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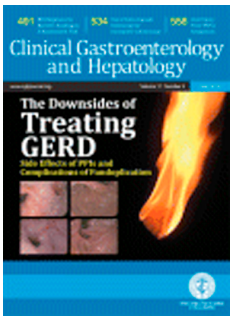

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Nonalcoholic Fatty Liver Disease and Fibrosis Associated With Increased Risk of Cardiovascular Events in a Prospective Study

Short title: Liver fibrosis and cardiovascular events

Francesco Baratta(1)*, Daniele Pastori(1)*, Francesco Angelico(2), Andrea Balla(3), Alessandro Maria Paganini(3), Nicholas Cocomello(1), Domenico Ferro(1), Francesco Violi(1,4), Arun J Sanyal (5), Maria Del Ben(1)**.**

***equal contribution; **joint senior authorship**

(1) I Clinica Medica, Department of Clinical Internal, Anesthetic and Cardiovascular Sciences,, Sapienza University of Rome, Viale del Policlinico 155, 00161 Rome, Italy.

(2) Department of Public Health and Infectious Diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy.

(3) Department of General Surgery and Surgical Specialties "Paride Stefanini", Sapienza University of Rome, Viale del Policlinico 155, 00161, Rome, Italy.

(4) Mediterranea Cardiocentro, Naples, Italy.

(5) Division of Gastroenterology, Department of Internal Medicine, Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA.

Correspondence to Dr Daniele Pastori, I Clinica Medica, Department of Internal Medicine and Medical Specialties, Sapienza University of Rome, Viale del Policlinico 155, 00161 Rome, Italy. Email: daniele,pastori@uniroma1.it

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List of abbreviations: AF: atrial fibrillation; ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUDIT: Alcohol Use Disorders Identification Test; BMI: body mass index: CABG: coronary artery bypass surgery; CVEs: cardiovascular events; FIB-4: fibrosis-4 score; GGT: gamma-glutamyl transferase; HDL: high density lipoprotein; HR: hazard ratio; IFG: impaired fasting glucose; IQR: interquartile range; LDL: low density lipoprotein; MELD: Model for End-Stage Liver Disease; MESA: Multi-Ethnic Study of Atherosclerosis; MetS: metabolic Syndrome; MI: myocardial infarction; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; NFS: NAFLD fibrosis score; PTA: percutaneous transluminal angioplasty; TIA; transitory ischemic attack; US: ultrasonography.

ABSTRACT

Background & Aims: Patients with non-alcoholic fatty liver disease (NAFLD) are at increased chance for cardiovascular events (CVEs). Severity of liver fibrosis is used to determine prognoses for patients with NAFLD, but little is known about the relationship between liver fibrosis and CVEs in the real world.

Methods: We analyzed data from the prospective observational progression of liver damage and cardiometabolic disorders in non-alcoholic fatty liver disease study, comprising 898 consecutive outpatients (mean age, 56.4±12.7 years; 37.5% women) screened for liver steatosis by ultrasound according to Hamagughi criteria. Liver fibrosis was defined as FIB-4 score greater than 2.67 and NAFLD fibrosis score greater than 0.676. After enrolment, patients were interviewed by phone every 6 months and examined every 12 months in the outpatient clinic, and CVEs were recorded (fatal or nonfatal ischemic stroke and myocardial infarction, cardiac or peripheral revascularization, new-onset arterial fibrillation and cardiovascular death). The primary outcomes were incidence rate of CVEs in patients with vs without NAFLD and factors associated with CVEs in patients with NAFLD.

Results: Over a median follow-up time of 41.4 months (3044.4 patient-years), 58 CVEs (1.9%/year) were registered. The rate of CVEs was higher in patients with (n=643, 2.1%/year) vs without NAFLD (n=255, 1.0%/year) (*P*=.066). In multivariable Cox proportional regression analysis, NAFLD increased risk for CVEs (hazard ratio [HR], 2.41; 95% CI, 1.06–5.47; *P*=.036), after adjustment for metabolic syndrome. Among patients with NAFLD, male sex, previous CVEs, metabolic syndrome and FIB-4 scores greater than 2.67 (HR, 4.02; 95% CI, 1.21–13.38; *P*=.023) were independently associated with risk of incident CVEs. NFS scores greater than 0.676 were also independently associated with risk of incident CVEs (HR, 2.35; 95% CI, 1.05–5.27; *P*=.038).

