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Prevalence of Subclinical Cardiovascular Disease in Patients with Nonalcoholic Fatty Liver Disease: Analysis of the Paracelsus 10,000 Cohort Study

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Highlights of the Study

- Fatty liver index, fibrotic nonalcoholic steatohepatitis index, and fibrosis-4 index were linked to increased odds of coronary artery calcification (CAC).
- Nonalcoholic fatty liver disease is linked to increased odds of CAC, regardless of confounding factors like age, sex, metabolic syndrome, and SCORE2.
- Liver fat and assessment of fibrosis offer additional insights for evaluation of risk of cardiovascular disease.

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Keywords
Nonalcoholic fatty liver disease · Coronary artery calcification · Fibrosis-4 index · Fatty liver index · FNI fibrotic nonalcoholic steatohepatitis index · Paracelsus 10,000 cohort study

Abstract
Background: In patients with nonalcoholic fatty liver disease (NAFLD), cardiovascular diseases are more often the cause of death than the liver disease itself. However, the prevalence of atherosclerotic manifestations in individuals with NAFLD is still uncertain. This study aimed to explore the association between NAFLD and coronary artery calcification (CAC) in a Central European population. ***Methods:*** A total of 1,743 participants from the Paracelsus 10,000 study were included. The participants underwent CAC scoring and were assessed for fatty liver index (FLI), fibrosing nonalcoholic steatohepatitis (NASH) index (FNI), and fibrosis-4 index (FIB-4 score), which are indicators for steatosis and fibrosis. Multivariable logistic regression models were calculated. ***Results:*** Results revealed an association between liver steatosis/fibrosis and CAC. An FLI >60 was associated with higher odds of NAFLD (odds ratio [OR] 3.38, 95% CI: 2.61–4.39, $p < 0.01$) and increased prevalence of CAC score >300 compared to FLI <30 (9% vs. 3%, $p < 0.01$), even after adjusting for traditional cardiometabolic risk factors. While the crude ORs of the FIB-4 scores ≥ 1.3 and FNI score were significantly associated with increased odds of CAC, they became nonsignificant after adjusting for age, sex, and MetS. ***Conclusion:*** This study reveals a significant association between NAFLD and CAC. The findings suggest that assessing liver fat and fibrosis could enhance the assessment of cardiovascular risk, but further research is needed to determine whether hepatic fat plays an independent role in the development of atherosclerosis and whether targeting liver steatosis can mitigate vascular risk.

Introduction

Recent studies have shown that nonalcoholic fatty liver disease (NAFLD) leads to increased liver-related morbidity and mortality [1]. Affecting up to 30% of the adult population in western countries, NAFLD is a worldwide public health problem, and there is ample evidence that its prevalence will increase in the future [1]. Recent research has identified metabolic dysfunction as a fundamental factor in fatty liver disease development [2]. This led to the introduction of the term "metabolic-dysfunction associated fatty liver disease" (MAFLD) to better reflect the underlying pathophysiology and associated risks of this condition [2]. MAFLD increases the risk of cardiovascular diseases (CVDs), including atherosclerosis and its complications [2, 3]. Chronic inflammation, oxidative stress, insulin resistance, and dyslipidaemia contribute to these risks [2, 3]. Given the rising prevalence of MAFLD and its impact on public health, screening for fatty liver and associated cardiovascular complications is crucial [3].

