Received: 20 April 2021 | Revised: 22 July 2021 | Accepted: 24 August 2021

DOI: 10.1002/hep.32131

ORIGINAL ARTICLE

Metabolic dysfunction–associated fatty liver disease improves detection of high liver stiffness: The Rotterdam Study

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Funding information
Erasmus Medical Center and Erasmus University (Rotterdam, Netherlands); Organization for the Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly; the Ministry of Education, Culture and Science; the Ministry for Health, Welfare and Sports; the European Commission (DG XII); the Municipality of Rotterdam; and the Foundation for Liver and Gastrointestinal Research

Abstract

Background and Aims: Recently metabolic dysfunction–associated fatty liver disease (MAFLD) has been introduced and was defined as hepatic steatosis with either overweight, diabetes, and/or a combination of other metabolic risk factors. We investigated the application of the MAFLD criteria as compared with NAFLD.

Approach and Results: We performed a cross-sectional analysis within the Rotterdam Study, a large prospective population-based cohort. Participants who attended the liver ultrasound and transient elastography program between 2009 and 2014 were eligible for inclusion. Subsequently, individuals with viral hepatitis, alcohol intake >60 g/day, missing alcohol data, and/or missing body mass index were excluded. According to their NAFLD and MAFLD status based on metadata and ultrasound, participants were allocated in overlap fatty liver disease (FLD), NAFLD-only, MAFLD-only, or no FLD. Fibrosis was defined as liver stiffness ≥8.0 kPa. In our analysis, 5445 participants were included: 1866 (34.3%) had MAFLD and 1604 (29.5%) [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "1623 (29.8%)"] had NAFLD. This resulted in 1547 (28.4%) [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "1566 (28.8%)"] individuals with overlap FLD, 319 (5.9%) [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "300 (5.5%)"] with MAFLD-only, 57 (1.0%) with NAFLD-only, and 3522 (64.7%) with no FLD. The MAFLD-only group was strongly associated with fibrosis (adjusted OR 5.30 [Correction added on December 27, 2021 after first online publication:

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FLD, fatty liver disease; GGT, gamma-glutamyl transpeptidase; HOMA-IR, homeostatic model assessment of insulin resistance; LSM, liver stiffness measurement; MAFLD, metabolic dysfunction–associated fatty liver disease; P25–P75, 25th to 75th percentile.

Laurens A. van Kleef and Ibrahim Ayada contributed equally to this work.

The preceding fragment was changed from "OR 5.27"], $p < 0.001$) and log-transformed liver stiffness (adjusted beta 0.116, $p < 0.001$), as opposed to the NAFLD-only group, in which no cases of fibrosis were identified and no association with liver stiffness (adjusted beta 0.006, $p = 0.90$) was found.

Conclusions: FLD is highly prevalent in the general population. However, not the NAFLD-only, but the MAFLD-only group was associated with fibrosis and higher liver stiffness—independent of demographic and lifestyle factors. We believe that using the MAFLD criteria will help improve the identification and treatment of patients with FLD at risk for fibrosis.

INTRODUCTION

Fatty liver disease (FLD) is increasingly common with an estimated adult prevalence of 25% worldwide. It has become one of the leading causes of cirrhosis and HCC in the Western world.[1] The global rise of this disease and its burden on health care outcome(s) has followed a worrisome and rapid increase in obesity and metabolic disorders.[2,3] Hepatic complications among individuals with FLD are relatively uncommon (0.77 deaths per 1000 person-years), compared with cardiovascular disease–specific mortality (4.79 per 1000 person-years).[1] Therefore, it is challenging to identify patients with advanced liver disease. Despite the low relative risk, absolute numbers of advanced FLD (driven by the sheer amount of individuals with FLD) have made FLD one of the leading causes for liver transplantation.[4] Because individuals with FLD have a significant cardiovascular risk, adequate multidisciplinary attention is key.[5,6] This includes focusing on improved lifestyle, treatment of hypertension, diabetes and lipid disorders, even in case of secondary causes of steatosis such as excessive alcohol consumption and steatogenic drug use.[7,8]

For this reason, an entity of metabolic dysfunction–associated fatty liver disease (MAFLD) was proposed, allowing the co-existence of secondary causes of steatosis. This definition consists of hepatic steatosis with diabetes, overweight, or at least two minor metabolic abnormalities.[9] The rationale for changing this definition is to acknowledge the primary drivers of NAFLD instead of ruling out other causes. Moreover, this definition provides guidance for screening and treating metabolic comorbidity by medical and lifestyle interventions.[10] Finally, from the patients' perspective, the current term "nonalcoholic" is not informative, suggesting the linkage with alcohol that can be stigmatizing and misleading.[11]

The shift from NAFLD to an etiology and inclusion-based definition of MAFLD has not yet been extensively studied, especially in European populations. Some recent publications already showed the usefulness and applicability of the MAFLD criteria in identifying individuals with impaired liver health and increased cardiovascular risk.[12–15] However, those were hampered by limited sample size, no access to transient elastography, and/or lack of up-to-date data from the general population that reflects the current extent of the fatty liver disease pandemic. As of yet, the association of MAFLD and liver stiffness in the general population needs to be determined. Therefore, this study addresses the differences in patient characteristics, liver stiffness, and fibrosis using both the MAFLD and the conventional NAFLD definition.

