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Prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) in a middle-aged population with overweight and normal liver enzymes, and diagnostic accuracy of noninvasive proxies

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Abstract

The prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) is increasing at an alarming rate. Elevated liver enzymes are a primary reason to refer patients for further testing. However, liver enzymes within the normal range do not exclude the presence of MASLD. We examined the prevalence of MASLD in a middle-aged population with overweight and normal liver enzymes. In addition, we examined the accuracy of 4 sets of noninvasive proxies for MASLD.

We included 1017 participants from the Netherlands epidemiology of obesity cohort study with body mass index ≥25 kg/m^2 and liver enzymes (asparate aminotransferase, alanine aminotransferase, gamma-glutamyltranspeptidase) within normal range. The diagnostic accuracy of biomarker scores (fatty liver index, liver fat score [LFS], STEATO-ELSA, and hepatic steatosis index) was determined against elevated hepatic triglyceride content measured by ^{1}proton magnetic resonance spectroscopy.

Participants (mean age 56 years, 49% women), had a median body mass index of 29.6 kg/m^2 and a median hepatic triglyceride content of 4.4%. MASLD was present in 42% of participants and was more common in men than women, with respectively 47% and 36% being affected. The LFS showed the highest accuracy with an area under the curve of 0.72. We identified metabolic syndrome as the prime predictor for MASLD with an odds ratio of 2.95 (95% confidence interval 2.20–3.98).

The prevalence of MASLD in middle-aged men and women with overweight and liver enzymes within the normal range is over 40%. LFS showed the highest accuracy to detect MASLD, but, overall, biomarker scores performed relatively poor. The presence of metabolic syndrome was the prime predictor of MASLD.

Abbreviations: ALT = alanine aminotransferase, AST = asparate aminotransferase, AUC = area under the curve, BMI = body mass index, CI = confidence interval, FLI = fatty liver index, HDL = high density lipoprotein, H-MRS = proton magnetic resonance spectroscopy, HSI = hepatic steatosis index, HTGC = hepatic triglyceride content, IQR = interquartile range, LFS = liver fat score, LUMC = Leiden university medical center, MASLD = metabolic dysfunction-associated steatotic liver disease, MRI = magnetic resonance imaging, NAFLD = non-alcoholic fatty liver disease, NEO = Netherlands epidemiology of obesity, OR = odds ratio, ULN = upper limit of normal, VCTE = vibration controlled transient elastography.

Keywords: biomarkers scores, MASLD, metabolic syndrome, NEO study, noninvasive tools

KCVS and LCTN-N have contributed equally to this work.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

Metabolic dysfunction-associated liver disease (MASLD), formerly known as nonalcoholic fatty liver disease (NAFLD),[1] is an increasingly prevalent disease and has been linked to metabolic, cardiovascular and malignant morbidity.[2] MASLD is defined as the presence of hepatic steatosis with the presence of metabolic risk factors, most notably type 2 diabetes mellitus and overweight.[1,3] It is considered to be more than a single entity, since it encompasses a range of phenotypes, starting from isolated steatosis, in which the predominant histological characteristic is lipid accumulation in hepatocytes, to metabolic dysfunction-associated steatohepatitis, characterized by the addition of hepatic inflammation and/or fibrosis, and metabolic dysfunction-associated steatohepatitis-related cirrhosis and hepatocellular carcinoma.[4,5] Abdominal, particularly visceral, obesity leading to insulin resistance is strongly associated with MASLD, both via increased delivery of free fatty acids to the liver and through increases of hepatic lipogenesis associated with hyperglycemia and hyperinsulinemia.[6–8] For this reason, MASLD is regarded as the hepatic manifestation of metabolic syndrome.[9–11] Metabolic syndrome is a cluster of metabolic abnormalities that increase the risk of cardiovascular disease and type 2 diabetes mellitus. It is typically diagnosed when an individual has at least 3 of the following 5 criteria: abdominal obesity, hypertension, elevated fasting glucose, high, and low high density lipoprotein cholesterol.[12]

The prevalence of MASLD ranges from 25% in the general population to 60% in high-risk populations, such as individuals with obesity and/or type 2 diabetes mellitus. The prevalence increases with age, obesity and lack of physical activity.[13,14] MASLD, when diagnosed at an early stage, is reversible when lifestyle modifications are implemented and causative factors are corrected.[15] Yet progression into fibrotic stages of MASLD is strongly associated with liver-related and overall mortality.[16,17] Thus, timely detection of MASLD is clearly called for. However, because MASLD commonly remains asymptomatic until the cirrhotic stage,[18] it is often overlooked and, consequently, undertreated.[19] Identifying individuals at high-risk of MASLD is needed to effectively treat patients and to prevent progression of disease, thus reducing liver-related and overall mortality.

To date, liver biopsy remains the gold standard for diagnosing and grading MASLD. However, liver biopsy is an invasive procedure that can be painful, has a risk of post-biopsy bleeding (up to 2%) and might convey a sampling error, due to only about 1/50.000th of the liver tissue being analyzed, while MASLD is often not equally distributed throughout the liver.[20,21] Proton magnetic resonance spectroscopy (^{1}H-MRS) is regarded as the most robust and accurate measure of hepatic triglyceride content (HTGC) and is consistent with liver biopsy in diagnosing and grading MASLD.[2,11,22–24] Although repeated assessments can be performed without safety concerns,[24,25] it is a time-consuming, costly procedure and only available in a few academic centers worldwide.[23] Many patients with MASLD express elevated serum liver enzymes, in particular alanine aminotransferase (ALT) and gamma-glutamyltranspeptidase (ϒGT). However, liver enzymes within the normal range do not exclude MASLD,[26] and although elevated liver enzymes may serve as a diagnostic clue for the presence of liver disease, they fail to predict the presence and severity of hepatic steatosis, inflammation and fibrosis.[5,27] The present European association for the study of the liver guideline recommends to screen for MASLD by liver enzymes and/or ultrasound as part of routine work-up in subjects with obesity or metabolic syndrome.[28] This may however underestimate the number of patients with MASLD.