Noninvasive methods like Systematic Coronary Risk Evaluation – Score 2 (SCORE2) and CT with the Agatston score can be valuable in risk assessment [4, 5]. If coronary artery calcification (CAC) detected through computer tomography indicates subclinical CVD in patients with fatty liver, further invasive diagnostic pathways are recommended for screening [6, 7]. This leads to a more aggressive approach in treating risk factors to prevent cardiovascular mortality [7, 8]. Further research is required to establish clear guidelines and criteria for identifying at-risk individuals and determining optimal screening strategies, addressing important clinical and mechanistic questions. Recent research has generated conflicting data regarding the screening of CVD [9–11] and these inconsistencies have led to varying recommendations from different expert groups regarding the screening of CVD in patients with NAFLD [8, 9]. Therefore, it is not surprising to see these divergent viewpoints considering the existing uncertainty surrounding this topic. The European Association for the Study of the Liver recommends routine screening for CVD in NAFLD patients, while the American Association for the Study of Liver Diseases highlights NAFLD as a cardiovascular risk marker without a specific recommendation for screening [9, 12]. However, a recent statement in their guidelines on NAFLD and CVD risk suggests that NAFLD should be considered as a cardiovascular risk marker and risk enhancer [13]. The Asia-Pacific Working Party on Nonalcoholic Fatty Liver Disease acknowledges CVD as an extrahepatic manifestation of NAFLD, but due to insufficient data, routine screening for patients with associated disorders is not supported, and risk assessment should be tailored to individual patient characteristics and needs [10, 11]. Additionally, recent studies on subclinical CVD have shown that the CAC score is a well-established risk factor and a surrogate marker for coronary atherosclerotic burden and an independent risk marker for coronary heart disease and events [6, 7]. According to the American College of Cardiology and the American Heart Association, the coronary artery calcium score was reported to be an appropriate marker for CVD risk [8].

The CAC score is readily available in local settings, including in smaller hospitals without invasive cardiology capabilities [14].

For further evaluation of risk, the European Society of Cardiology recommends the calculation of the SCORE2 to predict the 10-year risk of first-onset CVD in the European population [5]. This algorithm provides an estimation of the risk for both fatal and nonfatal CVD events combined [5].

While the exact strategy for diagnosis and risk stratification of NAFLD remains unclear, the fatty liver index (FLI) for evaluation of steatosis, the fibrosing nonalcoholic steatohepatitis (NASH) index (FNI) and the fibrosis-4 index (FIB-4) for evaluation of liver fibrosis have proven useful in population-based settings [15–17]. Therefore, we further evaluated the relationship between CVD and NAFLD by considering hepatic steatosis (expressed as FLI), steatohepatitis (expressed as FNI), and hepatic fibrosis (expressed as FIB-4). Investigating noninvasive markers as surrogate markers for NAFLD is important for identifying patients in clinical practice, and this study provides new insights by examining the association between these markers and CAC score, contributing to scientific knowledge and clinical recommendations for CVD risk assessment and prevention in NAFLD patients.

Methods

In this retrospective data analysis, the data used were obtained from the Paracelsus 10,000 cohort study. Data for this study were collected and entered from 10,044 participants into the Paracelsus 10,000 registry between 2013 and 2020 [18]. The study was designed as a prospective cohort study with the aim of investigating the health status of individuals aged between 40 and 70 years in and around the city of Salzburg [18]. For this analysis, the baseline data from the cohort were utilized. The research team has access to all data and warrants its integrity. The guidelines for ethics and data management corresponded to local standards and the study has been performed in accordance to the standards of the Declaration of Helsinki. The study was approved by the Local Ethics Committee (number: 415-E/1521/6-2012).

To obtain a representative sample, the subjects were randomly selected from the local population registry. The subjects were between 40 and 69 years old, with an equal distribution of women and men. Participation in the study included anthropometric measurements (height, weight, waist circumference, and blood pressure) as well as laboratory tests and stratification for CVD. Biochemical parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), hemoglobin A1c (HbA1c), total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and platelet count. Screening for CVD included coronary calcium scoring by computed tomography. This scan enables the quantification of CAC using the Agatston score, which also provides a measure of the extent of CAC [4]. The higher the Agatston score, the higher the risk of serious coronary events. Everyone in 5 participants was randomly selected for measurement of CAC due to limited resources [4]. Steatosis was defined by the FLI, which is calculated by body mass index (BMI), the waist circumference, the GGT, and the triglycerides [15]. The fibrotic NASH index (FNI) incorporates parameters such as AST, HDL, and HbA1c and was used to assess hepatic inflammation [16]. Liver fibrosis was estimated by the FIB-4 score [17]. Its calculation includes age, ALT, AST, and platelet count [17].

Inclusion/Exclusion Criteria

Figure 1 displays a flowchart outlining the inclusion and exclusion criteria, which were carefully defined to maintain the study's integrity. The study specifically focused on individuals aged 40–69 with complete assessments for FLI, FNI, FIB-4, and available CAC data, excluding those with missing scores.

