLD and MAFLD groups. Logistic regression modeling was performed. Cox proportional hazard model was used to examine the risk of developing CAC in participants without CAC at baseline and who had at least two CAC measurements.

Results

In cross-sectional analyses, 162,180 participants were included. Compared with either the no-NAFLD or no-MAFLD groups, the NAFLD and MAFLD groups were associated with a higher risk of having CAC (NAFLD: adjusted-OR 1.34, 95%CI 1.29-1.39; MAFLD: adjusted-OR 1.44, 95%CI 1.39-1.48). Among the four groups, the MAFLD-only group had the strongest association with risk of having CAC (adjusted-OR 1.60, 95%CI 1.52-1.69).

Conversely, the NAFLD-only group was associated with a lower risk of having CAC (adjusted-OR 0.76, 95%CI 0.66-0.87)

In longitudinal analyses, 34,233 participants were included. Compared with either the no-NAFLD or no-MAFLD groups, the NAFLD and MAFLD groups were associated with a higher risk of developing CAC (NAFLD: adjusted-HR 1.68, 95%CI 1.43-1.99; MAFLD: adjusted-HR 1.82, 95%CI 1.56-2.13). Among these four groups, the MAFLD-only group had the strongest independent associations with risk of developing CAC (adjusted-HR 2.03, 95%CI 1.62-2.55). The NAFLD-only group was not independently associated with risk of developing CAC (adjusted-HR 0.88, 95%CI 0.44-1.78)

Conclusions

Both NAFLD and MAFLD are significantly associated with an increased prevalence and incidence of CAC. These associations tended to be stronger for MAFLD.

Keywords: MAFLD, NAFLD, cardiovascular disease, coronary calcifications

ABBREVIATION LIST

NAFLD: non-alcoholic fatty liver diseases

CVD: cardiovascular disease

AHA: American Heart Association

MAFLD: metabolic dysfunction-associated fatty liver disease

FLD: fatty liver disease

KSHS: Kanbguk Samsung Health Study

CT: computed tomography

LDL: low-density lipoprotein

BMI: body mass index

HDL: high-density lipoprotein

HOMA-IR: homeostasis model assessment-estimated insulin resistance (HOMA-IR)

HbA1c: hemoglobin A1c

CAC: coronary artery calcification

CACs: coronary artery calcification score

IRB: Institutional Review Board

IQR: interquartile range

OR: odds ratio

HR: hazard ratio

CI: confidence interval

HBsAg: Hepatitis B surface antigen

HCV Ab: Anti-Hepatitis C antibody

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide (affecting up to nearly 30% of adults in the general population). NAFLD is now considered an emerging risk factor for atherosclerotic cardiovascular disease (CVD), even after adjustment for traditional CVD risk factors in many investigations[1-5]. With regard to this, a recent American Heart Association statement has highlighted the clinical importance of detecting NAFLD and providing early and appropriate lifestyle measures and pharmacological interventions to prevent both liver-related and CVD-related complications from NAFLD[1]. Many people with NAFLD have metabolic dysfunction and an international expert panel has recently proposed that metabolic dysfunction-associated fatty liver disease (MAFLD) should replace the old term "NAFLD", because MAFLD more appropriately defines this common liver disease and its related metabolic disorders[6,7]. This suggestion was promptly accepted by international and regional scientific societies, including the Asian Pacific Association for the Study of the Liver (APASL), the Latin American Association for the Study of Liver (ALEH), the Arabic Association for the Study of Diabetes and Metabolism (AASD), the Chinese Society of Hepatology (CSH), and organizations from the Middle East and North Africa[8-12]. In addition, the newly-proposed term MAFLD also better reflects the key role of cardiometabolic risk factors (such as overweight/obesity, type 2 diabetes mellitus, insulin resistance, and other features of the metabolic syndrome) in the pathophysiology of this common and burdensome liver disease[6,13].

For now, MAFLD and NAFLD are two increasingly common conditions that are under-diagnosed and under-appreciated as risk factors for CVD morbidity and mortality amongst the Cardiology community[1]. Emerging evidence suggests the existence of an association between MAFLD and risk of major adverse cardiac events[14]. However, it is currently uncertain whether

the MAFLD definition can better identify individuals at higher risk of developing CVD events, than the NAFLD definition.

Though coronary artery calcification (CAC) is a known marker of early CVD and CVD mortality, to date, to our knowledge, no studies have compared the association between NAFLD, MAFLD, and risk of having or developing CAC[15,16]. Therefore, in this study, we examined the comparative associations between NAFLD and MAFLD definitions with the presence of CAC at baseline in a cross-sectional study, as well as the risk of developing incident CAC in a retrospective cohort study, in a large cohort of South Korean individuals.