The objective of this study was to examine the prevalence of MASLD in middle-aged people with overweight and, most notably, liver enzymes within the normal range. Additionally, we will assess predictive factors for MASLD in this cohort, and assess the diagnostic accuracy of 4 distinct noninvasive biomarker scores for MASLD, namely fatty liver index (FLI),[29] liver fat score (LFS),[30] STEATO-ELSA[31] and hepatic steatosis index[32] compared to HTGC measured by ^{1}H-MRS.

2. Methods

2.1. Study design and study population

Data from the Netherlands epidemiology of obesity (NEO) study was used for this study. The NEO study is an ongoing population-based cohort study, with baseline measurements between 2008 and 2012. The study included 6671 participants living in the greater area of Leiden (in the west of the Netherlands), aged 45 to 65 years and predominantly with overweight (body mass index [BMI] ≥25 kg/m^2).[33]

Participants attended a baseline visit at the NEO study center at the Leiden university medical center (LUMC) after an overnight fast. Before this study visit, participants completed a general questionnaire at home to report the medical history of relevant morbidities and demographic, lifestyle, and clinical information. At the visit, participants underwent an extensive physical examination, including anthropometry, and blood sampling. Furthermore, they completed a screening form to rule out contra-indications for magnetic resonance imaging (MRI) (most notably a body circumference >170 cm, claustrophobia or metal implants). Of the participants who were eligible for MRI, approximately 40% were randomly selected for a direct assessment of ^{1}H-MRS. In the present analysis, we excluded participants without ^{1}H-MRS assessment, technical failure of the ^{1}H-MRS or because of missing data other than ^{1}H-MRS. A detailed description of the study design and data collection of the NEO study has previously been published.[33] The medical ethics committee of the LUMC approved the study and all participants gave written informed consent.

Presence of type 2 diabetes mellitus was defined as a self-reported history of type 2 diabetes mellitus, the use of glucose-lowering drugs or a fasting plasma glucose ≥7.0 mmol/L. Prediabetes was defined as fasting plasma glucose between 5.6 and 7.0 mmol/L. Cardiovascular disease was defined as (history of) myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. HTGC was quantified by ^{1}H-MRS of the liver (1.5 Tesla whole-body MR scanner, Philips Medical Systems, the Netherlands).[33] Participants with excessive alcohol consumption, defined as ≥30 g/day and ≥20 g/day for men and women, respectively, and participants with liver enzymes above the upper limit of normal (ULN) (ALT ≥45 U/L, asparate aminotransferase [AST] ≥35 U/L and/or ϒGT ≥55 U/L for men and ALT ≥34 U/L, AST ≥31 U/L and/or ϒGT ≥38 U/L for women[34]). The ULN of our academic hospital (LUMC) was used a reference. Participants without overweight (BMI <25 kg/m^2) were excluded from final analyses.

2.2. Literature search

A literature search was performed which identified 59 biomarker scores, developed between 1988 and 2018, that predicted NAFLD, the precursor of MASLD.[8,11,20,22,23,31,32,35–93] Fifty-four biomarker scores were excluded because they reported biomarkers not measured in the NEO study, had patented formulas, or had a lack of cutoff values for NAFLD. Finally, 4 combinations of biomarkers were suitable for investigation, namely the FLI,[29] LFS,[30] STEATO-ELSA[31] and hepatic steatosis index.[32] We use proxies for NAFLD to assess the presence of MASLD as previous studies have demonstrated that 98% to 99% of patients with NAFLD would fulfill the new criteria for MASLD.[1,94]

2.3. Statistical analyses

Baseline characteristics were expressed as a mean with standard deviation (SD) when normally distributed, as a median with interquartile range (IQR) when not-normally distributed, or as a frequency accompanied by a percentage in categorical data. HTGC measured by ^{1}H-MRS was dichotomized, where a cutoff value of 5.56% or higher indicated MASLD.[33] The following variables are reported for the entire study population and the groups after dichotomization for HTGC: age, sex, menopausal status, alcohol consumption, smoking behavior, height, body weight, BMI, waist and hip circumference, blood pressure, laboratory findings (fasting glucose, insulin, total cholesterol, low density lipoprotein and high density lipoprotein cholesterol, triglycerides and liver enzymes AST, ALT, and ϒGT), homeostatic model assessment for insulin resistance, and factors of comorbidity and drug usage. To examine the performance of the aforementioned biomarker scores to rule in or rule out MASLD, sensitivity, specificity, predictive values and area under the curve (AUC) were calculated. Published cutoff values were used for each set of biomarkers.[29–32,46] Both the lower (≥30) and higher (≥60) cutoffs were used for FLI.[29] We stratified for sex to examine the accuracy of the sets of biomarkers in men and women separately.[44,95] A logistic regression analysis was performed to evaluate predictive factors for MASLD.