Definition of Terms

NAFLD and associated steatosis were defined by the FLI score in the first step for continuous analysis. Secondly three groups were defined for categorial analysis according to Bedogni et al. [15] as follows: a score <30 was defined as a low FLI score (low FLI group); (i) ≥30 and <60 was defined as an intermediate FLI group and (ii) a score ≥60 was defined as an advanced FLI group. Steatohepatitis was defined by the FNI score as a continuous parameter [16]. The FNI was calculated as a percentage for continuous analysis. Liver fibrosis was defined by the FIB-4 score, a well-validated noninvasive assessment of liver fibrosis biomarker [17]. The FIB-4 score was calculated as published (age × AST [U/L]/platelet count [×109/L] × $\sqrt{}$ALT [U/L] [17]. The FIB-4 score was used both as continuous parameter and as a dichotomized parameter with a score <1.3 reliably excluding significant fibrosis, while >2.67 indicates a high likelihood of advanced fibrosis [17]. Subjects were categorized into a group reliably excluding fibrosis (FIB-4 <1.3) versus a group with fibrosis (elevated FIB-4 group, FIB-4 ≥1.3) [17].

The CAC score was taken as a surrogate marker for CVD and was calculated using the Agatston scoring method [4]. Subjects were dichotomized into a group with CAC (CAC score >0) and without (CAC score = 0) [4]. Further, SCORE2 was calculated according to European Society of Cardiology guidelines to estimate the 10-year risk of cardiovascular events [5].

Anthropometric Evaluation and Laboratory Data

BMI was calculated using weight and height to provide an estimate of body fat in males and females according to international standards. MetS was defined by 3 out of the following 5 criteria: (i) as central obesity with a waist circumference ≥102 cm in male participants or ≥ 88 cm in female, (ii) triglycerides ≥ 150 mg/dL (1.7 mmol/L), (iii) HDL < 40 mg/dL (1.04 mmol/L) in male or < 50 mg/dL (1.30 mmol/L) in female participants, (iv) blood pressure ≥130/≥ 85 mm Hg, and (v) fasting glucose ≥6.1 mmol/L [19]. Furthermore, the definition of dyslipidaemia was one or more of the following: (i) triglycerides ≥150 mg/dL (in fasting blood sample), (ii) total cholesterol >200 mg/dL, (iii) LDL >130 mg/dL and/or HDL <50 mg/dL in male and <40 mg/dL in female participants.