METHODS

The data supporting this study's findings are not publicly available due to the Institutional Review Board (IRB) restrictions, as the data were not collected in a way that could be distributed widely. However, the analytical methods are available from the corresponding author upon reasonable request.

Study population

We used the Kanbguk Samsung Health Study (KSHS) cohort to analyze the current study. The cohort description and data collection details have been extensively reported in previous studies[17,18]. Briefly, KSHS is cohort data established from annual or biannual health examinations at two health examination centers in South Korea. The examination comprises an extensive self-report questionnaire, laboratory data, and imaging studies.

The current study used the data collected from March 1, 2010, to December 30, 2019. To assess the cross-sectional associations between fatty liver disease and risk of prevalent CAC, participants who had liver ultrasound examinations and coronary artery computed tomography

(CT) scans were initially included in the study (n=167,295). The following participants were then excluded from the analysis: participants with a prior history of cancer (n=4,616), participants with missing data for some covariates (i.e., body mass index or plasma low-density lipoprotein [LDL]-cholesterol levels, n=68), and those with alcoholic fatty liver disease who did not meet MAFLD criteria[19] (n=443). As a consequence of these exclusion criteria, a total of 162,180 participants were included in the final cross-sectional analysis for MAFLD. For NAFLD evaluation, additional participants were excluded from the analysis due to missing variables (alcohol consumption data, n=12,595; anti-hepatitis C antibody [HCV Ab], n=11,145 or hepatitis B surface antigen [HBsAg], n=5,253). As a consequence of these exclusion criteria, a total of 149,585 participants were included in the final cross-sectional analysis for NAFLD (**Figure 1**).

To assess the longitudinal associations between NAFLD or MAFLD and risk of developing incident CAC, participants who had liver ultrasound examinations at baseline and who had least two CAC measurements, were recruited during the same study period (n=41,341). Among these participants, those who had CACs>0 at baseline were excluded from the analysis (n=7,108). Thus, a total of 34,233 participants were included in the final longitudinal analysis for MAFLD. For NAFLD evaluation, additional participants were also excluded due to missing variables (missing alcohol consumption data, n=2,449; missing HCV Ab or HBsAg, n=331). After these exclusions, a total of 31,510 participants were included in the final longitudinal analysis for NAFLD (**Figure 1**).

The Institutional Review Board of Kangbuk Samsung Hospital (IRB No.: 2022 -06-039) approved the study. The requirement for informed consent was waived due to the nature of non-identifiable data obtained during the health-screening examinations.

Definitions of MAFLD and NAFLD

We used standard definitions for diagnosing MAFLD and NAFLD[6,20]. Liver ultrasonography using a 3.5 MHz probe was performed in all participants by experienced radiologists, and fatty liver was diagnosed based on standard ultrasonographic features, including hepatorenal echo contrast, liver brightness, or vascular blurring[21]. Radiologists were blinded to all clinical details of participants, including CAC data. MAFLD was diagnosed if participants had evidence of hepatic steatosis on ultrasonography in the presence of one or more of the following three metabolic conditions: overweight or obesity (defined as body mass index [BMI] ≥23 kg/m^2), type 2 diabetes mellitus, or at least two of seven metabolic risk abnormalities [i.e., waist circumference ≥90 cm in men and ≥80 cm in women; serum triglyceride level ≥150 mg/dL; high-density lipoprotein (HDL) level <40 mg/dL in men or <50 mg/dL in women; systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or taking antihypertensive medications; prediabetes status based on levels of fasting glucose (100–125 mg/dL) or hemoglobin A1c (5.7%–6.4%); homeostasis model assessment-estimated insulin resistance (HOMA-IR) score ≥2.5, or plasma highly sensitive C-reactive protein (hs-CRP) level ≥0.2 mg/dL][6,20-24]. If participants did not meet the MAFLD criteria, they were categorized as the no-MAFLD group.

Separately, NAFLD was diagnosed if participants met the following criteria: evidence of hepatic steatosis on ultrasonography in the absence of excessive alcohol consumption (i.e., defined as >30 g/day for men and >20 g/day for women) and other coexisting liver diseases, including Hepatitis B or C infection, which were screened by HBsAg and anti-HCV tests [6,25,26]. If the participants did not meet the NAFLD criteria, they were categorized as the no-NAFLD group.