For all analyses, STATA statistical software (StataCorp, College Station, TX), version 14 was used.

3. Results

3.1. Baseline characteristics of the study population

From the 6671 participants included in the NEO study, a random sample of 2083 participants without contra-indications for MRI were invited for ^{1}H-MRS. After exclusion because of excessive alcohol consumption (n = 459) and missing data (n = 34), 711 (45%) participants had an elevated HTGC (n = 1590). When subsequently excluding individuals with BMI <25 kg/m^2 (n = 186), or with liver enzymes outside the normal range (n = 387), 686 (50%) and 444 (37%) participants had an elevated HTGC, respectively. After exclusion of participants with BMI <25 kg/m^2 and liver enzymes outside the normal range, a total of 1017 participants were eligible to be included in the final analyses, of whom 424 (42%) had an elevated HTGC. The flow of inclusion is shown in Figure 1. All baseline characteristics are shown in Table 1.

Table 1 also shows the participants divided into 2 groups; with HTGC <5.56% and HTGC ≥5.56%, defined as normal and elevated, respectively. In total, 424 (42%) participants had an elevated HTGC. There was a difference in age, with the group with elevated HTGC having a higher mean age, and the group with elevated HTGC had a higher percentage of men. Weight, BMI, and waist and hip circumference were higher in the participants with elevated HTGC. Median HTGC was 2.4% (IQR 1.6–3.7) and 11% (IQR 7.3–15.7) in the normal and elevated HTGC groups, respectively. More participants in the elevated HTGC group had metabolic syndrome (according to adult treatment panel III definition[12]), type 2 diabetes mellitus, prediabetes and hypertension, 64% compared to 29%, 11% compared to 5%, 40% compared to 26%, and 64% compared to 46% in the normal HTCG group, respectively.

3.2. Regression analysis

Table 2 shows the results of the multiple logistic regression analysis. The odds ratio (OR) of metabolic syndrome and BMI as a predictor for MASLD were 2.95 (95% confidence interval (CI) 2.20–3.98, $P < .00$) and 1.14 (95% CI 1.06–1.24, $P = .00$), respectively. Age and waist circumference were also statistically significant predictors of MASLD, both yielding an OR of 1.03 (95% CI 1.01–1.06, $P = .01$). The OR of type 2 diabetes mellitus as a predictor for MASLD was 1.18 (95% CI 0.69–2.03, $P = .55$) and thus not statistically significant. The OR of type 2 diabetes mellitus or prediabetes as a predictor for MASLD was 1.12 (95% CI 0.80–0.54, $P = .51$), see supplementary Table 3, http://links.lww.com/MD/J692.

3.3. Diagnostic accuracy

Table 3 shows the diagnostic accuracy of the 4 noninvasive biomarker scores compared to HTGC, for the entire studied population. Supplementary Tables 1, http://links.lww.com/MD/J693 and 2, http://links.lww.com/MD/J694 shows the diagnostic accuracy of the biomarker scores for men and women, respectively. The LFS had the biggest AUC both before (0.72 (95% CI 0.69–0.75)) and after stratification for sex (0.72 (95% CI 0.68–0.75) and 0.72 (95% CI 0.68–0.76) for men and women, respectively).

4. Discussion

Although liver enzymes above the ULN are currently an indication for referral by general practitioners, in our study population of middle-aged people with overweight and, notably, liver enzymes within the normal range, 42% of participants had HTGC ≥5.56% and could therefore be diagnosed with MASLD. This percentage was even higher among men, at 47%. Our findings demonstrate that relying upon liver enzymes as an indication for screening for MASLD will greatly underestimate the disease prevalence in this middle-aged population with overweight.

Our results complement the results of previously performed studies such as the Rotterdam study which sought to determine the prevalence of MASLD in an elderly population. In their cohort of 2811, 35.1% had MASLD, diagnosed using abdominal ultrasonography. Interestingly, in their cohort participants with MASLD had a median ALT of 21 U/L (IQR 16–17), whereas participants without MASLD had a median ALT of 17 U/L (IQR 14–21) which, although significantly different, is clinically irrelevant. AST was not significantly different between both groups.[14] These findings, together with the prevalence of 42% found in our study cohort of individuals without liver test abnormalities, underscore the inability of liver enzymes to exclude disease. Most screening programmes for MASLD aim to detect severe stages of disease, such as significant (≥F2) or even advanced (≥F3) fibrosis.[5,96] This follows

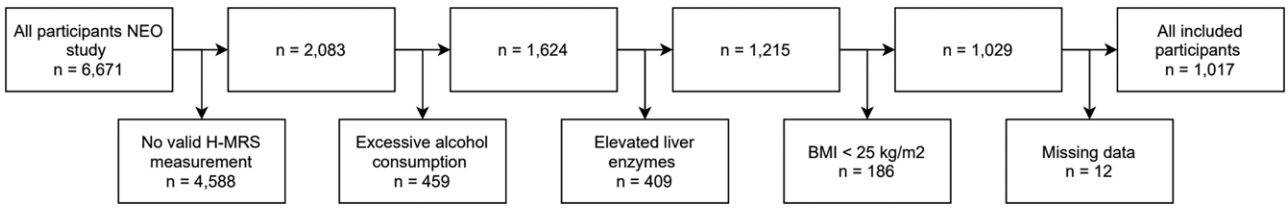

Figure 1. Flow chart of inclusion.

Table 1

Baseline demographics, clinical and histological characteristics of the participants, categorized by normal and increased HTGC.