Conclusions: In an analysis of data from a study of patients screened for NAFLD and followed, individuals with NAFLD had more than a 2-fold increase in risk of CVEs, and those with liver fibrosis had a 4-fold increase in risk. In patients with NAFLD, liver fibrosis indexes were independently associated with risk of incident CVEs. ClinicalTrials.gov no:NCT04036357.

KEY WORDS: PLINIO study, NFS, prognostic factor, heart disease

WHAT YOU NEED TO KNOW:

- **Background**: Little is known about the association between liver fibrosis and cardiovascular events in patients with NAFLD.
- **Findings**: We found a significant association between non-invasive scores of liver fibrosis (FIB-4 score and nonalcoholic fatty liver disease score), and cardiovascular events.
- **Implication for patients care**: Liver fibrosis scores can identify patients with NAFLD who have an increased risk of cardiovascular disease.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease worldwide. NAFLD includes a spectrum of diseases ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH) and may progress to cirrhosis and hepatocellular carcinoma[1]. In addition to liver-related complications, people with NAFLD have also an increased chance of developing cardiovascular events (CVEs), such as myocardial infarction and stroke[2-4], which are even more common than liver-related complications[5-7].

The presence of liver fibrosis, which represents the final common feature of chronic liver diseases of any aetiology, seems to be the most important factor worsening the prognosis of patients with NAFLD[8, 9], which seems to be dependent on the severity of liver fibrosis[8]. The problem is far-reaching considering the evidences reporting that a tenth of NAFLD patients may have advanced fibrosis (fibrosis stage ≥2), which is expected to increase in the general population by 2030 [10].

Despite the prognostic importance of liver fibrosis, a crucial issue is that not all patients can undergo a liver biopsy to assess the presence of liver fibrosis. At this purpose, much effort has been done to identify and validate non-invasive markers of liver fibrosis. The Fibrosis-4 score (FIB-4) and NAFLD fibrosis score (NFS)[11-14] are the two most studied and validated scoring systems in NAFLD patients to identify those with high probability to have advanced liver fibrosis, across all ages. The use of these scores may reduce the need for liver biopsy by identifying NAFLD patients at higher risk of having advanced liver fibrosis. Current guidelines suggest their use to detect or rule out severe fibrosis and monitor its progression in clinical practice as they can be easily calculated in large populations and repeated over time[15-17].

Recent evidence suggested that non-invasive markers of liver fibrosis may also have a prognostic value in different clinical settings. Data from the US NHANES demonstrated that in 11,154 participants, NFS and FIB-4 were significantly associated with mortality, mainly from cardiovascular causes (NFS: HR=3.46; FIB-4: HR=2.68)[16]. These data were also recently

confirmed in the general US population[18], and Young et al.[19] reported an independent association of liver fibrosis with albuminuria in Chinese diabetic patients.

However, there are few data on the predictive role of non-invasive score of fibrosis on CVEs and mostly are derived from post-hoc analyses[20].

Aim of the study was to evaluate the independent predictive value of FIB-4 and NFS scores for incident CVEs during the follow-up of a large cohort of patients with NAFLD.

MATERIAL AND METHODS

The occurrence of CVE is as a secondary pre-specified endpoint of the ongoing PLINIO study (Progression of LIver Damage and Cardiometabolic Disorders in Non-alcoholic Fatty Liver dIsease: an Observational Cohort study. ClinicalTrials.gov Identifier: NCT04036357).

This analysis was carried out in 898 consecutive outpatients referred to the Day Service of Internal Medicine and Metabolic Disorders of the Policlinico Umberto I University Hospital in Rome with at least one out of the following cardio-metabolic diseases: arterial hypertension, overweight/obesity, type 2 diabetes, dyslipidaemia, atrial fibrillation (AF), metabolic syndrome (MetS). Exclusion criteria were: average daily consumption of alcohol >20 g in women and of >30 g in men; excessive drinking and alcohol use were further confirmed by the use of Alcohol Use Disorders Identification Test, AUDIT[21] ; presence of hepatitis B surface antigen and antibody to hepatitis C virus; positive tests for autoimmune hepatitis; other chronic liver diseases; diagnosis of oncological diseases and concomitant therapy with drugs known to promote liver steatosis (e.g. amiodarone); other chronic infectious or autoimmune disease; clinical, biochemical or ultrasonography (US) signs of cirrhosis or portal hypertension[22].