PARTICIPANTS AND METHODS

Participants

We performed a cross-sectional investigative analysis within the Rotterdam Study, a large ongoing prospective population-based cohort. Citizens aged over 45 years and living in the Rotterdam suburb of Ommoord were eligible to participate and invited periodically for assessment. The department of Gastroenterology and Hepatology joined in 2009 and introduced a liver ultrasound and transient elastography program (FibroScan; Echosens, Paris, France). The rationale of the Rotterdam Study and detailed information were provided previously.[16]

Participants who attended the liver ultrasound program between March 2009 and June 2014 were eligible for inclusion. In line with previous studies, participants who had a major risk factor for fibrosis, other than FLD, were excluded.[12] This consisted of >60 g of daily alcohol consumption and viral hepatitis based on HBsAg or anti-hepatitis C (Roche Diagnostics, Mannheim, Germany). Additionally, participants were excluded in the case of (1) missing food frequency questionnaire (FFQ) data for the last two visits while drinking ≥4 days a week (because excessive alcohol could not be ruled out), or (2) missing body mass index (BMI) in the presence of steatosis and no other MAFLD inclusion criteria for persisting uncertainty about MAFLD diagnosis.

NAFLD diagnosis

NAFLD was defined as steatosis in the absence of well-known secondary causes of steatosis, consisting of steatogenic drug use (i.e., amiodarone, corticosteroids, and methotrexate) and excessive alcohol consumption, defined as >20 g daily in females or >30 g in males, on either the FFQ or the home interview.[17]

MAFLD diagnosis

According to Eslam et al.,[9] MAFLD was defined as steatosis in combination with metabolic dysfunction. This consists of overweight (BMI ≥ 25 kg/m^2), type 2 diabetes mellitus (defined as antidiabetic drug use or fasting plasma glucose ≥7.0 mmol/L), or a combination of at least two of the following metabolic abnormalities: (1) waist circumference ≥102 cm for males and ≥88 cm for females, (2) blood pressure ≥130/85 mmHg or antihypertensive drug use, (3) plasma triglycerides ≥1.70 mmol/L or lipid-lowering drug treatment, (4) HDL cholesterol < 1.0 mmol/L for men and <1.3 mmol/L for women or lipid-lowering drug treatment, (5) prediabetes defined as fasting plasma glucose of 5.6-6.9 mmol/L, or (6) homeostatic model assessment of insulin resistance (HOMA-IR) of ≥2.5. The last minor MAFLD criterium, C-reactive protein level >2 mg/L, could not be applied, as these data were unavailable.

We refer to original MAFLD when the entire cohort was included, regardless of viral hepatitis, alcohol-associated liver disease, or missing alcohol data. When excluding cases of viral hepatitis, alcohol-associated liver disease and missing alcohol data, we refer to this as modified MAFLD.

Subgroups

To study differences carefully, we allocated the participants into subgroups based on their NAFLD and modified-MAFLD status, resulting in the following groups: (1) neither NAFLD nor MAFLD (hereafter referred to as “no FLD”; (2) both NAFLD and MAFLD (hereafter referred to as “overlap FLD”; (3) NAFLD without impaired metabolic health, and as a result, no MAFLD inclusion (hereafter referred to as “NAFLD-only”; and (4) MAFLD, but no NAFLD, due to presence of secondary causes of steatosis (excessive alcohol or steatogenic drug use; hereafter referred to as “MAFLD-only”).

Liver ultrasound and liver stiffness

A single experienced sonographer performed the liver ultrasounds on a Hitachi Hi Vision 900 (PvW) (Tokyo, Japan). Steatosis was defined dichotomously on hyperechoic liver parenchyma in comparison with the kidney cortex or spleen.[18] Images were saved digitally and reassessed on request by a hepatologist with over 10 years of experience in liver sonography. Liver stiffness measurement (LSM) was performed using transient elastography (FibroScan). At least 10 measurements were obtained with the M or XL probe. Measurements were considered unreliable and were discarded in case of an interquartile range >30%, together with a LSM ≥ 7.1 kPa, according to the Boursier criteria.[19] Subsequently, hepatic fibrosis was defined as LSM ≥ 8.0 kPa.[20]