			All included participants (n = 1017)	HTGC < 5.56% (n = 593)	HTGC ≧ 5.56% (n = 424)	*P* value
Age, yr*			56 (50–61)	55 (49–60)	57 (51–62)	.00‡
Female, n (%)			503 (49.46)	323 (54.47)	180 (42.45)	.00§
Alcohol consumption	Alcohol intake per d, g/d*		5.06 (0.88;14.24)	4.73 (0.52–13.04)	5.84 (1.22–14.50)	.05‡
Smoking behavior	Never smoked, n (%)		414 (40.71)	261 (44.01)	153 (36.08)	.04§
	Former smoker, n (%)		479 (47.10)	266 (44.86)	213 (50.24)	
	Current smoker, n (%)		124 (12.19)	66 (11.13)	58 (13.68)	
Height, m*			1.73 (1.67–1.81)	1.72 (1.67–1.80)	1.75 (1.68–1.82)	.01‡
Weight, kg†			91.23 (13.12)	88.22 (11.94)	95.44 (13.54)	.00‖
BMI, kg/m^2*			29.59 (27.72–31.98)	28.90 (27.32–30.95)	30.73 (28.62–33.13)	.00‖
Hepatic triglyceride content, %*			4.37 (2.15–9.09)	2.44 (1.57–3.69)	10.65 (7.27–15.69)	.00‡
Waist circumference, cm†			102.05 (10.27)	99.23 (9.46)	105.99 (10.07)	.00‖
Hip circumference, cm†			110.65 (7.76)	109.62 (7.30)	112.09 (8.16)	.00‖
Blood pressure	Systolic blood pressure, mm Hg†		132.32 (16.51)	129.70 (16.35)	136.00 (16.03)	.00‖
	Diastolic blood pressure, mm Hg†		85.13 (10.30)	83.51 (10.40)	87.39 (9.72)	.00‖
Laboratory findings	Fasting glucose, mmol/L*		5.41 (5.11–5.87)	5.30 (5.01–5.69)	5.63 (5.24–6.10)	.00‡
	Fasting insulin, mU/L*		10.40 (7.07–15.00)	8.75 (6.09–12.10)	13.40 (9.68–18.80)	.00‡
	Fasting total cholesterol, mmol/L†		5.63 (1.06)	5.63 (1.04)	5.64 (1.10)	.94‖
	Fasting LDL cholesterol¶, mmol/L†		3.60 (0.98)	3.61 (0.96)	3.60 (1.00)	.83‖
	Fasting HDL cholesterol, mmol/L†		1.38 (0.38)	1.46 (0.39)	1.27 (0.34)	.00‖
	Fasting triglycerides, mmol/L*		1.27 (0.92–1.68)	1.11 (0.83–1.47)	1.46 (1.10–2.03)	.00‡
	ASAT, U/L*		22.10 (19.40–25.70)	21.80 (18.90–25.10)	22.70 (19.85–26.60)	.00‡
	ALAT, U/L*		22.60 (18.00–28.60)	20.80 (16.60–25.80)	25.80 (20.50–31.10)	.00‡
	ϒGT, U/L*		22 (16–30)	20 (14–27)	25 (20–33)	.00‡
HOMA-IR*			2.52 (1.68–3.74)	2.05 (1.43–2.99)	3.40 (2.39–4.97)	.00‡
Medical history	Metabolic syndrome#, n (%)		446 (43.85)	174 (29.34)	272 (64.15)	.00§
	Cardiovascular disease, n (%)		95 (9.34)	46 (7.76)	49 (11.56)	.04§
	Type 2 diabetes	Yes, n (%)	75 (7.37)	27 (4.55)	48 (11.32)	.00§
		Prediabetes, n (%)	323 (31.76)	154 (25.97)	169 (39.86)	
		No, n (%)	619 (60.08)	412 (69.48)	207 (48.82)	
	Hypertension, n (%)		543 (53.39)	272 (45.87)	271 (63.92)	.00§
Drugs usage	Lipid lowering drugs, n (%)		130 (12.78)	62 (10.46)	68 (16.04)	.01§
	Glucose lowering drugs, n (%)	None, n (%)	979 (96.26)	581 (97.98)	398 (93.87)	.00§
		Oral medication, n (%)	33 (3.24)	10 (1.69)	23 (5.42)	
		Oral medication and insulin, n (%)	5 (0.49)	2 (0.34)	3 (0.71)	
	Antihypertensive drugs, n (%)		267 (26.25)	128 (21.59)	139 (32.78)	.00§

HDL = high density lipoprotein, HOMA-IR = Homeostatic model assessment for insulin resistance, HTGC = hepatic triglyceride content, LDL = low density lipoprotein.

*Value not normally distributed, therefore presented as median with interquartile range (median [IQR]).

†Value normally distributed, therefore presented as means with standard deviation (mean [SD]).

‡Mann–Whitney *U* test.

§Chi-square test.

‖Unpaired *t* test.

¶Calculated using Friedewald formula.

#According to ATP III definition.[12]

Table 2

Multiple logistic regression analysis.