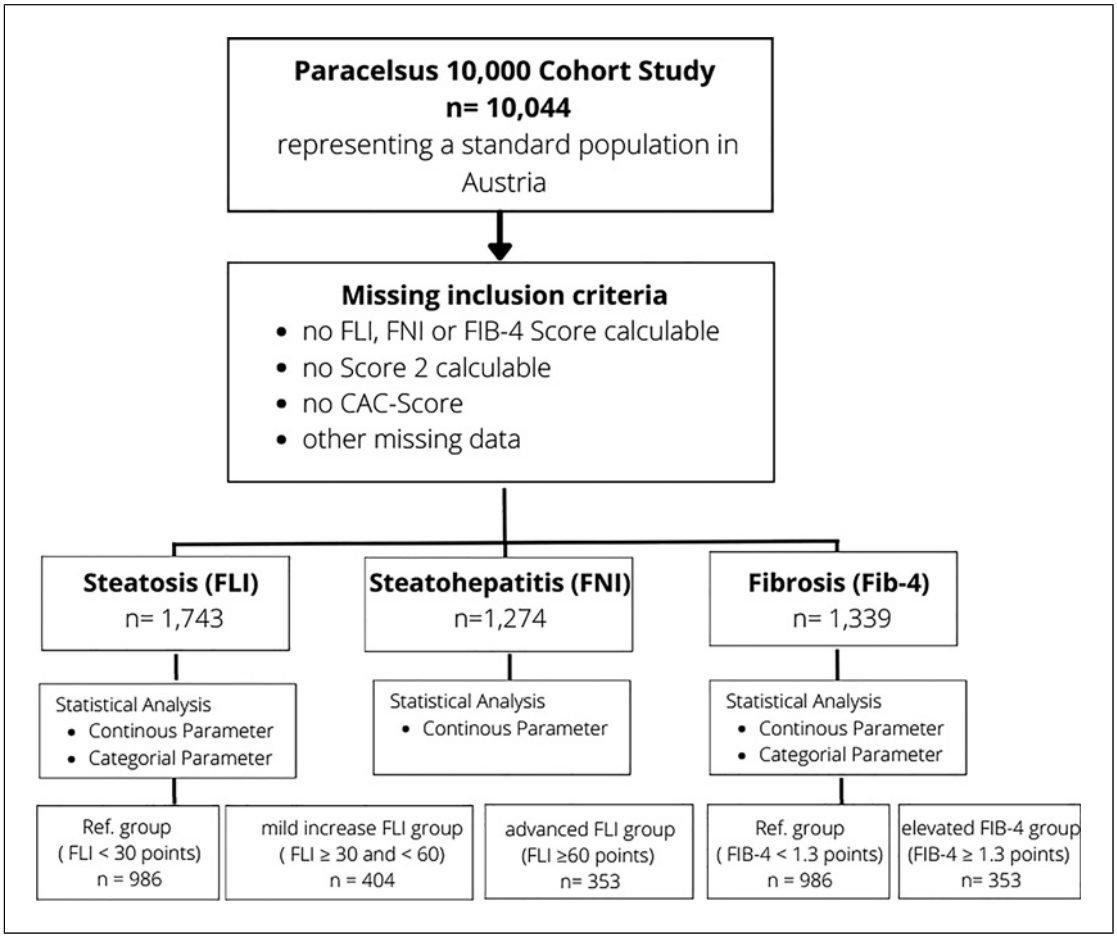

Fig. 1. Flowchart showing inclusion and exclusion criteria of retrospective data analysis. FLI, fatty liver index; FNI, fibrotic non-steatohepatitis index; FIB-4, fibrosis-4 index; SCORE2, systematic coronary risk evaluation; CAC, coronary artery calcification; *n*, number.

Diabetes was characterized as HbA1c > 6.4% or insulin or glucose-lowering medications. Hypertension was defined as either blood pressure >140 mm Hg systolic and 90 mm Hg diastolic or anti-hypertensive medication intake.

Statistical Analyses

A p value <0.05 was considered statistically significant. Continuous parameters were described by mean and standard deviation and binary variables by percentages (%). Significance levels were tested using Wilcoxon tests for continuous variables and χ^2 tests for binominal data to show differences in mean.

To show the association between NAFLD and CAC, multivariable logistic regression modules were performed. We used CAC >0 as binary dependent variable and co-variables were based on previous literature and our own clinical experience and forced the variables into the models. In the first step, we performed regression models with continuous variables for FLI, FNI, and FIB-4 and in the second with categorical outcomes, which were adjusted by four different models: model 1 describes the univariate logistic regression, model 2 was adjusted for SCORE2 alone, model 3 for age, sex, and MetS, and model 4 for age, sex, MetS, BMI, and alcohol intake in g/d.

Results

A total of 1,743 patients had FLI or FNI data available, with 1,274 patients having FNI data and 1,339 patients having FIB-4 data. All of these patients also had CAC scores available. Demographic characteristics are given in Tables 1 and 2. The study found significant differences in the presence of CAC score >300 among participants based on their FLI categories. In the FLI <30 group, 3% had a CAC score >300, while in the FLI >60 group, 9% had a CAC score >300, with a p value <0.01. Similar patterns were observed for CAC scores between 1–300,

Table 1. Baseline characteristics of the study population stratified according to their FLI score into a low, an intermediate FLI group, and an advanced FLI group