Categorization of participants according to fatty liver disease status and presence of coronary artery calcification

To compare the associations between the presence of MAFLD or NAFLD and the risk of having or developing CACs>0, participants were firstly categorized both into a MAFLD and no-MAFLD group (reference), and into a NAFLD and no-NAFLD group (reference), respectively, for cross-sectional and longitudinal analyses. In addition, participants were also categorized into four groups according to their fatty liver disease status, to compare the associations with risk of having or developing CACs>0 as follows: the no-FLD (fatty liver disease) group, the NAFLD-only group (defined as subjects having NAFLD but not MAFLD), the MAFLD-only group (defined as subjects having MAFLD but not NAFLD), and the both NAFLD and MAFLD group (defined as subjects having both conditions) (**Figure 1**).

In all participants, coronary artery calcification (CAC) was measured using a coronary computed tomography (CT) scan. A lightspeed VCT XTe-64 slice MDCT scanner (GE Healthcare, Tokyo, Japan) was used with a standard scanning protocol using 40*2.5-mm section collimation, 400 ms rotation time, 120 kV tube voltage, and 124 mAS (310 mA*0.4 seconds) tube current under ECG-gated dose modulation[24,27]. Expert radiologists, who were blinded to clinical and liver ultrasound data of participants, calculated the CAC burden using the Agatston method, which was expressed as coronary artery calcification score (CACs, AU)[27,28]. The presence of CAC was defined as CACs higher than 0. CACs ranged from 0-6282 in included participants (**Figure S1**). On a scale of 0 to 6282, participants were then categorized into the CACs=0 group and the CACs>0 group, respectively.

Statistical analysis

Continuous variables are expressed as the medians and interquartile ranges (IQR) or

mean ± standard deviations. Categorical variables are expressed as frequencies and percentages. The unpaired Student's *t*-test and the one-way analysis of variance (ANOVA) test were used to compare the means of continuous variables between the groups. Pearson's chi-squared test was used to compare categorical variables. To assess the cross-sectional associations of either MAFLD or NAFLD with CACs>0 at baseline, logistic regression modeling with confounding factor adjustment was undertaken. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were estimated. Model 1 was adjusted for age, sex, education level, smoking history, and regular exercise (≥ 3 times/week, a total of 75 min per week engaging in vigorous physical activities and 150 min per week of moderate physical activities)[29].

Model 2 was further adjusted for plasma LDL-cholesterol levels. Model 3 was further adjusted for prior history of coronary artery disease and the use of lipid-lowering medications, in addition to model 2's covariates. The same logistic regression models were also performed after stratifying participants into four groups according to their fatty liver disease status at baseline (i.e., the no-FLD group, the NAFLD-only group, the MAFLD-only group, and the both NAFLD and MAFLD group – as defined above).

Cox regression modeling was performed to assess the longitudinal associations of either MAFLD or NAFLD (or the aforementioned four groups with different fatty liver disease categories) with the risk of developing CAC in the subset of individuals who had CACs=0 at baseline. Adjusted hazard ratios (HR) with 95% confidence intervals (CIs) were estimated. The dependent variable in these Cox regression models was the hazard function of time to event (CACs>0). These Cox regression models were adjusted for the same covariates mentioned above. In the longitudinal analysis, all covariates including age were used as time-dependent, and the values were observed on the examination date. The time in the Cox regression model was defined as the time from baseline (initial health examination date) to the event (CACs>0), or time from

baseline to the end of the follow-up. STATA version 17.0 (StataCorp LP, College Station, TX, USA) was used for all statistical analyses. Two-sided *P*-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics of participants

Overall, among the 162,180 South Korean participants included in the study (74.23% men, mean age 41.55±8.82), the prevalence of MAFLD and NAFLD was 37.92% and 34.81%, respectively. A total of 21,958 (13.54%) participants had CACs>0 at baseline.

When participants were subdivided according to the presence or absence of CACs>0 at baseline (**Table 1**), individuals with CACs>0 were more likely to be older, of male sex, current smokers, to have higher alcohol consumption, lower education level, higher adiposity measures (BMI and waist circumference), higher blood pressure, and higher values of fasting glucose, HbA1c, HOMA-IR score, serum liver enzymes, and hs-CRP, as well as had a greater prevalence of metabolic risk abnormalities, compared to individuals with CACs=0 at baseline (all $p<0.001$). Furthermore, individuals with CACs>0 also had a greater prevalence of known type 2 diabetes and pre-existing CVD. Notably, they also had a markedly higher prevalence of NAFLD and MAFLD than subjects with CACs=0.