Variable	Units of increase	OR (95% CI)	Standard error	*P* value
BMI	1 kg/m^2	1.143 (1.057–1.236)	0.046	.00
Sex	0 = male; 1 = female	0.720 (0.498–1.041)	0.136	.08
Age	1 yr	1.034 (1.010–1.058)	0.012	.01
Metabolic syndrome	0 = no; 1 = yes	2.952 (2.201–3.978)	0.442	.00
Type 2 diabetes mellitus	0 = no; 1 = yes	1.181 (0.686–2.032)	0.327	.55
Waist circumference	1 cm	1.032 (1.008–1.057)	0.013	.01
Hip circumference	1 cm	0.971 (0.941–1.002)	0.016	.07

BMI = body mass index, CI = confidence interval, OR = odds ratio.

Table 3

Diagnostic performance of biomarker scores compared to proton magnetic resonance spectroscopy (^{1}H-MRS).

		MASLD							
		+	–	Total	Sens	Spec	PPV	NPV	AUC
FLI	+	405	479	884	0.955 (0.931–0.973)	0.192 (0.161–0.226)	0.458 (0.425–0.492)	0.857 (0.786–0.912)	0.574 (0.555–0.592)
cutoff: ≥ 30	–	19	114	133					
	Total	424	593	1017					
FLI	+	332	299	551	0.759 (0.716–0.799)	0.614 (0.573–0.653)	0.584 (0.542–0.626)	0.781 (0.741–0.818)	0.687 (0.645–0.726)
cutoff ≥ 60	–	102	364	466					
	Total	424	593	1017					
LFS	+	305	167	472	0.719 (0.674–0.762)	0.718 (0.680–0.754)	0.646 (0.601–0.689)	0.782 (0.745–0.816)	0.719 (0.691–0.747)
cutoff: ≥ −0.640	–	119	426	545					
	Total	424	593	1017					
STEATO-ELSA	+	394	420	814	0.929 (0.901–0.952)	0.292 (0.255–0.330)	0.484 (0.449–0.519)	0.852 (0.796–0.898)	0.610 (0.588–0.633)
cutoff: >0.386	–	30	173	203					
	Total	424	593	1017					
HSI	+	388	428	816	0.915 (0.884–0.940)	0.278 (0.243–0.316)	0.475 (0.441–0.510)	0.821 (0.761–0.871)	0.597 (0.574–0.619)
cutoff: ≥ 36	–	36	165	201					
	Total	424	593	1017					

AUC = area under the curve, FLI = fatty liver index, HIS = hepatic steatosis index, LFS = liver fat score, NPV = negative predictive value, PPV = positive predictive value.

the notion that progression into fibrotic stages is strongly correlated with liver-related and overall mortality.[16,17] Yet, MASLD is associated with adverse outcomes even before fibrosis occurs, and MASLD may be a precursor for the future development of metabolic syndrome components, including type 2 diabetes mellitus.[97,98] Ultrasonographic changes in MASLD status over time may affect the risk of incident type 2 diabetes mellitus, hypertension and other components of the metabolic syndrome.[98–101] This emphasizes the central role the liver plays within systemic homeostasis and underscores why MASLD should not be overlooked. Moreover, the finding of MASLD may encourage early lifestyle changes including increased physical activity, decreased caloric intake (especially fructose-containing beverages), and adherence to a healthier diet, such as a Mediterranean diet.[102,103]

We identified the presence of metabolic syndrome as the prime predictor for MASLD. Participants who met the criteria for metabolic syndrome were at almost 3 times higher risk of MASLD than those who did not meet the criteria.[12,104] BMI was also identified as a statistically significant predictor of MASLD with the chance of having MASLD increasing 1.14-fold for every added 1 kg/m^2. Surprisingly, type 2 diabetes mellitus and prediabetes were not statistically significant predictors for MASLD in our study population. Age, male sex and waist circumference were all statistically significant predictors of MASLD, but to a lesser extent than metabolic syndrome or BMI.

The LFS was best able to differentiate between participants with and without MASLD in our population with normal liver enzymes, as it yielded the largest AUC of the investigated biomarker scores. The other biomarker scores had low specificities and would thus lead to more excessive referral rates and diagnostic testing. It needs to be stressed that other diagnostic modalities, such as vibration controlled transient electrography (VCTE) which yields an AUC of 0.87 for the detection of >5% steatosis,[105] outperform the studied sets of biomarkers. Moreover, VCTE may provide health care providers with an additional point of care effect as it allows for direct counseling of the patient and thus may aid in eventual lifestyle changes. Unfortunately, these more intricate diagnostic tools have limited availability, especially for general practitioners.[96]

Of course, our study design comes with its limitations. Firstly, by excluding patients without overweight there is a clear oversampling of individuals with metabolic syndrome, type 2 diabetes mellitus and hypertension in our data compared to the general population. The study population, however, does represent a population at risk of MASLD. Enrollment for the NEO study was limited to people between the ages of 45 and 65 years old. This limits the possibility of translating recommendations to the wider public as findings cannot be extrapolated to other age groups. An important strength of our study is the large number of participants and the robust assessment of hepatic steatosis using ^{1}H-MRS.

5. Conclusion

MASLD is taking epidemic forms and remains mostly undiagnosed and, therefore, untreated which may lead to severe consequences. Our data indicate that over 40% of individuals with overweight and between the ages of 45 and 65 years old may have undiagnosed MASLD, even when liver enzymes are within the normal range. In our NEO study cohort, the presence of metabolic syndrome was the biggest predictor for MASLD, followed by BMI and waist circumference. The LFS appears to be best able to detect MASLD in our study population. However, performance remains relatively poor compared to other noninvasive testing modalities such as VCTE.