At first visit, all patients underwent a complete clinical and biochemical diagnostic work-up including routine clinical and biochemical evaluations. Anthropometric data (i.e. waist

circumference and body mass index, BMI) and information on concomitant treatment and co-morbidities were registered. Cardiovascular and metabolic risk factors were defined according to international guidelines (see supplementary file). Liver US scanning was performed to assess the presence of steatosis. All US were performed by the same operator who was blinded to laboratory values using a GE Vivid S6 apparatus equipped with a convex 3.5 MHz probe. Severity of liver steatosis was defined according to Hamaguchi score[23]. Informed written consent was obtained, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the local ethical board of Sapienza-University of Rome (Ref. 2277 prot. 873/11). All co-authors had access to the study data and had reviewed and approved the final manuscript.

Non-invasive markers of fibrosis

FIB4 was calculated as follows: (Age x AST) / (Platelets x √ (ALT)). FIB4 > 2.67 was considered representative of a high probability for F3/F4 in NAFLD[12, 24]. Thus, in patients aged 50–64, the FIB-4 threshold of 2.67 displayed optimal diagnostic accuracy with a c-index of 0.76[25].

NFS was calculated as follow: -1.675 + 0.037 x Age + 0.094 x BMI + 1.13 x IFG/diabetes + 0.99 x AST/ALT ratio – 0.013 x Platelets – 0.66 x Albumin. NFS>0.676 was considered representative of a high probability for fibrosis F3/F4[14] (c-index 0.71–0.76)[25].

Cardiovascular endpoints

Data on CVEs were prospectively collected during follow-up. After enrolment, patients underwent periodical phone calls (every six months) and visits (every 12 months) in the outpatient clinic. Only the first CVE registered during follow-up was used in the analysis. The type of CVE was confirmed by medical records (imaging or discharge letter). In case of a fatal event, information were obtained from relatives or general practitioners.

CVEs included a composite of fatal/nonfatal ischemic stroke and myocardial infarction (MI), cardiac (stent or coronary artery bypass surgery/CABG) or peripheral revascularization (carotid endarterectomy or lower limb percutaneous transluminal angioplasty, PTA), new-onset supraventricular arrhythmias (such as atrial fibrillation) and cardiovascular death. Diagnosis of MI was made according to the definition proposed by the Joint ESC/ACCF/AHA/WHF Task Force[26]. Ischemic stroke was determined on clinical manifestations and confirmed by radiological findings. If a patient died within 4 weeks of MI or stroke, this event was recorded as fatal MI/stroke. Transient ischemic attack (TIA) was defined according to the Classification of Cerebrovascular Diseases III[27]. Cardiovascular death included sudden death; progressive congestive heart failure; procedure-related death. Death was classified as cardiovascular unless an unequivocal non-cardiovascular cause of death was recorded.

Statistical analysis

Data are expressed as mean and standard deviation for normally distributed variables and median and interquartile range (IQR) for non-normally distributed data. Group comparisons were performed by unpaired Student's t-test or by Mann-Whitney test as appropriate. Proportions and categorical variables were tested by the χ^2 –test.

After dividing the whole population into two groups based on the presence/absence of NAFLD, a first descriptive analysis was performed, and the incidence rate of CVEs was calculated in patients with and without NAFLD and compared with the "test-based method"[28]. The association between the presence of NAFLD and CVEs was also investigated by univariate and multivariate Cox's proportional hazards regression analysis.

Then, we investigated factors associated with CVEs in the group of patients with NAFLD. At this aim, different models of Cox's proportional hazards regression analyses were built to calculate the adjusted relative hazard ratios (HR) of CVE by each clinical variable. In the first model (Model A) we used FIB-4 >2.67 as dependent variable to define liver fibrosis, while in the

second (Model B) we used NFS > 0.676. In addition, we repeated survival analysis by excluding new-onset AF from the composite outcome, and we also performed a subgroup analysis in patients without cardiovascular events at baseline.

All available variables were used as covariates in the multivariable models; for the multivariable models using FIB-4 we used the composite variable MetS instead of its single components, while in those with NFS, we used the single components, as MetS and NFS share some common items (BMI and IFG/diabetes). Final models were obtained from a stepwise forward selection procedure.

Only p values <0.05 were considered as statistically significant. All tests were two-tailed, and analyses were performed using computer software packages (SPSS-25.0, SPSS Inc. and MedCalc-19.1)

RESULTS

Mean age was 56.4±12.7 years and 37.5% of patients were women. Most patients were overweight and obese, arterial hypertension was present in 58.3%, type 2 diabetes mellitus in 25.7% and metabolic syndrome in 48.6%. Current treatment with statins was present in 38.6%. NAFLD was diagnosed in 643 (71.6%) patients. Clinical and biochemical characteristics of patients with and without NAFLD are reported in **Supplementary Table 1**. As compared to patients without, those with NAFLD had a higher prevalence of MetS and type 2 diabetes and significantly increased levels of blood glucose, triglycerides, waist circumference, BMI and serum liver enzymes.