Additional covariates

During each visit, research assistants measured anthropometrics, including waist circumference. Trained interviewers administered the questionnaires to ensure that questions were correctly interpreted and were completed accurately. Medication use was extracted from the pharmacy’s register to obtain accurate information on prescriptions of the participants and data on actual use was obtained during an interview. Blood samples were collected during fasting state. Glucose, blood lipids, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, and platelet count were assessed by automatic enzyme procedures and insulin with automatic immunoassay (Roche Diagnostics). HOMA-IR was calculated with glucose (mmol/L) multiplied by insulin (mmol/L) divided by 22.5.[21] The metabolic syndrome was defined according to the ATP-III criteria.[22]

Statistical analysis

Study characteristics were described with normally distributed variables provided as mean ± SD and non-normally distributed variables as median with 25th–75th percentile (P25–P75). ANOVA was used to study differences in normally distributed continuous data, Kruskal-Wallis for nonnormally distributed continuous data, and chi-squared test for categorical data. Logistic regression and linear regression were used to assess the association for the different subgroups (MAFLD-only, NAFLD-only, overlap FLD, and no FLD) and fibrosis or liver stiffness. In multivariable analysis, adjustments were made for demographics (age and sex), education level (low, moderate, or high), and intoxications (smoking [current/former or never] and alcohol consumption [grams/day]) and were selected based on prior research in this cohort.[23] Natural log transformation was applied to nonnormally distributed variables before being added to the models. The no-FLD participants functioned as the control group. In a sensitivity analysis, this control group was narrowed by excluding

participants with secondary causes for steatosis without meeting the MAFLD criteria.

Finally, the control group was replaced by participants without steatosis.

In an additional analysis, the same associations were investigated for original MAFLD, modified MAFLD, and NAFLD. The association between metabolic comorbidity and fibrosis was studied among participants with (both modified and original) MAFLD, and the role of concomitant excessive alcohol was explored. All analyses were performed in R version 4.0.3 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Participant characteristics

In the Rotterdam Study, 5967 participants underwent liver ultrasound between March 2009 and June 2014; among them, 522 were excluded, leaving 5445 participants for analysis (Figure 1). Baseline characteristics are provided for the included and excluded participants in Table S1. For the included participants, the mean age at study visit was 69.7 (SD 9.1) years; they were predominantly female (58.5%) and of European ancestry (97.4%). Metabolic comorbidities (i.e., hypertension, dyslipidemia, and [pre]diabetes) were common, resulting in a 42.0% prevalence of the metabolic syndrome. Hepatic steatosis was present in 35.5% (n = 1931). Reliable LSM were available in 72.7% (n = 3957) of the participants, and among them, 6.0% (n = 239) had fibrosis. The sensitivity and specificity for MAFLD to detect fibrosis was 59.4% and 69.9%, respectively, which was comparable to NAFLD (after exclusions for excessive alcohol and steatogenic drug use) of 55.7% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "56.1%"] and 69.2%. The positive and negative predictive value for MAFLD was 11.3% and 96.4%, and for NAFLD after exclusions, 8.8% and 96.7% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "10.2% and 96.2%"].

MAFLD identifies more individuals with FLD

NAFLD was diagnosed in 1604 of 5445 (29.5%) [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "1623 of 5445 (29.8%)"] participants, which represent 1604 of 4635 (34.6%) [Correction added on