Author contributions

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References

[1] Rinella ME, Lazarus JV, Ratziu V, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol. 2023.
[2] Ruissen MM, Mak AL, Beuers U, et al. Non-alcoholic fatty liver disease: a multidisciplinary approach towards a cardiometabolic liver disease. Eur J Endocrinol. 2020;183:R57–73.

[3] EASL-EASD-EASO. Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64:1388–402.
[4] Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34:274–85.
[5] Ruissen MM, Linde Mak A, Beuers U, et al. Non-alcoholic fatty liver disease: a multidisciplinary approach towards a cardiometabolic liver disease. 2020.
[6] Donnelly KL, Smith CI, Schwarzenberg SJ, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115:1343–51.
[7] Hardy T, Oakley F, Anstee QM, et al. Nonalcoholic fatty liver disease: pathogenesis and disease spectrum. Annu Rev Pathol. 2016;11:451–96.
[8] Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology. 2006;43(2 Suppl 1):S99–S112.
[9] Arab JP, Arrese M, Trauner M. Recent insights into the pathogenesis of nonalcoholic fatty liver disease. Annu Rev Pathol. 2018;13:321–50.
[10] Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. Nat Med. 2018;24:908–22.
[11] Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to treatment. Frontline Gastroenterol. 2014;5:277–86.
[12] Grundy SM, Brewer HB, Cleeman JI, et al. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Arterioscler Thromb Vasc Biol. 2004;109:433–8.
[13] Perumpail BJ, Khan MA, Yoo ER, et al. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J Gastroenterol. 2017;23:8263–76.
[14] Koehler EM, Schouten JN, Hansen BE, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in the elderly: results from the Rotterdam study. J Hepatol. 2012;57:1305–11.
[15] Martín-Domínguez V, González-Casas R, Mendoza-Jiménez-Ridruejo J, et al. Pathogenesis, diagnosis and treatment of non-alcoholic fatty liver disease. Rev Esp Enferm Dig. 2013;105:409–20.
[16] Taylor RJRS, Taylor RJRS, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology. 2020:1611–25.e12.
[17] Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology. 2017;65:1557–65.
[18] Arab JP, Barrera F, Gallego C, et al. High prevalence of undiagnosed liver cirrhosis and advanced fibrosis in type 2 diabetic patients. Ann Hepatol. 2016;15:721–8.
[19] Dyson JK, McPherson S, Anstee QM. Republished: non-alcoholic fatty liver disease: non-invasive investigation and risk stratification. Postgrad Med J. 2014;90:254–66.
[20] Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2014;20:475–85.
[21] Sanai FM, Keeffe EB. Liver biopsy for histological assessment: the case against. Saudi J Gastroenterol. 2010;16:124–32.
[22] Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab. 2005;288:E462–8.
[23] Ligabue G, Besutti G, Scaglioni R, et al. MR quantitative biomarkers of non-alcoholic fatty liver disease: technical evolutions and future trends. Quant Imaging Med Surg. 2013;3:192–5.
[24] Troelstra MA, Witjes JJ, van Dijk AM, et al. Assessment of imaging modalities against liver biopsy in nonalcoholic fatty liver disease: the Amsterdam NAFLD-NASH cohort. J Magn Reson Imaging. 2021;54:1937–49.
[25] Chin JL, Pavlides M, Moolla A, et al. Non-invasive markers of liver fibrosis: adjuncts or alternatives to liver biopsy? Front Pharmacol. 2016;7:159.
[26] Ma X, Liu S, Zhang J, et al. Proportion of NAFLD patients with normal ALT value in overall NAFLD patients: a systematic review and meta-analysis. BMC Gastroenterol. 2020;20:10.
[27] Wong VW, Wong GL, Tsang SW, et al. Metabolic and histological features of non-alcoholic fatty liver disease patients with different serum alanine aminotransferase levels. Aliment Pharmacol Ther. 2009;29:387–96.
[28] EASL-ALEH Clinical Practice Guidelines: non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol. 2015;63:237–64.
[29] Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006;6:33.
[30] Kotronen A, Peltonen M, Hakkarainen A, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology. 2009;137:865–72.
[31] Perazzo H, Benseñor I, Mill JG, et al. Prediction of liver steatosis applying a new score in subjects from the Brazilian longitudinal study of adult health. J Clin Gastroenterol. 2020;54:e1–e10.
[32] Lee JH, Kim D, Kim HJ, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis. 2010;42:503–8.
[33] de Mutsert R, den Heijer M, Rabelink TJ, et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol. 2013;28:513–23.
[34] Franck PFH SA, Postma J. Het Nederlandse Referentielaboratorium voor Enzymen. Ned Tijdschr Klin Chem. 2010;35:240–3.
[35] Eslamparast T, Tandon P, Raman M. Dietary composition independent of weight loss in the management of non-alcoholic fatty liver disease. Nutrients. 2017;9:800.
[36] Musso G, Gambino R, Cassader M, et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43:617–49.
[37] Cichoż-Lach H, Celiński K, Prozorow-Król B, et al. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. Med Sci Monit. 2012;18:CCR735–CR740.
[38] Lückhoff HK, Kruger FC, Kotze MJ. Composite prognostic models across the non-alcoholic fatty liver disease spectrum: clinical application in developing countries. World J Hepatol. 2015;7:1192–208.
[39] Zhang B, Ding F, Chen T, et al. Ultrasound hepatic/renal ratio and hepatic attenuation rate for quantifying liver fat content. World J Gastroenterol. 2014;20:17985–92.
[40] Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology. 2012;142:1592–609.
[41] Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2011;33:525–40.
[42] European Association for Study of L, Asociacion Latinoamericana para el Estudio del H. EASL-ALEH Clinical Practice Guidelines: non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol. 2015;63:237–64.
[43] Lee YH, Bang H, Park YM, et al. Non-laboratory-based self-assessment screening score for non-alcoholic fatty liver disease: development, validation and comparison with other scores. PLoS One. 2014;9:e107584.
[44] Perumpail BJ, Khan MA, Yoo ER, et al. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J Gastroenterol. 2017;23:8263–76.
[45] Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol. 2019;70:531–44.
[46] Otgonsuren M, Estep MJ, Hossain N, et al. Single non-invasive model to diagnose non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). J Gastroenterol Hepatol. 2014;29:2006–13.
[47] Vetter TR, Schober P, Mascha EJ. Diagnostic testing and decision-making: beauty is not just in the eye of the beholder. Anesth Analg. 2018;127:1085–91.
[48] Fedchuk L, Nascimbeni F, Pais R, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2014;40:1209–22.
[49] Kahl S, Straßburger K, Nowotny B, et al. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. PLoS One. 2014;9:e94059.
[50] Harrison SA, Oliver D, Arnold HL, et al. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut. 2008;57:1441–7.
[51] Cuthbertson DJ, Weickert MO, Lythgoe D, et al. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. Eur J Endocrinol. 2014;171:561–9.
[52] Angulo P, Bugianesi E, Bjornsson ES, et al. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology. 2013;145:782–9.e4.
[53] Wang J, Xu C, Xun Y, et al. ZJU index: a novel model for predicting nonalcoholic fatty liver disease in a Chinese population. Sci Rep. 2015;5:16494.

