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Erasmus University Rotterdam
Making Minds Matter

MASLD, cardiometabolic and inflammatory disorders

Alina N. Saidi

MASLD, cardiometabolic and inflammatory disorders
Thesis, Erasmus University, Rotterdam, the Netherlands

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MASLD, Cardiometabolic and Inflammatory Disorders

MASLD, cardiometabole en inflammatoire aandoeningen

Thesis

to obtain the degree of Doctor from
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by command of the
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Prof.dr.ir. A.J. Schuit

and in accordance with the decision of the Doctorate Board.
The public defence shall be held on

Friday 9 October 2026 at 13.00 hrs
by

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	Prof.dr. W.W. de Herder

Voor mijn ouders, wier onvoorwaardelijke liefde en steun mijn academische reis mogelijk hebben gemaakt.

.به والدین من، که عشق و حمایت بی قید و شرط آنها سفر اکادمیک من را ممکن ساخت

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Chapter 1

General introduction

Metabolic dysfunction associated steatotic liver disease (MASLD)

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the newly adopted term for what was previously known as non-alcoholic fatty liver disease (NAFLD). This change, introduced in June 2023 through an international, multi-society Delphi consensus process, marks a shift from a diagnosis of exclusion to one of inclusion. MASLD is defined by the accumulation of excess triglycerides in the liver in the presence of at least one cardiometabolic risk factor, and in the absence of excessive alcohol consumption (>20 g/day for women and >30 g/day for men) (1). The updated terminology underscores the metabolic nature of the disease and aims to support better recognition, diagnosis, and management.

Pathophysiology and risk factors

MASLD is a multifactorial disease that encompasses a spectrum of liver conditions, ranging from isolated steatosis to metabolic dysfunction-associated steatohepatitis (MASH), liver fibrosis, and cirrhosis (Figure 1). MASH is histologically defined by hepatic steatosis accompanied by lobular inflammation, hepatocyte ballooning, and/or fibrosis (1, 2).

The development and progression of MASLD are strongly driven by cardiometabolic risk factors. Among these, type 2 diabetes mellitus (T2DM) confers the highest risk, with studies showing faster rates of fibrosis progression, a more than twofold increase in the likelihood of advanced fibrosis (F3-F4), liver-related complications, and mortality (3-7). Other metabolic conditions, such as obesity (BMI ≥30 kg/m²), hypertension, and dyslipidemia (including hypertriglyceridemia and low HDL cholesterol), also contribute to disease progression, though their impact appears less pronounced than that of T2DM (3, 4).

The pathogenesis of MASLD involves a complex interplay between genetic, metabolic, dietary, and environmental factors. Among these, insulin resistance (IR) plays a central role. It promotes adipose tissue lipolysis and increased hepatic influx of free fatty acids, stimulates hepatic de novo lipogenesis (DNL), and impairs fatty acid oxidation. These processes drive hepatic fat accumulation, oxidative stress, and chronic low-grade inflammation, ultimately resulting in hepatocellular injury, steatohepatitis, and fibrosis (8-10).

Fibrosis and cardiovascular disease

MASLD and atherosclerotic cardiovascular disease (asCVD) are closely linked, as they share common pathophysiological mechanisms such as IR, endothelial dysfunction, and chronic low-grade inflammation. As a result, patients with MASLD are at increased risk of cardiovascular (CV) complications, including non-fatal cardiovascular disease (CVD) (HR 1.40), coronary heart disease (OR 1.33), and heart failure (HF) (OR 1.5) (11-14). MASLD has also been associated with markers of subclinical atherosclerosis, including increased carotid intima-media thickness (cIMT) and coronary artery calcification (CAC) (15).

While hepatic steatosis contributes to these risks, the presence and severity of liver fibrosis have emerged as the strongest predictors of CV outcomes in MASLD. Several studies have shown that liver stiffness is associated with subclinical atherosclerosis and incident CV events (16-20).

Given that CVD is now the leading cause of death in MASLD patients,(21-24) the identification of liver fibrosis carries not only hepatic significance but also important implications for CV risk stratification and coordinated management of liver and CV health.

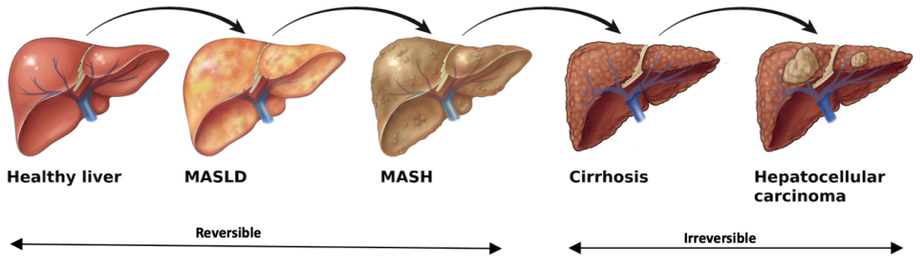


Figure 1. Different stages of metabolic dysfunction-associated steatotic liver disease.

Epidemiology

Over recent decades, MASLD has become the most common chronic liver disease worldwide, with its rising prevalence paralleling the global increase in obesity and T2DM. The estimated global prevalence of MASLD has increased from 25% in 2016 to over 30% today (25-28). Prevalence rates are particularly high in at-risk groups such as individuals with T2DM or obesity, reaching 60-75% (Figure 2) (28-30). Geographical variation exists, with the highest prevalence observed in Latin America (44%) and the lowest in Western Europe (25.1%) (31). Projections suggest that MASLD may affect up to 55% of the global population by 2040 (32). MASH is estimated to affect 5.3% of the global population, with fibrosis occurring in approximately one-third of the cases (25, 33). Current estimates suggest that significant fibrosis affects 8% of the population, while 1.5-3% have developed advanced stages (34-36). Looking ahead, modeling studies project a substantial rise in cirrhosis, hepatic decompensation, hepatocellular carcinoma (HCC), and MASLD-related mortality across most countries between 2015 and 2030 (37, 38). This projected increase is partly attributable to the rising prevalence of obesity, including in younger populations, which leads to earlier onset of MASLD and consequently a longer disease duration (39).

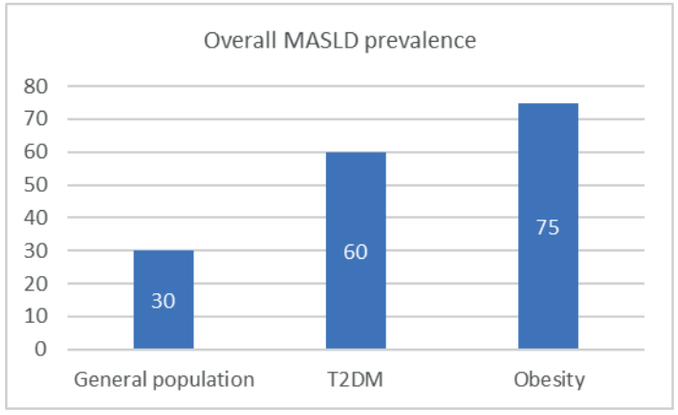


Figure 2. Prevalence of MASLD in the general population, among T2DM patients and patients with obesity.

Clinical impact

In its early stages, MASLD is asymptomatic and therefore often goes undiagnosed. Yet, timely intervention at this stage offers the potential to halt progression and even reverse the disease. Despite this, MASLD remains underdiagnosed, largely due to limited awareness among both healthcare providers and high-risk patients (40).

MASLD is now one of the leading indications for liver transplantation in the U.S. and Europe, underscoring its clinical severity (21-23). It is also strongly associated with reduced health-related quality of life (HRQoL) and imposes a substantial economic burden on healthcare systems, stemming from the costs of diagnosis, long-term management, and treatment of complications (41-43).

Tackling this challenge requires a multifaceted approach: raising awareness, systematically screening high-risk patients, and initiating timely treatment. This must be supported by close collaboration between general practitioners, hepatologists, diabetologists, and cardiologists. Together, these strategies can slow disease progression, improve outcomes, and ease the clinical and economic burden of MASLD.

Diagnosis and screening

In clinical practice, MASLD is often identified incidentally, either through elevated liver enzymes or the detection of hepatic steatosis on imaging which commonly prompts further diagnostic assessment. Although liver biopsy remains the gold standard for diagnosing MASLD and staging fibrosis, it is invasive, costly, and associated with potential complications, making it unsuitable for large-scale screening (44, 45).

Histological assessment of liver biopsies relies on several classification systems, such as the NAFLD Activity Score (NAS), which evaluates features including steatosis, inflammation,

hepatocellular ballooning, and fibrosis stage (46). However, interpretation of liver histology is subject to interobserver variability, limiting diagnostic consistency. Recent advances combining digital pathology (DP) with artificial intelligence (AI) have shown promise in reducing this variability (47).

To address these limitations, various non-invasive tests (NITs) have been developed (Table 1). These include serum-based biomarkers, such as the Fibrosis-4 (FIB-4) index for fibrosis assessment, and imaging techniques like vibration-controlled transient elastography (VCTE), offering safer and more accessible alternatives to invasive procedures.

Table 1. Overview of non-invasive tests (NITs) for assessing hepatic steatosis and hepatic fibrosis.

Steatosis NITs	**Sensitivity**	**Specificity**	**Reference standard**
Fatty Liver Index (FLI)(48)			
Rule out HS: cut-off value <30	87%	64%	Ultrasound fatty liver
Rule in HS: cut-off value ≥ 60	61%	86%	
Hepatic Steatosis Index (HSI)(49)			
Rule out HS: cut-off value <30	93%	40%	Ultrasound fatty liver
Rule in HS: cut-off value ≥ 36	46%	92%	
Liver Accumulation Product (LAP)(50)			
Rule in HSI in men: lnLAP >4.0	77%	75%	Ultrasound fatty liver
Rule in HSI in women: lnLAP >4.4	82%	79%	
NAFLD Liver Fat Score (LFS)(51)			
Rule out HS: ≤-0.640	86%	71%	^{1}H-MRS
Rule in HS: >-0.640	64%	86%	
Ultrasound			
≥ S1(52)	65%	77%	Histology
≥ S2(53)	90%	97%	
MRI-PDFF			
≥ S1(54)	86%	83%	Histology
≥ S2(55)	93%	85%	
CT			
≥ S1(52)	74%	70%	Histology
≥ S2(56)	91%	97%	
Vibration controlled transient elastography (CAP)(57)			
≥ S1 (mild)	87%	91%	Histology
≥ S2 (moderate)	85%	74%	
≥ S3 (severe)	76%	85%	
MR elastography (MRE)(58)			
≥ F2	66%	92%	Histology
≥ F3	86%	91%	
Fibrosis NITs	**Sensitivity**	**Specificity**	**Reference standard**
Fibrosis-4 (FIB-4) index(59)			
<F3: cut-off value <1.3	74%	71%	Histology
≥ F3: cut-off value >2.67	33%	98%	
NAFLD fibrosis score (NFS)(60)			
<F3: cut-off value <-1.455	82%	77%	Histology
≥ F3: cut-off value >0.676	51%	98%	

Metabolic Dysfunction-Associated Fibrosis 5 (MAF-5) index(61)			
<F3: cut-off value <0	80%	66%	Histology
≥ F3: cut-off value ≥1	69%	80%	
BARD(62)			
≥ F3: cut-off value ≥2	70%	60%	Histology
Vibration controlled transient elastography (LSM)(1, 63)			
≥ F2: cut-off 8.0 kPa	74-79%	74-86%	Histology
≥ F3: cut-off 12.0 kPa	75-81%	79-90%	

Abbreviations: HS, hepatic steatosis; 1H-MRS, Proton Magnetic Resonance Spectroscopy.

Vibration-controlled transient elastography

VCTE, commonly known by the device name Fibroscan® (Echosens, Paris), is a widely used, non-invasive method for assessing hepatic steatosis and fibrosis. This technique has been validated in various patient populations and is well-suited for use in outpatient clinical settings (64, 65). Fibroscan simultaneously measures liver stiffness (LSM) and estimates hepatic steatosis via the controlled attenuation parameter (CAP), providing immediate results (Figure 3). Probe selection depends on the distance from the skin to the liver capsule. The standard M-probe is used for most patients, while the XL-probe, which enables deeper tissue penetration, is preferred for patients with obesity. CAP values, expressed in decibels per meter (dB/m), quantify the extent of liver fat by measuring ultrasound signal attenuation; higher values indicate greater steatosis. LSM, measured in kilopascals (kPa), reflects liver stiffness by assessing the velocity of shear waves through liver tissue, with increased stiffness indicating more advanced fibrosis.

Due to its non-invasive nature, rapid results, and ability to simultaneously evaluate steatosis and fibrosis, Fibroscan is a practical and effective tool for the clinical assessment of MASLD.



Figure 3. Vibration-controlled transient elastography (Fibroscan) device.

Fibrosis-4 index

The FIB-4 index is one of the most widely used NITs for estimating the risk of liver fibrosis in patients with MASLD. It is a simple, cost-effective, and readily available tool based on four routinely collected laboratory parameters: age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and platelet count (66). Originally developed in patients with hepatitis C and Human Immunodeficiency Virus (HIV) co-infection (67), FIB-4 has since been validated in various populations, including those with MASLD (68). The index helps stratify patients into low, intermediate, or high risk of advanced fibrosis, thereby guiding clinical decision-making.

Treatment

Lifestyle modification

The cornerstone of MASLD management is lifestyle modification. Studies show that even modest weight loss (<5%) can lead to meaningful reductions in liver fat (69, 70). Greater weight loss, particularly ≥10%, is associated with significant improvements in liver health, including resolution of steatohepatitis and regression of fibrosis (70, 71).

Current clinical guidelines recommend lifestyle modification as the primary and only established treatment approach for MASLD. This includes individualized dietary changes, such as adopting

a Mediterranean-style diet, avoiding ultra-processed foods and sugar-sweetened beverages (SSBs), and engaging in regular physical activity, preferably ≥150 minutes per week of moderate or ≥75 minutes per week of vigorous-intensity exercise (1).
While weight loss remains the main driver of histological improvement, these interventions also improve hepatic steatosis and reduce cardiometabolic risk. Although the evidence for physical activity's effect on liver fibrosis is less robust, its benefits for metabolic and liver health support its central role in MASLD management.

Medication

In March 2024, resmetirom, a thyroid hormone receptor-β (THR-β) agonist developed specifically for patients with MASH, received Food and Drug Administration (FDA) approval, marking an important milestone in pharmacological treatment for more advanced MASLD (1, 72, 73). In addition to this, a broad range of therapeutic classes is being explored, including drugs acting on the fibroblast growth factor (FGF) system, inhibitors of DNL, and pan-PPAR agonists, which target multiple metabolic pathways involved in MASLD pathogenesis (74-76). Furthermore, incretin-based therapies, such as Glucagon-Like Peptide-1 (GLP-1) receptor agonists, and emerging combinations with glucose-dependent insulinotropic polypeptide (GIP), and glucagon receptor agonists have demonstrated beneficial effects on liver fat reduction and inflammation (77). Established medications used for T2DM and obesity management, including pioglitazone, SGLT2 inhibitors, and weight-loss drugs, show potential benefits in MASLD through improvements in insulin sensitivity, weight reduction, and cardiometabolic risk (78-80). However, the precise roles and long-term effects of many of these treatments in MASLD are not yet fully established, and ongoing clinical trials aim to clarify their efficacy and safety profiles.

Bariatric surgery

Besides lifestyle modifications and pharmacological interventions, bariatric surgery has emerged as a highly effective treatment option for patients with MASLD (81, 82). By facilitating substantial and sustained weight loss, bariatric surgery can lead to significant improvements not only in liver steatosis but also in overall cardiometabolic health (83). Studies have shown that this intervention reduces hepatic fat content, inflammation, and fibrosis, contributing to the reversal or mitigation of MASLD progression (84, 85). Importantly, the benefits of bariatric surgery extend beyond short-term outcomes; many patients experience long-lasting improvements in insulin sensitivity, glycemic control, and lipid profiles (86). This is particularly relevant for patients with morbid obesity and concurrent T2DM, who are at increased risk for both hepatic and CV complications. Moreover, bariatric surgery has been associated with reduced morbidity and mortality rates in this population, emphasizing its role as a critical component of comprehensive MASLD management (87).

Objectives of this thesis

This thesis addresses the underrecognized burden of MASLD and liver fibrosis in high-risk patient groups. Focusing primarily on individuals with cardiometabolic conditions, including T2DM, obesity, and arterial hypertension, as well as patients with rheumatoid arthritis (RA), a chronic inflammatory disease linked to elevated CV risk, the studies aim to fill critical gaps in understanding MASLD prevalence, fibrosis severity, and associated CV risk. Additionally, the thesis evaluates the impact of combined pharmacological and lifestyle interventions on liver health in patients with T2DM and overweight or obesity. Together, these investigations seek to improve identification, risk stratification, and management of MASLD in clinical practice, ultimately contributing to better patient outcomes.

Outline of this thesis

Chapter 1 provides a general introduction to MASLD, covering its definition, epidemiology, pathophysiology, diagnosis, treatment, clinical consequences, and the significance of liver fibrosis in CV-risk stratification.
Chapter 2 examines MASLD and liver fibrosis prevalence in T2DM, type 1 diabetes mellitus (T1DM), and high CV-risk non-diabetic patients at a Dutch cardiometabolic clinic, including clinical predictors and the link with CV risk.
Chapter 3 evaluates liver fibrosis in patients with morbid obesity and investigates its association with CV risk, using non-invasive diagnostics.
Chapter 4 reports on a randomized controlled trial (RCT) in patients with T2DM and overweight or obesity, assessing the effects of a combined pharmacological and lifestyle intervention on hepatic steatosis and fibrosis NITs.
Chapter 5 describes preliminary findings from a screening study of high-risk patients for MASLD and liver fibrosis in both primary and secondary care settings, using non-invasive diagnostics.
Chapter 6 examines the relationship between LAP-defined steatosis and subclinical vascular disease in patients with RA.
Chapter 7 investigates fibrosis and its relationship with CV risk factors in patients with RA.
Chapter 8 discusses the main findings, clinical implications, and directions for future research.

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Chapter 2

Prevalence of liver steatosis and fibrosis assessed by transient elastography in a high cardiovascular-risk outpatient cohort including T1DM and T2DM patients

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ABSTRACT

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is common in T2DM, likely due to insulin resistance and obesity. Although screening is recommended in high-risk patients, its prevalence in outpatient cardiovascular clinical settings remains unclear.

Methods: We analyzed data from 475 patients attending a cardiovascular outpatient clinic: 142 with T2DM, 78 with T1DM, and 255 non-diabetic individuals at elevated cardiovascular risk. Liver steatosis and fibrosis were assessed using vibration-controlled transient elastography (Fibroscan®): steatosis by controlled attenuation parameter (CAP ≥ 275 dB/m), and fibrosis risk by liver stiffness measurement (LSM ≥ 8.1 kPa). Carotid intima-media thickness (cIMT) was also measured.

Results: The cohort (47% women, mean age 53 years, BMI 29.8 kg/m2) showed MASLD in 39.2% and fibrosis risk in 18.3%. MASLD was most prevalent in T2DM (57.0%), followed by non-diabetics (35.3%) and T1DM (19.2%) ($p < 0.001$). Fibrosis risk was also highest in T2DM (22.5%) vs. T1DM (7.7%) and non-diabetics (19.2%) ($p = 0.02$). CAP values were higher in those with fibrosis risk. T2DM patients with MASLD had higher LSM (7.0 ± 3.0 kPa) compared to those without MASLD (5.1 ± 2.2 kPa; $p < 0.001$). cIMT was highest in T2DM (0.73 ± 0.12 mm; $p = 0.04$), but not associated with MASLD or fibrosis. BMI and triglycerides were the strongest predictors of both MASLD and fibrosis.

Conclusion: MASLD and risk of significant fibrosis were highest among T2DM patients. Within T2DM, those with MASLD had higher LSM, indicating increased risk of fibrosis. The presence of MASLD and risk of significant fibrosis was not associated with cIMT in this cardiometabolic cohort. BMI and plasma TG were consistent predictors across groups urging for more strict control by body weight reduction and lifestyle interventions.

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) encompasses a range of liver disease stages, from isolated steatosis, where lipid droplets accumulate in hepatocytes, to metabolic dysfunction-associated steatohepatitis (MASH), characterized by hepatocyte damage and hepatic inflammation (1). This can further progress to fibrosis, cirrhosis, and, in some cases, hepatocellular carcinoma (HCC), all in the absence of secondary causes such as excessive alcohol consumption or other liver diseases (1-3).

The global prevalence of MASLD is estimated at 30%, with higher rates observed in individuals with obesity and type 2 diabetes (T2DM) (4, 5). As global rates of obesity and T2DM rise, MASLD prevalence is expected to increase, likely becoming the leading indication for liver transplantation in the near future (6). Despite its growing prevalence, MASLD remains underdiagnosed due to limited awareness among both healthcare providers and patients (7-9). In addition to its hepatic implications, MASLD is closely linked to cardiovascular disease (CVD). Epidemiological studies show that MASLD is associated with increased carotid intima-media thickness (cIMT), a surrogate marker of arterial injury (10, 11). The underlying inflammatory and metabolic mechanisms, such as insulin resistance (IR) and systemic inflammation, contribute to vascular damage thereby increasing CVD risk (10). Moreover, MASLD's severity correlates with a higher incidence of atherosclerotic cardiovascular disease (asCVD) risk factors, such as hypertension and diabetes, making early detection and management essential (12, 13). Recent evidence suggests that MASLD contributes to asCVD beyond shared risk factors. In a cohort of 7507 adults in Eastern China, MASLD severity was independently associated with subclinical coronary atherosclerosis, particularly in individuals with severe MASLD or hypertension (14). Mechanistic and clinical data further highlight links through systemic inflammation, IR, and endothelial dysfunction (11), reinforcing its role as an independent cardiovascular (CV) risk factor. Different scientific organizations, including the American Heart Association (AHA) and the European Association for the Study of the Liver (EASL), have recommended MASLD screening in individuals with cardiometabolic disorders (13, 15, 16). Although liver biopsy is considered the diagnostic gold standard, its invasive nature, cost, and associated risks limit its utility in routine clinical care. To improve accessibility, non-invasive tests (NITs), such as vibration-controlled transient elastography (VCTE), have been developed (1, 17). VCTE is a non-invasive method that simultaneously assesses liver fibrosis and steatosis. Liver stiffness measurement (LSM), obtained from VCTE, stages fibrosis, while the controlled attenuation parameter (CAP) measures hepatic fat content. While MASLD has been widely studied in T2DM due to its strong association with IR, relatively little is known about MASLD in patients with type 1 diabetes mellitus (T1DM) (18, 19). Furthermore, few studies have explored MASLD and liver fibrosis in high CV risk populations attending cardiometabolic outpatient clinics; groups that are often overlooked despite sharing many metabolic risk factors. Therefore, this study aimed to evaluate the prevalence of MASLD

and risk of significant fibrosis in patients with T1DM and T2DM, compared to a high CV-risk non-diabetic group from a cardiometabolic outpatient clinic in the Netherlands. Secondary objectives include assessing differences in arterial injury between groups defined by MASLD and fibrosis status, and determining independent predictors of MASLD and significant fibrosis using backward stepwise logistic regression.

MATERIALS AND METHODS

Study design

This study was conducted at the Endocrinology and Diabetes Vascular Center of Franciscus Gasthuis Hospital in Rotterdam, the Netherlands. Patients attending the outpatient clinic for CV risk management were included. Data were collected from electronic health records (EHRs) of patients enrolled between January 2021 and July 2022, all of whom underwent a standard, protocolized CV assessment, including VCTE. The study received ethical approval from the local Medical Ethical Committee of the Franciscus Gasthuis & Vlietland Hospital and was conducted in accordance with the Declaration of Helsinki.

Study population

The study involved 475 adult patients: 142 with T2DM, 78 with T1DM, and 255 non-diabetic individuals, all of whom had cardiometabolic disorders. Patients were eligible if they visited the Endocrinology and Vascular Medicine department of Franciscus Gasthuis Hospital and underwent a FibroScan measurement between 1 January 2021, and 1 October 2022. Patients with previously diagnosed chronic liver disease were excluded, as well as those with conditions associated with secondary liver steatosis or fibrosis, such as HIV, viral hepatitis, hemochromatosis, Wilson's disease, sarcoidosis, or excessive alcohol consumption (≥3 units per day for men and ≥2 units per day for women).

Data collection

MASLD was defined as the presence of at least one cardiometabolic disorder and a CAP ≥ MASLD was defined as the presence of at least one cardiometabolic disorder and a controlled attenuation parameter (CAP) ≥ 275 dB/m (S1), in accordance with the 2021 European Association for the Study of the Liver (EASL) Clinical Practice Guidelines, indicating hepatic steatosis [19]. The cardiometabolic disorders considered were: overweight/obesity (BMI ≥ 25 kg/m2), hypertension (blood pressure ≥ 130/85 mmHg or use of antihypertensive medication), hypercholesterolemia (total cholesterol ≥ 5.0 mmol/L or use of lipid-lowering medication), dyslipidemia (LDL-C ≥ 3.0 mmol/L or HDL-C < 1.0 mmol/L), and diabetes (fasting glucose ≥ 7.0 mmol/L, HbA1c > 48 mmol/mol, or use of glucose-lowering medication). All variables were abstracted from the electronic health records. Risk of significant fibrosis was

defined as a liver stiffness measurement (LSM) ≥ 8.1 kPa (≥F2) [19]. CVD was defined as the presence of any of the following conditions: transient ischemic attack (TIA), cerebrovascular accident (CVA), peripheral arterial disease (PAD), or coronary artery disease (CAD). All data were encoded with a unique, non-identifiable code to maintain patient confidentiality.

Laboratory examination

Blood samples were analyzed for glucose, HbA1c, total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TGs), apolipoproteins (apo) B and AI, C-reactive protein (CRP), hemoglobin, thrombocytes, gamma-glutamyl transferase (GGT), creatinine, albumin, estimated glomerular filtration rate (eGFR), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) at the laboratory of Franciscus Gasthuis using standard procedures. All blood tests were conducted in a non-fasting state.

Vibration-controlled transient elastography

VCTE was performed using Fibroscan™ (Echosens, Paris, France) by trained personnel at the outpatient clinic. At this cardiometabolic clinic, the procedure is routinely applied to screen for hepatic steatosis and fibrosis. Patients were positioned supine with their right arm under their head. The probe (either M- or XL-probe) was placed in the intercostal space between the 10th and 12th ribs along the mid-axillary line. A minimum of 10 valid mea surements were required for the VCTE to be considered successful. Reliability was assessed by ensuring the interquartile range (IQR) from the median LSM value was ≤30% (20).

Carotid intima media thickness

Carotid ultrasound scans were performed using the ART-LAB (Esaote, Genoa, Italy) by trained personnel as described previously in detail (21). Imaging was conducted for each common carotid artery in three distinct projections. The cIMT was determined as the mean of six measurements, with at least two measurements per carotid artery required for inclusion.

Data analysis

Data are presented as mean ± standard deviation (SD) for normally distributed variables, or median and IQR for non-normally distributed variables. Comparisons of normally distributed variables were performed using Student's t-test, while non-normally distributed variables were analyzed using the Mann–Whitney U test or Kruskal–Wallis. ANOVA with post hoc analyses was used to explore differences between subgroups. Chi-square tests were employed to assess prevalence differences. To identify predictors of MASLD and risk of significant fibrosis, a backward stepwise logistic regression analysis was performed. Predictors included BMI, age, TGs, HDL-C, LDL-C, HbA1c, and sex. Collinearity was assessed using Variance Inflation Factor (VIF) values, with a threshold of VIF < 5 indicating acceptable levels of collinearity. As this study is exploratory in nature, analyses were primarily descriptive, and formal multivariable

adjustment was not performed. All statistical analyses were conducted using IBM SPSS Statistics version 28.0.0.0 (IBM SPSS Statistics, New York, NY, USA). A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The study population consisted of 475 patients (223 women, mean age 53 ± 14 years, mean BMI 29.8 ± 6.7 kg/m2) (Table 1). Of the total cohort, 39.2% (N = 186) had MASLD, and 18.3% (N = 87) were at risk of significant fibrosis. The prevalence of MASLD was 57.0%, 19.2%, and 35.3% in patients with T2DM, T1DM, and in those without diabetes, respectively (p < 0.001). 22.5%, 7.7%, and 19.2% of the T2DM, T1DM, and non-diabetic patients, respectively, were at risk of significant fibrosis (p = 0.02) (Figure 1). The mean cIMT in the overall cohort was 0.71 mm (±0.14), with T2DM patients showing the highest cIMT (Figure 2). T2DM patients also had the highest glucose levels, HbA1c, and TGs among the three subgroups. Additionally, T2DM patients exhibited significantly higher creatinine levels and reduced eGFR compared to non-diabetic patients. In contrast, T1DM patients had significantly lower BMI and higher HDL-C levels than non-diabetic patients. Non-diabetic patients had the highest LDL-C levels among all subgroups (Table 1). The majority of the cohort was Caucasian (67.7%), with smaller groups of Turkish (6.5%), North African (5.5%), and Hindustani (4.9%) patients. Further details on the ethnicity distribution of the cohort can be found in Supplementary Table S1. Baseline medication use is summarized in Supplementary Table S2.

Table 1. Baseline demographic and clinical characteristics for the overall group and subgroups. Data are expressed as mean ± standard deviation, median (IQR), or n (%), as appropriate.

Characteristics	Total (N = 475)	T2DM (N = 142)	T1DM (N = 78)	Non-DM (N = 255)	P-value
Age (years)	53 ± 14	59 ± 12***	52 ± 12	50 ± 14	<0.001
M/F	252/223	86/56	39/39	127/128	0.101
BMI (kg/m^2)	29.8 ± 6.7	30.4 ± 6.4	27.1 ± 5.0***	30.6 ± 7.3	<0.001
cIMT (mm)	0.71 ± 0.14	0.73 ± 0.12	0.69 ± 0.14	0.68 ± 0.16	0.036
Total cholesterol (mmol/L)	4.6 (3.8-5.5)	4.1 (3.7-5.3)	4.3 (3.7-5.0)	5.1 (4.5-5.7)	0.573
HDL-C (mmol/L)	1.3 ± 0.39	1.2 ± 0.32	1.5 ± 0.42***	1.3 ± 0.37	<0.001
LDL-C (mmol/L)	2.5 ± 0.92	2.3 ± 0.93***	2.3 ± 0.75***	3.0 ± 0.80	<0.001
Triglycerides (mmol/L)	1.6 (1.0-2.4)	2.1 (1.3-3.1) *	1.1 (0.8-1.7) **	1.5 (1.1-2.3)	<0.001
Glucose (mmol/L)	6.2 (5.4-7.9)	10.0 (7.4-14) ***	8.5 (5.6-11) **	5.8 (5.1-6.4)	<0.001
HbA1c (mmol/mol)	50.9 ± 15	59.0 ± 13***	57.3 ± 12***	36.4 ± 5.0	<0.001
Apo B (g/L)	1.05 ± 0.27	1.05 ± 0.38	0.82 ± 0.27	1.07 ± 0.20	0.330
Apo AI (g/L)	1.54 ± 0.26	1.49 ± 0.24	1.39 ± NA	1.59 ± 0.27	0.490
CRP (mg/L)	4.0 (2.0-7.0)	3.0 (2.0-6.0)	8.0 (4.0-25)	4.0 (2.0-7.0)	0.176
Hemoglobin (mmol/L)	8.7 ± 1.1	8.7 ± 1.1	8.2 ± 1.0*	8.8 ± 1.1	0.057
Thrombocytes (10^9/L)	258 ± 87	253 ± 93	305 ± 114	255 ± 82	0.099
GGT (U/L)	58 (31-106)	71 (38-177)	23 (14-140)	58 (29-97)	0.082
ALT (U/L)	38 (27-52)	38 (28-60)	22 (18-39)	39 (28-51)	0.158
AST (U/L)	39 (24-67)	38 (25-72)	29 (14-36)	41 (23-67)	0.131
Creatinine (mmol/L)	74 (65-87)	77 (67-96) **	76 (66-92)	72 (65-81)	0.006
Albumin (g/L)	44 (41-46)	44 (42-47)	46 (40-46)	44 (41-46)	0.850
eGFR (mL/min/1.73m^2)	80 (68-88)	77 (59-86) ***	78 (70-87)	82 (75-91)	<0.001
FIB-4	1.86 ± 1.50	1.94 ± 1.46	1.73 ± 1.12	1.85 ± 1.62	0.789
CAP (dB/m)	263 ± 59	282 ± 59***	238 ± 52*	259 ± 58	<0.001
LSM (kPa)	6.1 ± 3.0	6.2 ± 2.8	5.4 ± 1.8*	6.3 ± 3.3	0.039

Statistical significance is indicated by asterisks *, **, and ***, corresponding to probability values of <0.05, <0.01, and <0.001, respectively. These significance levels are in relation to the high CV-risk non-diabetic subgroup. P-values reflect ANOVA differences between groups. Abbreviations: BMI, body mass index; cIMT, carotid intima media thickness; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1C; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4 index; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

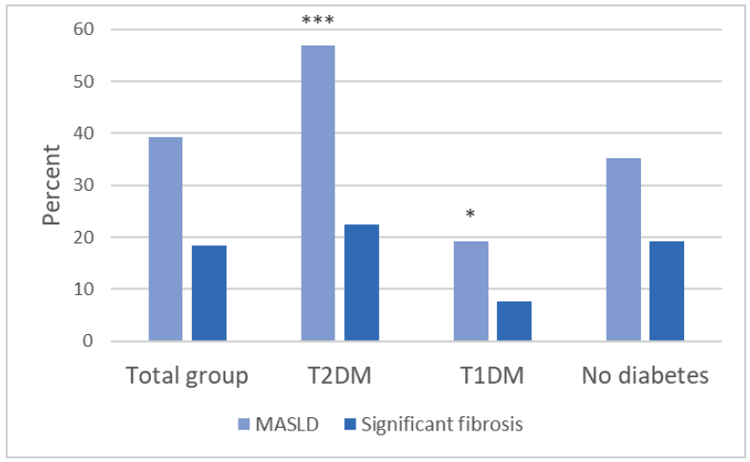


Figure 1. Prevalence of MASLD and risk of significant fibrosis in the total cohort and the among different subgroups. Statistical significance is indicated by asterisks *, **, and ***, corresponding to probability values of <0.05, <0.01, and <0.001, respectively, in relation to the high CV-risk non-diabetic subgroup.