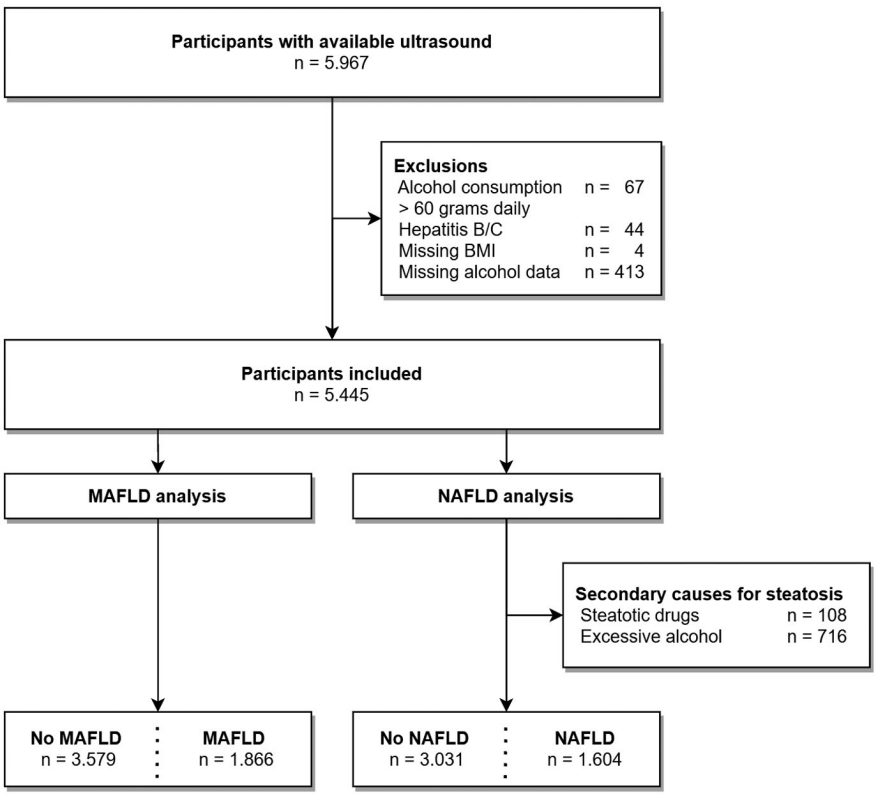

FIGURE 1 Participant selection. Flowchart of exclusions, NAFLD, and metabolic dysfunction–associated fatty liver disease (MAFLD) diagnosis. Participants can have multiple exclusion criteria or secondary causes for steatosis. Abbreviation: BMI, body mass index

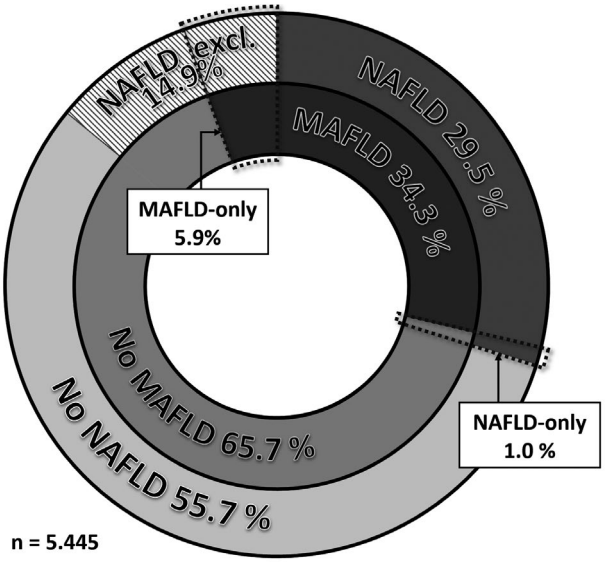

FIGURE 2 NAFLD and MAFLD distribution. The inner circle represents MAFLD, and the outer circle NAFLD. Non-overlapping groups are highlighted. The MAFLD-only criteria were present in 319 (5.9%) and NAFLD-only criteria in 57 (1.0%) participants. NAFLD-exclusion criteria were excessive alcohol and steatogenic drug use

December 27, 2021 after first online publication: The preceding fragment was changed from “1623 of 4737 (34.7%)”] of the participants without secondary causes of steatosis. Modified MAFLD was present in 1866 of 5445 (34.3%) participants. Diagnosis of modified MAFLD was based on steatosis together with overweight (*n* = 1740 of 1866), diabetes (*n* = 469 of 1866), and/or metabolic comorbidity (*n* = 1691 of 1866). Among the participants with modified MAFLD, 87% had >1 MAFLD criteria, and 22% had all MAFLD criteria. Table S2 provides the descriptive characteristics of the (modified and original) MAFLD and NAFLD populations, but no statistical tests were performed between them, given the extensive overlap. The individual associations among the original MAFLD, modified MAFLD, and NAFLD with fibrosis and liver stiffness are presented in Table S3. In general, associations for original MAFLD were significant and comparable with modified MAFLD, and observed effect sizes were more pronounced in the MAFLD groups, compared with NAFLD.

MAFLD was common in participants with NAFLD exclusion criteria

In our cohort, 1547 of 5445 (28.4%) [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from “1566 of 5445 (28.8%)”] had both NAFLD and modified-MAFLD, resulting in 96.4% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from “96.5%”] of individuals with NAFLD covered by the MAFLD criteria (Figure 2), and 3522 of 5445 (64.7%) had neither NAFLD nor MAFLD (no FLD). Finally, two non-overlapping groups were identified as MAFLD-only and NAFLD-only. The MAFLD-only criteria were present in 319 of 5445 (5.9%) [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from “300 of 5445 (5.5%)”] of the participants. This MAFLD-only group is characterized by having steatosis, but not fulfilling the criteria of NAFLD because of excessive alcohol consumption (90%) [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from “96%”] and/or steatogenic drug use (11%) [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from “4%”]. The NAFLD-only criteria were present in 57 of 5445 (1.0%) of the participants, and they did not comply with MAFLD, as no metabolic risk criteria were met. Of the participants with FLD (*n* = 1923), 80.4% had overlap FLD, 16.6% had MAFLD-only criteria, and 3.0% had NAFLD-only criteria [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "81.4% had overlap FLD, 15.6% had MAFLD-only criteria, and 3.0% had NAFLD-only criteria."].