The baseline clinical and biochemical characteristics of participants included in longitudinal analyses are presented in **Table S1**. Participants who developed CACs>0 during the follow-up were older and had a higher proportion of men, higher smoker rate and daily alcohol intake, lower education level, higher adiposity measures (BMI and waist circumference), higher blood pressure, and higher values of fasting glucose, HbA1c, HOMA-IR score, serum liver enzymes, and hs-CRP, as well as a greater prevalence of metabolic risk abnormalities, compared to those with CACs=0. Notably, participants who developed CACs>0 were more likely to have NAFLD or MAFLD at baseline compared to those with CACs=0 ($p<0.001$).

Cross-sectional associations of MAFLD or NAFLD with the presence of CACs>0 at baseline

As shown in **Table 2,** compared with the no-MAFLD group, individuals with MAFLD had a higher likelihood of having CAC at baseline (i.e., defined as the presence of CACs>0): age- and sex-adjusted OR 1.58 [95% CI 1.53-1.63]; model 1, adjusted-OR 1.58 [95% CI 1.53-1.63]; and model 2, adjusted-OR 1.51 [95% CI 1.46-1.56]; model 3, adjusted-OR 1.44 [95% CI 1.39-1.48]). Similarly, compared with the no-NAFLD group, individuals with NAFLD had a higher likelihood of having CACs>0, although the odds ratios tended to be lower than those observed in the MAFLD group: age- and sex-adjusted OR 1.47 [95% CI 1.42-1.52]; model 1, adjusted-OR 1.48 [95% CI 1.43-1.53]; model 2, adjusted-OR 1.41 [95% CI 1.36-1.46]; model 3, adjusted-OR 1.34 [95% CI 1.29-1.39]).

Cross-sectional associations between different fatty liver disease categories and presence of CACs>0 at baseline

We also assessed the associations between each fatty liver disease category and the presence of CACs>0 at baseline (**Table 3**). Compared with the no-FLD group, both the MAFLD-only group and the both NAFLD and MAFLD group were significantly associated with a higher likelihood of having CACs>0, even after adjustment for age, sex, education level, smoking history, regular exercise, plasma LDL-cholesterol, prior history of coronary artery disease and the use of lipid-lowering drugs (model 3, adjusted-OR 1.60 [95%CI 1.52-1.69] for the MAFLD-only group, and adjusted-OR 1.37 [1.33-1.42] for the both NAFLD and MAFLD group, respectively). Conversely, the NAFLD-only group was associated with a lower likelihood of having CACs>0 at baseline compared with the no-FLD group (model 3, adjusted-OR 0.76 [0.66-0.87]).

Risk of developing incident CAC according to fatty liver disease categories at baseline

As shown in **Table 4**, we assessed the associations between each fatty liver disease category and the risk of developing incident CACs>0 in the subgroups of subjects who had CACs=0 at baseline. The median follow-up duration was 8.76 years. Compared with either the no-MAFLD group or the no-NAFLD group, both the MAFLD group and the NAFLD group had a significantly higher risk of developing incident CACs>0, even after adjustment for potential confounding factors (model 3: MAFLD, adjusted-HR 1.82, 95%CI 1.56-2.13 vs. no-MAFLD; and NAFLD, adjusted-HR 1.68, 95%CI 1.43-1.99 vs. no-NAFLD).

When these participants were subdivided into four groups according to their fatty liver disease status at baseline (no-FLD, reference; NAFLD-only, MAFLD-only, both NAFLD and MAFLD group), the MAFLD-only group and the both NAFLD and MAFLD group were significantly associated with a higher risk of developing incident CACs>0, compared with the no-FLD group (model 3: MAFLD-only group, adjusted HR 2.03, 95% CI 1.62-2.55; and the both NAFLD and MAFLD group, adjusted HR 1.73, 95% CI 1.47-2.05), even after adjustment for potential confounders. Conversely, the NAFLD-only group was not independently associated with the risk of incident CACs>0 (**Table 5**).

DISCUSSION

To our knowledge, this is the first observational study to examine the comparative associations between NAFLD or MAFLD definitions and the risk of having or developing CAC (as a reliable marker of subclinical CVD) in a Korean cohort of middle-aged men and women.

Using the Kanbguk Samsung Health Study (KSHS) cohort database (n=162,180 participants), we found that NAFLD, and MAFLD were significantly associated with the

presence of CAC, even after adjusting for potential confounding factors, although the odds tended to be higher in the MAFLD group than in the NAFLD group. In addition, we found that compared with the no-FLD group, the MAFLD-only group and the both NAFLD and MAFLD group were associated with the presence of CAC, even after adjusting for age, sex, education level, smoking history, physical activity, pre-existing coronary artery disease, plasma LDL-cholesterol concentrations, or use of lipid-lowering medications.