	FLI <30 (*N* = 716)	FLI ≥30–<60 (*N* = 404)	FLI ≥60 (*N* = 550)	*p* value*
Age, years (SD)	55 (4)	55 (3)	56 (4)	0.09
Sex male, %	31	59	73	<0.01
ALT, U/L (SD)	20 (8)	25 (12)	34 (17)	0.02
AST, U/L (SD)	23 (6)	24 (7)	27 (10)	<0.01
GGT, U/L (SD)	19 (11)	33 (27)	50 (45)	<0.01
HbA1c, % (SD)	5.4 (0.3)	5.5 (0.4)	5.7 (0.7)	<0.01
Total cholesterol, mg/dL (SD)	211 (37)	215 (38)	219 (40)	<0.01
Triglycerides, mg/dL (SD)	81 (30)	115 (46)	177 (112)	<0.01
HDL, mg/dL (SD)	74 (17)	61 (15)	52 (13)	<0.01
LDL, mg/dL (SD)	136 (35)	147 (36)	152 (38)	<0.01
Platelets, G/L (SD)	251 (50)	252 (59)	249 (53)	0.53
BMI, kg/m^2 (SD)	23 (2)	27 (2)	31 (4)	<0.01
Diabetes mellitus, %	1	3	5	<0.01
Hypertension (yes), %	11	19	34	<0.01
Metabolic syndrome, %	0	7	41	<0.01
FIB-4 score >1.3 points, %	29	27	23	0.13
CAC score, % (*n*)				<0.001
CAC 0	71 (509)	62 (250)	48 (264)	
CAC 1–300	26 (183)	32 (131)	43 (238)	
CAC >300	3 (24)	6 (23)	9 (48)	

N, number; SD, standard deviation; HbA1c, hemoglobin A1c; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FIB-4 score, fibrosis-4 index; BMI, body mass index; CAC, coronary artery calcification score. **p* value describes the difference between low and advanced FLI groups.

with percentages of 26%, 32%, and 43% in the FLI <30, FLI 30–60, and FLI >60 groups, respectively. Participants with a FLI <30 also had a significantly higher proportion of individuals with a CAC score of zero compared to higher FLI scores. The same relationship was assessed using the FIB-4 score, with 5% of participants with an FIB-4 <1.3 having a CAC score >300 compared to 8% in the elevated score group. Additionally, 31% of participants with FIB-4 <1.3 had a CAC score in the 1–300 range, compared to 39% in the elevated score group, with a significantly higher proportion of individuals with a CAC score of zero in the FIB-4 <1.3 group compared to the elevated score group.

Association between FLI and CAC

Table 3 shows the association between FLI score/FIB-4 score and CAC. In the univariate analysis, the FLI score showed a significant association with an increased risk of CAC. This association persisted even after adjusting for confounding factors in models 2–3. However, after further adjustment for model 4, the association between FLI and CAC became nonsignificant. Categorical analysis revealed that in the intermediate FLI group, there was a twofold higher odds for CAC compared to the low FLI group, with an odds ratio (OR) of 1.98 (95% CI: 1.49–2.63, p value: <0.01) as shown in Table 4. The advanced FLI group had 3.38 higher odds for CAC compared to the low FLI group. In model 2 (adjustment for SCORE2) there was no association between the intermediate FLI group and CAC. In models 3 and 4 (adjustment for age, sex, and MetS) there was no association between the intermediate FLI group and CAC.

Association between FNI and CAC

In the univariate analysis, the FNI score was significantly associated with a higher risk of CAC (OR 1.05, 95% CI: 1.03–1.06, $p < 0.01$, Table 3). After adjusting for SCORE2 in model 2, and for age, sex, and metabolic syndrome in model 3, the association remained significant. However, in model 4, which included additional adjustments for age, sex, MetS, BMI, and alcohol intake, the association became nonsignificant (adjusted OR 1.01, 95% CI: 1.00–1.02, $p = 0.10$).

Association between FIB-4 and CAC

The FIB-4 score (OR, 1.88, 95% CI: 1.39–2.55, $p < 0.01$) was associated with higher odds for CAC in the univariate analysis. In model 2, the FIB-4 score remained associated

Table 2. Baseline characteristics of the study population stratified according to their FIB-4 score into low and elevated FLI groups