[54] Amato MC, Giordano C, Galia M, et al. Visceral Adiposity Index: a reliable indicator of visceral fat function associated with cardiometabolic risk. Diabetes Care. 2010;33:920–2.

[55] Chen G, Faris P, Hemmelgarn B, et al. Measuring agreement of administrative data with chart data using prevalence unadjusted and adjusted kappa. BMC Med Res Methodol. 2009;9:5.

[56] Ratziu V, Giral P, Charlotte F, et al. Liver fibrosis in overweight patients. Gastroenterology. 2000;118:1117–23.

[57] Sud A, Hui JM, Farrell GC, et al. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. Hepatology. 2004;39:1239–47.

[58] Miyaaki H, Ichikawa T, Nakao K, et al. Clinicopathological study of nonalcoholic fatty liver disease in Japan: the risk factors for fibrosis. Liver Int. 2008;28:519–24.

[59] Poynard T, Bedossa P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus METAVIR and CLINIVIR Cooperative Study Groups. J Viral Hepat. 1997;4:199–208.

[60] Kruger FC, Daniels CR, Kidd M, et al. APRI: a simple bedside marker for advanced fibrosis that can avoid liver biopsy in patients with NAFLD/NASH. S Afr Med J. 2011;101:477–80.

[61] Shoaei SD, Sali S, Karamipour M, et al. Non-invasive histologic markers of liver disease in patients with chronic hepatitis B. Hepat Mon. 2014;14:e14228.

[62] Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection comparison with liver biopsy and fibrotest. Hepatology. 2007;46:32–6.

[63] Ohta T, Sakaguchi K, Fujiwara A, et al. Simple surrogate index of the fibrosis stage in chronic hepatitis C patients using platelet count and serum albumin level. Acta Med Okayama. 2006;60:77–84.

[64] Calès P, Oberti F, Michalak S, et al. A novel panel of blood markers to assess the degree of liver fibrosis. Hepatology. 2005;42:1373–81.

[65] Demir M, Lang S, Schlattjan M, et al. NIKEI: a new inexpensive and non-invasive scoring system to exclude advanced fibrosis in patients with NAFLD. PLoS One. 2013;8:e58360.

[66] Lok AS, Ghany MG, Goodman ZD, et al. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. Hepatology. 2005;42:282–92.

[67] Zhang S, Du T, Li M, et al. Triglyceride glucose-body mass index is effective in identifying nonalcoholic fatty liver disease in nonobese subjects. Medicine (Baltim). 2017;96:e7041.

[68] Park YJ, Lim JH, Kwon ER, et al. Development and validation of a simple index system to predict nonalcoholic fatty liver disease. Korean J Hepatol. 2011;17:19–26.

[69] Yang H, Chen G, Song C, et al. A novel index including SNPs for the screening of nonalcoholic fatty liver disease among elder Chinese: a population-based study. Medicine (Baltim). 2018;97:e0272.

[70] Adams LA, Bulsara M, Rossi E, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem. 2005;51:1867–73.

[71] Campos GM, Bambha K, Vittinghoff E, et al. A clinical scoring system for predicting nonalcoholic steatohepatitis in morbidly obese patients. Hepatology. 2008;47:1916–23.

[72] Gholam PM, Flancbaum L, Machan JT, et al. Nonalcoholic fatty liver disease in severely obese subjects. Am J Gastroenterol. 2007;102:399–408.

[73] Seto WK, Lee CF, Lai CL, et al. A new model using routinely available clinical parameters to predict significant liver fibrosis in chronic hepatitis B. PLoS One. 2011;6:e23077.

[74] Bota S, Sirli R, Sporea I, et al. A new scoring system for prediction of fibrosis in chronic hepatitis C. Hepat Mon. 2011;11:548–55.