When we performed longitudinal analyses in the subset of participants who did not have CAC at baseline (i.e., those with CACs=0), we found that the associations were essentially comparable to those observed in the cross-sectional study. In fact, both NAFLD, and MAFLD were significantly associated with an increased risk of developing incident CAC even after adjusting for the aforementioned CVD risk factors and potential confounders, and the MAFLD group showed the numerically strongest association with incidental CAC. Similarly, when participants were subdivided into four subgroups, the MAFLD-only group and the both NAFLD and MAFLD group were independently associated with a higher risk of developing incident CAC. Conversely, the NAFLD-only group was not independently associated with incidental CAC.

Previous studies have proven that CAC is a reliable marker of early CVD and CVD mortality [15,16]. CAC >0 is associated with an increased risk of CVD[15]. In addition, CAC predicts CVD mortality, irrespective of the Framingham risk score factors[30]. A graded increase in CACs has been reported to be associated with an increase in CVD, and CACs greater than or equal to 1000 strongly predicts higher CVD risk[31]. Furthermore, the doubling of CACs resulted in a nearly 25% increase in the risk of CVD mortality[30]. Multiple factors may be involved in CAC development. Increased oxidative stress is associated with vascular calcification, and it may be a key link between low-grade chronic inflammation and vascular calcification[32].

Hyperlipidemia, dysglycemia and hyperinsulinemia may also promote vascular calcification[33-35]

To date, convincing evidence supports a strong association between NAFLD and increased risk of CVD morbidity and mortality[36-38]. For example, an updated and comprehensive meta-analysis of 36 longitudinal cohort studies (including about 5.8 million individuals from different countries) reported that NAFLD was significantly associated with a ~1.5-fold increased long-term risk of fatal or nonfatal CVD events over a median follow-up of 6.5 years. This risk markedly increased across the severity of NAFLD, especially the level of liver fibrosis[2]. In line with these findings, the American Heart Association has taken the first step in issuing a scientific statement about NAFLD and the risk of CVD in 2021. This statement places NAFLD as an underappreciated and independent risk factor for CVD[1].

Recently, international experts proposed redefining NAFLD as MAFLD[6]. After the introduction of this new term, multiple studies in various fields were conducted to find out if the MAFLD definition predicts the prognosis better than the NAFLD definition [7,39,40]. However, the long-term prognostic impact of this name change on CVD risk prediction is presently uncertain. We recently performed a meta-analysis of observational cohort studies that simultaneously used the NAFLD and MAFLD definitions for comparing the risk of fatal and nonfatal CVD events associated with both definitions[41]. In this meta-analysis of 7 cohort studies (including about 13 million individuals), we found that each of the two definitions was significantly associated with a higher risk of fatal or nonfatal CVD events over a median follow-up of 7.0 years (pooled random-effects hazard ratio 1.50, 95% CI 1.30-1.72 for MAFLD vs. no-MAFLD; and pooled random-effects hazard ratio 1.27, 95% CI 1.12-1.45 for NAFLD vs. no-NAFLD, respectively). Although MAFLD identified a greater number of incident CVD events than NAFLD, the risk for fatal or nonfatal CVD events associated with either definition

was not significantly different[41]. Collectively, the findings of this meta-analysis are in line with those reported in the present cohort study showing that both NAFLD and MAFLD definitions were significantly associated with a higher risk of having or developing CAC, even after adjusting for coexisting CVD risk factors, and the risk tended to be (slightly) greater in the MAFLD group than in the NAFLD group.

Interestingly, our study also showed that the NAFLD-only group was not independently associated with an increased risk of developing incident CAC during the follow-up period. Some factors might explain this finding. First, the NAFLD-only group includes lean subjects with NAFLD who do not have any common cardiometabolic risk factors[42]. NAFLD is known to be associated with the development of type 2 diabetes, atherogenic dyslipidemia and other cardiometabolic risk factors over time[43-45]. So, it is possible to hypothesize that NAFLD-only *itself* is not associated with CAC development, while coexisting cardiometabolic risk factors may, in large part, mediate the development of CAC[46]. Thus, we cannot exclude that the NAFLD-only status is a dynamic condition with a high risk of developing metabolic disorders (i.e., supporting a high risk of transition from NAFLD-only to MAFLD status over time). Second, as shown in Table 5, it should be noted that in the NAFLD-only group there were a small number of participants (n=600) and the number of subjects developing CACs>0 was very small (n=8) over the follow-up. The CIs were wide in this group overlapping unity (95%CIs 0.44 -1.78) and it is difficult to be certain whether the hazard was attenuated or increased.