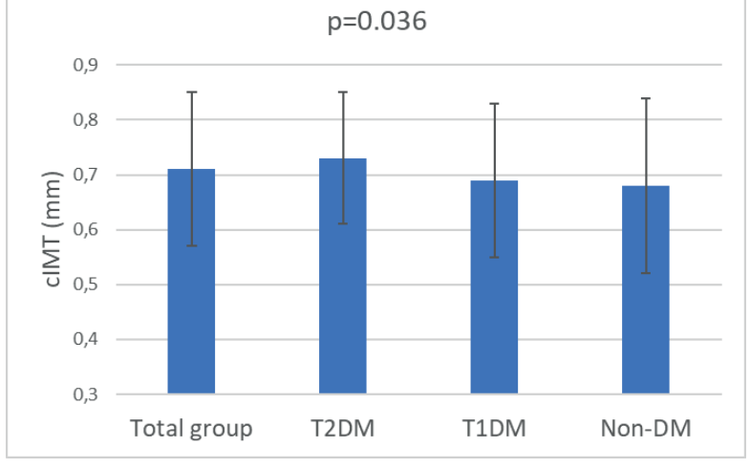


Figure 2. Mean carotid intima-media thickness across the total cohort and subgroups. P-value indicates ANOVA differences between groups. Abbreviations: T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; non-DM, high CV-risk non-diabetics.

Characteristics by MASLD and fibrosis status

Across the entire cohort and within each subgroup, BMI was significantly greater in patients with MASLD compared to those without. Additionally, in the overall population, individuals with MASLD showed significantly higher plasma TGs, as well as higher liver enzyme levels relative to those without MASLD (Table 2). Within the T2DM group, patients with MASLD showed significantly higher LSM compared to both T1DM patients and non-diabetic individuals. Although T2DM patients had higher cIMT than T1DM patients and high CV-risk non-diabetic patients, neither MASLD nor risk of significant liver fibrosis appeared to significantly affect cIMT within this group. Additionally, T2DM patients with MASLD showed higher LDL-C levels compared to those without MASLD (Table 2).

In the overall cohort, multivariable regression analysis, adjusted for age, sex, and LDL-C, revealed that MASLD was not significantly associated with cIMT (β = 0.012, 95% CI:−0.025 to 0.049, p = 0.516). Similarly, significant fibrosis did not show a significant association with cIMT (β = 0.044, 95% CI:−0.025 to 0.113, p = 0.211). However, age remained significantly positively associated with cIMT (β = 0.006, p < 0.001).

Subjects at higher risk of significant fibrosis had a higher BMI in the overall cohort as well as in the T2DM and T1DM groups (Table 3). Patients who were at risk of fibrosis also showed increased liver enzyme levels in the total population. Moreover, CAP values were significantly elevated in those with risk of fibrosis across both the entire cohort and the diabetic subgroups (Table 3).

Table 2. Demographic and clinical characteristics by MASLD status in the total population and subgroups.

Characteristics	Total (N = 475)			T2DM (N = 142)			T1DM (N = 78)			Non-DM (N = 255)		
	MASLD (N=186)	No MASLD (N=289)	p-value	MASLD (N=81)	No MASLD (N=61)	p-value	MASLD (N=15)	No MASLD (N=63)	p-value	MASLD (N=90)	No MASLD (N=165)	p-value
Age (years)	52 ± 14	54 ± 13	0.068	58 ± 12	61 ± 11	0.092	56 ± 11	51 ± 12	0.197	45 ± 14	52 ± 13	<0.001
M/F	100/86	152/137	0.803	49/32	37/24	0.984	4/11	35/28	0.044	47/43	80/50	0.568
BMI (kg/m^2)	33.1 ± 6.4	27.6 ± 6.0	<0.001	32.3 ± 6.2	27.8 ± 5.9	<0.001	31.9 ± 4.2	25.9 ± 4.4	<0.001	34.3 ± 6.8	28.3 ± 6.7	<0.001
cIMT (mm)	0.72 ± 0.12	0.70 ± 0.14	0.409	0.73 ± 0.12	0.74 ± 0.13	0.643	0.71 ± 0.12	0.68 ± 0.14	0.459	0.69 ± 0.15	0.68 ± 0.17	0.975
Total cholesterol (mmol/L)	5.5 ± 7.0	4.6 ± 1.2	0.129	6.0 ± 9.7	4.1 ± 1.2	0.207	4.5 ± 1.3	4.4 ± 0.91	0.696	5.1 ± 0.95	5.1 ± 1.2	0.958
HDL-C (mmol/L)	1.2 ± 0.36	1.3 ± 0.42	0.231	1.2 ± 0.31	1.1 ± 0.33	0.942	1.6 ± 0.39	1.5 ± 0.44	0.579	1.3 ± 0.35	1.3 ± 0.39	0.960
LDL-C (mmol/L)	2.6 ± 0.90	2.5 ± 0.93	0.155	2.4 ± 1.0	2.0 ± 0.87	0.042	2.4 ± 0.87	2.3 ± 0.72	0.634	3.0 ± 0.70	3.1 ± 0.87	0.850
Triglycerides (mmol/L)	2.2 ± 1.3	1.8 ± 1.3	0.035	2.5 ± 1.5	2.2 ± 1.6	0.324	1.1 ± 0.52	1.3 ± 0.74	0.455	2.0 ± 1.1	1.9 ± 1.4	0.611
Glucose (mmol/L)	7.1 ± 2.7	7.6 ± 3.6	0.441	9.8 ± 3.4	11.5 ± 4.6	0.222	8.1 ± 2.7	8.8 ± 3.7	0.806	5.9 ± 0.93	5.9 ± 1.1	0.911
HbA1c (mmol/mol)	51 ± 14	51 ± 15	0.900	59 ± 13	60 ± 12	0.680	56 ± 7.8	58 ± 13	0.612	37 ± 4.7	36 ± 5.3	0.365
ApoB (g/L)	1.1 ± 0.30	1.0 ± 0.23	0.209	1.1 ± 0.39	0.90 ± 0.31	0.207	0.87 ± 0.36	0.74 ± NA	NA	1.09 ± 0.21	1.05 ± 0.20	0.543
Apo AI (g/L)	1.6 ± 0.23	1.5 ± 0.27	0.726	1.6 ± 0.28	1.4± 0.17	0.169	NA	1.4 ± NA	NA	1.5 ± 0.12	1.6 ± 0.30	0.680
CRP (mg/L)	7.4 ± 9.3	8.0 ± 16	0.781	4.2 ± 4.1	4.7 ± 3.5	0.686	25.0 ± NA	10.2 ± 10.1	NA	8.6 ± 11	8.7 ± 19	0.970
Hemoglobin (mmol/L)	8.8 ± 1.2	8.7 ± 1.1	0.480	8.7 ± 1.1	8.8 ± 1.0	0.537	8.5 ± 0.57	8.1 ± 1.1	0.633	8.9 ± 1.2	8.7 ± 1.1	0.436
Thrombocytes (10^9/L)	266 ± 83	252 ± 90	0.230	264 ± 79	238 ± 110	0.283	296 ± 216	306 ± 106	0.910	266 ± 83	248 ± 81	0.194
GGT (U/L)	82.4 ± 76	106 ± 141	0.190	102 ± 98	173 ± 188	0.159	NA	66.0 ± 73.9	NA	73.9 ± 62.5	94.7 ± 130	0.202
ALT (U/L)	55 ± 37	40 ± 26	0.003	56 ± 37	45 ± 35	0.240	NA	31 ± 21	NA	53 ± 38	40 ± 24	0.010
AST (U/L)	65 ± 51	42 ± 30	<0.001	68 ± 63	44 ± 40	0.077	26 ± 13	38 ± 34	0.624	65 ± 43	41 ± 26	<0.001
Creatinine (mmol/L)	79.9 ± 21	81.3 ± 42	0.693	85.0 ± 24	91.7 ± 77	0.486	81.5 ± 20	81.5 ± 23	0.995	73.9 ± 15	76.0 ± 16	0.371
Albumin (g/L)	44.1 ± 3.3	46.7 ± 41	0.557	44.7 ± 3.2	42.3 ± 6.3	0.126	41.0 ± 1.4	43.8 ± 3.7	0.343	44.0 ± 3.3	48.1 ± 47	0.505
eGFR (mL/min/1.73m^2)	76.1 ± 15.3	75.6 ± 15.3	0.802	70.7 ± 16.7	71.8 ± 18.0	0.758	74.2 ± 13.9	74.7 ± 15.6	0.917	83.3 ± 10.5	78.4 ± 12.6	0.064
FIB-4	1.68 ± 1.3	1.98 ± 1.6	0.139	1.82 ± 1.2	2.10 ± 1.7	0.444	1.40 ± 0.56	1.79 ± 1.2	0.439	1.60 ± 1.5	2.01 ± 1.7	0.182
CAP (dB/m)	323 ± 30	223 ± 36	<0.001	325 ± 32	227 ± 37	<0.001	318 ± 31	219 ± 35	<0.001	323 ± 29	224 ± 36	<0.001
LSM (kPa)	6.7 ± 3.0	5.8 ± 2.9	<0.001	7.0 ± 3.0	5.1 ± 2.2	<0.001	5.8 ± 2.4	5.3 ± 1.6	0.436	6.6 ± 3.1	6.2 ± 3.4	0.329

* Data are given as mean (standard deviation), median (IQR), or numbers (%) where appropriate.
** Values shown as mean ± SD. NA = data not available due to small sample size or missing measurements.
Abbreviations: T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; non-DM, high CV-risk non-diabetics; BMI, body mass index; cIMT, carotid intima media thickness, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1C; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4 index; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

Table 3. Demographic and clinical characteristics by risk of significant fibrosis status in the total population and subgroups.

Characteristics	Total (N = 475)			T2DM (N = 142)			T1DM (N = 78)			Non-DM (N = 255)		
	Fibrosis (N=87)	No fibrosis (N=388)	p-value	Fibrosis (N=32)	No fibrosis (N=110)	p-value	Fibrosis (N=6)	No fibrosis (N=72)	p-value	Fibrosis (N=49)	No fibrosis (N=206)	p-value
Age (years)	54 ± 13	53 ± 14	0.453	56 ± 13	60 ± 11	0.124	56 ± 6.1	52 ± 12	0.144	52 ± 14	49 ± 14	0.187
M/F	54/33	198/190	0.062	21/11	65/45	0.506	3/3	36/36	1.000	30/19	97/109	0.075
BMI (kg/m^2)	31.9 ± 7.6	29.5 ± 6.5	0.023	34.2 ± 7.5	29.4 ± 5.8	0.004	31.7 ± 6.0	26.7 ± 4.7	0.017	29.7 ± 7.5	30.7 ± 7.3	0.523
cIMT (mm)	0.76 ± 0.11	0.70 ± 0.14	0.120	0.76 ± 0.10	0.73 ± 0.13	0.565	0.77 ± 0.16	0.68 ± 0.13	0.178	0.76 ± NA	0.68 ± 0.16	NA
Total cholesterol (mmol/L)	5.0 ± 1.6	5.0 ± 5.0	0.989	5.0 ± 8.1	5.2 ± 1.7	0.907	4.2 ± 0.81	4.4 ± 1.0	0.728	5.2 ± 1.6	5.1 ± 1.0	0.891
HDL-C (mmol/L)	1.2 ± 0.48	1.3 ± 0.38	0.134	1.1 ± 0.37	1.2 ± 0.31	0.748	1.5 ± 0.18	1.5 ± 0.44	0.989	1.1 ± 0.66	1.3 ± 0.31	0.506
LDL-C (mmol/L)	2.7 ± 1.2	2.5 ± 0.87	0.575	2.6 ± 1.3	2.2 ± 0.84	0.257	2.0 ± 0.55	2.3 ± 0.76	0.382	3.1 ± 1.2	3.0 ± 0.74	0.762
Triglycerides (mmol/L)	2.3 ± 1.2	1.9 ± 1.4	0.168	2.6 ± 1.2	2.3 ± 1.6	0.425	1.6 ± 0.78	1.3 ± 0.70	0.360	2.0 ± 1.1	1.9 ± 1.3	0.905
Glucose (mmol/L)	8.0 ± 3.0	7.3 ± 3.3	0.317	10.3 ± 3.4	10.9 ± 4.3	0.691	9.3 ± 1.1	8.5 ± 3.8	0.800	6.0 ± 0.76	5.9 ± 1.1	0.787
HbA1c (mmol/mol)	52 ± 15	51 ± 15	0.656	60 ± 16	59 ± 12	0.690	57 ± 6.5	57 ± 13	0.997	38 ± 4.2	36 ± 5.1	0.079
Apo B (g/L)	1.1 ± 0.32	1.0 ± 0.27	0.454	1.2 ± 0.38	1.0 ± 0.38	0.399	0.61 ± NA	0.93 ± 0.27	NA	1.2 ± 0.17	1.1 ± 0.20	0.351
Apo AI (g/L)	1.4 ± NA	1.5 ± 0.26	NA	1.4 ± NA	1.5 ± 0.25	NA	NA	1.4 ± NA	NA	NA	1.6 ± 0.27	NA
CRP (mg/L)	6.8 ± 8.6	8.0 ± 15	0.639	3.9 ± 2.2	4.6 ± 4.3	0.623	8.0 ± NA	13 ± 12	NA	7.9 ± 10	8.9 ± 17	0.786
Hemoglobin (mmol/L)	8.8 ± 1.1	8.7 ± 1.1	0.866	8.7 ± 0.98	8.7 ± 1.1	0.987	8.5 ± 0.57	8.1 ± 1.1	0.633	8.8 ± 1.2	8.8 ± 1.1	0.925
Thrombocytes (10^9/L)	240 ± 87	263 ± 87	0.085	240 ± 90	259 ± 95	0.469	193 ± 70.7	321 ± 111	0.143	243 ± 88	258 ± 80	0.308
GGT (U/L)	96.8 ± 80.2	96.4 ± 131	0.983	117 ± 89	143 ± 175	0.537	98 ± NA	58 ± 83	NA	86.3 ± 76.0	87.3 ± 118	0.967
ALT (U/L)	57 ± 45	42 ± 24	0.021	67 ± 47	43 ± 26	0.052	82 ± NA	25 ± 9.6	NA	51 ± 45	42 ± 23	0.277
AST (U/L)	65 ± 56	48 ± 35	0.025	91 ± 80	45 ± 37	0.018	66 ± 43	31 ± 28	0.156	51 ± 36	50 ± 35	0.793
Creatinine (mmol/L)	76.6 ± 17	81.6 ± 38	0.283	81.5 ± 19	89.7 ± 60	0.490	81.0 ± 27	81.5 ± 22	0.958	72.2 ± 11	76.0 ± 16	0.087
Albumin (g/L)	52.4 ± 61	43.4 ± 4.1	0.315	44.3 ± 2.9	43.4 ± 5.5	0.552	43.0 ± 4.2	43.3 ± 3.7	0.912	57.0 ± 76	43.3 ± 3.7	0.334
eGFR (mL/min/1.73m^2)	75.5 ± 12.5	75.8 ± 15.7	0.901	71.7 ± 14.7	71.2 ± 17.8	0.973	67.3 ± 11.9	75.1 ± 15.1	0.391	80.5 ± 8.7	80.2 ± 12.7	0.916
FIB-4	1.79 ± 1.3	1.88 ± 1.5	0.738	1.62 ± 1.1	2.08 ± 1.6	0.240	2.34 ± NA	1.72 ± 1.1	NA	1.90 ± 1.5	1.83 ± 1.7	0.840
CAP (dB/m)	283 ± 62	258 ± 58	<0.001	310 ± 46	275 ± 60	<0.001	286 ± 62	234 ± 50	0.019	266 ± 66	257 ± 56	0.308
LSM (kPa)	10.8 ± 3.6	5.1 ± 1.4	<0.001	10.5 ± 2.1	5.0 ± 1.5	<0.001	9.8 ± 1.3	5.0 ± 1.2	<0.001	11.1 ± 4.5	5.2 ± 1.4	<0.001

* Data are given as mean (standard deviation), median (IQR), or numbers (%) where appropriate.
** Values shown as mean ± SD. NA = data not available due to small sample size or missing measurements.
Abbreviations: T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; non-DM, high CV-risk non-diabetics; BMI, body mass index; cIMT, carotid intima media thickness, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1C; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4 index; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

Predictors of MASLD and fibrosis

Logistic regression analysis identified BMI as a significant predictor of MASLD across all patient groups (Table 4). In the total cohort, each 1 kg/m^2 increase in BMI was associated with a 13.3% increase in the odds of MASLD. Similarly, TGs were significantly associated with MASLD, with a 49.6% increase in the odds for each 1 mmol/L increase. Linearity of continuous predictors was assessed visually, and BMI and TGs showed clear linear trends, supporting their inclusion as continuous variables in the models. In subgroup analyses, BMI remained a crucial predictor of MASLD. In T2DM patients, each 1 kg/m^2 increase in BMI raised the odds of MASLD by 13.2%, while in T1DM patients, the odds increased by 22.3%, and by 11.7% in non-diabetic individuals with high CV-risk (Table 4). BMI was also identified as a predictor of risk for significant fibrosis in both T2DM and T1DM patients (Table 5). While BMI showed a positive association with risk of significant fibrosis in the overall cohort, the association did not reach statistical significance (p = 0.08). Additionally, TGs were found to be a strong predictor of risk of significant fibrosis in the overall cohort (Table 5). VIF values between independent variables were all below 5, indicating no significant collinearity.

Table 4. Independent predictors of MASLD from the final backward stepwise logistic regression model in the overall cohort and subgroups.

Group	Variable	B (SE)	Exp(B)	95% CI	p-value
Total cohort	Constant	-4.745 (0.849)	0.009		<0.001
	BMI (kg/m^2)	0.125 (0.026)	1.133	1.077 – 1.192	<0.001
	Triglycerides (mmol/L)	0.403 (0.174)	1.496	1.064 – 2.102	0.020
T2DM	Constant	-3.491 (1.403)	0.030		0.013
	BMI (kg/m^2)	0.125 (0.026)	1.132	1.033 – 1.240	0.008
T1DM	Constant	-7.001 (2.143)	0.001		0.001
	BMI (kg/m^2)	0.202 (0.071)	1.223	1.064 – 1.406	0.005
Non-diabetics	Constant	-5.152 (1.513)	0.006		<0.001
	BMI (kg/m^2)	0.111 (0.038)	1.117	1.037 – 1.203	0.004

Variables entered on step 1: Age, BMI, HbA1c, triglycerides, HDL-C, LDL-C, sex.
Abbreviations: T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; non-diabetics, high CV-risk non-diabetics.

Table 5. Independent predictors of significant fibrosis from the final backward stepwise logistic regression model in the overall cohort and subgroups.

Group	Variable	B (SE)	Exp(B)	95% CI	p-value
Total cohort	Constant	-4.337 (1.047)	0.013		<0.001
	Triglycerides (mmol/L)	0.422 (0.208)	1.524	1.015 – 2.289	0.042
T2DM	Constant	-4.746 (1.544)	0.009		0.002
	BMI (kg/m^2)	0.104 (0.047)	1.110	1.012 – 1.217	0.027
T1DM	Constant	-8.904 (3.238)	0.000		0.006
	BMI (kg/m^2)	0.212 (0.097)	1.236	1.022 – 1.494	0.029
Non-diabetics	Constant	-3.020 (0.724)	0.049		<0.001

Variables entered on step 1: Age, BMI, HbA1c, triglycerides, HDL-C, LDL-C, sex.
Abbreviations: T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; non-diabetics, high CV-risk non-diabetics.

MASLD and fibrosis in CVD patients

In the entire cohort, 86 patients (18.1%) had a history of CVD. Among these, 35 patients (40.7%) had MASLD, and 18 patients (20.9%) were at risk of significant fibrosis. Chi-square tests revealed no statistically significant differences in the prevalence of MASLD or risk of significant fibrosis between patients with and without CVD (p = 0.75 and p = 0.49, respectively).

DISCUSSION

In this cross-sectional study of patients attending a Dutch cardiometabolic outpatient clinic, we observed a high prevalence of MASLD (39.2%) and risk of significant fibrosis (18.3%), indicating a considerable burden of undiagnosed liver disease in this high-risk population. The prevalence of MASLD was highest among patients with T2DM followed by high CV-risk non diabetic individuals and patients with T1DM. A similar pattern was observed for the risk of significant fibrosis. Notably, T2DM patients with MASLD had a higher LSM, suggesting that MASLD may more strongly predispose this group to fibrosis development. Interestingly, neither MASLD nor risk of significant fibrosis were associated with cIMT. This may be explained that other CV risk factors may be operative in this high-risk population and therefore, MASLD does not differentiate in this respect. Of course, the number of subjects was limited, which may have influenced these results. Our diagnostic model identified BMI and TGs as the strongest predictors of MASLD and risk of significant fibrosis, reinforcing their role as key metabolic risk factors. These data clearly point at the relevance of weight control and lifestyle as main interventions in MASLD.

In line with existing literature, MASLD prevalence was higher in T2DM patients compared to the general population (18, 22, 23). Interestingly, MASLD was least prevalent in T1DM patients. This lower prevalence may reflect the unique metabolic profile of T1DM, including

absent endogenous insulin, reduced portal insulin levels, and increased GH and reduced IGF-1, fostering a catabolic, fasting-like state that limits hepatic fat accumulation (24). These distinct metabolic characteristics may confer protection against MASLD development and progression in T1DM. While the small sample size warrants cautious interpretation, similar prevalence rates were observed in another Dutch T1DM cohort (25).

Relative to global data, the MASLD prevalence observed in our cohort was lower than the 65.3% pooled prevalence reported in a recent meta-analysis of T2DM patients worldwide, which included over 2.2 million individuals. The meta-analysis also noted regional variations, with the highest prevalence in Eastern Europe (80.6%) and the Middle East (71.2%) (26). The lower prevalence in our study may partly reflect differences in diagnostic approaches, as the meta-analysis used estimated rates, whereas we used VCTE, providing more direct assessment of liver steatosis and fibrosis.

Few studies have assessed the prevalence of clinically significant liver fibrosis (defined as ≥8.1 kPa) in the general population, with reported rates ranging from 2% to 13.8% (27-31). In our cohort, the risk of significant fibrosis was substantially higher than these general population estimates, though still lower than rates reported in T2DM patients from Eastern European studies. For instance, a Romanian study of 424 T2DM patients using VCTE reported a significant fibrosis prevalence of 57.1%, while a Croatian study, applying a lower cutoff value of 7.0 kPa, found a prevalence of 46.6% in T2DM patients (32, 33). These differences likely reflect variations in study populations, diagnostic criteria, VCTE cutoff thresholds, as well as potential influences of healthcare practices, genetic predispositions, and lifestyle factors specific to the Netherlands. Notably, the prevalence of significant fibrosis observed among T1DM patients in our study (7.7%) is consistent with earlier Dutch findings, which reported rates around 6.7% in similar cohorts assessed with VCTE (25).

Previous Dutch MASLD research has primarily focused on general population cohorts, for example, the Lifelines study in Northern Holland, which used the Fatty Liver Index (FLI) for diagnosis (34), and the Rotterdam Study, which assessed an elderly population using ultrasound (35). De Vries et al. further compared MASLD prevalence between patients with T1DM and T2DM (25). Our study builds on this work by including non-diabetic patients with high CV risk, thereby offering a more comprehensive and representative overview of MASLD prevalence in routine cardiometabolic clinical practice.

In our cohort, VCTE provided quantitative LSM and CAP values to identify patients at risk of significant fibrosis. Although many studies focus primarily on advanced fibrosis (F3–F4), detecting significant fibrosis is crucial, as this population represents the main target for clinical trials and early intervention. VCTE enables reliable risk stratification in outpatient settings, and while traditional scores such as FIB-4 and NAFLD Fibrosis Score (NFS) can

complement it, they may underestimate F2–F3 prevalence. Recent studies have explored combined scoring systems, blood-based biomarkers, and machine learning approaches to improve identification of patients with significant fibrosis, supporting the integration of these tools into routine outpatient screening strategies (36-38).

Our findings reveal a substantial burden of undiagnosed liver disease in this cohort, particularly among T2DM patients, reinforcing the importance of incorporating NITs into routine screening. Early detection using these methods could help identify patients at risk of progression, leading to earlier interventions and potentially improving long-term outcomes. These results support current recommendations from several organizations that support non-invasive MASLD assessment in individuals with cardiometabolic risk (13, 15, 16, 39).

Nonetheless, some limitations should be noted. The cross-sectional design of the current study restricts our ability to establish causality or to evaluate the risk of long-term liver-related morbidity within the cohort. However, the presence of fibrosis has been consistently associated with adverse liver-related outcomes in longitudinal studies (40), underscoring the importance of its identification. Moreover, statistical power was limited by the small T1DM subgroup and the low number of fibrosis events, restricting the ability to draw definitive conclusions for these patients. Additionally, residual confounding could have influenced the observed null associations between cIMT, MASLD, and fibrosis, given the exploratory study design, incomplete covariate data, and small T1DM and T2DM subgroups that restricted fully adjusted analyses.

While VCTE is a reliable and non-invasive method for assessing steatosis and fibrosis, it cannot fully substitute for liver biopsy, the diagnostic gold standard. Some serum markers, such as FLI and routine abdominal ultrasound, were not assessed, and FIB-4 was available for only a subset of participants, possibly limiting detection of differences. Additionally, all blood samples were collected in the non-fasting state, which may particularly influence triglyceride values, although diabetes classification was primarily based on HbA1c and EHR diagnoses. The single-center, high CV-risk nature of the cohort may limit generalizability of our findings to broader populations. Furthermore, baseline medication use was recorded but not adjusted for, which may affect liver and cardiometabolic outcomes.

Future studies with larger cohorts, fasting laboratory assessments, and additional non-invasive markers alongside VCTE are needed to better evaluate MASLD and significant fibrosis.

CONCLUSION

In conclusion, this study highlights a substantial burden of undiagnosed MASLD and the risk of significant fibrosis in cardiometabolic outpatient populations, particularly among patients with T2DM. Elevated LSM values observed in T2DM patients with MASLD suggest an increased risk for fibrosis progression. The absence of an association between MASLD or fibrosis and cIMT may reflect distinct mechanisms driving hepatic and vascular risk in this cohort. BMI and plasma TG were consistent predictors across groups urging for stricter control through body weight reduction and lifestyle interventions.

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SUPPLEMENTARY DATA

Supplementary Table S1. Ethnicity distribution in the total cohort presented as n (%).

Ethnicity	Total cohort (N=470)
Caucasian	318 (67.7)
North African	26 (5.5)
Other African	11 (2.3)
Turkish	31 (6.6)
Hindustani	23 (4.9)
Asian	20 (4.3)
Latin-American	13 (2.8)
Other	28 (6.0)

*Ethnicity data were available for 470 of 475 participants; 5 participants had missing data.

Supplementary Table S2. Baseline medication use in the total cohort and subgroups presented as n (%).

Characteristics	Total (N = 475)	T2DM (N = 142)	T1DM (N = 78)	Non-DM (N = 255)
Metformin	102 (21.5)	90 (63.4) ***	11 (14.1) **	1 (0.4)
Statin	190 (40)	99 (69.7) ***	45 (57.7) ***	46 (18.0)
SU-derivative	27 (5.7)	26 (18.3) ***	1 (1.3)	0 (0)
GLP-1	19 (4.0)	16 (11.3) ***	1 (1.3)	2 (0.8)
SGLT2i	25 (5.3)	24 (16.9) ***	1 (1.3)	0 (0)
ACEi	53 (11.2)	30 (21.1) ***	10 (12.8)	13 (5.1)
ARB	109 (22.9)	59 (41.5) ***	20 (25.6) *	30 (11.8)
CCB	72 (15.2)	30 (21.1) *	14 (17.9)	28 (11.0)
Beta blocker	58 (12.2)	32 (22.5) ***	6 (7.7)	20 (7.8)
Diuretics	66 (13.9)	30 (21.1) **	9 (11.5)	27 (10.6)
DPP4-inhibitor	14 (2.9)	14 (9.9) ***	0 (0)	0 (0)
Alpha blocker	6 (1.3)	4 (2.8)	0 (0)	2 (0.8)

Statistical significance is indicated by asterisks *, **, and ***, corresponding to probability values of <0.05, <0.01, and <0.001, respectively. These significance levels are in relation to the non-diabetic subgroup. Abbreviations: T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; non-diabetics, high CV-risk non-diabetics; SU-derivative, sulfonylurea derivative; GLP-1, glucagon-like peptide-1; SGLT2i, sodium-glucose cotransporter 2 inhibitor; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CCB, calcium channel blocker; DPP4-inhibitor, dipeptidyl peptidase-4 inhibitor.

Chapter 3

Liver fibrosis as a predictor of cardiovascular risk in patients with severe obesity

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ABSTRACT

Background: Obesity is a substantial global health issue associated with increased risk of cardiovascular disease (CVD) and metabolic dysfunction-associated steatotic liver disease (MASLD). Despite the known link between obesity, CVD and MASLD, it remains unknown which factors contribute to higher cardiovascular (CV) risk in patients with obesity-induced liver fibrosis. Liver fibrosis, assessed by the Fibrosis-4 (FIB-4) index, may help to identify patients with obesity at increased CV risk.

Methods: Patients with severe obesity (Body Mass Index (BMI) ≥ 40 kg/m2) scheduled for bariatric surgery were subdivided into FIB-4 categories. Systemic leukocyte activation markers were measured by flow cytometry. Additionally, markers of vascular damage, namely the carotid intima media thickness (cIMT) and pulse wave velocity (PWV), were included.

Results: The cohort predominantly consisted of women (74%) with an average age of 41 years and mean BMI of 42.7 kg/m^2. Patients with an elevated FIB-4 (≥1.3) had higher systolic (146 ± 16 vs. 139 ± 15, p = 0.002) and diastolic blood pressure (91 ± 13 vs. 83 ± 12, p = 0.002), increased cIMT (0.66 ± 0.11 vs. 0.55 ± 0.10, p < 0.001), and higher PWV (8.2 ± 0.9 vs. 6.8 ± 1.1, p < 0.001) compared to those with a low FIB-4 (<1.3). Additionally, patients with a high FIB-4 tended to show increased expression of CD66b on granulocytes.

Conclusion: Patients with severe obesity who were at risk of liver fibrosis showed greater signs of vascular damage, insulin resistance, and systemic inflammation. This suggests that liver fibrosis can be a useful marker for identifying patients with obesity at high CV risk.

INTRODUCTION

Obesity has emerged as a significant global health problem, with its prevalence steadily rising in recent decades (1, 2). According to the World Health Organization (WHO), more than 2.5 billion adults with overweight were reported in 2022, of which over 890 million were classified as people with obesity (3). This increasing trend has significant implications for public health, given the numerous clinical complications associated with obesity, including cardiovascular disease (CVD) which remains the leading cause of morbidity and mortality worldwide, particularly among individuals with obesity (4).

Besides CVD, obesity is also strongly related to metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), a spectrum of liver diseases ranging from simple steatosis, in the presence of at least one cardiometabolic risk factor, to more severe stages such as metabolic dysfunction-associated steatohepatitis (MASH), which is characterized by steatosis, hepatocyte ballooning, and lobular inflammation with or without fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (5-9). The prevalence of MASLD among individuals with obesity is notably high and is expected to rise alongside the global obesity rates (10). Population studies indicate that MASLD can be found in 65% of patients with obesity (BMI 30-39.9 kg/m^2) and in approximately 85% of patients with severe obesity (BMI ≥ 40 kg/m^2) (11).

While obesity is closely linked to both CVD and MASLD, the factors determining individual susceptibility to CV complications remain unclear. Liver fibrosis, which can develop as a consequence of chronic inflammation in obesity, may be associated with an elevated CV risk. Excessive fat accumulation in adipose tissue drives chronic inflammation in obesity. This metabolic stress leads adipocytes to release adipokines. These signaling molecules recruit and activate innate immune cells, including granulocytes and monocytes (12-14). Upon activation, these cells express surface receptors like CD66b and CD11b. The increased expression of CD66b, in particular, serves as a reliable marker for neutrophil activation (15). Once activated, immune cells release pro-inflammatory cytokines and chemokines, which promote endothelial dysfunction and further perpetuate the inflammatory state in obesity (16, 17). This chronic inflammation also facilitates the infiltration of leukocytes into the liver, contributing to the development of liver fibrosis (18). Given the role of liver fibrosis in increasing CV risk, its presence in individuals with obesity may play a critical role in determining their higher susceptibility to CV complications. This study focuses on patients with severe obesity, a high-risk group that remains underrepresented in prior liver fibrosis research. Our hypothesis is that the presence of liver fibrosis, as indicated by the Fibrosis-4 (FIB-4) index, may distinguish patients with severe obesity at higher CV risk from their counterparts. The FIB-4 index is a non-invasive, cost-effective tool that estimates the degree of liver fibrosis and serves as an alternative to liver biopsy, which is invasive and carries potential risks (19, 20).

Therefore, this study aims to investigate the association between FIB-4 and CV risk factors in patients with severe obesity and to evaluate the relationship between FIB-4 and systemic leukocyte activation markers in this patient group.

MATERIALS AND METHODS

Study design

This study is a post hoc analysis of the ASSISI project, which was a prospective study with the primary aim to evaluate the effects of bariatric surgery on complement component 3 (C3) and systemic inflammatory markers associated with CV risk in patients with severe obesity (21-23). The study protocol was in accordance with the Declaration of Helsinki, approved by the Medical Ethical Committee Rotterdam (Maasstad Hospital, Rotterdam, the Netherlands; ABR no. NL47891.101.14, approval date: 29 April 2015) and registered in the Dutch Trial register (NTR5172) (24). Prior to collecting data written informed consent was obtained from each participant.

Study population

The study population consisted of 200 patients aged 18 to 65 who were scheduled for bariatric surgery. All patients met the criteria of the International Federation for the Surgery of Obesity (IFSO) for bariatric surgery (25). Inclusion criteria were: having a body mass index (BMI) of ≥40 kg/m^2, or a BMI of 35 kg/m^2 and obesity related clinical complications, including hypertension, type 2 diabetes mellitus (T2DM), dyslipidemia, respiratory disorders, severe joint disease, and significant obesity-related psychological or functional limitations. Patients were excluded if they were using immune-modulating medication, if they had an acute inflammatory disease within 6 weeks prior to inclusion, or if they previously underwent bariatric surgery or cholecystectomy. All data were collected at study entry, prior to the bariatric procedure.