During follow-up, 42 (4.6%) patients were lost and 856 were included in the survival analysis. The median follow-up time was 41.4 months (IQR: 23.2-62.8), yielding 3044.4 person-years of observation. At follow-up, 58 patients (1,9% year, 95%C.I. 1.5-2.5 year) experienced a CVE; a detailed description of events according to the presence of NAFLD is reported in **supplementary table 2**. A higher rate of CVEs was found in NAFLD patients (2.1%/year, 95%CI 1.6-2.8) compared to those without NAFLD (1.0%/year 95%CI 0.5-2.1, p=0.066) but did not meet a priori thresholds for statistical significance.

After excluding new-onset AF events, this difference became significant (NAFLD 1.9%/year 95%CI 1.4-2.6 vs without NAFLD 0.7%/year 95%CI 0.2-1.7, p=0.034).

Multivariate Cox regression analysis identified, age (HR: 1.07, 95%CI 1.04-1.10, p<0.001), male sex (HR: 3.20, 95%CI 1.57-6.54, p=0.001) and NAFLD (HR: 2.73, 95%CI 1.22-6.12, p=0.015) to be independently associated with the occurrence of CVEs (**Supplementary table 3**).

Patients with NAFLD

We analyzed characteristics of patients and the incidence of CVEs according to the presence of liver fibrosis, defined by NFS or FIB-4 score. **Table 1** reports clinical and biochemical characteristics of NAFLD patients with NFS above and below 0.676, respectively. Patients with

NFS >0.676 had higher prevalence of MetS and high blood pressure and lower levels of total and LDL cholesterol.

To evaluate predictors of CVEs in the group of patients with NAFLD, two separate Cox proportional regression analysis models were built using either NFS >0.676 or FIB-4 >2.67 as covariate. In Model A, NFS >0.676 was significantly associated with CVEs after adjustment for comorbidities (HR: 2.29, 95%CI 1.17-4.47, p=0.016) (**Table 2**; **Figure 1, Panel A**). Similarly, in Model B, a high FIB-4 >2.67 (HR: 4.57, 95%CI 1.61-12.98, p=0.004) was a predictor of incident CVEs (**Figure 1, Panel B**).

Similar findings were obtained when we excluded new-onset AF from the composite endpoint both for NFS (**Figure 2, Panel A;** adjusted HR: 2.42, 95%CI 1.19-4.91; p=0.014) and FIB-4 (**Figure 2, Panel B;** adjusted HR: 4.00, 95%CI 1.21-13.28; p=0.023).

Furthermore, when we analysed only patients without CVEs at baseline, we found a similar association between liver fibrosis and CVEs (**Supplementary Figure 1 Panel A** adjusted HR for NFS: 2.50, 95%CI 1.13-5.35; p=0.024); **Panel B** adjusted HR for FIB-4 4.28, 95%CI 1.29-14.20; p=0.017).

DISCUSSION

In this prospective study, we found a significant association between non-invasive markers of liver fibrosis and cardiovascular outcomes in patients with NAFLD. We assessed liver fibrosis using FIB-4 and NFS, two simple, non-invasive and validated biomarkers for identifying NAFLD patients with higher likelihood of having bridging fibrosis or cirrhosis.

The predictive role of non-invasive markers of liver fibrosis against CVEs has been scarcely investigated so far. The study by Vilar-Gomez et al. showed in 458 patients with biopsy confirmed NAFLD with bridging fibrosis or compensated cirrhosis, an incidence of vascular events of 0.9%/year[29]. Patients with pre-cirrhosis bridging fibrosis were more likely to suffer from vascular events than liver-related complications; of note, patients had a similar age and prevalence of cardiovascular disease compared to our cohort[29].

A large retrospective Korean study involving 111,492 adults showed that the presence of NAFLD was associated with an increased risk of myocardial infarction, which was similar in patients with and without liver fibrosis, evaluated by both NFS (≥ -1.455) or FIB-4 (≥ 1.3) score[30]. However, different cut-off values used to define the positivity of the scores and characteristics of the study population, such as the lower prevalence of cardio-metabolic risk factors compared to our patients, may account for this different result[30].

At multivariable analysis, we found that FIB-4 >2.67, history of cardiovascular disease and MetS were independent predictors of incident CVEs. Furthermore, when advanced fibrosis was defined using NFS, a score largely driven by metabolic factors (e.g. diabetes and obesity), NFS >0.676, male sex and history of cardiovascular disease predicted incident CVEs.