There are a few limitations to our study. First, this is a cohort study with an observational design that cannot definitively establish causality. Second, our results are not based on liver biopsy examination for diagnosing and staging NAFLD or MAFLD. Although liver biopsy remains the ‘gold standard’ for the diagnosis of this liver disease, it is an invasive

method that cannot be performed in large cohort studies. Fatty liver disease was assessed by liver ultrasonography that is considered the first-line imaging technique, both in clinical practice and large epidemiological studies. Finally, our study is limited to one ethnic group (South Korean people), and the distribution of CVD risk factors and the association between NAFLD/MAFLD and risk of CAC may differ by ethnic groups.

Conclusions

The findings of this large Korean cohort study further emphasize that clinicians should have a high index of suspicion that individuals with MAFLD and NAFLD are at increased risk of CVD. Our findings also suggest that the NAFLD-only group (who are individuals with fatty liver without any coexisting common metabolic risk factors) is not independently associated with higher risk of developing incident CAC. That said, further cohort studies from different countries are needed to examine the comparative associations of NAFLD or MAFLD definitions with the presence and development of CAC.

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AUTHOR CONTRIBUTIONS

K-CS: conceptualization, methodology, writing – review and editing, supervision, and project administration. **TKY**: writing – original draft, and writing – review and editing. **MYL:** Formal analysis and investigation. **CDB:** writing – review and editing; **M-HZ:** writing – review and editing; **GT:** conceptualization, writing – review and editing.

ETHICAL STATEMENT

The Institutional Review Board of Kangbuk Samsung Hospital (IRB No.: 2022 -06- 039) approved the current study. Informed consent was waived because we used only non-identifiable data obtained during the health-screening examinations.

DATA SHARING STATEMENT

The data that support the findings of this study are available on request from the corresponding author, Ki-Chul Sung, upon reasonable request.

Supplemental Materials

Table S1

Figures S1

Table 1. Baseline characteristics of participants stratified by CAC score at baseline.

	Overall	**CACs = 0**	**CACs > 0**	***P*-value**
Number	162,180	140,222	21,958	
Age, years	41.55±8.82	40.18±7.66	50.31±10.54	<0.001
Male sex, %	120,384 (74.23)	101,501 (72.39)	18,883 (86.0)	<0.001
Current smoker, %	34,842 (21.48)	28,753 (20.51)	6,089 (27.73)	<0.001
High alcohol intake*, %	25,062 (15.45)	20,439 (14.58)	4,623 (21.05)	<0.001
Higher education, %	126,084 (77.74)	110,951 (79.13)	15,133 (68.92)	<0.001
BMI, kg/m^2	24.39±3.34	24.25±3.35	25.25±3.15	<0.001
Waist circumference, cm	84.57±9.32	84.1±9.37	87.48±8.41	<0.001
Systolic BP, mmHg	112.94±12.63	112.18±12.42	117.77±12.86	<0.001
Diastolic BP, mmHg	72.83±9.76	72.24±9.63	76.59±9.7	<0.001
Fasting glucose, mg/dL	97.82±16.35	96.71±14.49	104.94±24.02	<0.001
Hemoglobin A1c, %	5.62±0.57	5.58±0.5	5.89±0.83	<0.001
HOMA-IR score	1.45 (0.96 - 2.18)	1.43 (0.95 - 2.14)	1.61 (1.04 - 2.5)	<0.001
ALT, U/L	21 (15 - 32)	21 (15 - 31)	24 (18 - 35)	<0.001
AST, U/L	21 (17 - 26)	20 (17 - 25)	23 (19 - 29)	<0.001
GGT, U/L	26 (17 - 43)	25 (16 - 41)	33 (21 - 54)	<0.001
Triglycerides, mg/dL	109 (76 - 160)	106 (74 - 156)	127 (90 - 184)	<0.001
HDL-cholesterol, mg/dL	55.63±14.66	56.17±14.77	52.19±13.43	<0.001
LDL-cholesterol, mg/dL	128.59±32.62	127.73±31.92	134.07±36.28	<0.001
Total cholesterol, mg/dL	197.62±34.97	196.89±34.1	202.26±39.75	<0.001