MAFLD-only criteria were associated with fibrosis

To further assess the difference between NAFLD and MAFLD, the non-overlapping groups were investigated. As a result of the differences in selection criteria, participants with MAFLD-only criteria, compared with the NAFLD-only group, had more metabolic comorbidities (i.e., metabolic syndrome; $p < 0.001$) and alcohol intake ($p < 0.001$) (Table 1). No statistically significant differences for age, sex, or education were found. However, the MAFLD-only group had higher AST ($p = 0.004$ [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "$p = 0.001$"]), ALT ($p = 0.001$), GGT ($p < 0.001$), and liver stiffness ($p = 0.015$) as compared with the NAFLD-only group. Moreover, fibrosis was common among MAFLD-only criteria, and not at all present in individuals with NAFLD-only criteria (14.9% vs. 0.0%; $p = 0.015$ [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "14.5% vs 0.0%; $p = 0.018$"]) (Table 1).

Compared to no FLD, the MAFLD-only group was associated with fibrosis (OR 4. 62 [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "OR 4.46"], $p < 0.001$), and this was persistent after adjusting for age, sex, alcohol consumption, smoking, and

TABLE 1 Characteristics of the MAFLD-only and NAFLD-only groups, compared with no fatty liver disease

Variable	Overlap FLD, *n* = 1547[d]	MAFLD-only (A), *n* = 319[e]	NAFLD-only (B), *n* = 57	No FLD (C), *n* = 3522	A vs. B, *p* value	A vs. C, *p* value	B vs. C, *p* value
Demographics							
Age (years)	69.9 (8.5)	68.8 (7.7)	68.5 (9.4)	69.7 (9.4)	0.815	0.082	0.334
Male, *n* (%)	641 (41.4)	166 (52.0)	26 (45.6)	1426 (40.5)	0.453	<0.001	0.518
European ancestry, *n* (%)	1323 (97.6)	294 (99.7)	51 (98.1)	3029 (97.1)	0.691	0.017	1.000
Education, *n* (%)					0.811	0.044	0.360
Low	847 (55.5)	130 (41.3)	25 (43.9)	1602 (45.9)			
Intermediate	424 (27.8)	118 (37.5)	22 (38.6)	1069 (30.6)			
High	255 (16.7)	67 (21.3)	10 (17.5)	818 (23.4)			
Current/former smoking, *n* (%)	1054 (68.3)	260 (81.5)	33 (58.9)	2088 (62.6)	<0.001	<0.001	0.451
Excessive alcohol intake,[a] *n* (%)	0 (0.0)	288 (90.3)	0 (0.0)	428 (12.2)	<0.001	<0.001	0.009
Physical examination							
High waist circumference,[b] *n* (%)	1132 (73.3)	230 (72.1)	0 (0.0)	1072 (30.4)	<0.001	<0.001	<0.001
BMI (kg/m^2)	30.4 (4.3)	29.8 (4.2)	23.4 (1.3)	26.2 (3.7)	<0.001	<0.001	<0.001
Comorbidity							
Hypertension, *n* (%)	1296 (83.8)	271 (85.0)	26 (45.6)	2416 (68.6)	<0.001	<0.001	<0.001
Diabetes, *n* (%)	406 (26.9)	63 (20.1)	0 (0.0)	361 (10.4)	<0.001	<0.001	0.019
Metabolic syndrome, *n* (%)	1040 (68.6)	218 (69.6)	0 (0.0)	984 (28.5)	<0.001	<0.001	<0.001
Biochemistry							
AST (U/L)	25 [21, 29]	26 [22, 31]	23 [21, 26]	24 [21, 28]	0.004	<0.001	0.351
ALT (U/L)	21 [16, 28]	23 [18, 29]	18 [15, 25]	17 [14, 22]	0.001	<0.001	0.250
GGT (U/L)	28 [20, 39]	34 [24, 50]	20.50 [15, 28]	21 [16, 31]	<0.001	<0.001	0.400
Alkaline phosphatase (U/L)	70 [59, 82]	67 [54, 79]	68 [60, 81]	68 [58, 80]	0.171	0.013	0.727
Platelets (10^9/L)	272 (66)	264 (67)	276 (51)	268 (69)	0.225	0.318	0.425
HDL-C (mmol/L)	1.31 (0.34)	1.44 (0.42)	1.61 (0.43)	1.55 (0.44)	0.004	<0.001	0.310
Triglycerides (mmol/L)	1.58 [1.20, 2.11]	1.54 [1.15, 2.13]	1.01 [0.75, 1.34]	1.16 [0.91, 1.53]	<0.001	<0.001	0.002
HOMA-IR	1.60 [1. 09, 2.41]	1.35 [0.98, 1.98]	0.79 [0.53, 1.05]	0.82 [0.58, 1.18]	<0.001	<0.001	0.232
Transient elastography							
Liver stiffness (kPa)	5.2 [4.1, 6.4]	5.1 [4.2, 6.6]	4.9 [3.9, 5.3]	4.6 [3.8, 5.7]	0.015	<0.001	0.931
Fibrosis,[c] *n* (%)	108 (10.4)	34 (14.9)	0 (0.0)	97 (3.7)	0.015	<0.001	0.399

Note: Data are presented as mean (SD), median (25th–75th percentile [P25–P75]), or *n* and percentage. The *p* values are calculated using ANOVA, Kruskal-Wallis, or chi-squared test.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; FLD, fatty liver disease; GGT, gamma- glutamyl transpeptidase; HDL-C, HDL cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance.