Baseline characteristics

Baseline characteristics were obtained during standard preoperative screening prior to bariatric surgery and included medical history and current medication profile. Anthropo-metric measurements included waist circumference, height, weight, and blood pressure. BMI was calculated using weight and height.

Laboratory measurements

Preoperative laboratory tests were performed on non-fasting blood samples following the hospital's standard procedures (22). Leukocyte activation markers (CD11b and CD66b) were measured by flow cytometry using blood samples collected in EDTA tubes. Fluorescent labeled antibodies against CD66b and CD11b were used to identify granulocytes and monocytes, with

CD45 used to distinguish leukocytes from other blood cells. Whole blood was incubated with antibodies for 15 min at room temperature in the dark, with isotype controls included to correct for nonspecific binding. Erythrocytes were lysed using a standard isotonic solution. Samples were analyzed using a Coulter Epics XL-MCL (Beckman Coulter, Miami, FL, USA) or Navios flow cytometer (Beckman Coulter, Miami, FL, USA), and data were processed with EXPO 32 or Kaluza 1.2 software. Marker expression was reported as mean fluorescence intensity in arbitrary units (26).

Carotid intima media thickness

Carotid intima media thickness (cIMT) measurements were performed by a trained and experienced sonographer using the ART-LAB (Esaote, Genoa, Italy) following procedures outlined in prior studies (22, 27). Ultrasound scans were performed with participants in a supine position, head resting comfortably and neck slightly hyperextended and rotated opposite to the probe. Images of the distal 1 cm of the far wall of each common carotid artery (CCA) were obtained using B-mode ultrasound, producing two echogenic lines representing the combined thickness of the intima and media layers. Each CCA was imaged in three views (right side: 90◦, 120◦, 150◦; left side: 210◦, 240◦, 270◦), and the segments were measured semi automatically in triplicate. The mean of these measurements was used for analyses.

Pulse wave velocity

Pulse wave velocity (PWV) was measured using the Mobil-O-Graph (I.E.M. Germany) [28]. To measure the PWV, an inflatable cuff was placed on the patient's bare left upper arm, with the cuff size chosen based on arm circumference. These cuffs detect fluctuations in cuff pressure caused by pulse waves when inflated to sub-diastolic pressures (28). PWV is calculated by analyzing the time delay between these fluctuations at different arterial locations. This value reflects the speed at which a mechanical wave propagates along the arterial wall, serving as an indicator of arterial stiffness. Higher PWV values are linked to increased vascular stiffness, which is a significant predictor of CV events (29). To ensure accuracy and reliability, PWV measurements were performed in triplicate.

Fibrosis-4 index

The FIB-4 is the most commonly used non-invasive test (NIT) to estimate the degree of hepatic fibrosis. The FIB-4 was calculated according to the following formula:

$$\mathrm{FIB-4} = \frac{\mathrm{Age\ (years)\ x\ AST\ (U/L)}}{\mathrm{Platelet\ count\ } \left(10^9\right) \mathrm{\ x\ } \sqrt{\mathrm{ALT}\ \left(\mathrm{U/L}\right)}}$$

Based on previously published literature, a cut-off of 1.3 was applied, as values ≥1.3 have been associated with an increased risk of advanced fibrosis (7, 30). Low fibrosis risk was defined as a FIB-4 < 1.3 and high fibrosis risk as a FIB-4 ≥1.3.

Statistical analyses

All statistical analyses were conducted using IBM SPSS statistics version 28.0.1.0 (IBM SPSS Statistics, New York, NY, USA). Continuous variables are presented as mean ±SD or median (IQR), and categorical variables as frequencies (%). Patients were stratified into a low FIB-4 group (<1.3) and a high FIB-4 group (≥1.3) based on the EASL recommendation on NITs (31-33). Between-group differences (low vs. high FIB-4) were assessed using independent samples t-tests or Mann–Whitney U tests as appropriate. Associations between medication use and FIB-4 categories were evaluated using Chi-square tests. Correlation analyses were performed using Pearson's correlation or Spearman's correlation where applicable. The primary aim of this post hoc analysis was to examine FIB-4 in relation to vascular and metabolic parameters. Established relationships between classical cardiometabolic risk factors and subclinical vascular measures in this cohort have been reported previously [22,28]. Associations between FIB-4 and vascular or metabolic parameters were assessed using multivariable regression analyses. For continuous FIB-4, a linear regression model included key demographic and cardiometabolic variables (age, cIMT, sex, BMI, systolic BP, HbA1c, LDL-C, HDL-C, triglycerides, creatinine, CRP). For dichotomized FIB-4 (<1.3 vs. ≥1.3), an exploratory logistic regression was applied using the same set of predictors (N = 92 with complete data). To account for potential non-linear effects of age, a separate sensitivity analysis modeled age using four linear spline terms (knots at 30, 40, and 50 years). Systemic leukocyte activation markers were included as exploratory outcomes, and no formal correction for multiple testing was applied. p-values of <0.05 were considered statistically significant.

RESULTS

The total cohort consisted of 200 patients, predominantly women (N = 148), with a mean age of 41 ±12 years and a mean BMI of 42.7 ±5.2 kg/m^2. FIB-4 values in the study population ranged from 0.23 to 2.5, with a mean of 1.02 ±0.44 (Table A1). Of the total cohort, 155 patients (77.5%) were classified as having a low FIB-4 score (<1.3), while 45 patients (22.5%) had a high FIB-4 score (≥1.3). Additional details can be found in Table A1. BMI and waist circumference were not significantly different between patients with low and high FIB-4 scores. The group with high FIB-4 scores was older (Table 1). Patients in the elevated FIB-4 group showed higher systolic and diastolic blood pressure compared to those with low FIB-4. They were also more frequently treated with antihypertensive medications (48.9% vs. 27.7%, p = 0.011) (Table 1). Significant positive correlations were observed between FIB-4 and both systolic and diastolic blood pressure (Figure 1). Additionally, those with a high FIB-4 showed increased cIMT and PWV, with positive correlations observed between FIB-4 and both cIMT and PWV (Figure 2). Moreover, those with a high FIB-4 showed increased levels of HbA1c and glucose (Table 1), with correlation analyses indicating significant positive associations between FIB-4 and both HbA1c and glucose levels (Figure 1). Classical CVD risk factors such as low HDL-C, high LDL-C, and high TG did not show significant differences between the groups (Table 1).

Table 1. Baseline characteristics by FIB-4 group: comparison of low FIB-4 (<1.3) versus high FIB-4 (≥1.3). Data presented as mean (standard deviation), median (interquartile range), or number (%).

	Low FIB-4 N = 155	High FIB-4 N = 45	*p*-Value
Demographic characteristics			
Age (years)	38 ± 11	53 ± 5.2	<0.001
Sex, n (%) female	123 (79)	25 (56)	0.001 *
Body weight (kg)	124 ± 19	126 ± 28	0.691
Body mass index (kg/m^2)	42.5 ± 4.4	43.3 ± 7.2	0.458
Waist circumference (cm)	129 ± 12	131 ± 16	0.341
Diabetes mellitus, n (%)	27 (17.4)	16 (35.6)	0.013 †
Clinical characteristics			
FIB-4	0.83 ± 0.26	1.67 ± 0.30	<0.001
Hematocrit (L/L)	0.42 ± 0.04	0.43 ± 0.03	0.524
Hemoglobin (mmol/L)	8.7 ± 0.8	8.8 ± 0.7	0.194
Systolic blood pressure (mmHg)	139 ± 15	146 ± 16	0.002
Diastolic blood pressure (mmHg)	83 ± 12	91 ± 13	0.002
Total cholesterol (mmol/L)	5.1 ± 1.0	5.4 ± 1.2	0.197
Triglycerides (g/L)	2.0 ± 1.1	2.2 ± 1.2	0.359
HDL-C (mmol/L)	1.2 ± 0.29	1.3 ± 0.31	0.278
LDL-C (mmol/L)	3.1 ± 0.92	3.3 ± 1.1	0.221
Apolipoprotein B (g/L)	1.1 ± 0.29	1.2 ± 0.33	0.069
HbA1c (mmol/mol)	41 ± 9.5	48 ± 14	0.002
Glucose (mmol/L)	5.7 ± 1.6	7.0 ± 2.9	0.006
Insulin (µIU/mL)	23 (17–44)	30 (18–47)	0.998
C-reactive protein (mg/L)	6 (3–10)	5 (3–9)	0.199
Creatinine (µmol/L)	65 ± 14	71 ± 16	0.038
Homocystein (µmol/L)	11.5 ± 4.1	14.0 ± 9.6	0.167
TSH (mU/l)	1.8 (1.4–2.3)	2.0 (1.5–2.6)	0.934
Medication			
Statins, n (%)	16 (10.3%)	15 (33.3%)	<0.001
Antihypertensives, n (%)	43 (27.7%)	22 (48.9%)	0.011
Antidiabetics, n (%)	22 (14.2%)	13 (28.9%)	0.027

*Abbreviations: FIB-4, Fibrosis-4 index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; TSH, thyroid stimulating hormone. * p-value for sex calculated using Pearson Chi-square. † Forty patients had a prior diagnosis of T2DM, and three were newly diagnosed at the time of inclusion.*

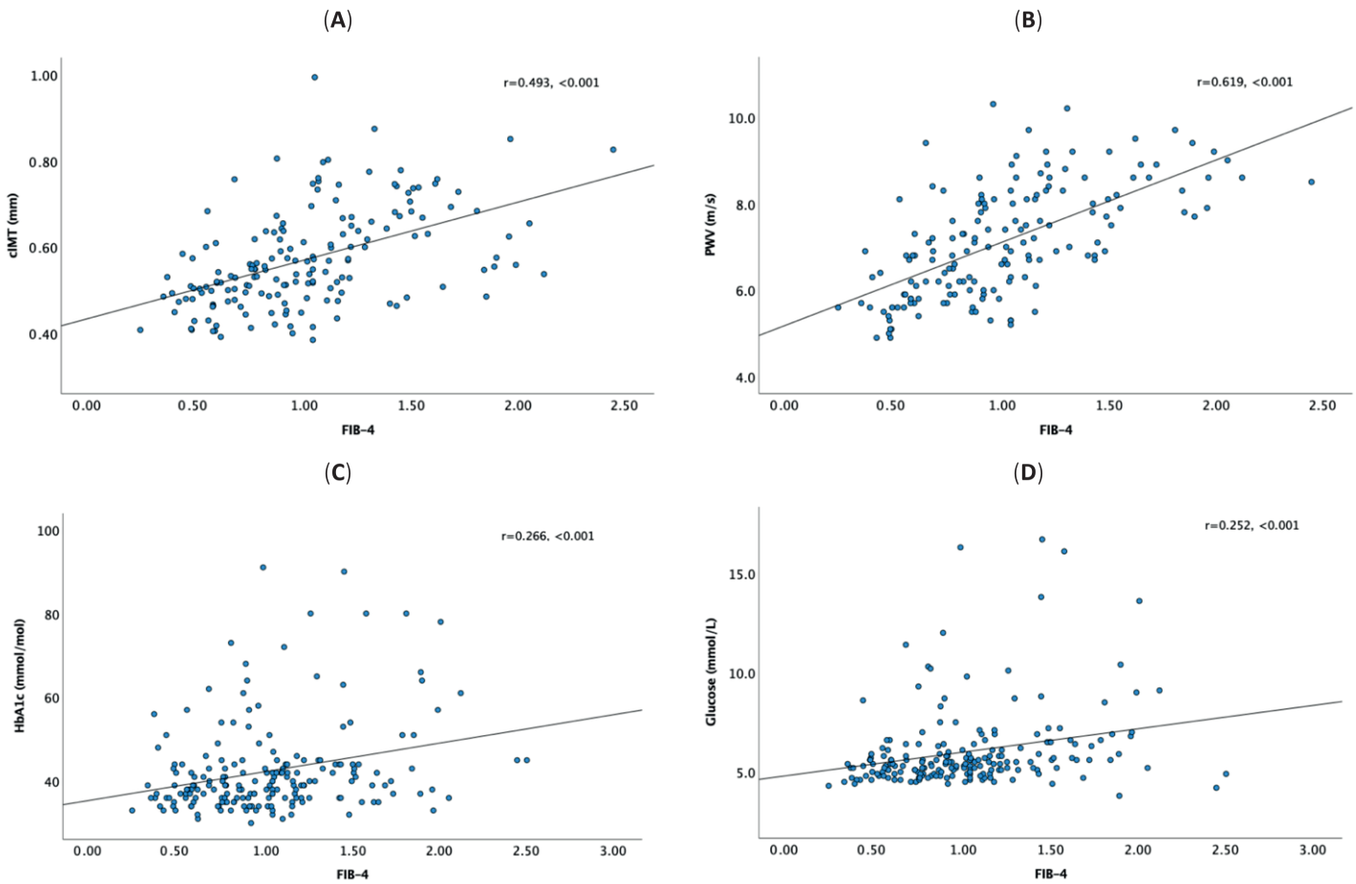


Figure 1. Correlations of the Fibrosis-4 (FIB-4) index with (A) carotid intima-media thickness (cIMT), (B) pulse wave velocity, (C) glycated hemoglobin A1c (HbA1c), (D) glucose levels, (E) sys-tolic blood pressure, and (F) diastolic blood pressure.

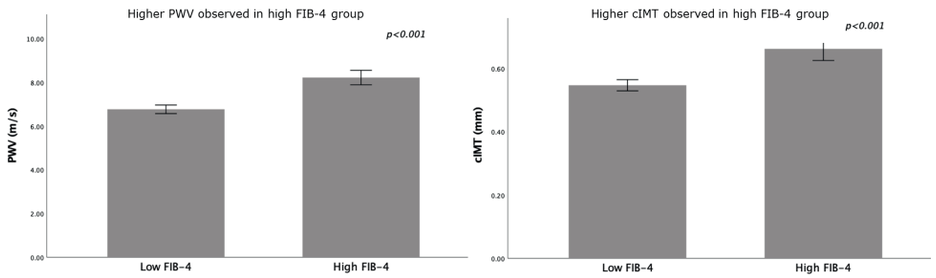


Figure 2. Mean pulse wave velocity (PWV) and carotid intima-media thickness (cIMT) values by FIB-4 group. Low FIB-4 (<1.3) vs. high FIB-4 (≥1.3): 6.8 ± 1.1 vs. 8.2 ± 0.9 m/s for PWV, and 0.55 ± 0.10 vs. 0.66 ± 0.11 mm for cIMT. Error bars indicate standard error of the mean (SE).

Subgroup analyses compared lipid levels in patients based on hypertension, statin use, and T2DM status. Overall, no major differences in lipid levels were observed between low and high FIB-4 across these subgroups. Patients in the high FIB-4 group showed higher use of statins, antihypertensive medications, and antidiabetic medication, including the use of insulin (Table 2). Those in the high FIB-4 group also tended to have an increased expression of granulocyte CD66b (Table 3), with a significant positive correlation between FIB-4 and granulocyte CD66b (rho = 0.177, p = 0.012). Conversely, these patients tended to have a decreased expression of monocyte CD11b surface markers (Table 3). Subgroup analyses (Table A2) showed that hypertensive patients with high FIB-4 had significantly higher cIMT and PWV than those with low FIB-4 (cIMT: 0.67 ±0.11 vs. 0.59 ±0.11 mm; PWV: 8.4 ±0.9 vs. 7.4 ±1.0 m/s; both p < 0.001), consistent with greater arterial stiffness. In diabetic patients, cIMT and PWV were numerically higher in the high FIB-4 group, but differences were not statistically significant (p = 0.29 and p = 0.07). Multivariable regression of baseline demographics and cardiometabolic factors, showed that only age and cIMT were independently associated with continuous FIB-4 (Table A3). Multivariable logistic regression of dichotomized FIB-4 (low vs. high) including 92 patients with complete data showed that age and HbA1c were independent predictors, whereas cIMT and CRP showed trends but were not significant (Table A4). Multivariable regression analysis identified age as the most significant predictor of cIMT (p < 0.001). CD66b on granulocytes also significantly influenced cIMT (p = 0.033), indicating its role in cIMT variation. Sex showed a significant negative association with cIMT (p = 0.003), with women exhibiting lower cIMT values than men. FIB-4 categories approached significance (p = 0.069), suggesting a potential, though less clear, link to cIMT. No significant associations were observed between cIMT and BMI, systolic blood pressure, or diastolic blood pressure (Table 4). To account for potential non-linear effects of age, age was modeled using four linear spline terms with knots at 30, 40, and 50 years. In this spline-adjusted model, FIB-4 remained significantly associated with cIMT (B = 0.054, p = 0.026), whereas the age spline segments were not statistically significant (Table A5).

Table 2. Chi-square test results for the association between low FIB-4 (<1.3) versus high FIB-4 (≥1.3) and medication use.

Medication	Low FIB-4 (%)	High FIB-4 (%)	X^2	*p*-Value
Statins	10.3	33.3	14.099	<0.001
Antihypertensives	27.7	48.9	7.109	0.011
Antidiabetics	14.2	28.9	5.217	0.027

Table 3. Flow cytometry results for leukocyte activation markers by FIB-4 group (low <1.3 vs. high ≥1.3).

Leukocyte Activation Marker	Low FIB-4	High FIB-4	*p*-Value
CD35 on monocytes (au)	7.3 ± 3.6	7.1 ± 3.0	0.680
CD35 on granulocytes (au)	10.7 ± 5.0	10.5 ± 6.5	0.815
CD11b on monocytes (au)	34.6 ± 10.2	31.6 ± 7.8	0.070
CD11b on granulocytes (au)	47.5 ± 15	44.8 ± 16	0.303
CD66b on granulocytes (au)	5.9 ± 1.8	6.7 ± 2.6	0.070

Table 4. Multivariable linear regression analysis of variables associated with carotid intima media thickness (cIMT).

	Standardized Coefficients Beta	VIF	*p*-Value
(Constant)	-	0.016	-
Age (years)	0.438	1.782	<0.001
CD66b on granulocytes (au)	0.163	1.356	0.033
CD11b on monocytes (au)	0.061	1.427	0.429
FIB-4	0.143	1.443	0.069
Sex (m/f)	−0.229	1.339	0.003
BMI (kg/m^2)	0.063	1.241	0.387
Systolic blood pressure (mmHg)	0.039	2.267	0.691
Diastolic blood pressure (mmHg)	−0.033	2.576	0.749

Abbreviations: FIB-4; Fibrosis-4 index; BMI, Body Mass Index; VIF, Variance Inflation Factor.

DISCUSSION

This study evaluated liver fibrosis, assessed by FIB-4, in patients with severe obesity and its association with CV risk markers, including cIMT and PWV. Patients with elevated FIB-4 exhibited early vascular changes, higher blood pressure, insulin resistance (IR), and systemic inflammation. Traditional CV risk factors, including BMI and lipid profiles, neither differentiated between groups nor showed independent associations with cIMT. Regression analyses revealed that sex and granulocyte CD66b expression were independently associated with cIMT, whereas FIB-4 showed a borderline association that became significant in spline-adjusted models accounting for age. Women had lower cIMT values, emphasizing sex as an important modifier of early vascular alterations. These results suggest that classical measures may have limited predictive value for early subclinical atherosclerosis in this population, which appears to be primarily mediated by fibrosis and inflammatory pathways.

Analyses treating FIB-4 as a continuous variable showed significant associations with age and cIMT, while exploratory logistic regression using dichotomized FIB-4 (≥1.3) revealed associations with age and HbA1c, with cIMT and CRP showing non-significant trends. The smaller number of individuals above the FIB-4 threshold likely reduced statistical power in the logistic analysis.

Patients with elevated FIB-4 had higher systolic and diastolic blood pressure despite more frequent use of antihypertensive medications, suggesting a greater burden of hypertension or CV comorbidity. This is consistent with the higher prevalence of hypertension in MASLD (34, 35) and a cross-sectional study of 4705 patients showing an association between hypertension and liver fibrosis (OR 2.0, 95% CI 1.3–3.0), which was stronger in obesity (OR 2.1, 95% CI 1.4–3.2) (36). Both hypertensive and non-hypertensive subgroups also showed that higher FIB-4 was associated with increased cIMT and PWV, reflecting greater arterial stiffness. These observations are reinforced by external cohorts, where in 8336 adults higher PWV was independently associated with MASLD (37), and in 2550 participants advanced liver fibrosis nearly doubled the risk of elevated cIMT and increased PWV (38), further underscoring the link between liver fibrosis and vascular dysfunction.

Metabolic factors further distinguished patients with elevated FIB-4. Glucose and HbA1c were elevated independently of BMI and despite more intensive antidiabetic therapy, highlighting the central role of IR and glycemic dysregulation in liver fibrosis. Most diabetic patients in the overall cohort had FIB-4<1.3, consistent with prior studies (39, 40), but T2DM was relatively more frequent among patients with elevated FIB-4, suggesting a link between glycemic status and fibrosis burden that warrants further investigation in larger cohorts.

Despite greater use of statins and other cardiometabolic medications, vascular damage in patients with elevated FIB-4 was not mitigated. While statins can induce vascular calcification, this generally reflects plaque stabilization rather than increased CV risk, and randomized trials demonstrate that statins reduce atherosclerotic progression (41-43). These findings imply that FIB-4 identifies individuals with severe obesity who remain at residual CV risk despite intensive preventive therapy.

Inflammatory profiling revealed higher CD66b expression on granulocytes in patients with elevated FIB-4, whereas CD11b expression on monocytes was decreased. This pattern suggests a shift toward granulocyte-driven inflammation, while monocyte-mediated pathways, typically involved in atherosclerosis (44, 45), appear less prominent. These results align with prior studies showing granulocyte activation contributes to systemic inflammation and vascular alterations in liver disease (46, 47).

Lipid profiles did not differ significantly between groups, likely due to the higher prevalence of statin use in patients with elevated FIB-4. This observation is consistent with the understanding that advanced liver disease can reduce total cholesterol and LDL-C via impaired hepatic cholesterol synthesis, whereas statin therapy may mask underlying dyslipidemia (46-49). Importantly, fibrosis has been linked to CV risk independent of lipid abnormalities (50, 51).

FIB-4 was selected for its widespread validation, guideline endorsement, and ease of clinical application (7). Although other non-invasive fibrosis scores, such as the NAFLD Fibrosis Score, are available, elevated FIB-4 effectively identifies patients with severe obesity at high CV risk. Future studies should assess how FIB-4 compares with alternative scores in predicting CV outcomes, which may further refine risk stratification in this population.

Collectively, these findings suggest that targeting liver fibrosis could prevent early vascular alterations independently of traditional risk factors, while granulocyte activation may mechanistically link liver fibrosis to vascular dysfunction. FIB-4 may identify patients with persistent CV risk despite optimal therapy, highlighting its potential as a clinically useful biomarker beyond traditional markers such as BMI, blood pressure, and lipid profiles. Given the exploratory nature of the study, these results are hypothesis-generating, and provide a basis for future larger, well-designed studies investigating the mechanisms linking liver fibrosis, inflammation, sex differences, and early CVD in this population.

The current study has several limitations. The study population was predominantly female, reflecting a common trend in research involving severe obesity. The relatively small, exploratory, hypothesis-generating design limited the scope of analyses and precluded exhaustive multivariable modeling. Non-fasting blood samples may have introduced variability but reflect real-world clinical conditions. Only a limited number of inflammatory markers were analyzed, and follow-up FIB-4 measurements or imaging-based assessments (e.g., ultrasound or elastography) were not available. Medication use was reported descriptively, without adjustment for confounding by indication, which could be addressed in larger, future studies. Importantly, this post hoc analysis evaluated multiple vascular outcomes without a pre-specified primary endpoint or formal sample size calculations, yet it provides novel, hypothesis generating insights into the links between liver fibrosis, systemic inflammation, and subclinical CVD in bariatric surgery candidates.

CONCLUSION

In conclusion, assessing liver fibrosis in individuals with severe obesity can help identify those with a very high CV risk. Our findings suggest that incorporating FIB-4 into routine CV risk assessment could improve patient stratification and support more tailored preventive strategies in this high-risk population.

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SUPPLEMENTARY DATA

Supplementary Table A1. Baseline clinical and demographic characteristics of the total cohort (N = 200). Data are given as mean (standard deviation), median (interquartile range) or number (%).

	Patients (N = 200)
Demographic characteristics	
Sex (f/m) [n, %]	148 (74)/52 (26)
Age (years)	41 ± 12
Body weight (kg)	125 ± 21
Body mass index (kg/m^2)	42.7 ± 5.2
Ethnicity	
Caucasian [n, %]	145 (73.2)
African [n, %]	20 (10.1)
Latin-American [n, %]	6 (3.0)
Indian [n, %]	8 (4.0)
Asian [n, %]	1 (0.5)
Clinical characteristics	
Hematocrit (L/L)	0.43 ± 0.04
Hemoglobin (mmol/L)	8.7 ± 0.81
Systolic blood pressure (mmHg)	140 ± 16
Diastolic blood pressure (mmHg)	85 ± 12
Total cholesterol (mmol/L)	5.2 ± 1.1
LDL-C (mmol/L)	3.1 ± 0.96
HDL-C (mmol/L)	1.2 ± 0.29
Triglycerides (mmol/L)	1.7 (1.2–2.7)
Apolipoprotein B (g/L)	1.1 ± 0.3
Glucose (mmol/L)	6.1 ± 2.4
C-reactive protein (mg/L)	6 (3.0–10)
Leukocytes (10^9/L)	8.7 ± 2.4
Carotid Intima Media thickness (mm)	0.57 ± 0.11
Medication	
Statins [n, %]	31 (15.5)
Antihypertensives [n, %]	65 (32.5)
Antidiabetics [n, %]	35 (17.5)
Medical history	
Diabetes [n, %]	43 (21.5)
Hypertension [n, %]	69 (34.5)
Hypercholesterolemia [n, %]	58 (29)
CVA or TIA [n, %]	5 (2.5)
Myocardial infarction	6 (3)
Fibrosis markers	
Fibrosis-4 index (FIB-4)	
Total population	1.02 ± 0.44
FIB-4 score <1.3 [n, %]	155 (77.5)
FIB-4 score ≥1.3 [n, %]	45 (22.5)

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CVA, cerebrovascular accident; TIA, transient ischemic attack; FIB-4, Fibrosis-4 index.

Supplementary Table A2. Subgroup analyses of cIMT and PWV according to FIB-4 category in hypertensive and diabetic patients.

Subgroup	FIB-4 Category	cIMT (mm)	N	*p*-Value cIMT	PWV (m/s)	N	*p*-Value PWV
Hypertensive	Low FIB-4	0.59 ± 0.11	66	<0.001	7.4 ± 1.0	64	<0.001
	High FIB-4	0.67 ± 0.11	28		8.4 ± 0.9	25	
Diabetic	Low FIB-4	0.60 ± 0.11	21	0.303	7.4 ± 1.0	20	0.079
	High FIB-4	0.64 ± 0.10	10		8.2 ± 0.9	8	

Notes: Values are mean ± SD. p-values compare low vs. high FIB-4 within each subgroup. Abbreviations: cIMT, carotid intima media thickness; PWV, pulse wave velocity; FIB-4, Fibrosis-4 index.

Supplementary Table A3. Multivariable linear regression of factors associated with continuous FIB-4.

Variable	B	Std. Error	Beta	*p*-Value	VIF
Constant	−0.566	0.660	-	0.394	-
Age (years)	0.021	0.004	0.501	<0.001	2.02
cIMT (mm)	0.832	0.416	0.208	0.049	2.04
Sex (female/male)	−0.130	0.100	−0.126	0.198	1.77
BMI (kg/m^2)	0.007	0.008	0.083	0.373	1.62
Systolic BP (mmHg)	−0.001	0.003	−0.018	0.831	1.35
HbA1c (mmol/mol)	0.005	0.004	0.105	0.215	1.32
LDL-c (mmol/L)	0.017	0.037	0.038	0.651	1.28
HDL-c (mmol/L)	0.029	0.172	0.017	0.865	1.80
Triglycerides (g/L)	0.008	0.048	0.014	0.877	1.63
Creatinine (µmol/L)	−0.002	0.004	−0.036	0.695	1.60
CRP (mg/L)	−0.007	0.005	−0.118	0.162	1.30

Supplementary Table A4. Multivariable logistic regression of factors associated with dichotomized FIB-4 (<1.3 vs. ≥1.3).

Predictor	OR	95% CI	*p*-Value
Age (years)	1.266	1.089–1.471	0.002
cIMT (per 0.1 mm)	1.947	0.871–4.354	0.107
Sex (female/male)	0.339	0.040–2.881	0.363
BMI (kg/m^2)	1.064	0.907–1.247	0.446
Systolic BP (mmHg)	1.011	0.960–1.065	0.692
HbA1c (mmol/mol)	1.100	1.000–1.210	0.049
LDL-C (mmol/L)	1.597	0.614–4.150	0.336
HDL-C (mmol/L)	1.902	0.116–31.198	0.758
Creatinine (µmol/L)	1.404	0.482–4.089	0.522
CRP (mg/L)	0.990	0.909–1.078	0.838

Note: cIMT was rescaled per 0.1 mm to provide interpretable odds ratios. Abbreviations: OR, odds ratio; CI, confidence interval; cIMT, carotid intima media thickness; BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein.

Supplementary Table A5. Regression of carotid intima-media thickness (cIMT) on FIB-4 and age modeled with splines.

Predictor	B	Standard Error	*p*-Value
Constant	0.431	0.088	<0.001
FIB-4	0.054	0.024	0.026
Age spline 1	0.001	0.003	0.839
Age spline 2	0.006	0.003	0.066
Age spline 3	0.004	0.003	0.166
Age spline 4	0.004	0.004	0.264

Note: Dependent variable: carotid intima-media thickness (cIMT). Age was modeled using four linear spline terms with knots at 30, 40, and 50 years (Age spline 1–4).

Chapter 4

Assessing the effects of naltrexone-bupropion on hepatic steatosis and fibrosis in patients with T2DM and overweight or obesity: Insights from a placebo-controlled trial

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ABSTRACT

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is prevalent among individuals with overweight or obesity and type 2 diabetes (T2DM), increasing the risk for liver-related and cardiovascular complications. Lifestyle changes to reduce body weight are the primary intervention to decrease the risk of MASLD and liver fibrosis. This study aimed to evaluate the impact of a pharmacological intervention with naltrexone/bupropion (NB) on hepatic steatosis and fibrosis risk in patients with T2DM using non-invasive tests.

Methods: In this post hoc analysis of a 56-week, randomized, double-blind, placebo-controlled trial 505 adults with T2DM and overweight or obesity were randomized to either NB or placebo (2:1), both combined with structured lifestyle counselling. Hepatic outcomes were measured using the Hepatic Steatosis Index (HSI), Metabolic Dysfunction Associated Fibrosis-5 (MAF-5) and Fibrosis-4 (FIB-4) indices. Changes in liver-related indices were analyzed by treatment arm and weight loss strata (<5%, ≥5%, ≥10%), and multivariable regression was used to identify predictors of hepatic improvement.

Results: At Week 56, the NB group achieved significantly greater body weight loss (6.3 ± 7.2 kg vs. 2.5 ± 5.2 kg, $p < 0.001$). Lifestyle intervention with NB treatment compared with placebo significantly reduced HSI (2.9 vs. 1.2, $p < 0.001$) and MAF-5 (0.80 vs. 0.31, $p= 0.003$), while FIB-4 scores remained unchanged in both groups. The best improvements in HSI and MAF-5 were observed in those achieving ≥5% or ≥ 10% weight loss compared with those with <5% (all $p < 0.001$). Weight change correlated strongly with HSI reduction ($r = 0.796$, $p < 0.001$) and moderately with MAF-5 ($r = 0.488$, $p < 0.001$), but not with FIB-4 ($r = 0.034$, $p= 0.590$). Multiple regression analysis identified weight change as the strongest predictor of improvements in hepatic markers (HSI, MAF-5, ALT and AST), with no significant contributions from other factors.

Conclusion: Weight loss was the key driver of hepatic improvements in patients with T2DM and overweight or obesity. Treatment with NB resulted in significantly greater weight reduction and significantly greater improvements in HSI and MAF-5 compared with placebo, suggesting an added benefit for liver health.

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), has emerged as a significant global health challenge, affecting approximately 30% of the general population. MASLD is defined by the presence of hepatic steatosis along with at least one cardiometabolic risk factor (1). MASLD encompasses a spectrum of liver conditions, starting with simple steatosis and potentially progressing to metabolic dysfunction-associated steatohepatitis (MASH), the inflammatory form of MASLD. This inflammatory stage can further advance to fibrosis, cirrhosis, and, in some cases, hepatocellular carcinoma (HCC), all in the absence of secondary factors like excessive alcohol use or other liver conditions (2-4).

MASLD is highly prevalent in patients with type 2 diabetes mellitus (T2DM), impacting over 65% of patients with T2DM (5). Cohorts with liver histology data available show that approximately 66% of patients with T2DM have MASH, underscoring the substantial burden of advanced liver disease in this group (6). Obesity, which commonly coexists with T2DM, further increases the likelihood of MASLD, with rates as high as 90% reported among obese patients (5). With the global rise in obesity and T2DM rates, the prevalence of MASLD is expected to increase as well, posing an ongoing and growing public health concern (5, 7, 8). A key driver of MASLD progression is liver fibrosis, which also serves as a strong predictor of serious complications such as liver failure and cardiovascular disease (CVD). Consequently, early detection and effective management of both MASLD and liver fibrosis are essential to prevent disease progression and reduce cardiovascular (CV) risk.

Currently, lifestyle interventions aimed at achieving weight loss remain the cornerstone of MASLD management, as even modest reductions in body weight can improve steatosis (6, 9, 10). In addition to lifestyle modification, various pharmacological treatments are currently under investigation for MASLD, such as incretins, PPAR agonists, and others (11). Among these, the combination of naltrexone sustained-release (SR) and bupropion SR (NB), which combines an opioid receptor antagonist and a dopamine/norepinephrine reuptake inhibitor, has been studied for its potential to reduce appetite by modulating the brain's reward system (12). This combination could offer a useful option for overweight or obese patients with T2DM and MASLD.