Lipid-lowering medications, %	7512 (4.63)	4380 (3.12)	3,132 (14.26)	<0.001
NAFLD, %	52,071 (32.11)	43,256 (30.85)	8,815 (40.14)	<0.001
MAFLD, %	61,493 (37.92)	50,214 (35.81)	11,279 (51.37)	<0.001
hs-CRP, mg/dL	0.05 (0.03 - 0.1)	0.05 (0.03 - 0.1)	0.06 (0.03 - 0.11)	<0.001
Type 2 diabetes, %	9,625 (5.93)	5,824 (4.15)	3,801 (17.31)	<0.001
Hypertension, %	26,676 (16.46)	18,048 (12.88)	8,628 (39.32)	<0.001
History of CAD, %	1,266 (0.78)	717 (0.51)	549 (2.5)	<0.001
History of dyslipidemia, %	31,270 (19.28)	23,450 (16.72)	7,820 (35.61)	<0.001
Regular exercise, %	22,498 (13.87)	18,731 (13.36)	3,767 (17.16)	<0.001
Metabolic risk abnormalities				
1) Waist ≥90/80 cm	50,831 (34.11)	42,008 (32.7)	8,823 (42.96)	<0.001
2) BP ≥130/85/drugs	34,507 (21.28)	24,788 (17.68)	9,719 (44.26)	<0.001
3) TG ≥150 mg/dL	46,797 (28.85)	38,391 (27.38)	8,406 (38.28)	<0.001
4) HDL ≤40/50 mg/dL	21,779 (14.62)	17,953 (13.97)	3,826 (18.63)	<0.001
5) Prediabetes status	73,572 (45.37)	60,729 (43.32)	12,843 (58.5)	<0.001
6) HOMA-IR score ≥2.5	29,997 (18.53)	24,544 (17.52)	5,453 (24.98)	<0.001
7) hs-CRP ≥0.2 mg/dL	17,489 (11.6)	14,973 (11.33)	2,516 (13.48)	<0.001

Values are expressed as means ± standard deviations, medians (interquartile ranges), or percentages. *High alcohol intake was defined as >30 g/day in men and >20 g/day in women.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease; BMI, body mass index; BP, blood pressure; HOMA-IR,

Homeostatic model assessment of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, highly sensitive C-reactive protein; CAD, coronary artery disease; BP, blood pressure; TG, triglycerides.

Table 2. Cross-sectional associations of either MAFLD or NAFLD with the presence of coronary artery calcification at baseline.

	Number	Event (CACs>0)	Age- and sex-adjusted Model OR (95% CI)	Adjusted Model 1 OR (95% CI)	Adjusted Model 2 OR (95% CI)	Adjusted Model 3 OR (95% CI)
MAFLD						
No-MAFLD	100,687	10,679	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
MAFLD	61,493	11,279	1.58 (1.53 - 1.63)	1.58 (1.53 - 1.63)	1.51 (1.46 - 1.56)	1.44 (1.39-1.48)
NAFLD						
No-NAFLD	97,514	10,399	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
NAFLD	52,071	8,815	1.47 (1.42 - 1.52)	1.48 (1.43 - 1.53)	1.41 (1.36 - 1.46)	1.34 (1.29-1.39)
Missing value	12,595	2,744				

Data are reported as odds ratios and 95% confidence intervals (in parenthesis). The dependent variable in these logistic regression models was the presence of CACs>0 at baseline.

Model 1: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week)

Model 2: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week), and plasma LDL-cholesterol levels

Model 3: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week), plasma LDL-cholesterol levels, prior history of coronary artery disease, and lipid-lowering medications

Table 3. Cross-sectional associations between different fatty liver disease subgroups and presence of coronary artery calcification at baseline.

Subgroup(s)	Number	Event (CACs>0)	Age- and sex-adjusted Model OR (95% CI)	Adjusted Model 1 OR (95% CI)	Adjusted Model 2 OR (95% CI)	Adjusted Model 3 OR (95% CI)
No-FLD	97,514	10,399	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
NAFLD-only	3,173	280	0.79 (0.69-0.9)	0.80 (0.7-0.91)	0.76 (0.66-0.87)	0.76 (0.66-0.87)
MAFLD-only	12,595	2,744	1.78 (1.69-1.88)	1.75 (1.65-1.84)	1.68 (1.60-1.77)	1.60 (1.52-1.69)
Both NAFLD & MAFLD	48,898	8,535	1.51 (1.46-1.57)	1.52 (1.47-1.58)	1.45 (1.40- 1.50)	1.37 (1.33-1.42)

Data are reported as odds ratios and 95% confidence intervals (in parenthesis). The dependent variable in these logistic regression models was the presence of CACs>0 at baseline.