[75] Poynard T, Munteanu M, Deckmyn O, et al. Validation of liver fibrosis biomarker (FibroTest) for assessing liver fibrosis progression: proof of concept and first application in a large population. J Hepatol. 2012;57:541–8.

[76] Hui AY, Chan HL, Wong VW, et al. Identification of chronic hepatitis B patients without significant liver fibrosis by a simple noninvasive predictive model. Am J Gastroenterol. 2005;100:616–23.

[77] Bonacini M, Hadi G, Govindarajan S, et al. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol. 1997;92:1302–4.

[78] Hsieh YY, Tung SY, Lee K, et al. Routine blood tests to predict liver fibrosis in chronic hepatitis C. World J Gastroenterol. 2012;18:746–53.

[79] Islam S, Antonsson L, Westin J, et al. Cirrhosis in hepatitis C virus-infected patients can be excluded using an index of standard biochemical serum markers. Scand J Gastroenterol. 2005;40:867–72.

[80] Ahmad W, Ijaz B, Javed FT, et al. A comparison of four fibrosis indexes in chronic HCV: development of new fibrosis-cirrhosis index (FCI). BMC Gastroenterol. 2011;11:44.

[81] Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology. 2004;127:1704–13.

[82] Guha IN, Myers RP, Patel K, et al. Biomarkers of liver fibrosis: what lies beneath the receiver operating characteristic curve? Hepatology. 2011;54:1454–62.

[83] Ratziu V, Massard J, Charlotte F, et al. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol. 2006;6:6.

[84] Kelleher TB, Mehta SH, Bhaskar R, et al. Prediction of hepatic fibrosis in HIV/HCV co-infected patients using serum fibrosis markers: the SHASTA index. J Hepatol. 2005;43:78–84.

[85] Poynard T, Ratziu V, Naveau S, et al. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. Comp Hepatol. 2005;4:10.

[86] Cross TJ, Rizzi P, Berry PA, et al. King's Score: an accurate marker of cirrhosis in chronic hepatitis C. Eur J Gastroenterol Hepatol. 2009;21:730–8.

[87] Younossi ZM, Page S, Rafiq N, et al. A biomarker panel for non-alcoholic steatohepatitis (NASH) and NASH-related fibrosis. Obes Surg. 2011;21:431–9.

[88] Ulitsky A, Ananthakrishnan AN, Komorowski R, et al. A noninvasive clinical scoring model predicts risk of nonalcoholic steatohepatitis in morbidly obese patients. Obes Surg. 2010;20:685–91.

[89] Fung J, Lai CL, Fong DY, et al. Correlation of liver biochemistry with liver stiffness in chronic hepatitis B and development of a predictive model for liver fibrosis. Liver Int. 2008;28:1408–16.

[90] Koda M, Matunaga Y, Kawakami M, et al. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. Hepatology. 2007;45:297–306.

[91] Lykiardopoulos B, Hagström H, Fredrikson M, et al. Development of serum marker models to increase diagnostic accuracy of advanced fibrosis in nonalcoholic fatty liver disease: the new LINKI Algorithm compared with Established Algorithms. PLoS One. 2016;11:e0167776.

[92] Zhou YJ, Zhou YF, Zheng JN, et al. NAFL screening score: a basic score identifying ultrasound-diagnosed non-alcoholic fatty liver. Clin Chim Acta. 2017;475:44–50.

[93] Goh GB, Issa D, Lopez R, et al. The development of a non-invasive model to predict the presence of non-alcoholic steatohepatitis in patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol. 2016;31:995–1000.

[94] Hagström H, Vessby J, Ekstedt M, et al. 99% of patients with NAFLD meet MASLD criteria and natural history is therefore identical. J Hepatol. 2023.

[95] Lonardo A, Nascimbeni F, Ballestri S, et al. Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. Hepatology. 2019;70:1457–69.

[96] Van Dijk A-M, Schattenberg JM, Holleboom AG, et al. Referral care paths for non-alcoholic fatty liver disease-Gearing up for an ever more prevalent and severe liver disease. 2021.

[97] Mikolasevic I, Milic S, Turk Wensveen T, et al. Nonalcoholic fatty liver disease - A multisystem disease? World J Gastroenterol. 2016;22:9488–505.

[98] Lonardo A, Nascimbeni F, Mantovani A, et al. Hypertension, diabetes, atherosclerosis and NASH: cause or consequence? J Hepatol. 2018;68:335–52.

[99] Sung KC, Wild SH, Byrne CD. Resolution of fatty liver and risk of incident diabetes. J Clin Endocrinol Metab. 2013;98:3637–43.

[100] Sung KC, Wild SH, Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. J Hepatol. 2014;60:1040–5.

[101] Yamazaki H, Tsuboya T, Tsuji K, et al. Independent association between improvement of nonalcoholic fatty liver disease and reduced incidence of type 2 diabetes. Diabetes Care. 2015;38:1673–9.

[102] Houttu V, Bouts J, Vali Y, et al. Does aerobic exercise reduce NASH and liver fibrosis in patients with non-alcoholic fatty liver disease? A systematic literature review and meta-analysis. Front Endocrinol (Lausanne). 2022;13:1032164.

[103] Houttu V, Csader S, Nieuwdorp M, et al. Dietary interventions in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis. Front Nutr. 2021;8:716783.

[104] Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23:469–80.

[105] Eddowes PJ, Sasso M, Allison M, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology. 2019;156:1717–30.