While liver biopsy remains the gold standard for MASLD diagnosis, its invasive nature, high cost, and potential complications make it unsuitable for routine screening or large-scale implementation (1, 13). To address this, several non-invasive tests (NITs) have been developed. These include imaging techniques such as vibration-controlled transient elastography (VCTE; FibroScan®), as well as various scoring algorithms (1). To estimate hepatic steatosis, the Hepatic Steatosis Index (HSI) has been validated and it has been widely adopted in both clinical and research contexts (14, 15). For fibrosis assessment, the recently validated Metabolic Dysfunction-Associated Fibrosis 5 (MAF-5) index, especially relevant for individuals with

metabolic disorders such as T2DM or obesity, or the widely utilized Fibrosis-4 (FIB-4) index could be utilized (13, 16-18).
This study aimed to investigate the effects of NB in combination with structured lifestyle counseling on hepatic steatosis and fibrosis risk in patients with T2DM and overweight or obesity, using NITs.

MATERIALS AND METHODS

Study design
This is a post-hoc analysis of 56-week, double-blind, placebo-controlled trial in which the effects of NB were evaluated (12). The study enrolled 505 patients with overweight or obesity and T2DM, all of whom received a standardized lifestyle intervention. The protocol was approved by each site's Institutional Review Board. Written informed consent was obtained from all participants, and the study adhered to the Declaration of Helsinki and the principles of Good Clinical Practice (GCP) standards (19).

Study population
The study included men and women aged 18 to 70 years with T2DM, a BMI between 27 and 45 kg/m², fasting blood glucose levels below <270 mg/dL, and hemoglobin A1c (HbA1c) levels ranging from 7% (53 mmol/mol) to 10% (86 mmol/mol). Patients were either not using diabetes medications or had maintained stable doses of oral antidiabetic drugs (OADs) for at least 3 months prior to enrollment. Patients had systolic blood pressure <145 mmHg and diastolic blood pressure <95 mmHg. A comprehensive list of inclusion and exclusion criteria is provided in a previous publication (12).

Data collection
Participants were randomized (2:1) via a computer-generated randomization schedule to receive either 32 mg/day naltrexone SR plus 360 mg/day bupropion SR (NB) or placebo, stratified by baseline HbA1c (≤64 mmol/mol or >64 mmol/mol) and sulfonylurea use. NB dosing was initiated at one tablet daily and escalated to two tablets twice daily by week 4. Adherence and adverse events were assessed via phone at week 2. Study visits occurred at screening, baseline, and every four weeks thereafter. Participants followed a hypocaloric diet (500 kcal/day deficit) with dietary and lifestyle counseling based on the World Health Organization (WHO) algorithm for calculating resting metabolic rate. Participants received nutrition counseling and instructional booklets adapted from the *Exchange Lists for Weight Management*, in alignment with guidelines established by the American Diabetes Association and the American Dietetic Association. Additionally, participants received written instructions on behavioral modification strategies, including recommendations to engage in at least 30 minutes of walking per day. Blood glucose was monitored twice daily for 4 weeks, with protocol-defined OAD adjustments if glycemic control deteriorated. Full details are available in the original publication (12).

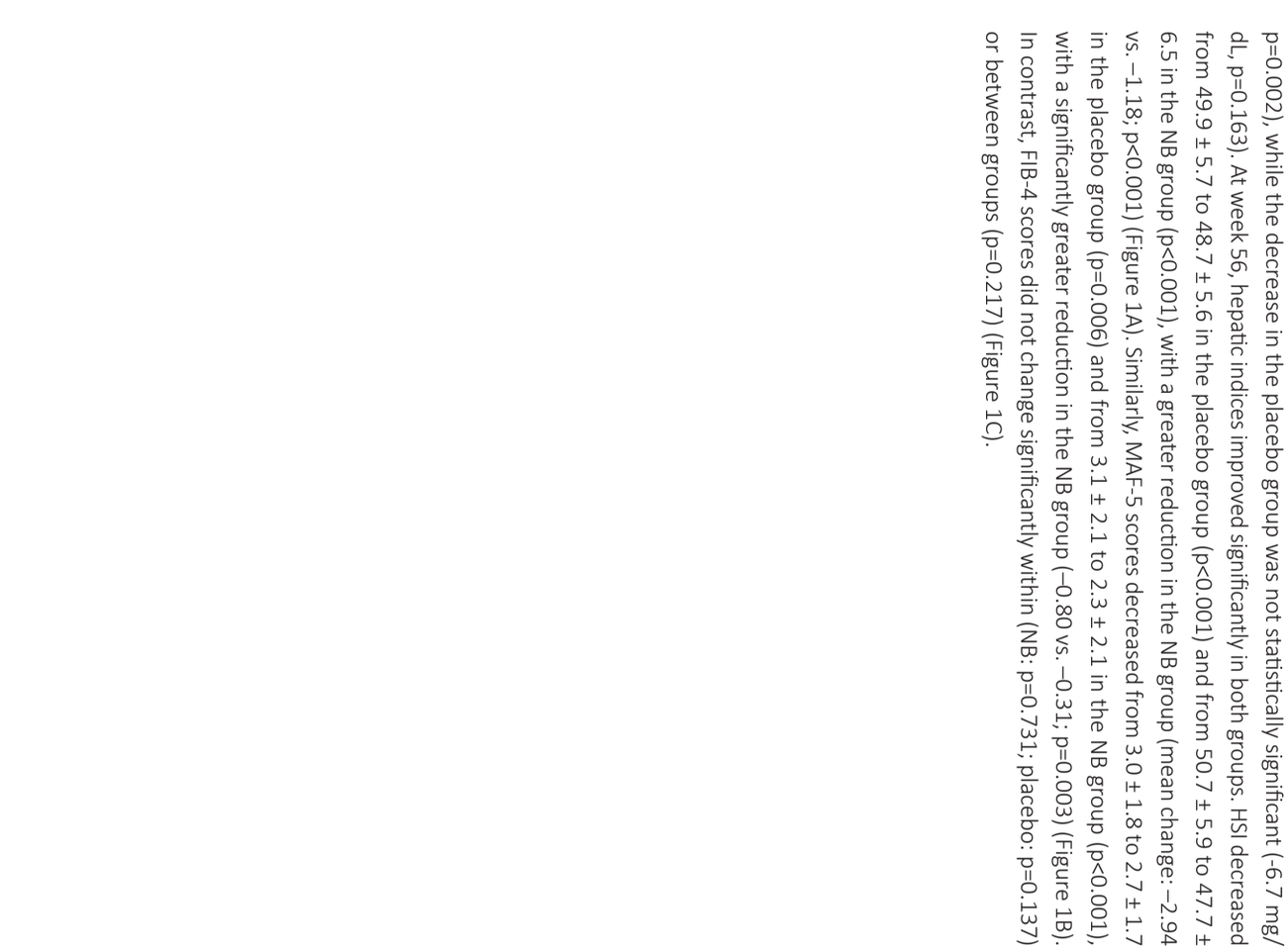
Outcome measures
The primary outcome measures were changes in hepatic steatosis and fibrosis, assessed using the HSI, MAF-5 and FIB-4 indices, respectively. Hepatic steatosis was evaluated at baseline and at week 56, with a reduction in HSI indicating improvement. HSI was calculated using the following formula: *HSI = 8 × AST/ALT+BMI (+2 if female, 0 if male).* HSI cut-off points were used to classify hepatic steatosis: values below 30 ruled out steatosis, and values above 36 ruled in steatosis. Risk of hepatic fibrosis was assessed at baseline and week 56 using the MAF-5 index, calculated as: *MAF–5 = -11.367 + WC × 0.0282 - BMI × 0.1761 + WC × BMI × 0.0019 + 2.0763 for diabetes + ln (AST) × 2.9207 – platelets × 0.0059.* A MAF-5 score of <0 indicated a low risk of fibrosis, while ≥1 indicated a high risk. FIB-4 was calculated as: age × AST / (platelets × √ALT), and interpreted using standard cut-offs: <1.3 (low risk), 1.3–2.67 (intermediate risk), and >2.67 (high risk) (1). Multiple regression models included factors such as baseline glucose levels, lipid parameters, and weight change, selected for their relevance to MASLD progression.

Statistical analysis
Data are presented as mean ± standard deviation (SD), unless otherwise specified. Paired samples t-tests were used to assess within-group changes in HSI, MAF-5, and FIB-4 from baseline to week 56 in both the treatment and placebo groups. Subgroup analyses, stratified by weight loss categories (<5%, ≥5%, and ≥10%) were conducted to evaluate differential responses in NITs. Correlation analyses were performed using Pearson's or Spearman's correlation coefficients, as appropriate. One outlier, identified based on extreme weight change, was excluded based on scatterplot analyses. Multiple regression analysis was performed to identify independent factors associated with changes in HSI, MAF-5, FIB-4 and liver enzymes. All analyses were conducted using IBM SPSS Statistics version 28.0.0.0 (IBM SPSS, New York, USA), with statistical significance set at $p<0.05$.

RESULTS

The study cohort included 285 females (56.5%) and 219 males (43.5%), with a mean age of 53.8 ± 9.3 years, mean weight of 104.4 ± 18.2 kg, and mean BMI of 36.4 ± 4.7 kg. All the participants fulfilled the criteria for MASLD at baseline. Baseline characteristics of the overall population are presented in Supplementary Table S1, and treatment group-specific baseline characteristics in Supplementary Table S2. The NB group showed a significantly larger weight

reduction compared to the placebo group (-6.3 ± 7.2 kg, vs.-2.5 ± 5.2 kg, p<0.001). In addition, fasting glucose levels decreased significantly in the NB group (mean change -10.9 mg/dL, p=0.002), while the decrease in the placebo group was not statistically significant (-6.7 mg/dL, p=0.163). At week 56, hepatic indices improved significantly in both groups. HSI decreased from 49.9 ± 5.7 to 48.7 ± 5.6 in the placebo group (p<0.001) and from 50.7 ± 5.9 to 47.7 ± 6.5 in the NB group (p<0.001), with a greater reduction in the NB group (mean change: −2.94 vs. −1.18; p<0.001) (Figure 1A). Similarly, MAF-5 scores decreased from 3.0 ± 1.8 to 2.7 ± 1.7 in the placebo group (p=0.006) and from 3.1 ± 2.1 to 2.3 ± 2.1 in the NB group (p<0.001), with a significantly greater reduction in the NB group (−0.80 vs. −0.31; p=0.003) (Figure 1B). In contrast, FIB-4 scores did not change significantly within (NB: p=0.731; placebo: p=0.137) or between groups (p=0.217) (Figure 1C).



Note:
(1) In figure A, the Y-axis starts at 44 for improved readability.
(2) Asterisks indicate statistical significance: p<0.05 for within-group changes (baseline vs. week 56).

Figure 1 – Comparison of (A) Hepatic Steatosis Index (HSI), (B) Metabolic Dysfunction-Associated Fibrosis-5 (MAF-5), and (C) Fibrosis-4 (FIB-4) index between the treatment and placebo groups at baseline and week 56.

Subgroup analyses hepatic steatosis and fibrosis

Baseline clinical and demographic characteristics by weight loss subgroups (<5%, ≥5%, and ≥10%) are presented in Table 1. From baseline to week 56, significant reductions in BMI were observed in participants with ≥5% and ≥10% weight loss in both the placebo and NB groups (all p<0.001). Among participants with <5% weight loss, BMI remained stable in the placebo group (p=0.490) and showed a small but statistically significant decrease in the NB group (p=0.045) (Supplementary Table S3). Improvements in HSI were significant among participants who achieved ≥5% or ≥10% weight loss, regardless of treatment group, with the largest reductions observed in those achieving ≥10% weight loss. In the placebo group, HSI significantly decreased in both the ≥5% (51.0 ± 5.9 to 46.3 ± 5.1, p<0.001) and ≥10% (50.4 ± 4.1 to 43.3 ± 4.9, p<0.001) subgroups, while no significant change was seen in the <5% subgroup (p=0.783). Similarly, in the NB group, significant reductions were seen in the ≥5% (51.1 ± 5.6 to 45.9 ± 5.8) and ≥10% (50.7 ± 5.2 to 43.9 ± 5.3) subgroups (both p<0.001), but not in the <5% subgroup (p=0.172) (Table 2). MAF-5 scores in the placebo group improved significantly in participants with ≥5% weight loss (p=0.002), whereas those with <5% weight loss showed no significant change (p=0.177). The ≥10% group showed a numerical improvement that did not reach statistical significance (p=0.112). In the NB group, MAF-5 improved significantly in both the ≥5% and ≥10% subgroups (both p<0.001), while the <5% group showed no significant change (p=0.258) (Table 3). FIB-4 scores remained unchanged across all weight loss categories in both the placebo and NB groups (placebo: p=0.388; NB: p=0.572) (Supplementary Table S4).

Table 1. Baseline clinical and demographic characteristics of the study participants by weight loss subgroup.

	<5% weight loss (N=157)		≥5% weight loss (N=117)		≥10% weight loss (N=53)	
	Placebo	**NB**	**Placebo**	**NB**	**Placebo**	**NB**
Sex (m/f)	43/33 (56.6/43.4)	45/36 (55.6/44.4)	8/16 (33.3/66.7)	40/53 (43/57)	2/6 (25/75)	15/30 (33.3/66.7)
Age (years)	55.0 ± 9.6	53.3 ± 9.2	57.2 ± 7.2	55.9 ± 7.7	59.4 ± 6.5	56.0 ± 8.8
Body weight (kg)	106.0 ± 17.0	107.0 ± 19.5	102.3 ± 16.6	106.5 ± 19.1	102.4 ± 13.9	104.0 ± 17.7
BMI (kg/m^2)	35.9 ± 4.6	36.3 ± 5.0	37.4 ± 4.1	37.0 ± 4.7	37.6 ± 3.5	36.7 ± 4.8
Waist circumference (cm)	115.0 ± 12.4	115.8 ± 13.4	113.8 ± 12.8	115.3 ± 12.1	114.4 ± 14.7	114.7 ± 11.9
Systolic blood pressure (mmHg)	125 ± 9	124 ± 11	127 ± 11	124 ± 11	125 ± 11	125 ± 10
Diastolic blood pressure (mmHg)	77 ± 7	78 ± 8	79 ± 8	77 ± 6	76 ± 8	77 ± 7
Total cholesterol (mg/dL)	177.3 ± 33.2	179.0 ± 43.3	187.3 ± 41.8	177.0 ± 41.6	200.6 ± 41.0	180.5 ± 36.3
LDL-C (mg/dL)	95.8 ± 31.6	100.7 ± 34.9	103.2 ± 35.2	96.1 ± 31.1	109.4 ± 23.3	99.3 ± 30.2
HDL-C (mg/dL)	44.6 ± 11.0	47.3 ± 10.7	49.7 ± 12.7	45.6 ± 9.3	51.0 ± 12.3	46.1 ± 8.7
Triglycerides (mg/dL)	158 (104 – 215)	134 (91 – 190)	163 (104 – 241)	148 (101 – 205)	202 (150 – 279)	162 (108 – 205)
Glucose (mg/dL)	158.7 ± 46.5	162.3 ± 44.4	156.7 ± 32.3	153.4 ± 34.0	149.9 ± 43.8	150.5 ± 29.2
Insulin (mIU/L)	15 (9.8 – 23)	15 (9 – 23)	14 (8.0 – 21)	15 (10 – 23)	10.5 (6.3 – 24)	15.0 (10 – 23)
ALT (U/L)	31.4 ± 15	31.4 ± 16	30.0 ± 15	30.4 ± 14	24.8 ± 14	30.2 ± 15
AST (U/L)	23.0 ± 8.2	23.1 ± 9.6	23.1 ± 8.7	22.1 ± 7.6	20.9 ± 10	22.7 ± 8.4
HSCRP (mg/L)	3.7 (1.6 – 6.7)	3.7 (1.8 – 8.3)	4.2 (2.0 – 8.1)	4.0 (1.5 – 7.1)	2.5 (1.7 – 7.1)	4.7 (1.5 – 7.6)

Data are presented as mean (standard deviation), number (%), or median (interquartile range). Abbreviations: NB, naltrexone-bupropion; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase, HSCRP, high-sensitivity C-reactive protein

Note: No statistically significant differences were observed between the placebo and naltrexone-bupropion groups across any weight loss categories.

Table 2. Subgroup analysis of changes in mean HSI between baseline and week 56 by weight loss category.

Treatment group	Weight loss group	Number of patients	HSI baseline	HSI week 56	P-value
Placebo	<5%	76	49.6 ± 5.6	49.5 ± 5.5	0.783
	≥5%	24	51.0 ± 5.9	46.3 ± 5.1	<0.001
	≥10%	8	50.4 ± 4.1	43.3 ± 4.9	<0.001
Treatment	<5%	79	50.2 ± 6.3	49.7 ± 6.5	0.172
	≥5%	93	51.1 ± 5.6	45.9 ± 5.8	<0.001
	≥10%	45	50.7 ± 5.2	43.9 ± 5.3	<0.001

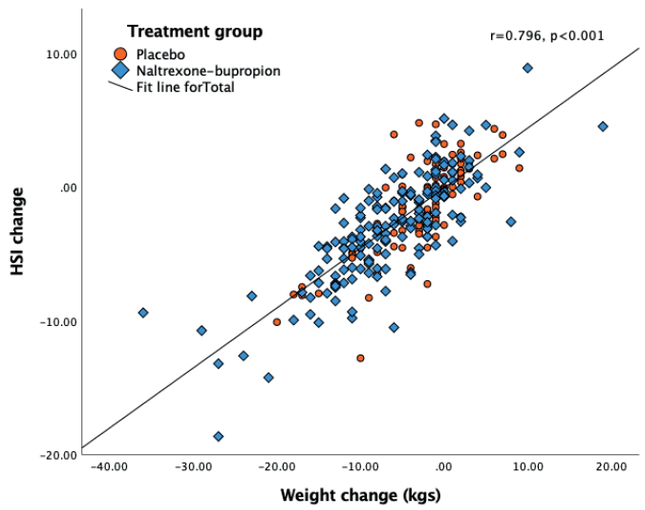
Table 3. Subgroup analysis of changes in mean MAF-5 between baseline and week 56 by weight loss category.

Treatment group	Weight loss group	Number of patients	MAF-5 baseline	MAF-5 week 56	P-value
Placebo	<5%	71	3.0 ± 1.8	2.9 ± 1.7	0.177
	≥5%	22	2.7 ± 1.9	2.0 ± 1.7	0.002
	≥10%	6	1.9 ± 1.7	1.1 ± 1.6	0.112
Treatment	<5%	74	3.2 ± 2.1	3.0 ± 2.2	0.258
	≥5%	84	3.0 ± 1.9	1.7 ± 1.7	<0.001
	≥10%	39	3.0 ± 2.1	1.3 ± 1.6	<0.001

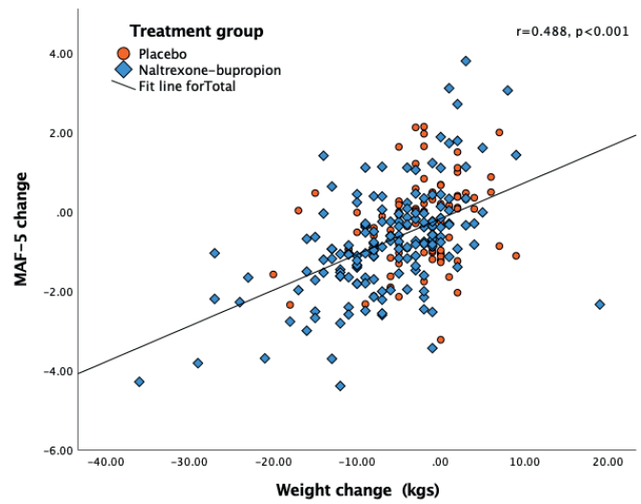
Correlation and regression analyses

Weight change was strongly correlated with reductions in HSI (r=0.796, p<0.001) and moderately with MAF-5 (r=0.488, p<0.001) (Fig. 2A–B). In contrast, no significant correlation was found between weight change and FIB-4 score (r=0.034, p=0.590) (Fig. 2C). Multiple regression analyses confirmed weight change as the strongest independent predictor of changes in HSI (β=0.441, p<0.001), MAF-5 (β =0.096, p<0.001) (Supplementary Tables S5 and S6), ALT (β=0.559, p<0.001), and AST (β=0.202, p=0.011) (Tables 4 and 5). Other variables, including age, sex, and baseline lipid levels, were not significant predictors (all p>0.05).

A.



B.



C.

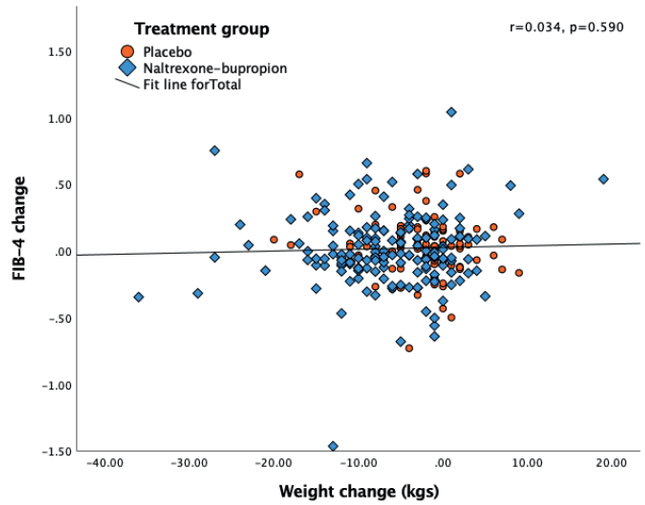


Figure 2 – Correlations between weight change and (A) HSI change, (B) MAF-5 change, and (C) FIB-4 change.

Table 4. Multiple regression analysis of factors associated with changes in ALT.

Variable	B	95% CI	P-value
Constant	0.853	-19.987 – 21.568	0.937
Weight change (kg)	0.559	0.329 – 0.789	<0.001
Age (years)	-0.085	-0.275 – 0.106	0.384
Sex (f/m)	1.376	-2.544 – 5.296	0.490
Triglycerides (mg/dL)	0.004	-0.021 – 0.028	0.771
HDL-C (mg/dL)	0.065	-0.115 – 0.246	0.475
LDL-C (mg/dL)	0.017	-0.032 – 0.066	0.493
Glucose (mg/dL)	0.016	-0.025 – 0.056	0.445
Weight (kg)	-0.042	-0.143 – 0.058	0.407

Note: Dependent variable: Change in ALT
Abbreviations: High-Density Lipoprotein Cholesterol; HDL-C, Low-Density Lipoprotein Cholesterol; LDL-C.

Table 5. Multiple regression analysis of factors associated with changes in AST.

Variable	B	95% CI	P-value
Constant	-0.617	-14.685 – 13.451	0.931
Weight change (kg)	0.202	0.047 – 0.357	0.047
Age (years)	-0.126	-0.255– 0.003	0.055
Sex (f/m)	0.704	-1.944 – 3.353	0.601
Triglycerides (mg/dL)	0.013	-0.003 – 0.030	0.119
HDL-C (mg/dL)	0.070	-0.052 – 0.191	0.261
LDL-C (mg/dL)	0.015	-0.018 – 0.048	0.363
Glucose (mg/dL)	0.014	-0.014 – 0.041	0.329
Weight (kg)	-0.015	-0.083 – 0.053	0.660

Note: Dependent variable: Change in AST
Abbreviations: High-Density Lipoprotein Cholesterol; HDL-C, Low-Density Lipoprotein Cholesterol; LDL-C.

DISCUSSION

This study assessed the effect of NB combined with structured lifestyle counseling on liver health in patients with T2DM and overweight or obesity. The original study demonstrated that NB results in greater weight loss compared to lifestyle modification alone (12). Building upon these findings, our study shows that NB may also improve hepatic steatosis and fibrosis, as measured by HSI and MAF-5. The current study identified weight loss as the primary determinant of improvements in hepatic steatosis and fibrosis, reinforcing its central role in the management of MASLD, aligning with existing literature (6, 9, 10). Improvements in hepatic steatosis were observed primarily in participants who achieved at least 5% weight loss, with greater benefits seen at ≥10%. In contrast, weight loss below 5% was not sufficient to produce meaningful changes in hepatic steatosis in either group. Similar trends were observed for hepatic fibrosis, although the ≥10% weight loss placebo subgroup did not reach statistical significance, likely due to a small sample size. The observed differences are unlikely

to be explained by the use of concomitant antidiabetic medications such as metformin or thiazolidinediones (TZDs), as these were similarly distributed between groups, as reported in the original study (12).

In comparison with existing literature, studies investigating GLP-1 receptor agonists such as semaglutide and liraglutide similarly emphasize the central role of weight loss in improving liver outcomes in MASLD. For instance, a phase 3 trial of semaglutide in patients with biopsy-confirmed MASH and significant fibrosis demonstrated substantial resolution of steatohepatitis and fibrosis alongside an average weight loss exceeding 10% (20). Similarly, a recent study comparing liraglutide to matched lifestyle-induced weight loss in non-diabetic MASLD patients found that comparable weight reductions yielded similar improvements in hepatic steatosis and disease activity, highlighting weight loss as the primary mediator of liver benefit (21). Notably, liraglutide also provided additional metabolic advantages, such as improved glucose regulation, underscoring potential incretin-related effects beyond weight reduction (22).

In contrast, resmetirom, a thyroid hormone receptor-β agonist, exerts its therapeutic effects through direct modulation of hepatic lipid metabolism. While resmetirom is not a weight-loss agent per se, in a phase 2 trial of resmetirom, patients who achieved ≥5% weight loss experienced greater hepatic improvements, suggesting that weight loss amplifies its liver-specific benefits (23). These findings underscore weight loss as a central driver of hepatic improvement across therapies, while also illustrating that agents like resmetirom may exert additional, weight-independent benefits.

To the best of our knowledge, this is among the first analyses reporting beneficial effects of NB on hepatic outcomes in patients with T2DM and overweight or obesity using NITs. A prior pooled RCT reported ALT improvements with NB but did not focus solely on patients with T2DM and used FIB-4 (12), a marker known to have limited accuracy in metabolic populations (24-28). Consistent with evidence suggesting FIB-4's suboptimal performance in patients with T2DM, our study did not show any changes, while the metabolic-specific NIT MAF-5 was more responsive to weight-related hepatic changes, supporting its use in patients with T2DM (16).

Several limitations should be considered. Although HSI and MAF-5 include BMI and waist circumference and may partly reflect weight-related changes, the fact that weight loss was the strongest predictor of ALT and AST changes supports our conclusion of hepatic improvement. Additionally, while validated, these non-invasive markers cannot replace liver biopsy or advanced imaging for definitive diagnosis and staging. Furthermore, our analysis was limited by the absence of data necessary to calculate other established non-invasive steatosis and fibrosis markers, which restricted the scope of comparative assessments in this study. Future research should consider a wider variety of NITs. Moreover, this study is a post-hoc analysis of a trial primarily designed for other endpoints than MASLD-related outcomes. Given this, the findings should be considered hypothesis-generating and warrant

confirmation in prospective studies. Finally, while the NITs used to assess hepatic steatosis and fibrosis were not originally developed for longitudinal follow-up, emerging evidence supports their potential utility in this context (29-31). Future studies with longer follow-up periods and larger, more diverse cohorts are needed to confirm the durability of NB's benefits and to further validate these markers against histological or imaging endpoints. Such studies are essential to establish the role of NB as a potential adjunctive treatment in the metabolic management of MASLD.

CONCLUSION

In conclusion, this study demonstrates that weight loss is the primary factor driving improvements in hepatic steatosis and fibrosis, as assessed by HSI and MAF-5, among patients with T2DM and overweight or obesity. Importantly, treatment with NB resulted in significantly greater weight loss compared to lifestyle intervention alone, which was accompanied by more pronounced reductions in hepatic indices. These findings suggest that NB provides an added benefit by facilitating further weight loss, thereby enhancing liver health beyond lifestyle measures.

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Chapter 5

Metabolic dysfunction-associated steatotic liver disease and liver fibrosis in primary and secondary care in the Netherlands: preliminary results from the GRIPonMASH Consortium

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ABSTRACT

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) and its progressive stages often go unrecognized, particularly in high-risk individuals. We evaluated the prevalence of MASLD and liver fibrosis in Dutch primary and secondary care settings.

Methods: This analysis includes preliminary data from the Dutch cohort of the GRIPonMASH study, a European study screening 10,000 high-risk individuals (with type 2 diabetes (T2DM), obesity, hypertension, or metabolic syndrome (MetS)) using non-invasive tests (NITs), including vibration-controlled transient elastography (VCTE) and the Fibrosis-4 (FIB-4) index. MASLD was defined as a controlled attenuation parameter (CAP) value of ≥275 dB/m or higher. Significant liver fibrosis as a liver stiffness measurement (LSM) of ≥8.0 kPa.

Results: 174 patients (43% female; mean age 58 ± 12 years; mean BMI 31.7 ± 5.6 kg/m²) were included from primary (54%) and secondary care (46%). T2DM was the most frequent inclusion criterion (50%), followed by hypertension (26%), obesity (20%), and MetS (4%). MASLD prevalence was 59% overall, with rates of 58% in T2DM, 66% in obesity, 56% in hypertension, and 57% in MetS groups. Significant liver fibrosis was observed in 17% of patients, with similar prevalence in both care settings. MASLD was associated with higher BMI and weight in both care levels. In primary care, MASLD patients also had lower HDL-C (1.1 vs. 1.3 mmol/L, p=0.004) and higher triglycerides (1.9 vs. 1.3 mmol/L, p=0.003). In secondary care, MASLD was linked to higher HbA1c (65 vs. 53 mmol/mol, p=0.007). BMI (OR=1.14, p=0.008) and triglycerides (OR=2.63, p=0.002) were the strongest predictors of MASLD. FIB-4 scores did not differ significantly between care settings (p=0.47).

Conclusion: This preliminary analysis found a high prevalence of MASLD and fibrosis among cardiometabolic patients in both primary and secondary care. The comparable disease burden across settings underscores the need for awareness and targeted evaluation of high-risk individuals. Early identification using cost-effective NITs in selected patients- particularly those with higher BMI or triglycerides- may enable timely intervention, support lifestyle-based management, and contribute to optimized referral pathways.

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) is increasingly recognized as a major contributor to both liver-related and cardiometabolic morbidity (1-3). About 30% of the global population is affected by MASLD, with even higher rates observed in people with metabolic comorbidities such as type 2 diabetes mellitus (T2DM), obesity, hypertension, and metabolic syndrome (MetS)(4, 5). Among these high-risk groups, MASLD affects up to 55% of those with T2DM and up to 75% of individuals with obesity (6-8).

MASLD can progress to metabolic dysfunction-associated steatohepatitis (MASH), characterized by ballooning and hepatic inflammation, which can lead to fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma (HCC) (9). Approximately one in five patients with MASLD develop MASH, with general population estimates in Europe ranging between 6% and 7%. Among people with T2DM or obesity, the estimated prevalence of MASH may be as high as 65% and 30%, respectively (10). The severity of MASH largely depends on the extent of liver fibrosis, which is a known predictor of liver related complications and all-cause mortality (11, 12).

Despite its high burden, MASLD remains underdiagnosed in routine clinical settings due to its asymptomatic nature during early disease stages (13). It's usually identified in its advanced stages, when complications have developed. However, the disease is reversible in its early phases, highlighting the importance of early detection.

While liver biopsy remains the gold standard for MASLD diagnosing and staging, non-invasive tests (NITs), such as serological markers and vibration controlled transient elastography (VCTE), are increasingly used to assess the disease severity and to guide clinical decision-making (14, 15).

Management of MASLD primarily focuses on lifestyle modifications, which remain the cornerstone of treatment. Weight loss through dietary changes and increased physical activity has been shown to reduce liver fat, improve inflammation, and even reverse fibrosis in early stages of the disease (16-18). In more advanced cases, a multidisciplinary approach is often required, including structured lifestyle programs, bariatric surgery, and participation in clinical trials. Pharmacological treatment options are also emerging (19, 20). In March 2024, the U.S. Food and Drug Administration (FDA) approved resmetirom, the first medication for MASLD with fibrosis, offering a new avenue for patients at risk of disease progression (21). Since June 2024, resmetirom has also become available in the Netherlands, marking an important step forward in the clinical management of MASLD.

Since 2016, several leading scientific societies, including EASL, EASD, and EASO, have provided guidance on managing MASLD (22). In 2021, these recommendations were expanded to include noninvasive tests (NITs), which help assess disease severity without the need for liver biopsy. NITs generally fall into two groups: blood-based markers, such as enzyme scores and serum panels, and imaging methods like ultrasound, MRI, or transient elastography (TE) (23).

The most recent update, in June 2024, introduced the MASLD terminology and incorporated the latest evidence on disease management (15). Despite these advances, MASLD remains under-recognized in routine practice, largely because the disease is often silent and clinician awareness is limited.