	FIB-4 <1.3 (*N* = 986)	FIB-4 ≥1.3 (*N* = 353)	*p* value
Age, years (SD)	55 (4)	56 (3)	<0.01
Sex male, %	51	61	<0.01
ALT, U/L (SD)	25 (11)	28 (19)	0.02
AST, U/L (SD)	23 (5)	30 (11)	<0.01
GGT, U/L (SD)	32 (26)	38 (51)	0.05
HbA1c, % (SD)	5.5 (0.4)	5.5 (0.5)	<0.01
Total cholesterol, mg/dL (SD)	215 (38)	212 (38)	0.14
Triglycerides, mg/dL (SD)	114 (72)	117 (80)	0.09
HDL, mg/dL (SD)	62 (18)	65 (19)	0.02
LDL, mg/dL (SD)	145 (37)	140 (35)	0.18
Platelets, G/L (SD)	269 (49)	206 (36)	<0.01
BMI, kg/m^2 (SD)	27 (5)	26 (5)	0.12
Diabetes mellitus, %	3	3	0.57
Hypertension (yes), %	20	22	0.33
Metabolic syndrome, %	16	14	0.38
FLI score [points] (SD)	45 (30)	42 (30)	0.04
CAC score, % (*N*)			<0.01
CAC 0	64 (632)	53 (187)	
CAC 1–300	31 (305)	39 (139)	
CAC >300	5 (49)	8 (27)	

N, number; SD, standard deviation; HbA1c, hemoglobin A1c; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FLI, fatty liver index; BMI, body mass index; CAC score, coronary artery calcification score.

Table 3. Association between FLI score/FIB-4 score and CAC: continuous variables

	FLI score	FNI score	FIB-4 score
	OR (95% CI, *p* value)	OR (95% CI, *p* value)	OR (95% CI, *p* value)
Model 1	1.02 (1.017–1.02, <0.01)	1.05 (1.04–1.06, <0.01)	1.88 (1.39–2.55, <0.01)
Model 2	1.01 (1.00–1.01, <0.01)	1.01 (1.00–1.02, 0.05)	1.78 (1.26–2.51, <0.01)
Model 3	1.01 (1.00–1.01, <0.01)	1.02 (1.00–1.03, 0.02)	1.21 (0.86–1.70, 0.28)
Model 4	1.00 (1.00–1.01, 0.07)	1.01 (1.00–1.02, 0.10)	1.29 (0.91–1.83; 0.15)

Model 1 shows the univariate crude OR for CAC. Model 2 shows aOR after adjustment for SCORE2. Model 3 shows aOR after adjustment for age, sex, and MetS. Model 4: shows aOR after adjustment for age, sex, MetS, BMI, and alcohol intake in grams per day. FLI, fatty liver Index; FIB-4, fibrosis-4 index; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; BMI, body mass index.

with CAC. However, when adjusting for age, sex, and metabolic syndrome (model 3), and further adjusting for BMI and alcohol intake (model 4), no independent association between FIB-4 score and CAC was observed. Participants with a FIB-4 ≥1.3 had a significant higher risk for CAC as compared to the low FIB-4 group (OR, 1.55, 95% CI: 1.21–1.98, $p < 0.01$). After adjusting for model 3 and 4, the association between an FIB-4 score ≥1.3 and CAC became nonsignificant (Table 4).

Discussion

This study showed that individuals with steatosis, as determined by the FLI, are at an elevated overall risk for CAC. Higher FLI scores were associated with higher odds of subclinical CAC. Even after adjustment for cardiometabolic risk factors using SCORE2, there was still an independent association between liver steatosis and CAC. In the stratified analysis, when comparing patients with

Table 4. Association between FLI score/FIB-4 score and CAC: categorial variables model 1 shows the univariate crude OR for CAC

	FLI <30	FLI: ≥30–<60	FLI: ≥60	FIB-4 <1.3	FIB-4: ≥1.3
		OR (95% CI, *p* value)	OR (95% CI, *p* value)		OR (95% CI, *p* value)
Model 1	Ref	1.98 (1.49–2.63, <0.01)	3.38 (2.61–4.39, <0.01)	Ref	1.55 (1.21–1.98, <0.01)
Model 2	Ref	1.28 (0.94–1.75, 0.12)	1.44 (1.05–1.97, 0.02)	Ref	1.54 (1.17–2.02, <0.01)
Model 3	Ref	1.29 (0.95–1.77, 0.11)	1.67 (1.20–2.33, <0.01)	Ref	1.21 (0.92–1.59, 0.18)
Model 4	Ref	1.18 (0.90–1.60, 0.27)	1.44 (1.00–2.07, 0.05)		1.19 (0.89–1.60, 0.24)