[a]Daily alcohol consumption > 30 g for male and >20 g for female.

[b]Waist circumference >102 cm for male and >88 cm for female.

[c]Defined as liver stiffness ≥ 8.0 kPa.

[d][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "n = 1566". This in turn lead to minor insignificant changes in the characteristics.]

[e][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "n = 300". This in turn lead to minor insignificant changes in the characteristics.]

education level (adjusted OR [aOR] 5.30 [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "5.27"], $p < 0.001$). Similar results were obtained for overlap FLD (aOR 3.29 [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "aOR 3.31"], $p < 0.001$) (Table 2). This did not apply to the NAFLD-only criteria, as no cases of fibrosis were identified and logistic regression analysis was not possible. Therefore, with linear regression (Table 3), no association between NAFLD-only and (natural) log-transformed LSM could be demonstrated compared with no FLD in multivariable analysis (adjusted beta

TABLE 2 Association of MAFLD-only criteria and overlap FLD with fibrosis (LSM ≥ 8.0 kPa) compared with no FLD

		Unadjusted			Adjusted		
	Cases	OR	95% CI	*p* value	OR	95% CI	*p* value
MAFLD-only	34 of 228[a]	4.62[b]	3.01–6.94	<0.001	5.30[c]	3.12–8.89	<0.001
NAFLD-only	0 of 42		NA			NA	
Overlap FLD	108 of 1034[d]	3.07[e]	2.31–4.09	<0.001	3.29[f]	2.44–4.42	<0.001

Note: Results were obtained with logistic regression and given as OR and 95% CI for fibrosis as outcome, the reference group had no FLD (cases = 97 of 2653). For NAFLD-only criteria, no cases of fibrosis were observed; therefore, logistic regression was not possible. Multivariable analyses were adjusted for age, sex, alcohol consumption, smoking, and education.

Abbreviations: LSM, liver stiffness measurement; NA, not available.

[a][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "31 of 214"]

[b][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "4.46 (2.86 - 6.80)"]

[c][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "5.27 (2.99 - 9.16)"]

[d][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "111 of 1048"]

[e][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "3.12 (2.35 - 4.15)"]

[f][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "3.31 (2.47 - 4.45)"]

TABLE 3 Association of MAFLD-only, NAFLD-only, and overlap-FLD criteria with log-transformed liver stiffness (kPa) compared with no FLD

		Unadjusted			Adjusted		
	n	beta	95% CI	*p* value	beta	95% CI	*p* value
MAFLD only	228[a]	0.134[b]	0.091–0.176	<0.001	0.116[c]	0.072–0.160	<0.001
NAFLD only	42	–0.002	–0.096 to 0.092	0.963	0.006	–0.083 to 0.095	0.900
Overlap FLD	1034[d]	0.111[e]	0.087–0.134	<0.001	0.106	0.083–0.128	<0.001

Note: Results were obtained with linear regression and given as beta with 95% CI for (natural) log-transformed liver stiffness (kPa) as outcome; the reference group had no FLD (*n* = 2653). Multivariable analyses were adjusted for age, sex, alcohol consumption, smoking, and education.

[a][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "214"]

[b][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "0.131 (0.088 - 0.175)"]

[c][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "0.116 (0.070 - 0.161)"]

[d][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "1048"]

[e][Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "0.111 (0.088 - 0.135)"]

0.006, $p = 0.90$). In contrast, this was clearly present for MALFD-only (adjusted beta 0.116, $p < 0.001$) and overlap FLD (adjusted beta 0.106, $p < 0.001$). Results from linear and logistic regression were consistent when the control group was replaced by participants without steatosis nor secondary causes for steatosis and available liver stiffness measurement (control group A; *n* = 2262) or by participants without steatosis (control group B; *n* = 2647) (Table S4).