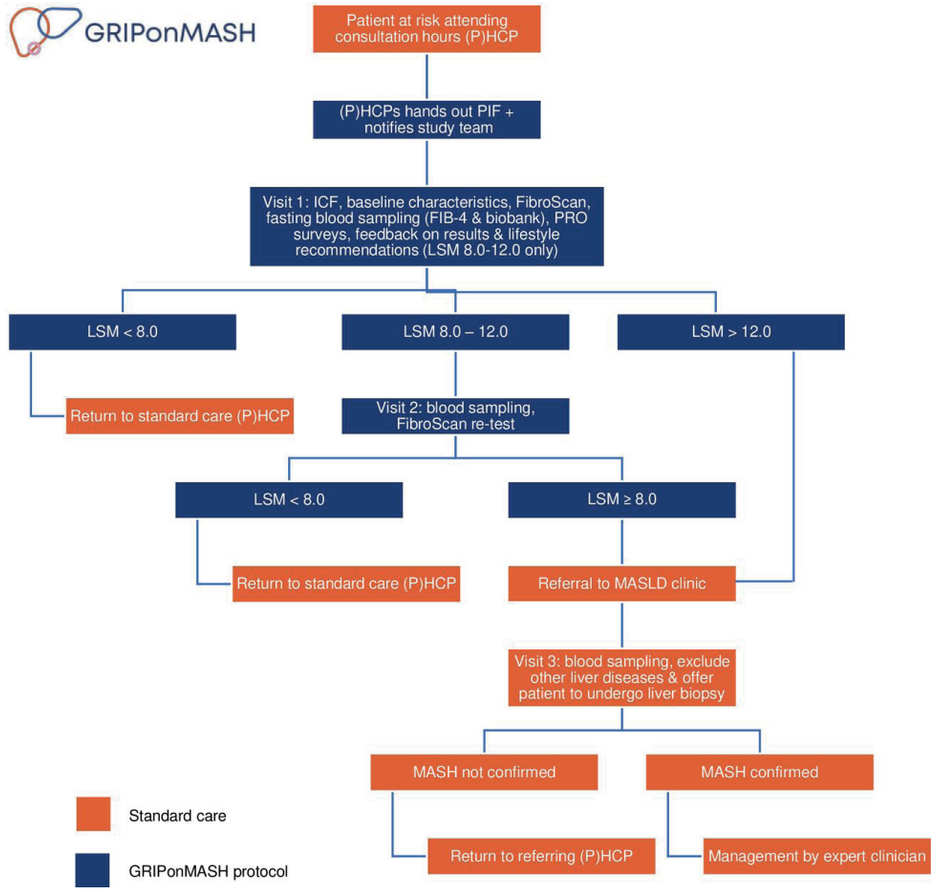
In order to support healthcare professionals in adopting the recommended EASL patient care pathways, the Global Research Initiative for Patient Screening on MASH (GRIPonMASH) study was initiated (24). This international multicenter study screens high-risk individuals identified in primary or secondary care for MASLD and liver fibrosis across 10 European countries, using at least two NITs. In addition to screening, GRIPonMASH seeks to raise awareness and promote the implementation of systematic screening practices among healthcare providers, with the ultimate goal of improving patient outcomes.

In this preliminary analysis, we aimed to determine the prevalence of MASLD and liver fibrosis in Dutch primary and secondary care settings among high-risk populations. Factors associated with MASLD were examined using univariate analyses, with significant variables entered into multivariable regression to assess independent effects.

METHODS AND MATERIALS

Study design

This analysis is based on preliminary data from the GRIPonMASH study, an ongoing multicenter international study aiming to enroll 10.000 patients across 10 European countries (Figure 1). For the current analysis, we included Dutch participants recruited from general practitioner clinics in Rotterdam and Amsterdam, as well as from outpatient clinics at the Franciscus Gasthuis Hospital, Amsterdam University Medical Center, and two affiliated regional hospitals.



Abbreviations: FIB-4, Fibrosis4 index; ICF, informed consent form; LSM, liver stiffness measurement; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction- associated steatotic liver disease; (P)HCP, (primary) healthcare provider; PIF, patient information folder.

Figure 1. Flowchart of study design and patient flow.

Study population

The study included 174 patients between the age of 18 to 75 years. Patients were eligible if they had a current diagnosis of T2DM, obesity (BMI ≥30 kg/m²), arterial hypertension, or MetS, following the definitions listed in Supplementary Table S2. Patients with chronic liver diseases, high alcohol consumption (>3 units/day for men, >2 units/day for women), recent bariatric surgery, or any condition likely to affect liver status or adherence to study procedures were excluded. Full inclusion and exclusion criteria are detailed in Supplementary Table S3.

Data collection

During the baseline visit, anthropometric parameters (weight, blood pressure (BP), waist circumference (WC), and height) were recorded. Hepatic fibrosis was measured via vibration-controlled transient elastography (VCTE; FibroScan®), and hepatic steatosis was quantified using the controlled attenuation parameter (CAP). MASLD was defined as a CAP value ≥275 dB/m, in the absence of other causes of liver disease or excessive alcohol consumption. Fasting blood samples were collected and ALT, AST and platelet count were determined in the local laboratory. The Fibrosis-4 (FIB-4) index is a validated marker of liver fibrosis risk.(25) FIB-4 was calculated using the following formula:

FIB-4 = (Age [years] × AST [U/L]) / (Platelets [10^9/L] × √ALT [U/L]). FIB-4 values were categorized as follows: <1.3 indicating low fibrosis risk, 1.3-<2.67 indicating intermediate fibrosis risk, and ≥2.67 indicating high fibrosis risk. Additional laboratory data were retrieved from medical and general practitioner records. Fibrosis risk was also assessed using liver stiffness measurement (LSM) obtained via FibroScan®. LSM values were classified as follows: <8.0 kPa indicating low fibrosis risk, 8.0-12.0 kPa intermediate fibrosis risk, and ≥12.0 kPa high fibrosis risk.

The subsequent follow-up of each patient was determined based on their LSM result. Patients with LSM <8.0 kPa remained in standard care. Those with LSM between 8.0 and 12.0 kPa received lifestyle advice focused on weight loss, supported by Dutch Heart Foundation brochures, and underwent a Fibroscan re-test after 3 months. Patients with LSM ≥12.0 kPa were referred to a MASLD outpatient clinic for further evaluation, including liver biopsy if clinically indicated. Written informed consent was obtained prior to collecting data, and all procedures were conducted in accordance with Good Clinical Practice and the Declaration of Helsinki (26).

Data analysis

Data are presented as mean ± standard deviation (SD), unless otherwise specified. Comparisons between primary and secondary care settings were performed using independent samples t-tests for continuous variables and chi-square tests for categorical variables. For within-subject comparisons (e.g., baseline vs. follow-up), paired samples t-tests were conducted. Correlation analyses were performed to assess relationships between continuous variables using Pearson's correlation for normally distributed data or Spearman's rank correlation for non-normally distributed data. Univariate logistic regression was used to assess the association between each independent variable and the outcome; variables with p<0.10 were included in the multivariable logistic regression model to avoid excluding potentially important predictors. All statistical analyses were performed using IBM SPSS Statistics version 28.0.0.0 (IBM SPSS Statistics, New York, United States). A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

A total of 174 patients were included (43.4% female and 56.6% male), of whom 54.0% were recruited from primary care and 46.0% from outpatient clinics. The mean age was 58 ± 12 years, and the mean body mass index (BMI) was 31.7 ± 5.6 kg/m². A detailed description of baseline clinical and demographic characteristics, as well as medication use in the total population, is provided in Supplementary Table S1.

When comparing patients by care setting, those in secondary care had significantly higher diastolic blood pressure (89 ± 11 vs. 86 ± 10 mmHg, p=0.043) and HbA1c levels (59 ± 9.4 vs. 53 ± 16 mmol/mol, p=0.021) than those in primary care.

Regarding medication use, insulin was used substantially more often in secondary care (45.0% vs. 8.5%, p<0.001). Use of angiotensin II receptor blockers (ARBs, p=0.026), GLP-1 receptor agonists (p=0.049), fibrates (p=0.043), statins (p=0.033), and ezetimibe (p=0.045) were also significantly more common among secondary care patients (Table 1).

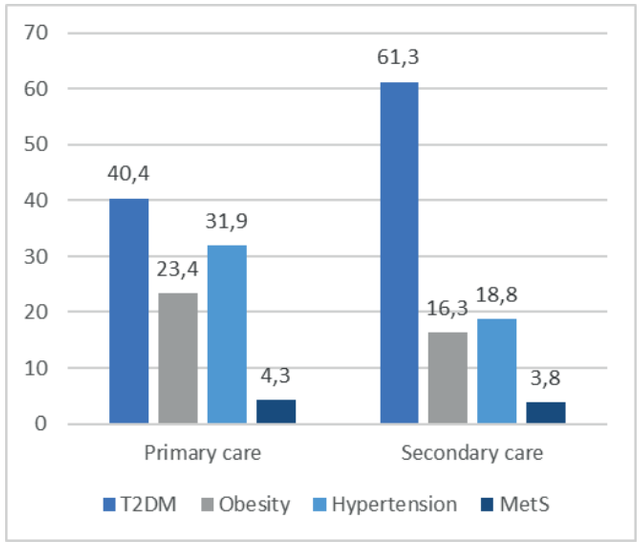
In the overall study population, T2DM was the most prevalent inclusion criterion (50.0%), followed by hypertension (25.9%), obesity (20.1%), and MetS (4.0%). An overview of most common inclusion criteria by care setting is provided in Figure 2.

Table 1. Baseline clinical and demographic characteristics by care setting. Data are expressed as mean ± standard deviation, median (IQR), or n (%), as appropriate.

Characteristics	Primary care (N=94)	Secondary care (N=80)	P-value
Demographic characteristics			
Age (years)	59 ± 12	58 ± 12	0.187
Sex (female/male)	38(40.4)/56(59.6)	37(46.8)/42(53.2)	0.387
Weight (kg)	95 ± 17	94 ± 18	0.700
BMI (kg/m²)	31.4 ± 5.6	32.0 ± 5.7	0.446
Clinical characteristics			
Systolic BP (mmHg)	145 ± 22	142 ± 19	0.436
Diastolic BP (mmHg)	89 ± 11	86 ± 10	0.043
Total cholesterol (mmol/L)	4.7 ± 1.2	4.4 ± 1.3	0.138
LDL-C (mmol/L)	2.7 ± 1.1	2.4 ± 1.1	0.092
HDL-C (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	0.986
Triglycerides (mmol/L)	1.6 (1.1 – 2.5)	1.4 (1.1 – 2.6)	0.842
HbA1c (mmol/mol)	53 ± 9.4	59 ± 16	0.021
Creatinine (mmol/L)	80 ± 19	82 ± 22	0.641
eGFR (mL/min/1.73 m²)	84 (75 – 90)	86 (66 – 90)	0.624
TSH (mIU/L)	1.7 (0.9 – 2.6)	1.1 (0.8 – 2.2)	0.258
ALT (U/L)	35 ± 26	33 ± 33	0.629

Characteristics	Primary care (N=94)	Secondary care (N=80)	P-value
AST (U/L)	28 ± 21	27 ± 18	0.769
Medication use			
ACE inhibitor	15 (16.0)	11 (13.8)	0.832
ARB	19 (20.2)	29 (36.3)	0.026
Loop diuretics	1 (1.1)	1 (1.3)	1.000
Antialdosterine diuretics	0	0	N/A
Thiazide diuretics	9 (9.6)	3 (3.8)	0.148
Other diuretics	2 (2.1)	4 (5.0)	0.415
Antiplatelets	5 (5.3)	6 (7.5)	0.756
Anticoagulants	9 (9.6)	10 (12.5)	0.628
Insulin s.c.	8 (8.5)	36 (45.0)	<0.001
NSAID	3 (3.2)	3 (3.8)	1.000
PPI	25 (26.6)	29 (36.3)	0.191
GLP1 analogue	1 (1.1)	6 (7.5)	0.049
SGLT2 inhibitor	8 (8.5)	12 (15.0)	0.234
Pioglitazone	0	0	N/A
Fibrates	0	4 (5.0)	0.043
Statins	39 (41.5)	47 (58.8)	0.033
Ezetimibe	8 (8.5)	16 (20.0)	0.045
PCSK9 inhibitor	0	3 (3.8)	0.095
Metformin	35 (37.2)	33 (41.3)	0.641
SU derivative	6 (6.4)	11 (13.8)	0.127
Calcium antagonist	15 (16.0)	20 (25.0)	0.184
Betablockers	23 (24.5)	17 (21.3)	0.718
Other medication	42 (44.7)	50 (62.5)	0.023
No medication	12 (12.8)	6 (7.5)	0.322

Abbreviations: Systolic BP, systolic blood pressure; diastolic BP, diastolic blood pressure; LDL-c, low density cholesterol; HDL-c, high density cholesterol; HbA1c, Hemoglobin A1c; eGFR, estimated Glomerular Filtration Rate; TSH, Thyroid-Stimulating Hormone; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ACE inhibitor, Angiotensin-Converting Enzyme inhibitor; ARB, Angiotensin II Receptor Blocker; Insulin s.c., Insulin subcutaneous; NSAID, Non-Steroidal Anti-Inflammatory Drug; PPI, Proton Pump Inhibitor; GLP1, Glucagon-Like Peptide-1; SGLT2, Sodium-Glucose Cotransporter 2; PCSK9, Proprotein Convertase Subtilisin/Kexin Type 9; SU derivative, Sulfonylurea derivative.



Abbreviations: T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome.

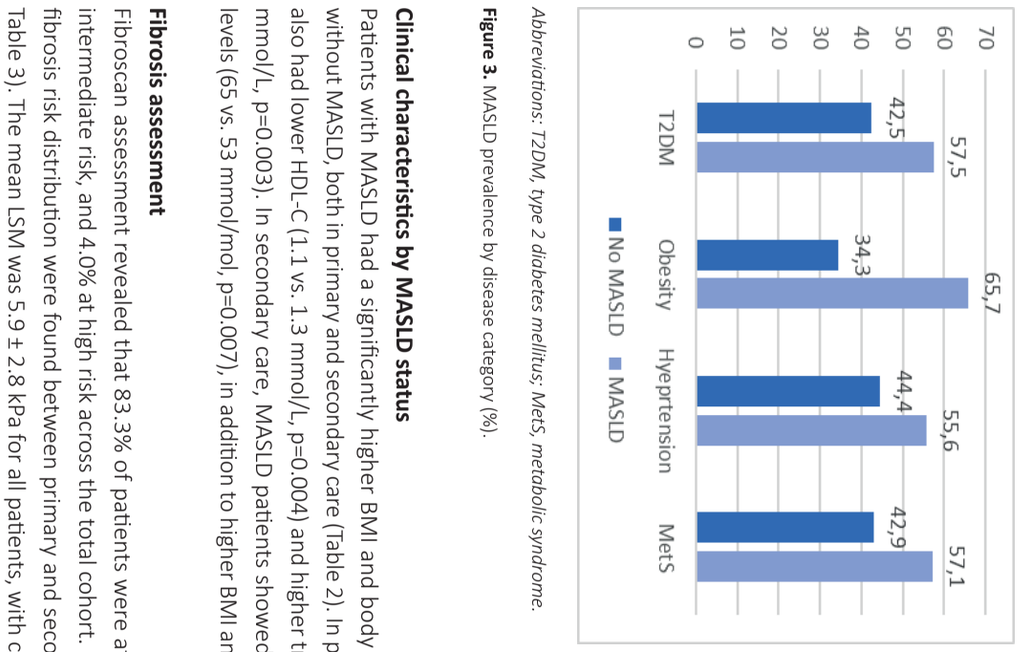
Figure 2. Inclusion criteria by care setting (%).

MASLD prevalence

The prevalence of MASLD in the overall study cohort was 58.6%. When stratified by healthcare setting, prevalence was 61.7% among patients recruited from primary care and 55.0% among those from secondary care, with no statistically significant difference between the two settings (p=0.37). Complementing these findings, the mean CAP value for the total cohort was 288 ± 54 dB/m, with no significant difference between primary (292 ± 53 dB/m) and secondary care (282 ± 56 dB/m) (p=0.12).

Stratification by primary diagnosis revealed a high and relatively uniform MASLD burden across the cardiometabolic subgroups: 57.5% in patients with T2DM, 65.7% in those with obesity, 55.6% in patients with hypertension, and 57.1% in those with MetS (Figure 3). Although MASLD prevalence was numerically highest in the obesity group, differences across diagnostic categories were not statistically significant (p=0.81).

No significant sex differences in MASLD prevalence were observed, either in the overall study population (p=0.68) or within any of the primary diagnostic subgroups (all p>0.05).



Abbreviations: T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome.

Figure 3. MASLD prevalence by disease category (%).

Clinical characteristics by MASLD status

Patients with MASLD had a significantly higher BMI and body weight compared to those without MASLD, both in primary and secondary care (Table 2). In primary care, MASLD patients also had lower HDL-C (1.1 vs. 1.3 mmol/L, p=0.004) and higher triglyceride levels (1.9 vs. 1.3 mmol/L, p=0.003). In secondary care, MASLD patients showed significantly higher HbA1c levels (65 vs. 53 mmol/mol, p=0.007), in addition to higher BMI and weight (p=0.004 for both).

Fibrosis assessment

Fibroscan assessment revealed that 83.3% of patients were at low fibrosis risk, 12.6% at intermediate risk, and 4.0% at high risk across the total cohort. No significant differences in fibrosis risk distribution were found between primary and secondary care groups (p=0.27; Table 3). The mean LSM was 5.9 ± 2.8 kPa for all patients, with comparable values observed in primary (6.0 ± 2.6 kPa) and secondary care (5.8 ± 3.0 kPa; p=0.65).

Table 2. Baseline clinical and demographic characteristics by care setting and MASLD status. Data are expressed as mean ± standard deviation, median (IQR), or n (%), as appropriate.

Characteristics	Primary care (N=94)			Secondary care (N=80)		
	No MASLD	MASLD	P-value	No MASLD	MASLD	P-value
Demographic characteristics						
Age (years)	62 ± 12	57 ± 11	0.073	57 ± 10	57 ± 13	0.978
Sex (female/male)	14(38.9)/22(61.1)	24(41.4)/34(58.6)	0.832	18(50)/18(50)	19(43.2)/25(56.8)	0.578
Weight (kg)	87 ± 16	100 ± 17	<0.001	87 ± 16	99 ± 19	0.004
BMI (kg/m^2)	28.5 ± 4.7	33.2 ± 5.3	<0.001	30.0 ± 4.9	33.7 ± 5.8	0.004
Clinical characteristics						
Systolic BP (mmHg)	142 ± 20	147 ± 23	0.340	141 ± 19	144 ± 19	0.467
Diastolic BP (mmHg)	89 ± 11	90 ± 11	0.394	86 ± 10	85 ± 10	0.834
Total cholesterol (mmol/L)	4.4 ± 0.9	4.9 ± 1.3	0.207	4.1 ± 1.1	4.6 ± 1.4	0.120
LDL-C (mmol/L)	2.5 ± 0.8	2.9 ± 1.2	0.270	2.3 ± 1.0	2.4 ± 1.2	0.622
HDL-C (mmol/L)	1.3 ± 0.3	1.1 ± 0.2	0.004	1.2 ± 0.2	1.1 ± 0.4	0.454
Triglycerides (mmol/L)	1.3 (0.9 – 1.8)	1.9 (1.2 – 2.7)	0.003	1.3 (1.0 – 1.7)	1.9 (1.1 – 3.9)	0.075
HbA1c (mmol/mol)	54 ± 10	53 ± 9.0	0.526	53 ± 14	65 ± 16	0.007
Creatinine (mmol/L)	82 ± 22	79 ± 18	0.533	82 ± 18	82 ± 26	0.981
eGFR (mL/min/1.73 m^2)	82 (72 – 89)	89 (75 – 90)	0.199	86 (65 – 90)	89 (71 – 90)	0.982
TSH (mIU/L)	1.7 (0.9 – 2.4)	1.7 (0.9 – 2.9)	0.678	1.3 (0.6 – 2.3)	1.0 (0.8 – 1.9)	0.953
ALT (U/L)	32 ± 34	36 ± 19	0.500	32 ± 38	33 ± 30	0.933
AST (U/L)	29 ± 21	27 ± 21	0.668	26 ± 9.0	27 ± 22	0.726

Abbreviations: Systolic BP, systolic blood pressure; diastolic BP, diastolic blood pressure; LDL-c, low density cholesterol; HDL-c, high density cholesterol; HbA1c, Hemoglobin A1c; eGFR, estimated Glomerular Filtration Rate; TSH, Thyroid-Stimulating Hormone; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase.

Table 3. Fibrosis risk stratification by Fibroscan in primary and secondary care.

Fibrosis risk	Primary care (N=94)	Secondary care (N=80)
Low risk (<8.0 kPa)	78 (83%)	67 (83.8%)
Intermediate risk (8.0- 12.0 kPa)	14 (14.9%)	8 (10%)
High risk (≥ 12.0 kPa)	2 (2.1%)	5 (6.3%)

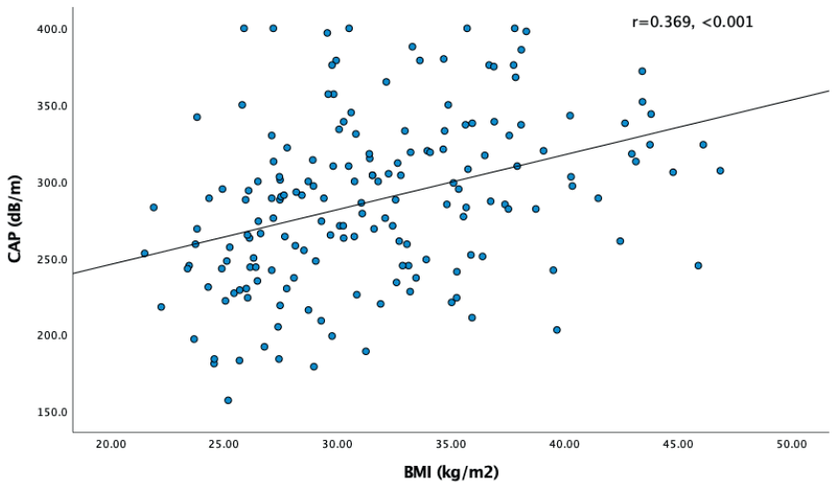
Follow-up Fibroscan results

In a subset of 23 patients who underwent Fibroscan retesting, no statistically significant difference was observed in CAP values between baseline and follow-up (mean difference: -2.83 dB/m ± 43.4, p=0.758). Conversely, LSM values demonstrated a significant reduction at follow-up compared to baseline (mean difference: 1.75 kPa ± 2.53, p=0.003). Concurrently, modest but statistically significant decreases were observed in anthropometric parameters, including BMI (mean difference: 0.55 kg/m² ± 1.2, p=0.031), body weight (1.67 kg ± 3.7, p=0.043), and WC (3.0 cm ± 6.9, p=0.050). No significant changes were detected in systolic or diastolic blood pressure (all p>0.05).

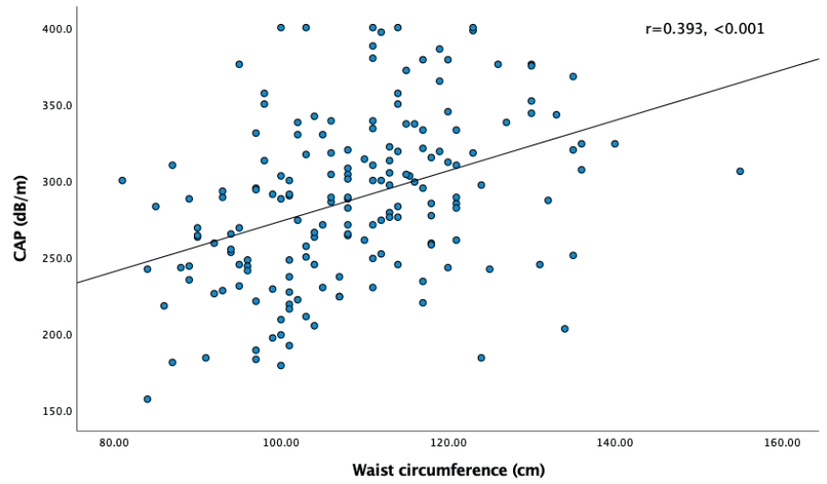
Correlation analyses

Significant positive correlations were observed between hepatic steatosis, assessed by CAP, and key anthropometric measures including BMI (r=0.37, p<0.001), body weight (r=0.36, p<0.001), and waist circumference (r=0.39, p<0.001) (Figure 4). LSM was also positively correlated with CAP (r=0.39, p<0.001) and systolic blood pressure (r=0.18, p=0.018). No significant correlations were observed between LSM and FIB-4 (r=-0.003, p=0.971) or diastolic blood pressure (r=0.10, p=0.211).

A.



B.



C.

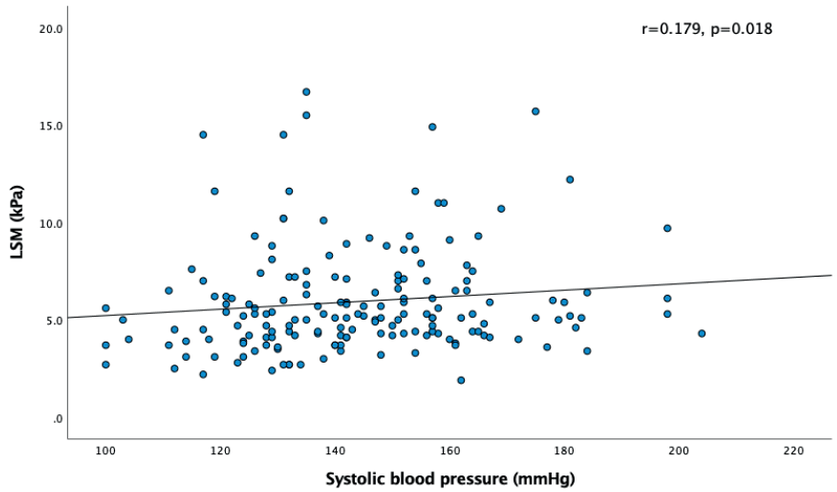


Figure 4. Correlation analyses between CAP and BMI (A), CAP and waist circumference (B), and LSM and systolic blood pressure (C).

FIB-4 analyses

FIB-4 data were available for 150 patients. The mean FIB-4 score in the total population was 1.25 ± 0.49, with similar values between primary (1.25 ± 0.53) and secondary care (1.26 ± 0.44) groups (p=0.47). Comparison of FIB-4 scores between patients with and without MASLD showed no significant differences in the total population (1.3 ± 0.4 vs. 1.2 ± 0.5, p=0.21) or in subgroups with T2DM, obesity, and MetS. However, among patients with hypertension, those with MASLD surprisingly had a lower mean FIB-4 score compared to those without MASLD (1.1 ± 0.5 vs. 1.4 ± 0.5, p=0.04) (Supplementary Table S4).

Logistic regression analyses

Univariate logistic regression analyses showed that BMI, weight, total cholesterol, HDL cholesterol, triglycerides, and sex were associated with MASLD at a significance level of p<0.10 (Supplementary Table S5). To identify independent predictors, these variables were entered into a backward stepwise multivariate logistic regression model. After stepwise removal of non-significant variables, BMI (OR=1.14, 95% CI 1.03–1.25, p=0.008) and triglycerides (OR=2.63, 95% CI 1.43–4.86, p=0.002) remained significant independent predictors of MASLD (Supplementary Table S6). Other variables, including weight, total cholesterol, HDL cholesterol, and sex, did not contribute significantly and were excluded from the final model.

DISCUSSION

This study reveals a high prevalence of MASLD and liver fibrosis among patients with cardiometabolic risk factor across both primary and secondary care settings. The comparable prevalence between care levels underscores MASLD as a widespread and under-recognized condition, warranting systematic liver assessment in high-risk populations regardless of healthcare setting. MASLD was consistently associated with elevated BMI and triglycerides, but metabolic characteristics varied by care level. Patients in primary care exhibited a more atherogenic lipid profile, characterized by lower HDL-C and higher triglycerides, whereas those in secondary care showed poorer glycemic control. These differences likely reflect variations in patient populations, as secondary care predominantly manages patients with established diabetes. However, we cannot rule out that some of these differences may be influenced by fasting status during blood sampling, since blood samples in secondary care are more often collected after fasting, which can affect glucose and lipid levels. Further research is needed to better understand and confirm these differences. In addition, our preliminary Fibroscan follow-up data suggest that weight loss can significantly improve liver stiffness, highlighting its central role in MASLD management.

In line with global modelling data by Estes et al., which projects a rising trend in MASLD particularly in Western countries, our study shows a high burden of MASLD in our cardiometabolic population (27). This trend is mainly driven by the increased prevalence of obesity, T2DM and MetS, key morbidities included in our cohort. The MASLD prevalence in our T2DM subgroup (57.5%) and among patients with obesity (65.7%) corresponds closely with previously reported ranges (7, 28-30). Given this substantial disease burden among high-risk individuals, effective identification strategies are essential.

Notably, we found that MASLD prevalence and fibrosis risk were comparable between patients managed in primary and secondary care. This finding challenges the assumption that MASLD is predominantly managed within primary care and referred to hepatology only in cases of advanced disease (31, 32). Instead, our findings suggest that high-risk individuals in general practice may carry a disease burden equivalent to those already under specialist care, indicating a potential gap in early recognition and structured screening pathways in primary care.

This is supported by Theodoreson et al., who emphasize that despite primary care's strategic position for early MASLD detection, its effectiveness is often limited by the absence of standardized care pathways and the underuse of NITs such as FIB-4 and VCTE (33). Together, these observations underscore the urgent need for systematic MASLD identification strategies to enable timely intervention and prevent progression to advanced liver disease.

Previous studies have shown that structured fibrosis risk stratification in primary care is cost-effective, reducing unnecessary specialist referrals and invasive procedures while accurately identifying patients at risk for advanced fibrosis (34, 35). For example, a recent UK cost-utility analysis of sequential NIT screening strategies (FIB-4 followed by ELF or TE) versus standard care in primary care MASLD patients found these approaches reduced false positives and unnecessary referrals by up to 40%, while lowering costs and improving healthcare efficiency.[4] These findings support implementing structured NIT pathways for earlier, more accurate MASLD detection and cost-effective management.

Our study also demonstrated that lifestyle-driven weight loss can significantly improve liver health, aligning with previous research showing lifestyle interventions reduce fibrosis and liver disease severity. For example, a year-long lifestyle program in patients with morbid obesity lowered fibrosis prevalence from approximately 12% to 0%, particularly in those who lost over 10% of their body weight (36). Similarly, Vilar-Gomez et al. reported that patients with MASH who lost ≥10% of their weight experienced significant fibrosis regression and resolution of liver inflammation (37). Together, these findings reinforce the crucial role of weight loss in improving MASLD outcomes.

Finally, an exploratory finding in our cohort revealed an unexpectedly lower FIB-4 score among hypertensive MASLD patients relative to hypertensive non-MASLD patients. This counterintuitive result is likely influenced by sample size constraints and should be further examined in the larger GRIPonMASH cohort.

This study has several strengths, including a well-characterized cohort drawn from both primary and secondary care, and the use of guideline-recommended Fibroscan technology in real-world clinical settings. In addition, preliminary follow-up data suggest that liver fibrosis may be reversible with weight loss, underscoring the importance of lifestyle interventions. Nevertheless, several limitations should be acknowledged. First, given the preliminary nature of the analyses, the overall sample size was relatively small. Consequently, subgroup sizes were also limited, and liver enzyme assessments were only available at baseline, preventing evaluation of changes over time and identification of patients at higher risk of MASLD progression. Second, although follow-up Fibroscan assessments showed decreased liver stiffness, the findings are based on a limited patient subset. Third, liver disease was not confirmed by advanced imaging or liver biopsy, which remains the gold standard. Finally, voluntary participation may have introduced selection bias, limiting the generalizability of our findings to the broader high-risk population.

Our findings demonstrate a high prevalence of MASLD and fibrosis among patients with cardiometabolic risk factors across both primary and secondary care settings, highlighting the importance of awareness of this condition among healthcare providers. Higher BMI and elevated triglycerides were identified as independent predictors of MASLD, suggesting these factors may help identify individuals at greater risk. These results underscore the need for increased clinical awareness and targeted assessment in high-risk populations, enabling timely intervention. Furthermore, the observed reductions in liver stiffness with modest weight loss emphasize lifestyle modification as a key component of effective MASLD management.

Building on these insights, the ongoing GRIPonMASH study, which aims to enroll 10,000 European patients, will further investigate MASLD and fibrosis in diverse cardiometabolic populations. This extensive cohort will allow for a more detailed understanding of risk factors, disease progression, and opportunities for targeted assessment, supporting more informed clinical awareness and interventions.

CONCLUSION

This preliminary analysis demonstrates a high prevalence of MASLD and significant fibrosis among high-risk patients in both primary and secondary care settings in the Netherlands. The comparable disease burden across these settings highlights that MASLD is not confined to specialist care and underscores the importance of awareness and targeted evaluation of high-risk individuals. Early identification using NITs, such as Fibroscan, in selected patients -particularly those with higher BMI or triglyceride levels- can facilitate timely intervention, while education and management of metabolic risk factors and lifestyle remain central to preventing disease progression. Such an approach may optimize outcomes, improve liver health, and ensure efficient use of healthcare resources.

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SUPPLEMENTARY DATA

Supplementary Table S1. Baseline clinical and demographic characteristics. Data are expressed as mean ± standard deviation, median (IQR), or n (%), as appropriate.

Characteristics	Total group (N=174)
Demographic characteristics	
Age (years)	58 ± 12
Sex (female/male)	75(43.4)/98(56.6)
Weight (kg)	94 ± 18
BMI (kg/m^2)	31.7 ± 5.6
Clinical characteristics	
Systolic blood pressure (mmHg)	144 ± 20.7
Diastolic blood pressures (mmHg)	87 ± 11
Total cholesterol (mmol/L)	4.5 ± 1.3
LDL-C (mmol/L)	2.5 ± 1.1
HDL-C (mmol/L)	1.1 ± 0.30
Triglycerides (mmol/L)	1.4 (1.1 – 2.4)
HbA1c (mmol/mol)	56.4 ± 13.4
Creatinine (mmol/L)	81.1 ± 20.8
eGFR (mL/min/1.73 m²)	85 (72 – 90)
TSH (mIU/L)	1.4 (0.81 – 2.3)
ALT (U/L)	34 ± 29
AST (U/L)	27 ± 19
Medication use	
ACE inhibitor	26 (14.9)
ARB	48 (27.6)
Loop diuretics	2 (1.1)
Antialdosterine diuretics	0
Thiazide diuretics	12 (6.9)
Other diuretics	6 (3.4)
Antiplatelets	19 (10.9)
Anticoagulants	11 (6.3)
Insulin s.c.	44 (25.3)
NSAID	6 (3.4)
PPI	54 (31.0)
GLP1 analogue	7 (4.0)
SGLT2 inhibitor	20 (11.5)
Pioglitazone	0
Fibrates	4 (2.3)
Statins	86 (49.4)
Ezetimibe	24 (13.8)
PCSK9 inhibitor	3 (1.7)
Metformin	68 (39.1)
SU derivative	17 (9.8)
Calcium antagonist	35 (20.1)

Characteristics	Total group (N=174)
Betablockers	40 (23.0)
Other medication	92 (52.9)
No medication	18 (10.3)

Abbreviations: LDL-C, low density cholesterol; HDL-C, high density cholesterol; HbA1c, Hemoglobin A1c; eGFR, estimated Glomerular Filtration Rate; TSH, Thyroid-Stimulating Hormone; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ACE inhibitor, Angiotensin-Converting Enzyme inhibitor; ARB, Angiotensin II Receptor Blocker; Insulin s.c., Insulin subcutaneous; NSAID, Non-Steroidal Anti-Inflammatory Drug; PPI, Proton Pump Inhibitor; GLP1, Glucagon-Like Peptide-1; SGLT2, Sodium-Glucose Cotransporter 2; PCSK9, Proprotein Convertase Subtilisin/Kexin Type 9; SU derivative, Sulfonylurea derivative.