FLI, fatty liver index; FIB-4, fibrosis-4 index; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; Ref, reference; BMI, body mass index. Model 2 shows aOR after adjustment for SCORE2. Model 3 shows aOR after adjustment for age, sex, and MetS. Model 4 shows aOR after adjustment for age, sex, MetS, BMI, and alcohol intake in grams per day.

low FLI scores to those with medium and high FLI scores, we observed that mild steatosis was not associated with an increased cardiovascular risk after adjustment for SCORE2. However, advanced steatosis was significantly associated with a significantly higher risk for CVD. This risk remained even after adjustment for both the SCORE2 and the presence of metabolic syndrome. The study findings indicate a positive association between hepatic steatosis and the risk of CVD, independent of traditional cardiac risk factors (SCORE2) and cardiometabolic risk factors (metabolic syndrome). Furthermore, there was an initial association between the (FNI) score and an increased risk of CAC. However, after adjusting for various confounding factors, this association became nonsignificant. These results highlight the complex interplay between steatohepatitis and cardiometabolic risk factors. Similarly, liver fibrosis, as assessed by FIB-4, was associated with an increased risk for CAC. This positive association between liver fibrosis and CVD risk remained statistically significant even after adjusting for SCORE2. However, after accounting for cardiometabolic risk factors through multivariable adjustment, there was no significant association between the FIB-4 score and CAC. Therefore, out data suggest that liver fibrosis may be independently associated with CVD risk, specifically in relation to traditional cardiac (SCORE2), but not cardiometabolic risk factors (metabolic syndrome). It is important to note that none of our patients had an FIB-4 score greater than 3.25. Consequently, we acknowledge that our data may be underpowered to conclusively assess the relationship between advanced fibrosis and CVD.

Our findings support the concept that in addition to traditional CV risk factors such as hypertension, dyslipidaemia or smoking, NAFLD is a further independent risk for CAC. Results align with recent studies that have demonstrated an increased risk for CVD as NAFLD progresses to advanced fibrosis [20, 21]. Moreover, Lee et al. [20] conducted a study indicating that patients with more severe fibrosis stages and higher FIB-4 scores have a heightened risk for CAC progression compared to those in the normal FIB-4 group.

The study included 1,173 asymptomatic adults with CAC scores from 2007 to 2013, and found that 25% of participants developed CAC during the follow-up period [20]. Higher FIB-4 scores were associated with CAC progression, particularly in individuals with NAFLD. The OR for CAC progression was higher in subjects with NAFLD and intermediate/high FIB-4 scores compared to those without NAFLD [20]. Similar results were observed using the NAFLD fibrosis score (NFS) [20]. In contrast to the study by Lee et al. [20] , the current study expanded the analysis by also incorporating the FLI score and the FNI score to assess the risk for CAC. Ballestri et al. [21] demonstrated the association between liver fibrosis biomarkers and CAC, highlighting the complex relationship between liver fibrosis, cardiovascular risk, and CAC progression in patients with NAFLD.

However, expert groups have explained these findings by attributing them to higher plasma markers of oxidative stress and inflammation originating from the diseased liver, which contributes to a systemic inflammatory and prothrombotic state [3]. Chronic inflammation in patients with NAFLD has been shown to trigger endothelial dysfunction, which promotes the development of coronary atherosclerosis [3]. Nonalcoholic steatohepatitis has also been associated with prothrombotic factors stimulated by inflammatory cytokines, leading to an abnormal lipid metabolism, chronic inflammation, and oxidative stress [3].

Taking all these factors in consideration, it may be justified to screen for CVD in patients with NAFLD. The Danish Cardiovascular Screening Trial conducted a randomized trial with over 45,000 patients, which demonstrated that cardiovascular screening, including cardiac imaging and screening for cardiometabolic risk

factors followed by appropriate treatment, if necessary, reduced the incidence of stroke, myocardial infarction or death by 11% in individuals aged 65–69 years ($p = 0.007$) [22]. The study also found that screening for CVD resulted in higher rates of prophylactic treatment with antithrombotic agents (22.9% vs. 8.3%; HR 3.12; 95% CI: 2.97–3.28; $p < 0.001$) and lipid-lowering agents (20.7% vs. 9.0%; HR 2.54; 95% CI: 2.42–2.67; $p < 0.001$) [22].