Metabolic comorbidity is associated with fibrosis in participants with MAFLD

Participants with modified MAFLD were categorized for the number of present inclusion criteria, consisting of overweight, diabetes mellitus, and two or more minor metabolic comorbidities. Fibrosis prevalence increased from 8.3% and 8.9% for one and two criteria present to 20.5% for meeting all three MAFLD inclusion criteria. By logistic regression, fibrosis presence increased for having three (OR 2.43, $p < 0.001$) compared with having only one or two inclusion criteria. This result was persistent after adjusting for age, sex, education level, smoking status, and alcohol consumption (aOR 2.42, $p < 0.001$). One could argue that this association is driven primarily by the group of excessive alcohol consumers. Importantly, however, we observed that among participants with MAFLD, having all MAFLD criteria was associated with increased risk of fibrosis, regardless of the presence of excessive alcohol consumption (adjusted HR [aHR] 2.30, 95% CI 1.49-3.53, without concomitant excessive alcohol consumption, compared with aHR 3.63, 95% CI 1.51-8.10, for concomitant excessive alcohol consumption) (Table S5). Similar results were obtained for the metabolic syndrome, which was also associated with an increased risk for fibrosis (aOR 1.86, $p = 0.004$) among individuals with MAFLD. Despite larger ORs among subjects with superimposed excessive alcohol consumption, no statistical significance was reached. This was in line with additional analysis: among subjects with MAFLD, higher log-transformed liver stiffness was observed for excessive alcohol consumption (beta 0.026, $p = 0.344$) but was not statistical significant.

DISCUSSION

In this large ongoing population-based cohort study, we examined the consequences of adopting the MAFLD criteria on identifying fibrosis and liver stiffness as compared with the conventional NAFLD definition. The prevalence of modified MAFLD was higher than that of NAFLD in this elderly population (34.3% and 29.5% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "29.8%"], respectively), and among the participants with NAFLD, 96.4% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "96.5%"] also complied with the MAFLD criteria. These results are consistent with other studies, which showed prevalences of 26%–37% for MAFLD,[13–15,24,25] with >94% of the NAFLD cases also being identified with MAFLD.[14,15,25]

In our cohort, 1.0% was classified as NAFLD-only (i.e., having FLD without impaired metabolic health, thus not meeting the MAFLD definition). This implies that few participants are missed when applying the MAFLD criteria (most participants are in the overlap FLD) (Figure 2). A similar NAFLD-only prevalence was reported by Niriella et al.[14] (1.3%) and Wong et al.[15] (1.7%), whereas Lin et al.[13] reported 4.7% of individuals having NAFLD-only criteria in the NHANES-III cohort. The difference in prevalence of the latter study as compared with ours may be explained by the younger population (43.7 vs. 69.7 years), which was notably less prone to hypertension (24.9% vs. 73.6%). Moreover, given the fact that NHANES-III data were collected between 1988 and 1994, demographics, comorbidity, and disease characteristics may have changed.

Interestingly, baseline characteristics of NAFLD-only criteria were similar to those of the no-FLD population, an observation in agreement with previous literature.[13,15] Moreover, multivariable analysis did not show an association with LSM for the NAFLD-only population, compared with no FLD. This suggests that not including NAFLD-only criteria with the MAFLD criteria does not impair good patient care, meaning patients with an elevated LSM are not being missed. However, it is essential to assess the long-term outcomes of this group with further follow-up studies before firm conclusions can be made. Given the good metabolic health of the NAFLD-only group, genetic predisposition needs to be investigated. Variations in PNPLA3 (patatin-like phospholipase domain containing 3) and TM6SF2 (transmembrane 6 superfamily member 20), for example, have been linked to severe steatosis, steatohepatitis, and fibrosis even without overt metabolic comorbidities, driven by impaired hepatic lipid metabolism.[26–28] However, false-positive results from abdominal ultrasound should be considered, given the imperfect test characteristics compared with the golden standard, liver biopsy.[29]

MAFLD-only criteria were common, with a prevalence of 5.9% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "5.5%"] in this cohort, and represented 16.6% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "15.6%"] of the participants with FLD. Identification of this MAFLD-only group is important, as it was significantly associated with higher liver stiffness and fibrosis, independent of alcohol consumption, similar to previous reported results.[12,15] Therefore, adapting the MAFLD criteria will enable better identification of individuals at risk for having fibrosis, which is an important predictor for liver-related events, including HCC.[30]

Fibrosis prevalence was higher among participants with MAFLD who had more metabolic comorbidity, such as when all MAFLD inclusion criteria or the metabolic syndrome were present. A similar approach was reported by Yamamura et al., showing an association with fibrosis and metabolic abnormalities, in Japanese participants with FLD.[12] These consistent findings suggest that metabolic comorbidity, along with steatosis, are the main drivers of fibrogenesis. This observation supports the call for intensifying multidisciplinary management of MAFLD and lifestyle programs to improve metabolic health and alcohol awareness, in addition to careful assessment of liver health by a hepatologist, regardless of the presence of secondary causes of steatosis.[8]