Supplementary Table S2. Criteria for diagnosis of type 2 diabetes mellitus, obesity, arterial hypertension and metabolic syndrome.

Condition	Criteria
Type 2 diabetes mellitus	At least 2 times a fasting glucose > 7,0 mmol/L
	Or elevated non-fasting glucose >11,1 mmol/L 2 hours after OGTT
	Or HbA1c ≥48 mmol/mol (≥6.5%)
	Or being actively treated for previously diagnosed type 2 diabetes by an HCP
Obesity	Body mass index (BMI) > 30
	Or waist circumferences: Caucasian: male ≥ 94 cm, female ≥ 80 cm South-Asian/Chinese: male ≥90 cm, female ≥80 cm Japanese: male ≥85 cm, female ≥90 cm
Arterial hypertension	Systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg
	Or being actively treated for previously diagnosed arterial hypertension by an HCP
Metabolic syndrome	Central obesity defined as waist circumference (see above)

Supplementary Table S3. Inclusion and exclusion criteria

Inclusion criteria

- The subject must be willing to provide written informed consent before any study-specific procedures are performed;
- The subject must be aged 18-75 years old;
- The subject must have a current diagnosis of at least one of the following four conditions: type 2 diabetes mellitus, obesity, arterial hypertension, or metabolic syndrome (following the definitions given in Supplementary Table S2).

Exclusion criteria

- The patient is known with hepatitis B, C or HIV or any other liver condition (like hemochromatosis, sarcoidosis, Wilson's disease etc.);
- The patient is known with any other condition that may lead to liver fibrosis or cirrhosis;
- The patient engages in excessive alcohol use (defined as: >3 units/day in males [30 grams/day] and >2 units/day in females [20 grams/day]);
- The patient has a history or evidence of any other clinically significant condition or planned or expected procedure that in the opinion of the Investigator, may compromise the patient's safety or ability to be included in this study;

- The patient is an employee or contractor of the facility that is conducting the study or is a family member of the Investigator, sub-Investigator, or any Sponsor personnel;
- The patient is not able to understand the details of the protocol and/or is not able to provide written informed consent;
- The patient is pregnant or breastfeeding;
- The patient underwent bariatric surgery in the last 12 months.

Supplementary Table S4. Comparison of FIB-4 scores between patients with and without MASLD in the total population and across clinical subgroups

	No MASLD (N=64)	MASLD (N=86)	P-value
Total (N=150)	1.3 ± 0.4	1.2 ± 0.5	0.206
T2DM (N=76)	1.3 ± 0.4	1.4 ± 0.5	0.314
Obesity (N=29)	1.3 ± 0.5	1.0 ± 0.5	0.073
Hypertension (N=40)	1.4 ± 0.5	1.1 ± 0.5	0.044
MetS (N=5)	1.2 ± 0.5	1.1 ± 0.4	0.642

Abbreviations: T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome.

Supplementary Table S5. Univariate logistic regression analyses of factors associated with metabolic dysfunction-associated steatotic liver disease.

Variable	B	Odds Ratio (Exp(B))	95% CI for OR	P-value
BMI (kg/m^2)	0.159	1.17	(1.09 – 1.25)	<0.001
Weight (kg)	0.045	1.05	(1.02 – 1.07)	<0.001
Age (years)	-0.016	0.98	(0.95 – 1.01)	0.237
Sex (male/female)	0.128	1.14	(0.63 – 2.05)	0.675
Primary diagnosis	-0.016	0.99	(0.72 – 1.35)	0.924
Systolic BP (mmHg)	0.010	1.01	(0.99 – 1.03)	0.210
Diastolic BP (mmHg)	0.009	1.01	(0.97 – 1.04)	0.525
ALT (U/L)	0.003	1.00	(0.99 – 1.01)	0.608
AST (U/L)	-0.001	1.00	(0.98 – 1.02)	0.918
Total cholesterol (mmol/L)	0.368	1.45	(1.04 – 2.01)	0.030
HDL (mmol/L)	-1.745	0.18	(0.04 – 0.73)	0.016
LDL (mmol/L)	0.267	1.31	(0.89 – 1.92)	0.169
Triglycerides (mmol/L)	0.955	2.60	(1.47 – 4.60)	0.001

Abbreviations: Systolic BP, systolic blood pressure; diastolic BP, diastolic blood pressure; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; HDL-c, high density cholesterol; LDL-c, low density cholesterol.

Supplementary Table S6. Final model of backward stepwise multivariate logistic regression for predictors of metabolic dysfunction-associated steatotic liver disease.

Variable	Odds ratio	95% CI for OR	P-value
BMI (kg/m^2)	1.135	1.033 – 1.247	0.008
Triglycerides (mmol/L)	2.631	1.425 – 4.858	0.002
Constant	-	-	0.001

Chapter 6

LAP-defined steatosis and subclinical vascular disease in rheumatoid arthritis: a post-hoc analysis of the FRANCIS trial

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease associated with metabolic dysfunction-associated steatotic liver disease (MASLD), which may contribute to cardiovascular (CV) risk. This study aimed to examine the association between hepatic steatosis, measured by a non-invasive test, and CV risk markers in RA patients.

Methods: This post-hoc analysis used cross-sectional and longitudinal data from the Franciscus Rheumatoid Arthritis and Cardiovascular Intervention Study (FRANCIS), an open-label randomized controlled trial of intensive cardiovascular risk management in patients with rheumatoid arthritis without cardiovascular disease (CVD) or type 2 diabetes. Hepatic steatosis was assessed using the Lipid Accumulation Product (LAP) with sex-specific thresholds for rule-in and rule-out of severe steatosis. Associations with carotid intima media thickness (cIMT) were evaluated using correlation analyses and independent-samples t-tests. Independent relationships were further assessed using multivariable linear regression adjusting for age, sex, BMI, and systolic blood pressure. Longitudinal changes in cIMT over 3 years were analyzed according to LAP-defined steatosis and treatment strategy.

Results: Among 236 RA patients (66% female; mean age 54 ± 10 years), those with LAP-defined steatosis had a more adverse cardiometabolic profile and a trend toward higher baseline cIMT (0.61 ± 0.10 vs 0.57 ± 0.11 mm; p=0.072). In the crude regression, lnLAP showed a trend toward an association with cIMT (p=0.072), which was no longer observed after adjustment for age, sex, BMI, and systolic blood pressure (p=0.365). Over 3 years, intensive treatment reduced cIMT progression compared with standard treatment in patients without LAP-defined steatosis (ΔcIMT 0.03 ± 0.07 vs 0.06 ± 0.12 mm; p=0.013), whereas no treatment effect was observed in patients with LAP-defined steatosis (p=0.696). Intensive treatment also produced greater improvements in lipid parameters, including reductions in total cholesterol and apoB in both groups, and increased apoAI mainly in participants with LAP-defined steatosis, while changes in liver enzymes, CRP, and glycemic markers were minimal.

Conclusion: In this exploratory post-hoc analysis, LAP-defined hepatic steatosis identified RA patients with a more adverse cardiometabolic profile and trends toward greater subclinical vascular burden. Intensive CV risk management was associated with lower cIMT progression in patients without LAP-defined steatosis. These findings suggest that RA patients with hepatic steatosis may require alternative or more intensive strategies to reduce CV risk.

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD) (1-3), affects approximately 30% of the general population and is characterized by excessive hepatic fat accumulation in the presence of at least one cardiometabolic risk factor, in the absence of secondary causes of hepatic steatosis, or excessive alcohol intake (4, 5). While early-stage steatosis is reversible, progression to liver fibrosis is a key determinant of MASLD prognosis and is associated with increased morbidity and mortality (6-9).

MASLD is also common in patients with rheumatoid arthritis (RA), a chronic autoimmune disease with elevated cardiovascular (CV) risk (1). Approximately one-third of RA patients are affected by MASLD (10, 11), which can range from simple steatosis to more severe forms such as metabolic dysfunction-associated steatohepatitis (MASH), potentially progressing to cirrhosis or hepatocellular carcinoma (HCC) (12). Fibrosis, in particular, is an important CV risk factor, both in RA and non-RA populations, as demonstrated in our previous work (13).

RA is associated with increased cardiovascular disease (CVD) risk, driven by systemic inflammation and often undertreated traditional CV risk factors (6–9). Patients with RA have a 1.5-fold higher risk of CVD and increased CVD-related morbidity and mortality compared with the general population (6, 8, 9, 14, 15). Subclinical vascular damage is also evident, as carotid intima-media thickness (cIMT), a marker of atherosclerosis, is frequently elevated in RA patients (13, 16, 17).

Despite the close interconnection between MASLD and CVD, and the central role of inflammation in both conditions, the specific contribution of hepatic steatosis to CV risk in RA remains unclear.

While liver biopsy remains the gold standard for assessing hepatic fat content and fibrosis, its invasive nature, potential complications, and high cost limit its routine use (18). Non-invasive tests (NITs), such as the lipid accumulation product (LAP) and the Fibrosis-4 index (FIB-4), provide validated measures of hepatic steatosis and fibrosis without the need for imaging or biopsy and are recommended by the European Association for the Study of the Liver (EASL) guidelines (4, 10, 11).

The present study therefore investigates the association between hepatic steatosis, assessed using LAP, and CV risk markers, including cIMT, in patients with RA, and evaluates whether longitudinal changes in cIMT differ according to treatment strategy and LAP-defined steatosis categories.

METHODS AND MATERIALS

Study design

This is a post-hoc analysis of the Franciscus Rheumatoid Arthritis and Cardiovascular Intervention Study (FRANCIS), an open-label randomized controlled trial designed to assess the impact of intensive CV risk management in patients with RA (9, 19, 20). Patients were randomized to receive either standard treatment or an intensive treat-to-target treatment regarding CV risk factors, as previously described (9). In short, patients within the treat-to-target group were treated according to a pre-specified protocol for anti-hypertensive treatment, lipid lowering drugs, metformin, and life style advice including diet and smoking cessation. Patients with the standard treatment were only evaluated and their general physician received written advice based on results of the visits, however, initiation of treatment was based on their own judgement and CVD risk guidelines. The study protocol was approved by the Medical Ethical Committee of Maasstad Hospital, Rotterdam, and conducted in accordance with the Declaration of Helsinki (21). The trial was registered with The Dutch Trial Register (NTR3873; ABR no. NL32669.101.10).

Study population

The study cohort included 236 RA patients attending the outpatient clinic of the Department of Rheumatology at Franciscus Gasthuis Hospital, Rotterdam, the Netherlands. Eligible participants were younger than 70 years and had a confirmed diagnosis of RA according to the American College of Rheumatology criteria (22). Patients with type 1 or type 2 diabetes mellitus at baseline- defined by fasting glucose >7.0 mmol/L, HbA1c >48 mmol/mol, or ongoing treatment for diabetes- were excluded. Individuals with a history of CVD, defined as prior myocardial infarction, cerebrovascular events, intermittent claudication, peripheral artery disease requiring amputation, coronary artery bypass grafting, or percutaneous coronary interventions, were also excluded. Additional exclusion criteria comprised chronic kidney disease, defined as an estimated glomerular filtration rate (eGFR) below 40 mL/min/1.73 m².

Data collection

Baseline assessments were performed during outpatient visits between 2009 and 2012. Blood samples were collected following an overnight fast, and standard anthropometric measurements, including weight, height, and blood pressure, were recorded. Medication use was documented for disease modifying anti-rheumatic drugs (DMARDs) (including methotrexate, hydroxychloroquine, and leflunomide), biologicals (including rituximab and anti-TNF agents), steroids, non-steroidal anti-inflammatory drugs (NSAIDs), statins, and antihypertensives, RA disease activity was assessed using the 28-joint Disease Activity Score (DAS28) (23). Longitudinal analyses of cIMT progression included patients with complete baseline and 3-year follow-up data for LAP and cIMT.

Lipid Accumulation Product (LAP)

The natural logarithm of LAP (lnLAP) was computed using sex-specific formulas based on waist circumference (WC, cm) and fasting triglycerides (TGs, mmol/L):

$$\mathrm{LAPmen} = (\mathrm{WC} - 65) \times \mathrm{TG}$$

$$\mathrm{LAPwomen} = (\mathrm{WC} - 58) \times \mathrm{TG}$$

Severe steatosis was defined using sex-specific thresholds of lnLAP > 4.0 for men and lnLAP > 4.4 for women, which were used to categorize patients as 'rule-in' or 'rule-out' for hepatic steatosis. LAP has been validated as a surrogate marker for hepatic fat content and metabolic risk in general and RA populations (24).

Carotid intima media thickness

cIMT was assessed by trained sonographers using the ART-LAB ultrasound system (Esaote, Italy), following previously described protocols (19). B-mode imaging was performed to capture the far wall of each common carotid artery, producing two echogenic lines corresponding to the intima and media layers. The distance between these lines was measured to determine the cIMT. Atherosclerotic plaque was defined as a localized thickening with a cIMT ≥ 1.0 mm.

Laboratory measurements

For all participants, a standardized panel of laboratory tests was performed at the Department of Clinical Chemistry, Franciscus Gasthuis Hospital. Blood samples were collected following routine procedures. Liver enzymes, renal function markers, total cholesterol, HDL-C, glucose, C-reactive protein (CRP), and triglycerides (TGs) were analyzed using DxC analyzers or Synchron LX20 systems (Beckman Coulter, USA) (25, 26). LDL-C concentrations were calculated using the Friedewald formula when TG levels were below 4.00 mmol/L.

Data analysis

Data are presented as mean ± standard deviation (SD), unless otherwise specified. Normally distributed continuous variables were compared using independent-samples t-tests, while non-normally distributed variables were analyzed with Mann-Whitney U or Kruskal-Wallis tests. Associations between lnLAP and CV risk markers, cIMT, were assessed using Pearson or Spearman correlation coefficients, as appropriate. Differences in these markers across LAP-defined steatosis categories were evaluated with t-tests or ANOVA, with Levene's test applied to assess equality of variances. Multivariable linear regression was used to assess the independent association between LAP-defined steatosis and cIMT, adjusting for age, sex, BMI, and systolic blood pressure. Variance inflation factors (VIFs) were calculated to assess multicollinearity, with VIF values <5 considered acceptable. Two-way ANOVA was used to

test the interaction between treatment group and LAP-defined steatosis for longitudinal cIMT changes. Categorical variables were compared using chi-square tests. Changes in ΔcIMT from baseline to 3-year follow-up were analyzed using independent-samples t-tests, stratified by treatment group and LAP-defined steatosis. Statistical significance was defined as $p<0.05$. Observations with missing data were excluded from the analyses. Analyses were performed using IBM SPSS Statistics version 28.0.0.0 (IBM, New York, USA).

RESULTS

Baseline characteristics

A total of 236 patients with RA were assessed at baseline (66% female; mean age 54 ± 10 years; mean BMI 26.4 ± 4.6 kg/m²). Baseline characteristics according to LAP-defined hepatic steatosis ("rule-in" vs "rule-out") are summarized in Table 1. Median lnLAP in the total cohort was 3.6 (IQR 3.1±3.9), with 3.4 (3.0±3.8) in the rule-out group and 4.5 (4.3±4.9) in the rule-in group ($p<0.001$). Baseline characteristics were generally comparable between treatment groups within each LAP-defined steatosis group (Supplementary Table S1). Patients with LAP-defined steatosis were older, had higher BMI and WC, and exhibited a more atherogenic lipid profile, including higher total cholesterol, TGs, apoB, and apoB48, and lower HDL-C and apoAI (all $p<0.05$; see Table 1). They also had higher systolic blood pressure, and showed a non-significant trend toward higher diastolic pressure ($p=0.091$), as well as higher fasting glucose compared with those without LAP-defined steatosis. Mean cIMT tended to be higher in the rule-in group (0.61 ± 0.10 mm vs 0.57 ± 0.11 mm; $p=0.072$), and lnLAP correlated positively with cIMT ($r=0.187$, $p=0.004$; Figure 1). Antihypertensive therapy was more frequently used among patients with steatosis (35.3% vs 15.4%; $p=0.006$), whereas the use of statins, hydroxychloroquine, anti-TNF agents, prednisone, methotrexate, and NSAIDs did not differ between groups.

Association between LAP-defined steatosis and cIMT

In the crude linear regression, LAP-defined steatosis showed a non-significant trend toward higher cIMT (B = 0.036, 95% CI-0.003 to 0.076, $p=0.072$). After adjustment for age, sex, BMI, and systolic blood pressure, the association was no longer observed (B =-0.016, 95% CI-0.050 to 0.019, $p=0.365$) (Supplementary Table S2). Regression diagnostics showed no major deviations from model assumptions, and no relevant multicollinearity was observed among included covariates (all VIFs <5).

Table 1. Baseline characteristics of RA patients (N=236) according to the Lipid Accumulation Product (LAP). Data are expressed as mean ± standard deviation, median (IQR), or n (%), as appropriate. P-values indicate differences between "rule out" and "rule in" groups for each marker.

Variable	LAP rule-out (N=202)	LAP rule-in (N=34)	P-value
lnLAP	3.4 (3.0 – 3.8)	4.5 (4.3 – 4.9)	<0.001
cIMT (mm)	0.57 ± 0.11	0.61 ± 0.10	0.072
Age (years)	54 ± 11	57 ± 7	0.024
Sex (f/m)	140(69.3)/62(30.7)	15(44.1)/19(55.9)	0.004
BMI (kg/m²)	25.9 ± 4	30.0 ± 5	<0.001
WC (cm)	92 ± 11	109 ± 12	<0.001
Total cholesterol (mmol/L)	5.4 ± 1.0	5.8 ± 1.0	0.027
HDL-C (mmol/L)	1.6 ± 0.4	1.2 ± 0.3	<0.001
LDL-C (mmol/L)	3.4 ± 0.9	3.6 ± 0.9	0.133
TG (mmol/L)	0.98 (0.71 – 1.2)	2.0 (1.6 – 2.6)	<0.001
ApoB (g/L)	0.98 ± 0.25	1.2 ± 0.26	<0.001
ApoAI (g/L)	1.7 ± 0.4	1.5 ± 0.4	0.009
ApoB48 (mg/L)	8.0 (4.8 – 12)	14 (11 – 23)	<0.001
Systolic BP (mmHg)	132 ± 18	139 ± 17	0.031
Diastolic BP (mmHg)	79 ± 10	82 ± 10	0.091
Fasting glucose (mmol/L)	5.4 ± 0.52	5.7 ± 0.48	0.032
HbA1c (mmol/mol)	35 ± 4.2	36 ± 5.4	0.473
CRP (mg/L)	3.0 (1.0 – 6.0)	2.0 (1.0 – 5.0)	0.303
ALT (U/L)	25 (18 – 31)	29 (23 – 37)	0.007
AST (U/L)	17 (13 – 21)	19 (15 – 22)	0.227
DAS28CRP	2.5 ± 1.1	2.3 ± 0.98	0.502
DAS28ESR	2.6 ± 1.2	2.3 ± 1.2	0.261
FIB-4	0.80 (0.54 – 1.1)	0.79 (0.67 – 1.1)	0.650
Antihypertensives (%)	31 (15.4)	12 (35.3)	0.006
Statins (%)	14 (7.0)	0 (0)	0.113
Hydroxychloroquine (%)	48 (23.8)	8 (23.5)	0.976
Anti-TNF (%)	71 (35.3)	12 (35.3)	0.997
Prednisone (%)	25 (12.4)	5 (14.7)	0.714
Methotrexate (%)	149 (74.5)	25 (73.5)	0.905
NSAID (%)	83 (41.3)	15 (44.1)	0.757

Abbreviations: lnLAP, natural logarithm of the Lipid Accumulation Product (LAP); cIMT, carotid intima media thickness; BMI, body mass index; WC, waist circumference; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; ApoB, apolipoprotein B; ApoAI, apolipoprotein AI; ApoB48, apolipoprotein B48; systolic BP, systolic blood pressure; diastolic BP, diastolic blood pressure; HbA1c, hemoglobin A1C; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; DAS28CRP, Disease Activity Score using 28 joints with C-Reactive Protein; DAS28ESR, Disease Activity Score using 28 joints with Erythrocyte Sedimentation Rate; Anti-TNF, anti-tumor necrosis factor; NSAID, non-steroidal anti-inflammatory drug.

** P-values derived from independent-samples t-test for normally distributed variables, Mann–Whitney U test for non-normally distributed variables, and chi-square test for categorical variables. Observations with missing values for medication variables (1–2 per variable) were excluded from the calculation of percentages and chi-square analyses.*

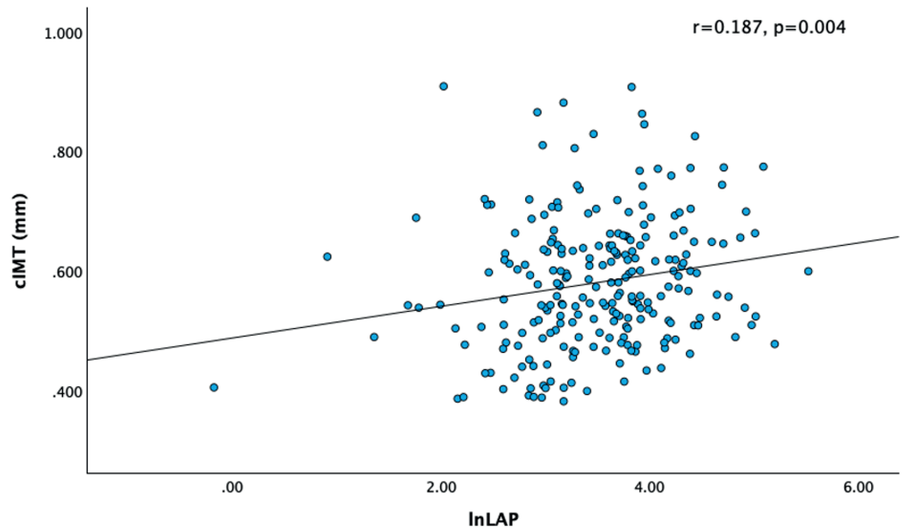


Figure 1. Correlation between carotid intima-media thickness and log-transformed Lipid Accumulation Product.

Abbreviations: cIMT, carotid intima media thickness; lnLAP, natural logarithm of lipid accumulation product.
Note: Log-transformation was applied due to skewed distribution of LAP.

Pre- and post-intervention changes in biochemical parameters

Pre- and post-intervention biochemical outcomes at 3 years are summarized in Table 2, stratified by baseline steatosis status and treatment group. Among patients without LAP-defined steatosis, body weight increased slightly in the intensive treatment group (75 ± 14 → 76 ± 14 kg, p=0.033) but remained unchanged in the standard treatment group (p=0.246). No significant weight changes were observed in participants with LAP-defined steatosis. Total cholesterol decreased significantly in participants without LAP-defined steatosis in both treatment groups (both p<0.001). In participants with LAP-defined steatosis, a significant reduction occurred only in the intensive treatment group (p=0.002), whereas no significant change was observed with standard treatment (p=0.131). HDL-C increased across treatment groups and steatosis categories (all p≤0.005), and LDL-C decreased significantly in all groups (all p≤0.035). TGs increased significantly in the standard treatment group among participants without LAP-defined steatosis (p<0.001), while remaining stable with intensive treatment (p=0.185), leading to a significant between-group difference (p=0.002). ApoB decreased significantly with intensive treatment in both steatosis categories, whereas standard treatment led to a small but statistically significant increase among participants without LAP-defined steatosis, resulting in a significant between-group difference. ApoAI increased significantly only in participants with LAP-defined steatosis receiving intensive treatment (p=0.041), while

remaining largely unchanged in other groups. Fasting glucose and HbA1c increased modestly, primarily in participants without LAP-defined steatosis, while remaining stable in those with LAP-defined steatosis, with values remaining within the non-diabetic range. Inflammatory and hepatic markers (CRP, ALT, AST) did not change significantly in any group.

cIMT progression by treatment and LAP category

Changes in cIMT over 3 years varied according to LAP-defined steatosis and treatment strategy (Table 3; Figure 2). In patients without LAP-defined steatosis, cIMT progression was lower in the intensive treatment group compared with the standard group (0.03 ± 0.07 mm vs 0.06 ± 0.12 mm; p=0.013). In patients with LAP-defined steatosis, cIMT progression did not differ between treatment groups (0.05 ± 0.05 mm vs 0.04 ± 0.05 mm; p=0.696). Two-way ANOVA showed no significant interaction between treatment strategy and LAP-defined steatosis (p=0.192), with post-hoc simple effects indicating a significant treatment effect only in the rule-out steatosis group.

DISCUSSION

In this exploratory study, LAP-defined hepatic steatosis identified RA patients with a more adverse cardiometabolic profile and a trend toward higher baseline cIMT. This association was no longer observed after adjustment for classical CV risk factors, suggesting that the vascular burden associated with steatosis in RA is largely mediated through established metabolic risk factors rather than steatosis itself. Over three years, intensive risk-factor management was associated with lower cIMT progression compared with standard treatment in patients without LAP-defined steatosis. In contrast, patients with LAP-defined steatosis showed metabolic improvement, particularly in lipid profiles, but without detectable vascular benefit. Glycemic, hepatic, and inflammatory markers remained largely unchanged in both groups, and body weight changes were minimal. The absence of a vascular effect in the LAP-defined steatosis subgroup may reflect limited statistical power due to the small sample size, increasing the risk of type II error.

In non-RA populations, a sub analysis of the CLEAR Outcomes trial reported that hepatic steatosis was independently associated with increased CV risk, and that LDL-cholesterol lowering with bempedoic acid reduced events in high-risk individuals (27). Differences with our findings likely relate to higher baseline CV risk, larger sample size, and the use of hard clinical endpoints rather than surrogate vascular measures.

Within our cohort, intensive CV risk management attenuated cIMT progression compared with standard care, particularly through favorable effects on lipid metabolism. Specifically, it prevented the rise in triglycerides observed under standard treatment, reduced apoB levels, and modestly increased apoAI in patients with LAP-defined steatosis, consistent with improved

lipoprotein profiles. However, these metabolic changes did not translate into detectable differences in vascular structure over follow-up. Overall, these findings suggest that the observed differences in vascular outcomes are primarily driven by classical cardiometabolic risk factors, consistent with the absence of an independent association between LAP-defined steatosis and cIMT after adjustment, although residual confounding cannot be fully excluded. These results align with previous studies showing that MASLD is common in RA and closely linked to IR and cardiometabolic dysfunction (28, 29). In the general population, MASLD is associated with increased CV risk, but this relationship appears stronger in more advanced stages of disease, particularly in the presence of fibrosis (30-33). In the present cohort, LAP-defined steatosis clustered with obesity, dyslipidemia, elevated blood pressure, and higher fasting glucose, all established determinants of vascular disease, which likely explains the attenuation of its association with cIMT after multivariable adjustment.

Our previous work similarly demonstrated that liver fibrosis, rather than isolated steatosis, is more strongly associated with subclinical atherosclerosis in RA (13).

Together, these findings suggest that early metabolic liver changes primarily reflect systemic cardiometabolic dysfunction, whereas more advanced stages of MASLD may be more directly involved in vascular injury and cardiovascular risk. Consistent observations have been reported in non-RA populations, where associations with CV outcomes are stronger in more advanced stages of MASLD (30-33).

Strengths of this study include the well-characterized RA cohort, longitudinal vascular assessment, and standardized CV evaluation within a randomized trial framework. Limitations should also be acknowledged. First, this was a post-hoc exploratory analysis not powered for steatosis-defined subgroup comparisons. Second, hepatic steatosis was assessed using LAP rather than imaging or histology, which may limit the accuracy of hepatic fat assessment despite its validated association with metabolic risk. Third, the relatively small number of patients with LAP-defined steatosis likely limited power to detect subgroup-specific associations and treatment effects. Finally, the single-center design may limit generalizability.

From a clinical perspective, LAP may serve as a simple and inexpensive tool to identify RA patients with an adverse cardiometabolic profile who may benefit from intensified CV risk management. Future studies using imaging-based assessments of hepatic steatosis and fibrosis in larger RA cohorts are needed to clarify whether hepatic steatosis independently contributes to CV risk and whether targeting metabolic dysfunction can improve vascular outcomes in this population.

CONCLUSION

In conclusion, in RA, LAP-defined hepatic steatosis identifies patients with a more adverse cardiometabolic profile and a trend toward higher baseline cIMT, an effect largely explained by traditional CV risk factors linked to IR. These findings suggest that steatosis itself is not independently associated with CV risk and emphasize the importance of managing classical cardiometabolic risk factors. Intensive CV risk management was associated with lower cIMT progression in patients without LAP-defined steatosis, whereas patients with LAP-defined steatosis showed metabolic improvements but no significant vascular benefit. Given the post-hoc nature of this analysis and limited subgroup size, these findings should be interpreted as exploratory. Larger prospective studies are needed to clarify the role of steatosis as a CV risk modifier in RA and to further investigate the contribution of metabolic dysfunction to vascular outcomes in this population.

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Table 2. Pre- and post-intervention changes in blood markers by steatosis category and treatment strategy.

Variable	Treatment group	Rule-out steatosis (N=202)				Rule-in steatosis (N=34)			
		Baseline	Follow-up	N	P-value	Baseline	Follow-up	N	P-value
Weight (kg)	Standard	75 ± 13	76 ± 16	103	0.246	91 ± 18	89 ± 16	16	0.430
	Intensive	75 ± 14	76 ± 14	99	0.033	91 ± 16	92 ± 20	17	0.504
Total cholesterol (mmol/L)	Standard	5.5 ± 1.0	5.1 ± 0.8	103	<0.001	6.0 ± 0.9	5.4 ± 0.9	16	0.131
	Intensive	5.3 ± 1.1	4.7 ± 1.0	97	<0.001**	5.7 ± 1.2	4.7 ± 1.1	17	0.002
HDL-C (mmol/L)	Standard	1.5 ± 0.4	1.7 ± 0.4	103	<0.001	1.2 ± 0.3	1.4 ± 0.3	16	0.005
	Intensive	1.6 ± 0.4	1.7 ± 0.4	97	<0.001	1.1 ± 0.3	1.4 ± 0.4	17	<0.001
LDL-C (mmol/L)	Standard	3.4 ± 0.9	2.9 ± 0.8	103	<0.001	3.8 ± 0.7	3.0 ± 0.7	13	0.035
	Intensive	3.3 ± 1.0	2.5 ± 0.7	96	<0.001*	3.5 ± 1.0	2.5 ± 0.6	16	<0.001
Triglycerides (mmol/L)	Standard	1.1 (0.7 – 1.3)	1.2 (0.9 – 1.8)	103	<0.001	1.7 (1.6 – 3.3)	1.7 (1.2 – 2.3)	16	0.088
	Intensive	0.9 (0.7 – 1.2)	1.0 (0.7 – 1.4)	97	0.185**	2.3 (1.8 – 2.7)	2.0 (1.5 – 2.6)	17	0.381
ApoB (g/L)	Standard	0.99 ± 0.3	1.0 ± 0.3	103	0.024	1.2 ± 0.3	1.2 ± 0.3	16	0.540
	Intensive	0.97 ± 0.3	0.90 ± 0.3	97	0.012**	1.2 ± 0.3	1.0 ± 0.2	17	0.025
ApoAI (g/L)	Standard	1.7 ± 0.4	1.7 ± 0.4	103	0.091	1.5 ± 0.3	1.5 ± 0.2	16	0.928
	Intensive	1.7 ± 0.4	1.7 ± 0.3	97	0.854	1.6 ± 0.4	1.7 ± 0.5	17	0.041
Fasting glucose (mmol/L)	Standard	5.4 ± 0.5	5.9 ± 1.4	102	<0.001	5.7 ± 0.6	5.8 ± 0.7	17	0.715
	Intensive	5.5 ± 0.6	5.7 ± 1.4	98	0.092	5.6 ± 0.3	5.9 ± 0.9	17	0.147
HbA1c (mmol/mol)	Standard	35 ± 4.2	37 ± 4.1	101	<0.001	36 ± 5.3	37 ± 4.3	17	0.337
	Intensive	35 ± 4.2	37 ± 5.4	97	<0.001	36 ± 5.6	37 ± 5.0	17	0.289
CRP (mg/L)	Standard	3.0 (1.0 – 6.0)	2.0 (1.0 – 5.0)	103	0.380	1.0 (1.0 – 4.0)	1.0 (1.0 – 4.3)	17	0.373
	Intensive	3.0 (1.0 – 6.0)	2.0 (1.0 – 5.0)	99	0.519	3.0 (1.0 – 6.0)	2.0 (1.0 – 5.0)	17	0.058
ALT (U/L)	Standard	24 (18 – 31)	24 (18 – 30)	102	0.176	31 (26 – 38)	33 (23 – 41)	16	0.877
	Intensive	25 (19 – 32)	24 (16 – 30)	98	0.263	28 (21 – 36)	26 (19 – 34)	17	0.636
AST (U/L)	Standard	18 (13 – 21)	17 (13 – 22)	103	0.256	20 (17 – 28)	22 (16 – 26)	17	0.977
	Intensive	17 (13 – 21)	16 (13 – 23)	99	0.703	18 (13 – 21)	16 (13 – 27)	17	0.635

Abbreviations: HDL-C, high density cholesterol; LDL-C, low density cholesterol; ApoB, apolipoprotein B; ApoAI, apolipoprotein AI; HbA1c, glycated hemoglobin; CRP, C-reactive protein; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase.

*Notes: Total N per steatosis category (rule-out 202, rule-in 34); Ns for individual variables may be lower due to missing data. Data are presented as mean ± SD for normally distributed variables and median (IQR) for non-normally distributed variables. P-values reflect within-group changes from baseline to follow-up. *p < 0.05 and **p < 0.01 indicate differences in change between standard and intensive treatment.*

Table 3. Carotid intima-media thickness (cIMT) progression over 3 years by treatment strategy and baseline lnLAP category.