Model 1: Adjusted for age, sex, education level, smoking history, regular exercise (3 times/week)

Model 2: Adjusted for age, sex, education level, smoking history, regular exercise (3 times/week), and plasma LDL-cholesterol levels

Model 3: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week), plasma LDL-cholesterol levels, prior history of coronary artery disease, and lipid-lowering medications

Table 4. Risk of developing incident CAC according to the presence or absence of either MAFLD or NAFLD at baseline.

	Number	PY	Event (CACs>0)	Incident Rate (95% CI)	Adjusted Model 1 HR (95% CI)	Adjusted Model 2 HR (95% CI)	Adjusted Model 3 HR (95% CI)
MAFLD		193178.64	688	35.61 (33.05 – 38.38)			
No MAFLD	22069 (64.47)	125685.77	295	23.47 (20.94 – 26.31)	1 (ref.)	1 (ref.)	1 (ref..)
MAFLD	12164 (35.53)	67492.87	393	58.23 (52.75 – 64.28)	1.97 (1.69 – 2.30)	1.92 (1.65 – 2.24)	1.82 (1.56 – 2.13)
NAFLD		177367.55	580	32.70 (30.14 – 35.47)			
No NAFLD	21364 (62.41)	121666.27	287	23.59 (21.01 – 26.48)	1 (ref.)	1 (ref.)	1 (ref.)
NAFLD	10146 (29.64)	55701.27	293	52.60 (46.91 – 58.98)	1.83 (1.55 – 2.16)	1.77 (1.5 – 2.09)	1.68 (1.43 – 1.99)
Missing value	2,723 (7.95)						

Data are reported as hazard ratios and 95% confidence intervals (in parenthesis).The dependent variable in these Cox regression models was the hazard function of time to event (CACs>0).

Model 1: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week)

Model 2: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week), plasma LDL-cholesterol levels

Model 3: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week), plasma LDL-cholesterol levels, prior history of coronary artery disease, and lipid-lowering medications

Abbreviations: PY, per 10^4 person-year; FLD, fatty liver disease; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease.

Table 5. Risk of developing incident CAC according to different fatty liver disease subgroups at baseline.

Subgroup(s)	Number	PY	Event (CACs>0)	Incident Rate (95% CI)	Adjusted Model 1 HR (95% CI)	Adjusted Model 2 HR (95% CI)	Adjusted Model 3 HR (95% CI)
No-FLD	21364 (62.6)	121666.27	287	23.59 (21.01 - 26.48)	1 (ref.)	1 (ref.)	1 (ref.)
NAFLD-only	600 (1.76)	3362.87	8	23.79 (11.9 - 47.57)	0.89 (0.44 - 1.79)	0.87 (0.43 - 1.76)	0.88 (0.44 -1.78)
MAFLD-only	2618 (7.67)	15154.47	108	71.27 (59.02 - 86.06)	2.18 (1.74 - 2.73)	2.15 (1.72 - 2.69)	2.03 (1.62 - 2.55)
Both NAFLD & MAFLD	9546 (27.97)	52338.41	285	54.45 (48.48 - 61.16)	1.88 (1.59 - 2.21)	1.82 (1.54 - 2.14)	1.73 (1.47 - 2.05)

Data are reported as hazard ratios and 95% confidence intervals (in parenthesis). The dependent variable in these Cox regression models was the hazard function of time to event (CACs>0).

Model 1: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week)

Model 2: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week), plasma LDL-cholesterol levels

Model 3: adjusted for age, sex, education level, smoking history, regular exercise (3 times/week), plasma LDL-cholesterol levels, prior history of coronary artery disease, and lipid-lowering medications

Abbreviations: PY, per 10^4 person-year; CI, confidence interval; FLD, fatty liver disease; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease.

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Highlights

-Prior to this study, potential association between metabolic dysfunction-associated fatty liver disease (MAFLD) and coronary artery calcification (CAC) was unknown.

-We conducted cross-sectional and longitudinal analyses to examine the comparative associations of MAFLD or NAFLD with the risk of having or developing CAC.

-MAFLD showed a stronger numerical association with risk of having or developing CAC than NAFLD, in both cross-sectional and longitudinal analyses.

-Our results emphasize that individuals with MAFLD are at increased risk of CVD.

Figure 1. Flow diagram and categorization of study participants in cross-sectional and longitudinal analysis.

A: Flow diagram of cross-sectional analysis and categorization of participants

B: Flow diagram of longitudinal analysis and categorization of participants.

Abbreviations: AFLD, alcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; US, ultrasound; CAC, coronary artery calcification