Although this is a large ongoing European cohort investigating MAFLD with access to detailed metabolic health data alongside liver ultrasound and transient elastography, there are some limitations that need mentioning. First, our cohort of participants is an aging cohort with a mean age of 69.7 years and predominantly Caucasian. Therefore, it may not be entirely representative for the whole population. In particular, our results may not be generalizable to a multi-ethnic, younger population. Second, this is a cross-sectional study; therefore, causal relations and long-term outcomes could not be studied. This is of particular concern for the NAFLD-only group, which had no cross-sectional association with fibrosis, but might be at risk in the long term. A third and unavoidable factor is that 96.4% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "96.5%"] of the participants with NAFLD had overlap FLD. As a consequence, the only comparison between NAFLD and MAFLD was possible using the non-overlapping “only” groups with relatively small numbers. This might have resulted in insufficient statistical power, especially in the NAFLD-only group (n = 57). However, the effect sizes found in this group were very small, and might not have clinical relevance,

even if it was significant. Fourth, although liver ultrasound is the diagnostic modality mostly used in the assessment of liver steatosis, it should be noticed that liver ultrasound has limited sensitivity for detecting mild steatosis.[29] Fifth, LSM is a noninvasive approach to assess the presence of liver fibrosis and has a strong correlation with histologically staged liver fibrosis. Nonetheless, the gold standard for both steatosis and fibrosis remains liver biopsy, despite being invasive and prone to sampling error.[31,32] Sixth, in our cohort we had no information on C-reactive protein, which is one of the minor metabolic inclusion criteria for MAFLD diagnosis.[9] Despite missing this information, we already had a low rate of NAFLD-only criteria, it is therefore unlikely that this had a major impact on our results. Seventh, the Rotterdam Study was not designed to study alcohol consumption specifically. Therefore, additional studies focusing on alcohol consumption are required in larger cohorts with more detailed alcohol data to further elucidate the potential synergistic risk with metabolic dysfunction. Finally, because chronic viral hepatitis and alcohol-associated liver disease were excluded for modified MAFLD (to allow for a fair comparison between MAFLD and NAFLD), these results might not be entirely generalizable to the entire MAFLD population. However, comparable associations were found for original-MAFLD and modified-MAFLD criteria, indicating that the impact of this selection on our results is limited.

In conclusion, we found that 96.4% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "96.5%"] of the participants with NAFLD were also identified with the MAFLD criteria (i.e., overlap FLD). NAFLD without impaired metabolic health, NAFLD-only, was present in only 1.0% of the population. It had similar characteristics as no FLD, and was not associated with liver stiffness. Hence, use of the MAFLD definition does not appear to lead to exclusion of patients with FLD at risk for fibrosis. In contrast, 5.9% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "5.5%"] of our cohort had MAFLD-only criteria, representing 16.6% [Correction added on December 27, 2021 after first online publication: The preceding fragment was changed from "15.6%"] of the participants with FLD. This MAFLD-only group was associated with both higher LSM and more prevalent fibrosis, which is an important predictor of hepatic complications. Moreover, among the participants with MAFLD, metabolic comorbidity (e.g., metabolic syndrome) was associated with fibrosis, which underlines the importance of this definition. It also encourages adequate multidisciplinary treatment and lifestyle interventions between disciplines. To identify the MAFLD-only group, which would not have been identified by using the NAFLD criteria, we recommend considering using the MAFLD criteria.

ETHICS

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare, and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (www.trialregister.nl) and into the World Health Organization International Clinical Trials Registry Platform (www.who.int/ictrp/network/primary/en/) under shared catalog number NTR6831. All participants provided written, informed consent to participate in the study and to have their information obtained from treating physicians. All authors had access to the study data and reviewed and approved the final manuscript.

CLINICAL TRIAL NUMBER

NTR6831.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contribution of the participants of the Rotterdam Study, research assistants (particularly Paulien van Wijngaarden for performing the liver ultrasounds), the general practitioners, hospitals, and pharmacies in Rotterdam. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University (Rotterdam, Netherlands), Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. Financial support was also provided by the Foundation for Liver and Gastrointestinal Research (Rotterdam, the Netherlands). The funding sources did not influence study design, data collection, analysis and interpretation of the data, nor the writing of the report and decision to submit for publication.

CONFLICT OF INTEREST

Dr. de Knegt consults and received grants from AbbVie. He is on the speakers' bureau for Echosens. He received grants from Gilead and Janssen.

AUTHOR CONTRIBUTIONS

Data analysis and writing of the manuscript: Laurens A. van Kleef, Ibrahim Ayada, and Robert de Knegt. *Study design, critical review of the manuscript, and approval of final version:* Laurens A. van Kleef, Ibrahim Ayada, Louise J.M. Alferink, Qiuwei Pan, and Robert de Knegt. All authors approved submission of the manuscript.

DATA AVAILABILITY STATEMENT

Data can be obtained upon request. Requests should be directed toward the management team of the Rotterdam Study (secretariat.epi@erasmusmc.nl), which has a protocol for approving data requests. Because of restrictions based on privacy regulations and informed consent of the participants, data cannot be made freely available in a public repository.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: van Kleef LA, Ayada I, Alferink LJM, Pan Q, de Knegt RJ. Metabolic dysfunction–associated fatty liver disease improves detection of high liver stiffness: The Rotterdam Study. Hepatology. 2022;75:419–429. https://doi.org/10.1002/hep.32131