Baseline lnLAP category	Treatment group	N	ΔcIMT	P-value
Rule-out steatosis	Standard	103	0.06 ± 0.12	0.013
	Intensive	99	0.03 ± 0.07	
Rule-in steatosis	Standard	17	0.04 ± 0.05	0.696
	Intensive	17	0.05 ± 0.05	

Notes: ΔcIMT = change in carotid intima-media thickness from baseline to 3-year follow-up (mm); values are mean ± SD. Steatosis categories were based on baseline lnLAP. P-values represent post-hoc simple effects from the two-way ANOVA (treatment × lnLAP category) comparing standard versus intensive treatment within each category. No significant interaction was observed.

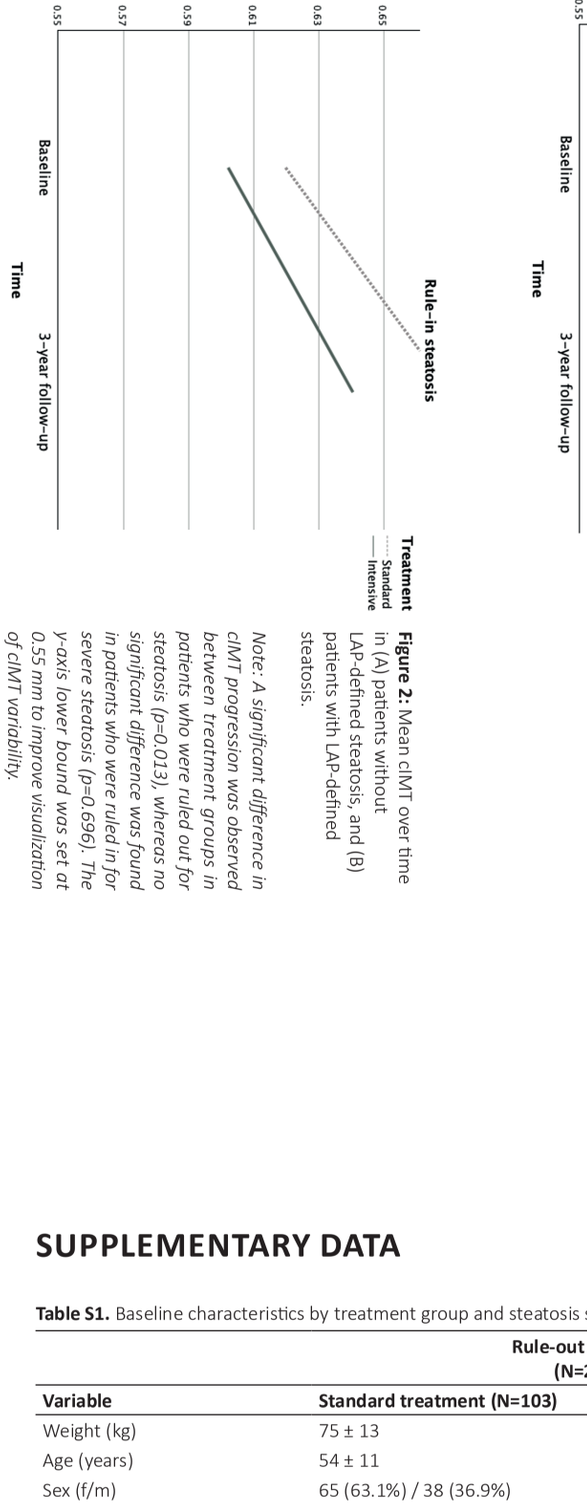


Figure 2: Mean cIMT over time in (A) patients without LAP-defined steatosis, and (B) patients with LAP-defined steatosis.

Note: A significant difference in cIMT progression was observed between treatment groups in patients who were ruled out for steatosis (p=0.013), whereas no significant difference was found in patients who were ruled in for severe steatosis (p=0.696). The y-axis lower bound was set at 0.55 mm to improve visualization of cIMT variability.

SUPPLEMENTARY DATA

Table S1. Baseline characteristics by treatment group and steatosis status.

	Rule-out steatosis (N=202)		Rule-in steatosis (N=34)	
Variable	**Standard treatment (N=103)**	**Intensive treatment (N=99)**	**Standard treatment (N=17)**	**Intensive treatment (N=17)**
Weight (kg)	75 ± 13	75 ± 14	91 ± 18	91 ± 16
Age (years)	54 ± 11	54 ± 11	56 ± 7.4	58 ± 6.9
Sex (f/m)	65 (63.1%) / 38 (36.9%)	75 (75.8%) / 24 (24.2%)	6 (35.3%) /11 (64.7%)	9 (52.9%) / 8 (47.1%)
WC (cm)	91 ± 12	92 ± 11	108 ± 12	110 ± 12
Systolic BP (mmHg)	133 ± 19	131 ± 17	137 ± 12	141 ± 21
Total cholesterol (mmol/L)	5.5 ± 1.0	5.3 ± 1.1	5.9 ± 0.8	5.7 ± 1.2
HDL-C (mmol/L)	1.5 ± 0.4	1.6 ± 0.4	1.2 ± 0.3	1.1 ± 0.3
LDL-C (mmol/L)	3.4 ± 0.9	3.3 ± 1.0	3.8 ± 0.7	3.5 ± 1.0
Triglycerides (mmol/L)	1.1 (0.7 – 1.3)	0.9 (0.7 – 1.2)	1.7 (1.6 – 3.3)	2.3 (1.8 – 2.7)
CRP (mg/L)	3 (1 – 6)	3 (1 – 6)	1 (1 – 4.5)	3 (1 – 6.5)

Abbreviations: WC, waist circumference; systolic BP, systolic blood pressure; HDL-C, high density cholesterol; LDL-C, low density cholesterol.

Table S2. Multivariable regression analysis of cIMT and log-transformed LAP (lnLAP), adjusted for confounding variables.

Variable	B	95% CI	p-value
Model 1	0.036	-0.003 – 0.076	0.072
Model 2	-0.016	-0.050 – 0.019	0.365

Model 1, crude model; model 2, adjusted for age, sex, BMI, and systolic blood pressure. Abbreviations: cIMT, carotid intima media thickness; LAP, Lipid Accumulation Product.

Chapter 7

Metabolic dysfunction-associated steatotic liver disease and cardiovascular risk factors in rheumatoid arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease linked with metabolic dysfunction-associated steatotic liver disease (MASLD), which may increase cardiovascular (CV) risk. This study explores the association between liver fibrosis, assessed by the Fibrosis-4 (FIB-4) index, and CV risk factors in RA patients

Methods: Cross-sectional data from the Franciscus Rheumatoid Arthritis and Cardiovascular Intervention Study (FRANCIS), a randomized, cardiovascular single center, intervention study involving RA patients without cardiovascular disease (CVD) or type 2 diabetes (T2DM), were analyzed. Liver fibrosis was assessed using FIB-4, with a cut-off point of ≥1.3 to define high fibrosis risk, and its relationship with CV risk factors, medication use, and subclinical atherosclerosis, measured by carotid intima-media thickness (cIMT), was evaluated.

Results: Among 326 patients (68.4% female, age 53 ± 11 years, BMI 26.5 ± 4.5 kg/m^2), those with high FIB-4 (n=49) had higher cIMT (p=0.002), apolipoprotein B48 (p=0.04), systolic blood pressure (p=0.007), alkaline phosphatase (p=0.002), and anti-CCP levels (p=0.02). High FIB-4 was associated with lower leukocyte count and complement component 3. Statin use was linked to higher FIB-4 (OR=4.49, p=0.014), while hydroxychloroquine use was associated with lower FIB-4 (OR=0.11, p=0.004). Disease activity scores did not differ between low and high FIB-4 groups.

Conclusion: Elevated FIB-4 in RA patients is associated with increased cIMT, higher blood pressure and elevated atherogenic remnants. Incorporating FIB-4 measurements into routine clinical care for RA populations could effectively identify individuals at the highest CV risk, enabling the implementation of more intensive CV risk management strategies.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune and inflammatory disease which can lead to significant disability and reduced quality of life (1). Beyond joint involvement, RA has notable extra-articular manifestations, impacting the lungs, skin, eyes, heart, kidneys, nervous system, and gastrointestinal tract (2, 3). One potential clinical outcome of RA is liver involvement, which can manifest as autoimmune biliary disease, abnormal liver test results, or metabolic dysfunction-associated steatotic liver disease (MASLD) (4-6). MASLD is characterized by excess fat accumulation in the liver along with at least one cardiometabolic risk factor, in the absence of secondary causes of hepatic steatosis or excessive alcohol consumption (7). Approximately one-third of RA patients are affected by MASLD (8, 9), which can range from simple steatosis to more severe forms like metabolic dysfunction-associated steatohepatitis (MASH), potentially advancing to cirrhosis and hepatocellular carcinoma (HCC) (10). Furthermore, MASLD is associated with an increased risk for CVD (11).

Treatment with disease-modifying antirheumatic drugs (DMARDs) may also contribute to the risk of MASLD in RA. Methotrexate (MTX), the most frequently used DMARD for RA, has been linked to various hepatic side effects, including elevated liver enzymes and an increased risk of liver fibrosis, especially with long-term use or higher doses (12, 13). Other RA medications, such as biologicals, have also been associated with hepatotoxicity, although the extent of their contribution to liver fibrosis remains unclear (14). Given the widespread use of these medications in RA management, it is crucial to evaluate their potential impact on liver health, particularly on the progression of liver fibrosis.

RA is closely linked to CVD, primarily due to the combined effects of systemic inflammation, potentially causing harm to the arterial wall, and the presence of traditional CV risk factors, which are often undertreated in this population (15). Patients with RA have a 1.5-fold increased risk of CVD and experience higher rates of CVD-related morbidity and mortality compared to the general population (16, 17). The increased risk of premature mortality in RA patients is attributed to both persistent inflammation and inadequate management of traditional CV risk factors (18-22).

In addition to traditional CV risk factors, the carotid intima-media thickness (cIMT), a surrogate marker for subclinical atherosclerosis, has also been shown to be increased in RA patients (23, 24). Recent studies also suggested that the increased CVD risk in RA may be associated with accumulation of atherogenic intestinal remnants carrying apolipoprotein (apo) B48 (25, 26).

Although CVD and MASLD are closely interconnected and inflammation plays a central role in both, this has not been studied in detail in a typical systemic inflammatory condition like RA. We hypothesized that the presence of MASLD, particularly liver fibrosis, a key determinant of MASLD prognosis, has been associated with increased CV risk [25].

While liver biopsy is considered the gold standard for diagnosing liver fibrosis, it is an invasive and costly procedure which is not routinely performed unless there is a clear clinical indication (27). Therefore, we used the Fibrosis-4 (FIB-4) index, a non-invasive blood test recommended by the European Association for the Study of the Liver (EASL) guidelines, for the assessment of liver fibrosis (7, 28). The aims of this study were to investigate the association between liver fibrosis, as estimated by the FIB-4, and CV risk factors, including hypertension, lipid levels, and cIMT in patients with RA. Additionally, it aimed to investigate the association between liver fibrosis and medication use in this patient population.

MATERIALS AND METHODS

Study design and study population

This is a post-hoc analysis of the Franciscus Rheumatoid Arthritis and Cardiovascular Intervention Study (FRANCIS), an open-label randomized controlled trial that aimed to investigate the effectiveness of a strict treatment of CV risk factors in RA (26, 29, 30). The study population (n=326) consisted of patients attending the outpatient clinic of the Department of Rheumatology at the Franciscus Gasthuis Hospital in Rotterdam, the Netherlands. Participants were included if they were less than 70 years of age and if they had been diagnosed with rheumatoid arthritis, defined according to the criteria of the American College of Rheumatology (31). Patients were excluded if they had type 1 diabetes mellitus (T1DM) or T2DM at baseline, defined as a fasting glucose of >7.0 mmol or HbA1c >48 mmol/mol, or being treated for these conditions or if they had a history of CVD. The latter of which was defined as a prior myocardial infarction, cerebrovascular event, intermittent claudication, amputation due to peripheral artery disease (PAD), coronary artery bypass graft (CABG), or percutaneous transluminal coronary angioplasty (PTCA). Additionally, patients were excluded if they had kidney disease, defined as an estimated glomerular filtration rate (eGFR) of <40 ml/min/1.73m^2. The study was approved by the medical ethical committee of the Maasstad hospital in Rotterdam, the Netherlands, and was conducted in accordance with the Declaration of Helsinki (The Dutch Trial register, NTR3873; ABR no. NL32669.101.10)(32).

Data collection

Baseline data was collected during outpatient clinic visits between 2009 and 2012. Blood samples were obtained after an overnight fast. In addition, anthropomorphic measurements (e.g., weight, height, blood pressure) were recorded. Variables included for medication use were methotrexate (MTX), NSAIDs, biologicals, statins, antihypertensives, leflunomide, hydroxychloroquine (HCQ), prednisone, and anti-TNF therapy. Furthermore, RA disease activity was evaluated using the Disease Activity Score (DAS28) wherein 28 joints are evaluated (33).

Fibrosis-4 index

The FIB-4 was calculated according to the following formula: age(years)× AST(U/L)/platelet (PLT) (10^9/L) × √ALT(U/L). Low hepatic fibrosis risk was defined as a FIB-4 <1.3 and high fibrosis risk as a FIB-4 ≥1.3. The FIB-4 has been extensively validated in a broad spectrum of MASLD subjects and in RA patients (7, 28).

Carotid intima media thickness

cIMT was measured by trained and experienced sonographers using the ART-LAB (Esaote, Italy) as described earlier (29). Using B-mode ultrasounds which produced two echogenic lines, ultrasound images were made of the far wall of each common carotid artery (CCA). These lines represented the overall thickness of the media and intima layers of the arterial wall. Atherosclerotic plaque was defined as a lesion with a focal cIMT ≥ 1.0 mm.

Laboratory measurements

In each patient, a standard set of laboratory measurements was performed at the Department of Clinical Chemistry of the Franciscus Gasthuis hospital. Blood samples were drawn following the standard procedures. Liver enzymes and renal tests as well as total cholesterol, HDL-C, glucose, C-reactive protein (CRP), and triglycerides (TGs) were measured using DxC analyzers (Beckman Coulter, United States) or Synchron LX20 (Beckman Coulter, United States) (34, 35). LDL-C was measured using the Friedewald formula if TGs were below 4.00 mmol/L. Complement C3 levels were measured using the Atellica® Solution (Siemens Healthineers, Germany). Furthermore, using an ELISA, apoB48 serum levels were quantified as previously described (36).

Statistical analysis

Data are presented as mean ± standard deviation (SD), unless otherwise specified. Normally distributed variables were analyzed using the independent T-test, while non-normally distributed parameters were assessed with the Mann-Whitney U test or Kruskal-Wallis test. Correlation analyses were conducted using Pearson's or Spearman's correlation coefficients, where appropriate. Multivariate binary logistic regression was performed with FIB-4 categories (low vs. high) as the dependent variable and medication use as the independent variables. Chi-square tests were also conducted. A multiple regression analysis was performed to investigate the association between FIB-4 (low vs. high) and cIMT adjusting for hypertension and lipid levels. All statistical analyses were performed using IBM SPSS Statistics version 28.0.0.0 (IBM SPSS Statistics, New York, United States), with statistical significance set at a p-value < 0.05.

RESULTS

The study population included 223 women and 103 men, with an average age of 53 years (± 11) and an average BMI of 26.5 kg/m² (± 4.5). The majority of the cohort had a low FIB-4 score (n=277, 85.0%). Patients with an elevated FIB-4 score were significantly older and had higher systolic blood pressure, higher apoB48 concentrations, higher cIMT values, higher alkaline phosphatase, and higher anti-cyclic citrullinated peptide (anti-CCP) concentrations (Table 1; Fig. 1). In addition, they had statistically significant lower BMI, as well as lower levels of thrombocytes and a lower leukocyte count, particularly neutrophils and lymphocytes, and lower levels of complement C3. There was no difference in disease activity score between groups (Table 1).

Table 1 Total group (n=326). Baseline characteristics in the low FIB-4 versus high FIB-4 groups. P-value is for comparison between FIB-4 groups. Data are expressed as mean ± standard deviation, median (IQR), or n (%), as appropriate

	Low FIB-4 N= 277	High FIB-4 N= 49	p-value
Demographic characteristics			
Age (years)	52 ± 11	62 ± 7.2	<0.001
Sex (m/f)	83/194 (30/70)	20/29 (41/59)	0.132
Smoking (no/yes)	207/55 (79/21)	39/9 (81/19)	0.724
Clinical characteristics			
Body mass index (kg/m^2)	26.7 ± 4.6	25.2 ± 3.6	0.031
Systolic blood pressure (mmHg)	131 ± 18	139 ± 22	0.007
Diastolic blood pressure (mmHg)	78.9 ± 10	81 ± 12	0.142
Total cholesterol (mmol/L)	5.4 ± 1.1	5.6 ± 1.1	0.224
HDL-C (mmol/L)	1.5 ± 0.41	1.6 ± 0.43	0.069
LDL-C (mmol/L)	3.4 ± 0.94	3.4 ± 0.90	0.537
Triglycerides (mmol/L)	1.04 (0.72- 1.55)	1.05 (0.76- 1.49)	0.757
ApoAI (g/L)	1.68 ± 0.37	1.73 ± 0.37	0.441
Apo B (g/L)	1.00 ± 0.26	1.00 ± 0.25	1.000
Apo B48 (mg/L)	8.2 (5.0- 12.1)	9.6 (6.8- 15.5)	0.036
Fasting glucose (mmol/L)	5.5 ± 0.54	5.5 ± 0.63	0.350
HbA1c (mmol/mol)	35.3 ± 4.5	35.1 ± 3.9	0.839
CRP (mg/L)	3.0 (1.0 – 6.0)	2.0 (1.0 – 5.0)	0.181
Hemoglobin (g/dL)	8.5 ± 0.8	8.7 ± 0.5	0.002
Thrombocytes (10^9/L)	255 ± 63.2	172 ± 30.9	<0.001
Leukocyte counts (10^9/L)	6.7 ± 2.0	5.2 ± 1.5	<0.001
Neutrophils (10^9/L)	4.1 ± 1.7	3.1 ± 1.2	<0.001
Lymphocytes (10^9/L)	1.8 (1.4- 2.28)	1.5 (1.1- 1.75)	0.024
Monocytes (10^9/L)	0.55 ± 0.19	0.49 ± 0.18	0.057
Eosinophils (10^9/L)	0.16 ± 0.11	0.16 ± 0.16	0.979
Basophils (10^9/L)	0.03 ± 0.05	0.02 ± 0.04	0.128
Complement C3 (g/L)	1.19 ± 0.23	1.08 ± 0.18	<0.001
Complement C4 (g/L)	0.21 ± 0.06	0.19 ± 0.05	0.053

	Low FIB-4 N= 277	High FIB-4 N= 49	p-value
Alkaline phosphatase (U/L)	70.5 ± 23.1	83.1 ± 38.2	0.002
Gamma-GT (U/L)	21 (16 – 29)	21 (16 – 32)	0.399
Creatinine (µmol/L)	69.5 ± 14.7	73.3 ± 13.9	0.092
Microalbumin (mg/L)	7.0 (5.0 – 14)	7.0 (5.0 – 15)	0.249
Uric acid (mmol/L)	0.29 (0.25 – 0.34)	0.32 (0.26 – 0.36)	0.878
TSH (mU/L)	1.9 (1.3 – 2.7)	2.0 (1.3 – 3.8)	0.221
Anti-CCP (U/mL)	0.64 ± 0.48	0.80 ± 0.41	0.027
DAS28CRP	2.5 ± 1.1	2.2 ± 0.93	0.171
DAS28BSE	2.6 ± 1.3	2.4 ± 0.98	0.287
ALAT (U/L)	16 (13- 20)	23 (19.5- 27)	<0.001
ASAT (U/L)	25 (19 – 32)	27 (20 – 35)	0.086
FIB-4	0.73 ± 0.28	1.75 ± 0.45	<0.001

	Low FIB-4 N= 277	High FIB-4 N= 49	p-value
Medication use			
Antihypertensives	43 (15.6)	10 (20.4)	0.405
Statins	9 (3.3)	6 (12.2)	0.015
Leflunomide	3 (1.1)	2 (4.1)	0.166
Hydroxychloroquine	71 (25.8)	2 (4.1)	<0.001
Anti-TNF	97 (35.3)	19 (38.8)	0.747
Prednisone	34 (12.4)	5 (10.2)	0.814
Methotrexate	206 (74.9)	38 (77.6)	0.589
NSAID	120 (43.6)	20 (40.8)	0.755

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; remnant-C, remnant cholesterol; apoAI, apolipoprotein AI; apoB, apolipoprotein B; apoB48, apolipoprotein B48; HbA1c, hemoglobin A1C; CRP, C-reactive protein; Gamma-GT, gamma-glutamyl transferase; TSH, thyroid-stimulating hormone; anti-CCP, anti-cyclic citrullinated peptide; Disease Activity Score 28-joint count with C-Reactive Protein; DAS28CRP, Disease Activity Score 28-joint count with Erythrocyte Sedimentation Rate; DAS28BSE, Alanine Aminotransferase; ALT, Aspartate Aminotransferase; AST, FIB-4, fibrosis-4 index

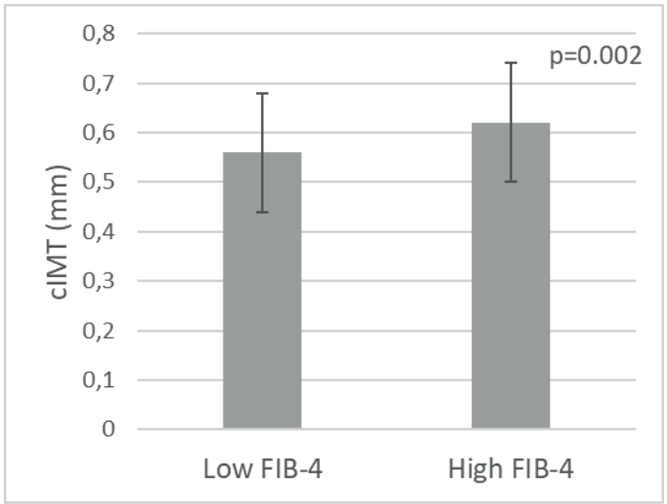


Figure 1. Mean carotid intima media thickness (cIMT) in the low FIB-4 versus high FIB-4 group; 0.57 mm ± 0.12 in the low FIB-4 group vrs. 0.62 mm ± 0.12 in the high FIB-4 group (p=0.002)

Correlation analyses showed significant positive associations between FIB-4 and systolic blood pressure (rho=0.301, p<0.001), cIMT (rho=0.409, p<0.001), total cholesterol (rho=0.212, p<0.001), LDL-C (rho=0.190, p<0.001) and apoB48 (rho=0.141, p=0.011). In contrast, negative correlations were found between FIB-4 and complement C3 (rho=-0.161; p=0.004) and leukocyte count (rho=-0.399, p<0.001). There was no statistically significant correlation between FIB-4 and triglycerides (rho=0.060, p=0.278).

Logistic regression analysis showed that statin use was associated with high FIB-4, while HCQ use was associated with lower FIB-4 (Table 2). To further explore these associations, we conducted univariate logistic regression analyses for each medication separately (Supplementary Table S1). These analyses confirmed that statin use was significantly associated with high FIB-4, while HCQ use was significantly associated with lower FIB-4. No significant associations were observed for antihypertensives, leflunomide, anti-TNF agents, prednisone, MTX, NSAIDs, or other biologics (all p>0.05). In terms of medication use, 12.2% of the patients in the high FIB-4 group used statins, compared to only 3.3% in the low FIB-4 group (Table 1). Similarly, HCQ use showed a statistically significant difference between the two groups, with 4.1% of the patients in the high FIB-4 group using HCQ, compared to 25.8% in the low FIB-4 group. These associations were found independently of the use of other medications included in the analysis. No significant differences were found for other medications, including antihypertensives, leflunomide, anti-TNF agents, prednisone, MTX, NSAIDs, and biologicals (all p>0.05) (Table 1).

Higher FIB-4 scores are associated with increased cIMT. This relationship persists even when adjusting for potential confounders, including systolic blood pressure and LDL-C (Table 3).

While adjustments were also made for HDL-C and diastolic blood pressure, the linearity with these variables was weaker than that of those included in the final model.

Table 2. Logistic regression analysis of medication effects on FIB-4 (low versus high).

Variable	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for Exp(B)
Constant	-1.826	0.461	15.686	1	<0.001	0.161	
Antihypertensives	0.257	0.423	0.370	1	0.543	1.293	0.565 – 2.962
Statins	1.501	0.612	6.019	1	0.014	4.488	1.352 – 14.890
Hydroxychloroquine	-2.176	0.749	8.436	1	0.004	0.113	0.026 – 0.493
Anti-TNF	-0.070	0.343	0.041	1	0.839	0.933	0.476 – 1.828
Prednisone	-0.265	0.536	0.244	1	0.621	0.767	0.269 – 2.193
Methotrexate	0.403	0.420	0.921	1	0.337	1.497	0.657 – 3.411
NSAID	-0.096	0.335	0.082	1	0.774	0.909	0.472 – 1.750

Table 3. Multiple regression analysis of cIMT and FIB-4, adjusted for confounding variables.

Variable	B	95% CI	P-value
Model 1	0.055	0.021 – 0.089	0.002
Model 2	0.037	0.005 – 0.068	0.024

Model 1: Crude model; Model 2: Adjusted for systolic blood pressure and LDL-C

DISCUSSION

The results from the current study have extended the findings from our previous observations reporting on the association between RA and CV risk factors, by also showing an association with liver fibrosis as estimated by FIB-4 (25, 26, 37). The findings of the current study showed that RA patients with a high FIB-4 have higher cIMT values, even after adjusting for potential confounders, reinforcing the notion that liver fibrosis may independently contribute to CV risk in RA patients. This underscores FIB-4's potential as a marker for both liver health and CV risk stratification in RA. Additionally, high systolic blood pressure was found to be significantly associated with increased cIMT, regardless of FIB-4 levels. This highlights the importance of managing blood pressure in RA patients, particularly in those with liver fibrosis, to reduce CV risk. There was no difference in disease activity score between those with low and high FIB-4, suggesting that liver fibrosis, as measured by FIB-4, may be independent of overall RA disease activity, which highlights the need to consider additional factors when assessing liver health in RA patients.

Previously, we reported elevated apoB48 concentrations in RA patients compared to controls (25, 26). The present study expands that observation by also demonstrating an association with liver fibrosis. It is well known that chylomicrons and their remnants can directly infiltrate intact endothelium, become trapped and induce an inflammatory response along with the formation of foam cells. This process plays a crucial role in the initiation and progression of

atherosclerosis (37, 38). This mechanism may explain the increased cIMT observed in RA patients with liver fibrosis. While the relationship between cIMT and liver fibrosis is evident, the pathophysiology of the accumulation of chylomicron remnants remains unclear. A key factor in the clearance of chylomicron remnants is a receptor-mediated pathway in the liver. Liver fibrosis could disrupt this process by reducing the number or function of these receptors. One could speculate that this impairment may lead to higher levels of circulating chylomicron remnants due to reduced hepatic clearance. Another important consideration is that RA patients with high FIB-4 levels also were more frequently on statins. Normally, statin use increases LDL-receptor expression, thereby enhancing the clearance of lipoproteins. This could either mean that these subjects had already been identified as having a higher CV risk and were therefore on appropriate treatment or that liver fibrosis might reduce statin effectiveness by impairing LDL-receptor activity, thus hindering the clearance of atherogenic lipoproteins despite statin treatment. At this stage there is no clinical or experimental evidence supporting the latter hypothesis.

Another factor that may contribute to liver complications in RA is the presence of anti-CCP antibodies. While these antibodies are well-known for targeting various cell types in RA, their impact on liver cells is less understood. Recent laboratory studies have revealed that anti-CCP antibodies can influence hepatocytes by triggering the release of inflammatory proteins, impairing fat and glucose metabolism, and ultimately causing liver damage and apoptosis (39). This mechanism could potentially explain the increased anti-CCP levels observed in patients with high FIB-4 scores.

Our study demonstrated a significant association between HCQ use and lower FIB-4 scores, a finding consistent with recent research suggesting that HCQ may have a protective effect against the development of MASLD (40). HCQ is known to influence adiponectin levels, which play a key role in regulating metabolic processes. Higher adiponectin levels, linked to HCQ use, are associated with reduced systemic inflammation and improved insulin sensitivity, both of which can help mitigate liver damage (41). This may explain the lower FIB-4 scores observed in patients using HCQ. However, it is important to consider that this association may be confounded by indication. The protective effect of HCQ against liver fibrosis observed in this study may, in part, be attributed to its use in patients with lower RA disease activity. As low disease activity is inherently associated with reduced systemic inflammation, it is possible that the observed benefit reflects better-controlled RA rather than a direct pharmacological effect of HCQ. Given these considerations, the findings need to be interpreted with caution, and further studies are warranted to explore these potential mechanisms and establish the independent role of HCQ in liver fibrosis prevention.

Moreover, in our study, we did not find any significant associations between MTX use and liver fibrosis. Similarly, a recent multicenter study involving around 1,000 patients with RA or psoriasis also found no association between the cumulative dose or duration of MTX exposure and liver fibrosis (42). Although MTX has been previously linked to hepatotoxicity

and an increased risk of liver fibrosis (13, 43), these newer findings suggest that the actual risk may be lower than previously believed. This discrepancy underscores the need for further research to clarify MTX's impact on liver health and to better understand the factors that may influence these outcomes.

The lower inflammatory markers observed in patients with high FIB-4 levels could be attributed to the effects of advanced liver fibrosis. Advanced liver fibrosis often leads to portal hypertension and resultant hypersplenism, causing the sequestration of leukocytes and thrombocytes in the spleen, thereby reducing their levels in the bloodstream. Alternatively, the inverse relationship between leukocyte count and FIB-4 may also be attributed to MTX use, which can cause myelosuppression and reduce leukocyte counts, often leading to neutropenia, anemia, and thrombocytopenia (43, 44).

Additionally, in contrast with previous studies, our study did not find significant associations between glucocorticoid use and liver fibrosis (40, 45). This may be due to differences in glucocorticoid dosage, treatment duration, or comorbidities within our cohort. Further research with larger samples and more detailed data on glucocorticoid use is needed to clarify its potential impact on liver fibrosis in patients with RA.

This study had some limitations. Firstly, the cross-sectional design limits our ability to determine causality and observe changes over time. Longitudinal studies are needed to explore these aspects further. Secondly, while FIB-4 provides valuable insights, it is not the gold standard for liver fibrosis assessment; liver biopsy remains the preferred method. Additionally, only a small portion of the study population had a high FIB-4. Another limitation is the lack of data on cumulative steroid use. As this information was not collected prospectively, it was not feasible to include it in our analysis. However, given the primary focus of this study on the associations between liver fibrosis and CVD risk factors, as well as medication use in patients with RA, we believe the absence of this data is unlikely to have impacted the validity of our key findings though it is an important consideration for future research. Moreover, some patients in this study were prescribed medications such as HCQ, prednisolone, and NSAIDs, which are not currently recommended as first-line treatments for rheumatoid arthritis. These prescriptions likely reflect individual clinical decisions, including intolerance or contraindications to recommended therapies like methotrexate, or historical prescribing practices. Such variations highlight the complexity of managing rheumatoid arthritis in real-world settings and should be considered when interpreting the study's findings.

CONCLUSION

In conclusion, RA patients with a high FIB-4 showed increased cIMT, indicating higher CV risk. This relationship appears to be influenced by hypertension and the accumulation of intestinal atherogenic chylomicron remnants. The observed inverse relationship between FIB-4 with systemic inflammatory markers suggests a complex interaction that warrants further research. Hydroxychloroquine may have the potential to offer some protective effect against liver fibrosis in RA patients, though further research is needed to confirm this finding**.**

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Chapter 8

General Discussion

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a common yet often underrecognized liver disorder and represents the hepatic manifestation of metabolic syndrome. Its spectrum, from simple steatosis to fibrosis, carries an increased risk of cirrhosis and cardiovascular disease (CVD). High-risk populations, including individuals with type 2 diabetes (T2DM), obesity, or systemic inflammatory conditions, are particularly affected but frequently remain undiagnosed. Despite its clinical significance, important questions remain regarding the prevalence, key determinants, and cardiovascular (CV) consequences of MASLD, especially the interplay between metabolic and inflammatory pathways.

This thesis explores MASLD and liver fibrosis across diverse high-risk populations characterized by systemic inflammation, including patients T2DM and type 1 diabetes (T1DM), obesity, arterial hypertension, and rheumatoid arthritis (RA). It aims to expand our understanding of MASLD beyond the general population and to emphasize the critical role of liver health in comprehensive cardiometabolic care.

2. Reflection on chapters

In Chapter 2 we investigated subjects attending an outpatient cardiometabolic clinic. Until then, liver health had not been included in the clinical evaluation of these subjects, although arising evidence already suggested that MASLD, and especially liver fibrosis, was an additive factor to total CV risk. Here we showed that MASLD was highly prevalent among these patients, particularly in those with T2DM, with approximately 57% fulfilling the diagnosis of MASLD. BMI and triglycerides (TGs) were the strongest predictors of steatosis and fibrosis. Interestingly, MASLD and fibrosis risk were not associated with carotid intima-media thickness (cIMT), suggesting that vascular disease depends on extrahepatic factors or that treatment of classic risk factors already is sufficient to improve vascular status. In order to evaluate the contribution of liver health to CV risk, long term prospective studies will be needed including liver specific interventions. This study provided novel information on MASLD prevalence in such a high CV-risk population. In this high-risk cohort MASLD prevalence was substantially higher than in general population studies, such as the Lifelines and the Rotterdam Study, where prevalence ranges from 22-30% (1-3). Prevalence in T1DM patients was comparatively low, highlighting population-specific differences in liver fat accumulation.

Across all subgroups in this chapter, BMI and TG levels consistently emerged as the strongest determinants of both liver steatosis and fibrosis, emphasizing the central role of metabolic factors in MASLD pathogenesis, especially those associated to insulin resistance (IR). These findings align with recent results from Leite et al., who reported that BMI and TGs independently predicted liver stiffness and steatosis in a vibration controlled transient elastography (VCTE) assessed T2DM cohort (4). Similarly, our data are consistent with the longitudinal study by Song et al., showing that higher BMI and TG levels predict both the development of MASLD and its progression to fibrosis (5). Together, these observations reinforce the notion that metabolic impairment associated with IR, rather than vascular or inflammatory pathways alone, is a primary driver of MASLD in high-risk cardiometabolic populations.