However, other studies have reported contrasting findings in this regard [23]. Kirby et al. [23] found no relationship between CVD and NAFLD when diagnosed by imaging techniques. They observed associations between BMI >30 and HbA1c > 6.5% with CVD [23]. Nevertheless, a subgroup analysis revealed a connection between FIB-4 and CAC [23]. This finding further strengthens our observation that participants with an FIB-4 score ≥1.3 had a higher risk for CAC after adjustment for Score2.

Limitations of our study include the lack of liver biopsies or imaging studies due to their impracticality in larger populations [24]. However, we utilized the FLI, FNI, and FIB-4 scores as accessible and noninvasive screening tools for liver fibrosis, considering their high safety, cost-effectiveness, and ease of use [15, 17]. Another limitation of our study is the possibility that medications used for diabetes and hypertension may also be prescribed for other indications, which could introduce confounding effects. While we believe that the impact of such cases is expected to be minimal given the large sample size, it is important to consider this potential confounding. Furthermore, we did not collect data on other aetiologies of chronic liver disease, such as autoimmune diseases or medication-induced liver diseases. By interpreting the results of this study, overfitting should also be considered, as both the FIB-4 and the SCORE are calculated using patients' age. The retrospective cross-sectional study design limits the generalizability of the correlation between NAFLD and CVD observed in our findings. Strengths of our study include the representative sample of an apparently healthy general population, the large number of high-quality Agatston scores, and the broad baseline characteristics, which made multivariable adjustment for known covariates possible.

Conclusions

This study demonstrates that NAFLD is associated with an increased risk of CVD, The severity of liver fat, steatohepatitis, and fibrosis, assessed by FLI, FNI, and FIB-4 scores, independently predict CAC. Our data suggest that assessment of liver fat and possibly fibrosis may serve as additional information in the assessment of CV risk in addition to risk assessment by SCORE2 variables. Further research is needed to establish causality and explore underlying mechanisms.

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Statement of Ethics

This study was conducted in accordance with the World Medical Association Declaration of Helsinki and was approved by the National Review Board 415-E/1521/6-2012. Written informed consent was obtained from all participants, and their identities were kept confidential. Informed consent was obtained from all subjects involved in the study.

Conflict of Interest Statement

Eugen Trinka has received personal fees from Arvelle Therapeutics, Inc., Argenx, Bial, Biogen, Biocodex, Böhringer Ingelheim, Eisai, Epilog, Everpharma, GlaxoSmithKline, GW Pharma, Jazz Pharmaceuticals, LivaNova PLC, Marinus Pharmaceuticals, Inc., Medtronic, NewBridge Pharmaceuticals, Novartis, Sandoz, Sanofi, Sunovion Pharmaceuticals, Inc., Takeda, UCB Pharma, and Xenon; grants from Austrian Science Fund (FWF), Bayer, Biogen, Eisai, European Union, GlaxoSmithKline, Novartis, Österreichische Nationalbank, Red Bull, and UCB Pharma; He is the CEO of NeuroConsult GmbH.; and has been a trial investigator for Eisai, GlaxoSmithKline, Marinus, Pfizer, and UCB Pharma; no COI related to the study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Conceptualization: Florian Koutny, Bernhard Wernly, Elmar Aigner, and Andreas Maieron; methodology: Florian Koutny, Bernhard Wernly, and Elmar Aigner; formal analysis: Bernhard

Wernly; investigation: Florian Koutny, Bernhard Wernly, and Elmar Aigner; preparation of the original draft: Florian Koutny; review and editing: Bernhard Wernly, Elmar Aigner, Andreas Maieron, Christian Datz, Sophie Gensluckner, Andrea Mega, Bernhard Iglseder, Patrick Langthaler, Vanessa Frey, Bernhard Paulweber, and Eugen Trinka; visualization: Florian Koutny; Supervision: Bernhard Wernly, Elmar Aigner, and Andreas Maieron; all authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. The data are not publicly available due to restrictions, which apply to the availability of these data, which were used under license for this study. Data are available with permission from LIFE Child, Leipzig, and the Leipzig Childhood Obesity Consortium.

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