Chapter 3 extended these findings to patients with morbid obesity, showing that elevated FIB-4 scores (reflecting liver fibrosis risk) were associated with adverse CV profiles, including higher blood pressure, arterial stiffness, and systemic inflammation. These results indicate that FIB-4 may serve as a practical marker to capture both hepatic and CV risk in patients with morbid obesity, consistent with meta-analytic evidence that higher FIB-4 predicts CV events independently of MASLD (6).

MASLD and atherosclerotic cardiovascular disease (ASCVD) share a common pathophysiological mechanism, namely IR, with high blood pressure, systemic inflammation, and dyslipidemia (7-9) as major determinants. IR not only promotes fat accumulation and fibrosis in the liver but also contributes to blood vessel dysfunction and atherosclerosis. Hepatokines, such as fibroblast growth factor 21 (FGF21), and increased procoagulant activity may further link MASLD to vascular damage (10, 11). Epidemiological studies support this liver-heart connection, showing associations between MASLD, subclinical atherosclerosis, and higher rates of CV events, although the relationship with CV mortality is less consistent (12-14). Advanced liver fibrosis may further increase CV risk via procoagulant and atherogenic mechanisms, highlighting the value of non-invasive markers like FIB-4 to identify patients at high risk.

Chapter 4 investigated lifestyle counselling combined with naltrexone-bupropion (NB) therapy in subjects with T2DM. This was a post-hoc analysis of the COR-DM Study (15). Weight loss emerged as the strongest predictor of hepatic improvements in line with previous studies (16-18). This analysis showed that improvements of hepatic outcomes were most pronounced in patients achieving at least 5% body weight loss, with greater improvement in non-invasive markers of steatosis and fibrosis with greater weight loss. Among non-invasive fibrosis markers, the MAF-5 score, which combines metabolic and clinical parameters, was evaluated alongside FIB-4 (19). MAF-5 was associated with improvements in fibrosis following weight loss, whereas FIB-4 showed non-significant changes, highlighting MAF-5's potential as a sensitive tool for detecting early metabolic-driven liver changes and guiding intervention in patients with T2DM. Modest weight loss (<5%) may not sufficiently decrease hepatic TG content or restore insulin sensitivity, limiting improvements in liver enzymes and fibrosis markers (16, 20). In addition to enhancing the effects of lifestyle intervention by facilitating weight loss, NB therapy may also influence hepatic stellate cell (HSC) activity, as suggested by experimental studies (19, 21-23). While in this chapter weight loss was the dominant driver of hepatic improvement, we also believe that qualitative improvements in diet composition, such as increased intake of vegetables, fiber, and unsaturated fats, may also contribute to liver health improvement independent of weight change, highlighting mechanisms beyond weight loss that warrant further study (24, 25).

Chapter 5 used data from the GRIPonMASH study. This is a European IHI sponsored (IHI no. 101132946) study including participants from both primary and secondary care with at least

one of the following conditions: T2DM, obesity, hypertension or metabolic syndrome (26). This preliminary analysis revealed that MASLD affected nearly 60% of patients and significant liver fibrosis was present in 17%, with comparable prevalence across care settings. This was unexpected since one may have expected that subjects in secondary care may have more risk factors or be at higher risk of fatty liver disease. These findings emphasize that liver disease is not confined to secondary care and underscore the importance of systematic assessment in high-risk populations, consistent with Chapter 2, where a substantial portion of cardiometabolic patients had MASLD and significant liver fibrosis, often undiagnosed in routine practice. They also align with previous reports in T2DM and obesity, supporting projections of a rising disease burden in Western populations (18, 27). BMI and TGs again emerged as the strongest independent predictors of MASLD, highlighting the central role of metabolic factors associated to IR in liver fat accumulation. In a subset of patients with follow-up Fibroscan data, modest reductions in weight, BMI, and waist circumference were associated with improvements in liver stiffness, suggesting that even small lifestyle changes can positively impact the risk of liver fibrosis as assessed by VCTE. Overall, these findings highlight the importance of including liver health evaluation using NITs to detect MASLD and liver fibrosis, enabling early identification, risk stratification, and timely intervention across both primary and secondary care settings.

Chapter 6 examined LAP-defined steatosis in a completely different cohort of patients characterized by systemic inflammation, namely RA. This cohort was included to evaluate the effect of a chronic inflammatory condition on liver health. Earlier studies had already suggested that in this group of patients, liver steatosis may be more prominent than in the general population (28, 29). However, not many studies have evaluated this condition in relation to CV risk against a background of liver health. For this purpose, we used data from the FRANCIS study which is a randomized CV risk intervention study with a long follow up in a well-defined RA cohort (30, 31). Earlier data from this study already had shown that multiple risk intervention in RA resulted in improvements of vascular status as estimated by cIMT measurements (30). However, no data had been published so far on liver health. Liver steatosis in this analysis was associated with a more adverse cardiometabolic profile, including higher BMI, dyslipidemia, and elevated blood pressure, paralleling patterns observed in cardiometabolic populations. However, once traditional CV risk factors were accounted for, steatosis itself was not independently associated with cIMT. This suggests that in RA, metabolic dysfunction, rather than hepatic fat accumulation per se, is likely the main driver of early vascular changes. While intensive CV risk management significantly reduced cIMT progression only in patients without liver steatosis. The data may suggest that in this group of patients, classical CV risk intervention is not sufficient to improve vascular health when MASLD is present. Whether specific liver-health interventions may result in improved vascular health or decrease cIMT progression, remains to be studied. However, it needs to be underlined that the small size of the rule-in steatosis group precluded definitive conclusions of treatment

effects in this subgroup. In addition, more specific measures reflecting liver health should be applied like VCTE for example, in order to better characterize MASLD status**.**

Chapter 7 focused on liver fibrosis in the same FRANCIS RA cohort, estimated using FIB-4. In contrast to liver steatosis, liver fibrosis risk was independently associated with higher cIMT and apolipoprotein B48 (ApoB48) levels, reinforcing the concept that disease stage within MASLD has implications for vascular changes. Notably, hydroxychloroquine (HCQ) use was linked to lower FIB-4 scores, suggesting potential protective effects through modulation of systemic inflammation. These findings indicate that in RA, fibrosis may serve as a more sensitive marker of cumulative metabolic and inflammatory burden, with clearer implications for early vascular alterations than liver steatosis alone.

Collectively, Chapters 2-7 demonstrate that MASLD and liver fibrosis are prevalent across diverse cardiometabolic and inflammatory conditions, with metabolic risk factors such as BMI and TGs reflecting IR and consistently associated with liver disease severity. Importantly, liver fibrosis repeatedly emerged as the component most strongly linked to cIMT, which may be considered to reflect subclinical atherosclerosis, highlighting its role as a central prognostic risk factor.

3. Synthesis of findings

Across the studies in this thesis, a consistent picture emerges: MASLD is highly prevalent in diverse high-risk populations, including those with T2DM, obesity, hypertension, and metabolic syndrome (MetS). Prevalence rates in these groups were high and, when compared with those reported in general population cohorts from previous studies, they appear substantially higher (2, 32). Importantly, the burden of MASLD and clinically relevant fibrosis was similar in primary and secondary care, showing that liver disease is not confined to subjects attending outpatient clinics who in theory may have a higher CV risk burden than those being seen in primary care settings. Although one might expect a higher prevalence in secondary care, the comparable rates suggested that metabolic dysfunction underlying MASLD is already highly prevalent in the general cardiometabolic population, even before specialist referral.

Several shared determinants of MASLD and fibrosis were identified. Elevated BMI and TGs consistently predicted the presence and severity of liver disease across cardiometabolic populations. Elevated TGs reflect underlying dyslipidemia and are associated with hepatic fat accumulation. Hypertriglyceridemia may contribute directly to liver inflammation and fibrosis through lipotoxicity and IR pathways (33-35). Studies show that TG-based indices predict fibrosis and mortality in MASLD, and that reduction of hypertriglyceridemia improves hepatic outcomes (36, 37).

Given the central role of BMI, TGs, and IR in MASLD and fibrosis, weight loss remains the most effective intervention (17, 38). Even modest reductions in weight or waist circumference have been linked to improvements in liver stiffness, suggesting that lifestyle interventions can provide early benefits, particularly in patients with fibrosis (39).

Building on this, pharmacological adjuncts such as NB may provide additional hepatic benefits by promoting weight loss, supporting a combined lifestyle and pharmacological approach in selected patients. Other weight-loss–driven therapies, including GLP-1 receptor agonists such as semaglutide and dual or triple receptor agonists like tirzepatide and survodutide, similarly improve liver outcomes primarily through reductions in body weight and systemic metabolic improvements. Phase 2 and phase 3 trials have demonstrated that these therapies lead to significant reductions in liver fat, improvements in steatohepatitis resolution, and beneficial effects on insulin sensitivity and broader metabolic profiles in patients with MASLD (40-42). By contrast, therapies designed to target hepatic metabolism directly, such as THR-β agonists and experimental inhibitors of de novo lipogenesis, exert liver-specific effects that are largely independent of body weight (43).

Based on the findings of this thesis, it can be hypothesized that weight-loss–driven therapies reduce systemic inflammation and CV risk through improvements in adiposity and insulin sensitivity. Liver-targeted therapies may provide benefits by reducing hepatic fat and inflammation, which could indirectly influence systemic outcomes. Future studies are needed to test whether these mechanistic effects translate into measurable improvements in CV risk and systemic inflammatory markers.

Across the studies in this thesis, distinct mechanisms connecting MASLD to CV risk emerged. In cardiometabolic populations, MASLD appeared to drive CV risk mainly via metabolic dysfunction, such as dyslipidemia and visceral adiposity, rather than direct vascular damage. In RA, hepatic steatosis reflected adverse metabolic profiles but was not independently associated with subclinical CV risk after adjustment for traditional risk factors. In contrast, liver fibrosis was independently associated with subclinical CV risk, highlighting the importance of disease stage in determining CV relevance in RA populations. Systemic inflammation and altered lipid metabolism appeared to contribute to this increased CV risk, as reflected by associations with subclinical atherosclerosis and elevated ApoB48. The plasma concentrations of ApoB48 reflect intestinally derived chylomicron remnants. These lipoproteins are highly atherogenic and they tend to accumulate in insulin-resistant and inflammatory states. ApoB48 elevation in RA suggests impaired postprandial lipid clearance, potentially linking systemic inflammation and hepatic fat accumulation to vascular injury (44, 45).

This raises the question of whether modulation of systemic inflammation could influence liver fibrosis and its CV consequences in RA. In line with this hypothesis, HCQ use in RA patients was associated with a lower risk of fibrosis, suggesting a potential protective effect mediated through anti-inflammatory pathways. To date, however, no large prospective randomized clinical trials of anti-inflammatory interventions have demonstrated improved liver outcomes.

Beyond RA, emerging evidence suggests that pharmacological interventions targeting lipid metabolism may provide benefits for both liver and CV outcomes in high-risk populations. For example, post-hoc analyses of the PROSPER trial and other studies have shown that

pravastatin reduced all-cause mortality associated with higher fibrosis scores in elderly adults, highlighting a potential dual role in supporting liver and CV health (46, 47).

Collectively, these studies demonstrate a high and consistent burden of MASLD and fibrosis across diverse high-risk populations, with shared determinants such as BMI and TGs, as well as population-specific mechanisms including systemic inflammation in RA. Importantly, liver fibrosis emerged as a consistent marker of increased CV risk, suggesting that it reflects an integrative signal of cumulative metabolic and inflammatory burden, rather than serving merely as a surrogate for conventional risk factors.

4. Clinical implications

The findings of this thesis have implications for clinical practice. MASLD and fibrosis are common in patients with T2DM, obesity, hypertension, and MetS, independent of care level. Systematic screening using validated non-invasive tools such as FIB-4 or MAF-5 should be incorporated into standard care pathways. Lifestyle modification, particularly body weight reduction, remains the cornerstone of MASLD management, as even modest improvements can reduce fibrosis risk. Pharmacological adjuncts, such as NB, may provide additional benefit and warrant further investigation. Finally, these findings emphasize the importance of multidisciplinary collaboration between hepatology, cardiology, endocrinology, and primary care to provide holistic management of patients with MASLD and high CV risk.

5. Limitations

Several limitations should be acknowledged. Most studies were cross-sectional in design, which precludes causal inference and limits the ability to assess long-term outcomes. Additionally, all studies relied on non-invasive assessments of liver fibrosis, such as VCTE, FIB-4, and MAF-5, which, while validated, are less precise than histological examination. Some cohorts were relatively homogeneous, such as the predominantly female obesity cohort, and biomarker availability was limited in certain populations. These factors may reduce generalizability. Nonetheless, the studies provide valuable real-world insights into MASLD and fibrosis across diverse high-risk populations.

6. Future directions

Future research should continue to explore the natural history of MASLD and fibrosis, including their contribution to CV outcomes, and develop improved non-invasive diagnostic tools to enhance risk stratification. Mechanistic and interventional studies are needed to clarify pathways linking MASLD to vascular dysfunction and evaluate the long-term effects of lifestyle and pharmacological interventions. While MASLD contributes to CV complications, it also represents a major long-term liver risk, with rising numbers of patients developing advanced fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma (HCC), which often leads to liver transplantation or liver-related mortality. Public health strategies should therefore consider

both CV and liver-related outcomes to adequately prepare healthcare systems for the increasing burden of advanced liver disease (48-50).

From a public health perspective, routine screening for MASLD in high-risk populations, particularly patients with T2DM, should be implemented to enable early detection and timely intervention. Complementary strategies include preventing obesity and T2DM, raising awareness among healthcare providers and at-risk individuals. Given the increasing prevalence of obesity among children and adolescents, consideration of screening younger populations may be warranted (51, 52). Integrating these approaches into broader cardiometabolic health initiatives can help reduce disease burden and healthcare costs at the population level (53).

7. Conclusion

This thesis advances understanding of MASLD and liver fibrosis in high-risk populations. It demonstrates that MASLD is highly prevalent in cardiometabolic and inflammatory conditions, and that fibrosis is associated with increased CV risk. The disease burden is comparable in primary and secondary care settings, emphasizing the need for screening in both care levels. While metabolic dysfunction underpins most associations, inflammatory and lipid-mediated mechanisms amplify risk in specific populations. Clinically, these findings support integrating liver health assessment into routine cardiometabolic care and using tailored strategies that combine lifestyle change, pharmacotherapy aimed to reduce body weight and improve insulin sensitivity, and, where appropriate, anti-inflammatory therapies. Embedding liver health into routine cardiometabolic care represents both a challenge and an opportunity to prevent liver disease progression, to reduce CV risk, and to improve long-term outcomes, while also addressing the substantial healthcare and economic burden of MASLD through prevention, early detection, and timely intervention that can ease strain on healthcare systems.

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Appendix

APPENDIX A. ENGLISH SUMMARY

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent yet often underrecognized condition that increases the risk of liver fibrosis and cardiovascular (CV) complications. This thesis investigates the prevalence, risk factors, and clinical implications of MASLD across diverse high-risk populations, and evaluates strategies to improve hepatic and cardiometabolic health.

In a cohort of cardiometabolic outpatients, MASLD was most prevalent among patients with type 2 diabetes (T2DM), with over one-fifth showing a risk of significant fibrosis. Interestingly, neither MASLD nor fibrosis was associated with carotid intima-media thickness (cIMT), suggesting that liver-related CV risk may not be captured by conventional carotid measures, potentially reflecting extra-carotid vascular involvement. Body mass index (BMI) and plasma triglyceride (TG) levels were the strongest predictors of MASLD and fibrosis risk, emphasizing the central role of metabolic control and lifestyle interventions in disease management.

Among patients with severe obesity, liver fibrosis assessed by FIB-4 identified individuals at higher CV risk, as indicated by increased arterial stiffness (pulse wave velocity) and cIMT, elevated blood pressure, insulin resistance (IR), and systemic inflammation. Traditional risk factors such as BMI and lipid profiles did not differentiate fibrosis risk groups, highlighting the value of liver fibrosis as an early marker of CV vulnerability in this population. These findings support the use of FIB-4 in routine CV risk assessment to enable more precise identification of high-risk individuals and tailored preventive strategies.

In patients with T2DM and overweight or obesity, pharmacological intervention with naltrexone/bupropion (NB) combined with structured lifestyle counseling resulted in greater weight loss compared with lifestyle intervention alone. Weight reduction emerged as the primary driver of improvements in hepatic steatosis and fibrosis, as measured by HSI and the T2DM-specific MAF-5 index, whereas the general fibrosis score FIB-4 remained uninformative. Participants achieving ≥5% weight loss experienced meaningful liver improvements, with the most pronounced benefits observed at ≥10% weight loss. These results suggest that NB may enhance liver health by facilitating greater weight loss beyond lifestyle measures alone, reinforcing the central role of weight management in MASLD treatment.

Screening of high-risk individuals across both primary and secondary care in the Netherlands revealed a high prevalence of MASLD and liver fibrosis, with comparable disease burden between both care settings. MASLD was consistently associated with elevated BMI and TGs, although primary care patients showed more atherogenic lipid profiles and secondary care patients had poorer glycemic control. Preliminary follow-up data demonstrated that lifestyle-driven weight loss can significantly improve liver stiffness, underscoring the importance of systematic, non-invasive liver assessment in primary care to enable timely intervention, improve hepatic outcomes, and optimize healthcare efficiency. Structured screening pathways can reduce unnecessary specialist referrals while targeting care to those most at risk.

In patients with rheumatoid arthritis (RA) without T2DM, LAP-defined hepatic steatosis identified individuals with a more adverse cardiometabolic profile and higher baseline cIMT. However, after adjusting for traditional CV risk factors, including age, sex, BMI, and blood pressure, steatosis was not independently associated with cIMT, suggesting that classical cardiometabolic risk factors, rather than hepatic fat accumulation per se, drive vascular changes in RA. Longitudinal follow-up over three years showed that intensive CV risk management attenuated cIMT progression in patients without steatosis, whereas those with LAP-defined steatosis showed minimal vascular improvement. These findings highlight that steatosis identifies higher-risk metabolic phenotypes but is not an independent CV risk factor in RA, emphasizing the need to manage traditional cardiometabolic risk factors.

Unlike steatosis, fibrosis was independently associated with higher CV risk in patients with RA. Those with an elevated FIB-4 had higher cIMT, increased systolic blood pressure, and elevated atherogenic lipoprotein remnants, suggesting that liver fibrosis contributes to CV risk even in non-obese, non-diabetic populations. Notably, hydroxychloroquine (HCQ) use was associated with lower FIB-4 scores, indicating a potential protective effect against liver fibrosis. These findings highlight FIB-4 as a valuable non-invasive marker for identifying RA patients at increased CV risk and suggest that pharmacological interventions may positively influence liver health in this population.

Taken together, this thesis demonstrates that MASLD and liver fibrosis are highly prevalent and clinically relevant across multiple high-risk populations. Early detection through non-invasive testing, coupled with lifestyle and pharmacological interventions, can improve both hepatic and CV outcomes. These results provide a foundation for personalized risk assessment, preventive strategies, and the integration of structured liver screening in both primary and specialist care, emphasizing the central role of weight management in mitigating MASLD and its cardiometabolic consequences.

APPENDIX B. NEDERLANDSE SAMENVATTING

Metabole disfunctie-gerelateerde steatotische leverziekte (MASLD), beter bekend als leververvetting, is een veelvoorkomende aandoening die vaak niet wordt herkend. Leververvetting verhoogt het risico op leververlittekening en hart- en vaatziekten (HVZ). In dit proefschrift onderzoeken we hoe vaak MASLD voorkomt, welke factoren het risico verhogen, en wat dit betekent voor de gezondheid van de lever en het hart in hoogrisico populaties. Ook bekijken we strategieën om de lever- en hartgezondheid te verbeteren.

Bij patiënten die poliklinisch werden behandeld voor metabole aandoeningen, kwam MASLD het vaakst voor bij mensen met type 2 diabetes (T2D). Meer dan een vijfde van hen had een verhoogd risico op ernstige lever verlittekening. Interessant was dat leververvetting en lever verlittekening niet samenhingen met de dikte van de halsslagader (cIMT), een veelgebruikte maat voor HVZ-risico. Dit suggereert dat het risico op HVZ bij leververvetting mogelijk niet volledig zichtbaar is via traditionele metingen van de bloedvaten in de hals. Body mass index (BMI) en het triglyceridengehalte in het bloed bleken de belangrijkste voorspellers van MASLD en lever verlittekening, wat het belang van een gezonde leefstijl en goede metabole controle benadrukt.

Bij patiënten met ernstig overgewicht kon lever verlittekening, gemeten met de FIB-4-score, helpen om mensen met een hoger risico op HVZ te identificeren. Deze patiënten hadden vaker stijvere bloedvaten (pulse wave velocity), dikkere halsslagaders (cIMT), hogere bloeddruk, insulineresistentie (IR) en meer ontstekingsverschijnselen in het lichaam. Traditionele risicofactoren, zoals BMI en lipiden in het bloed, konden deze hoogrisicogroepen niet goed onderscheiden, wat laat zien dat lever verlittekening een belangrijke vroege aanwijzing kan zijn voor kwetsbaarheid van het hart en de bloedvaten. Deze bevindingen ondersteunen het gebruik van FIB-4 bij routinematige beoordeling van het HVZ-risico, zodat hoogrisicopatiënten beter kunnen worden opgespoord en gerichte preventieve maatregelen kunnen krijgen.

Bij patiënten met T2D en overgewicht of obesitas leidde een behandeling met het medicijn naltrexon/bupropion (NB), gecombineerd met gestructureerde leefstijlbegeleiding, tot meer gewichtsverlies dan leefstijlbegeleiding alleen. Gewichtsverlies bleek de belangrijkste factor voor verbetering van leververvetting en lever verlittekening, gemeten met de Hepatic Steatosis Index (HSI) en de MAF-5-score, terwijl de algemene fibrose-score, FIB-4, niet veranderde. Patiënten die minstens 5% van hun lichaamsgewicht verloren, lieten duidelijke verbeteringen in de lever zien, met de grootste effecten bij ≥10% gewichtsverlies. Deze resultaten laten zien dat NB de levergezondheid kan verbeteren door extra gewichtsverlies bovenop leefstijlaanpassingen te bevorderen, en benadrukken dat gewichtsverlies een centrale rol speelt bij de behandeling van MASLD.

Screening van hoogrisicopatiënten in zowel de eerstelijns- als tweedelijnszorg in Nederland liet een hoge prevalentie van MASLD en lever verlittekening zien, met een vergelijkbare ziektelast binnen beide zorgniveaus. MASLD was consequent geassocieerd met een hogere BMI en hogere triglyceridenwaarden. Preliminaire follow-upgegevens toonden aan dat gewichtsverlies door leefstijlinterventies de leverstijfheid aanzienlijk kan verbeteren. Dit benadrukt het belang van systematische, niet-invasieve leverbeoordeling in de eerstelijnszorg, zodat tijdig kan worden ingegrepen, de levergezondheid kan worden verbeterd en de efficiëntie van de zorg kan worden verhoogd. Gestructureerde screeningspaden kunnen bovendien onnodige verwijzingen naar specialisten verminderen en de zorg richten op patiënten met het hoogste risico.

Bij patiënten met reumatoïde artritis (RA) zonder T2D bleek dat leververvetting, gedefinieerd via de Lipid Accumulation Product (LAP), samenhing met een slechter cardiometabool profiel en een hogere basiswaarde van de cIMT. Na correctie voor klassieke risicofactoren zoals leeftijd, geslacht, BMI en bloeddruk, was leververvetting echter niet meer onafhankelijk geassocieerd met cIMT. Dit geeft aan dat vooral traditionele risicofactoren de vaatveranderingen bij RA bepalen. Longitudinale follow-up over drie jaar liet zien dat intensief HVZ-risicomanagement vooral bij patiënten zonder leververvetting de cIMT-progressie verminderde, terwijl patiënten met leververvetting minder verbetering lieten zien.

In tegenstelling tot leververvetting was lever verlittekening wél onafhankelijk geassocieerd met een hoger risico op HVZ onder RA-patiënten. Patiënten met hogere FIB-4-scores hadden hogere cIMT-waarden, een hogere systolische bloeddruk en hogere waarden van atherogene lipoproteïneresten. Dit suggereert dat lever verlittekening bijdraagt aan het cardiovasculaire risico, zelfs bij patiënten die niet obees zijn en geen diabetes hebben. Opvallend was dat het gebruik van hydroxychloroquine (HCQ) samenhing met lagere FIB-4-scores, wat wijst op een mogelijk beschermend effect van het medicijn tegen lever verlittekening. Deze bevindingen laten zien dat FIB-4 een nuttige marker is om RA-patiënten met verhoogd HVZ-risico te identificeren en dat medicamenteuze behandeling de levergezondheid in deze populatie kan beïnvloeden.

Samengevat toont dit proefschrift dat MASLD en lever verlittekening niet alleen veel voorkomen, maar ook klinisch relevant zijn voor diverse hoogrisicopatiënten. Vroege opsporing met niet-invasieve tests, gecombineerd met leefstijl- en medicamenteuze interventies, kan zowel de levergezondheid als het HVZ-risico verbeteren. Deze bevindingen bieden een basis voor gepersonaliseerde risicobeoordeling, preventieve strategieën en de integratie van gestructureerde leverscreening in zowel de eerstelijns- als de specialistische zorg. Daarbij ligt het belang van gewichtsbeheersing centraal om MASLD en het bijbehorende cardiometabole risico te verminderen.

APPENDIX C. LIST OF ABBREVIATIONS

1H-MRS	Proton Magnetic Resonance Spectroscopy
ACE inhibitor	Angiotensin-Converting Enzyme inhibitor
AHA	American Heart Association
AI	artificial intelligence
ALT	alanine aminotransferase
Apo-AI	apolipoprotein AI
ApoB	apolipoprotein B
ApoB-48	apolipoprotein B48
anti-CCP	anti-cyclic citrullinated peptide
anti-TNF	anti-tumor necrosis factor
ARB	angiotensin II receptor blocker
AST	aspartate aminotransferase
asCVD	atherosclerotic cardiovascular disease
BMI	body mass index
BP	blood pressure
C3	complement component 3
CABG	coronary artery bypass graft
CAC	coronary artery calcification
CAD	coronary artery disease
CAP	controlled attenuation parameter
CCA	common carotid artery
CI	confidence interval
cIMT	carotid intima media thickness
CRP	C-reactive protein
CT	computed tomography
CV	cardiovascular
CVA	cerebrovascular accident
CVD	cardiovascular disease
DAS28	Disease Activity Score wherein 28 joints are evaluated
dB/m	decibel/meter
DMARDs	disease-modifying antirheumatic drugs
DNL	de novo lipogenesis
DP	digital pathology
EASD	European Association for the Study of Diabetes
EASL	European Association for the Study of the Liver
EASO	European Association for the Study of Obesity
eGFR	estimated glomerular filtration rate

EHRs	electronic health records
ELF	Enhanced Liver Fibrosis
FDA	Food and Drug Administration
FGF21	fibroblast growth factor 21
FIB-4	fibrosis-4 index
FLI	Fatty Liver Index
FRANCIS	Franciscus Rheumatoid Arthritis and Cardiovascular Intervention Study
GCP	Good Clinical Practice
GH	growth hormone
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-Like Peptide-1
GGT	gamma-glutamyl transferase
GRIPonMASH	Global Research Initiative for Patient Screening on MASH
HbA1c	Hemoglobin A1c
HCC	hepatocellular carcinoma
HCQ	hydroxychloroquine
HSCs	hepatic stellate cells
HDL-c	high density cholesterol
HF	heart failure
HIV	Human Immunodeficiency Virus
HR	hazard ratio
HRQoL	health-related quality of life
HS	hepatic steatosis
HSI	Hepatic Steatosis Index
ICF	informed consent form
IFSO	International Federation for the Surgery of Obesity
IGF-1	Insulin-Like Growth Factor 1.
Insulin s.c.	subcutaneous insulin
IPAQ-SF	International Physical Activity Questionnaire Short Form
IQR	interquartile range
IR	insulin resistance
kPa	kilopascal
LAP	Lipid Accumulation Product
LDL-c	low density cholesterol
LFS	Liver Fat Score
lnLAP	natural logarithm of the Lipid Accumulation Product (LAP)
LSM	liver stiffness measurement
MAF-5	Metabolic Dysfunction-Associated Fibrosis-5 score
MASH	metabolic dysfunction-associated steatohepatitis

MASLD	metabolic dysfunction-associated steatotic liver disease
MetS	metabolic syndrome
MRE	Magnetic Resonance Elastography
MRI	Magnetic Resonance Imaging
MRI-PDFF	MRI-PDFF – Magnetic Resonance Imaging – Proton Density Fat Fraction
MTX	methotrexate
NB	naltrexone/bupropion
NA	not available
NAFLD	non-alcoholic fatty liver disease
NAS	NAFLD Activity Score
NFS	NAFLD Fibrosis Score
NIT	non-invasive test
NSAID	non-steroidal anti-inflammatory drug
OADs	oral antidiabetic drugs
OR	odds ratio
PAD	peripheral arterial disease
PPAR	Peroxisome Proliferator-Activated Receptor
PCSK9	Proprotein Convertase Subtilisin/Kexin Type 9
(P)HCP	(primary) healthcare provider
PIF	patient information folder
PPI	proton pump inhibitor
PTCA	percutaneous transluminal coronary angioplasty
PWV	pulse wave velocity
RA	rheumatoid arthritis
RCT	randomized controlled trial
SD	standard deviation
SGLT2	Sodium-Glucose Cotransporter 2
SR	sustained-release
SSB	sugar-sweetened beverages
SU derivate	sulfonylurea derivative
T1DM	type 1 diabetes mellitus
T2D	type 2 diabetes
T2DM	type 2 diabetes mellitus
TE	transient elastography
TG	triglycerides
THR-β	thyroid hormone receptor-β
TIA	transient ischemic attack
TSH	Thyroid-Stimulating Hormone
TZDs	thiazolidinediones
VCTE	vibration-controlled transient elastography
VIF	Variance Inflation Factor
WC	waist circumference
WHO	World Health Organization

APPENDIX D. PHD PORTFOLIO

PhD candidate: Alina N. Saidi
Promotor: Professor dr. A.J. van der Lelij
Promotor: Dr. M. Castro Cabezas
Department: Internal Medicine

Courses		**ECT**
2024	Fibroscan Training, Echosens	**1**
2023	Scientific Integrity, Erasmus MC	**0.3**
2023	Biostatistics, Amsterdam AMC	**1**
2023	PhD Career Event: Shaping your future, Erasmus MC	**1**
2022	Fibroscan Training, Echosens	**1**
2022	Good Clinical Practice course, Franciscus Gasthuis & Vlietland	**1**

Conferences		**ECT**
2025	European Association for the Study of Diabetes (EASD), Vienna	**1**
2025	European Association for the Study of the Liver (EASL), Amsterdam	**1**
2025	GRIPonMASH annual meeting, Utrecht	**1**
2024	MASH symposium, Rotterdam	**1**
2024	Annual Dutch Diabetes Research Meeting (ADDRM), Wageningen	**1**
2024	European Association for the Study of the Liver (EASL), Milan	**1**
2024	92nd European Atherosclerosis Society (EAS), Lyon	**1**
2024	GRIPonMASH kick-off meeting, Sevilla	**1**
2023	European Association for the Study of the Liver (EASL), Prague	**1**
2023	European Association for the Study of the Liver (EASL), Vienna	**1**
2022	European Association for the Study of the Liver (EASL), Dublin	**1**

Presentations		**ECT**
2025	Oral presentation EASD Vienna	**1**
2025	Oral presentation Annual GRIPonMASH meeting Utrecht	**1**
2025	Online informatiebijeenkomst huisartsen GRIPonMASH	**1**
2025	Journal club, P3N network	**1**
2024	Oral presentation ADDRM	**1**
2024	Oral presentation EAS Lyon	**1**
2024	Poster presentation EASL Milan	**1**
2024	Poster presentation Wetenschapsdag Franciscus Gasthuis & Vlietland	**1**
2024	Online informatiebijeenkomst huisartsen GRIP on MASH	**1**
2024	Presentatie Beatrixzaal internisten en arts-assistenten, Franciscus Gasthuis & Vlietland	**1**
2024	Presentatie huisartsenpraktijk GRIPonMASH, locatie huisartsenpraktijk De Kleine Heemtuin	**1**
2024	Presentatie huisartsenpraktijk GRIPonMASH, locatie huisartsenpraktijk Damzicht	**1**
2024	Online informatiebijeenkomst huisartsen GRIPonMASH	**1**
2024	Journal club, P3N network	**1**
2023	Poster presentation EASL Prague (2 posters)	**2**
2023	Presentatie huisartsenpraktijk GRIPonMASH, locatie huisartsenpraktijk Huisartsen Schiedam Noord	**1**
2023	Presentatie huisartsenpraktijk GRIPonMASH, locatie huisartsenpraktijk GC Ommoord	**1**
2023	Online informatiebijeenkomst huisartsen GRIPonMASH	**1**
2023	Journal club, P3N network	**1**
2022	Presentatie pathologen Pathan, Franciscus Gasthuis & Vlietland	**1**

Grants and bursaries	
2024	Registration bursary, European Association for the Study of the Liver (EASL) *Included congress registration fee waiver and free EASL membership for one year.*
2023	Full bursary for poster presentations, European Association for the Study of the Liver (EASL), Prague *Included €650 travel grant, congress registration fee waiver and free EASL membership for one year.*

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APPENDIX F. ABOUT THE AUTHOR

Alina Nilab Saidi is geboren in Kabul en groeide op in Leiderdorp. Na het Stedelijk Gymnasium in Leiden volgde zij zowel de bachelor als de master Gezondheidswetenschappen aan de Vrije Universiteit Amsterdam. Tijdens haar master specialiseerde zij zich in Infectieziekten en Publieke Gezondheid. Hierna startte zij een promotietraject aan het Franciscus Gasthuis & Vlietland, in samenwerking met het Erasmus Medisch Centrum. Tijdens de afronding van haar promotietraject is zij gestart als postdoctoraal onderzoeker aan het Leids Universitair Medisch Centrum.