In addition, in our study, MetS was also an independent predictor of CVE in NAFLD patients. This finding is in keeping with previous data showing that MetS increases cardiovascular risk also in other cardiovascular settings, such as hypertension[31], AF[32], and obstructive sleep apnea syndrome[33].

Thus, our data suggest that liver fibrosis development in patients with NAFLD may be the result of a long-term exposure to cardio-metabolic risk factors such as diabetes. Thus, the concomitant presence of multiple cardio-metabolic conditions may induce a chronic low-grade pro-inflammatory and pro-oxidant status[34] leading to liver inflammation (i.e. macrophage activation) and collagen deposition[35, 36].

The study may also have clinical implications. The association between liver fibrosis and cardiovascular risk supports a potential role for statin treatment in patients with NAFLD[37]. Recently, the use of NFS among 14,819 patients enrolled in IMPROVE-IT trial, identified an independent population of patients who were at highest risk of recurrent events, who benefit from combination therapy with ezetimibe/simvastatin with a 3.7% absolute reduction of risk of CVEs[17]. In addition, a recent metanalysis showed that statins may delay the development of fibrosis in patients with chronic liver disease of different aetiologies[38]. Thus, statins may favourably affect both liver-related and cardiovascular outcomes in patients with NAFLD. Despite these evidences, it has been reported a poor attitude to prescribe statins in NAFLD patients, mainly due to the concern for its possible toxicity in liver disease [39]. Finally, our results might also suggest a rationale for targeting fibrosis in multiple end organs.

This study has several limitations that should be mentioned. Fatty liver was assessed by US and patients had no liver biopsy, nor evaluation by magnetic resonance spectroscopy. The absence of histological data did not allow to investigate the possible association between liver inflammation and CVEs. The observational design does not allow finding a cause-effect relationship between liver fibrosis and the other variables of interest. Besides, the small number of individual events, did not allow us to evaluate the association between FIB-4 and NFS and specific types of cardiovascular events. Moreover, it is important to underline that, although well-validated and widely used, surrogate serum biomarkers, such as the FIB-4 and NFS, may not accurately assess fibrosis stage. A further limitation is that this is a single centre study performed in an hospital-based

setting. Finally, given the very low number of patients on aspirin for primary prevention and no information on familial history of CVEs or on the presence of carotid plaques at baseline, larger studies need to address the role of these variables in cardiovascular risk of NAFLD patients.

In conclusion**,** non-invasive markers of liver fibrosis in patients with NAFLD are associated with an increased risk of cardiovascular events, independently from the presence of metabolic syndrome.

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Table 1. Clinical and biochemical characteristics of patients with NAFLD Fibrosis Score (NFS) ≤0.676 and >0.676.

	NAFLD Fibrosis Score ≤0.676 n=550	NAFLD Fibrosis Score >0.676 n=93	p
Men	62.5 %	71.9 %	0.195
Smoking	23.2 %	14.0 %	0.134
Metabolic syndrome	56.7 %	64.3 %	<0.001
Previous coronary heart disease	4.5 %	11.5 %	0.013
Previous cerebrovascular disease	1.3 %	6.3 %	0.006
Atrial Fibrillation	0.8 %	32.3 %	<0.001
Statins	40.5 %	45.6 %	0.481
High blood pressure (≥ 130 / ≥85 mm/Hg or use of antihypertensive drugs)	70.9 %	87.5 %	0.007
Waist circumference (cm)	107.1 ± 11.8	108.8 ± 12.7	0.316
Abdominal obesity (waist circumference ≥102 cm in men and ≥88 cm in women)	80.7 %	78.9 %	0.727
Fasting blood glucose (mg/dl)	105.7 ± 29.8	109.4 ± 22.3	0.398
Diabetes	28.0 %	43.2 %	0.004
Total cholesterol (mg/dl)	198.9 ± 40.5	185.4 ± 42.2	0.019
HDL cholesterol (mg/dl)	48.1 ± 14.0	46.2 ± 12.1	0.331
LDL cholesterol (mg/dl)	118.9 ± 35.2	108.7 ± 38.5	0.049
Triglycerides (mg/dl)	136.0 [101.0-185.0]	132.5 [97.2-164.5]	0.627
Hamaguchi Score	3.0 [2.0-5.0]	4.0 [3.0-5.0]	0.016

Table 2. Stepwise multivariate Cox proportional hazard regression analysis of factors associated with cardiovascular events (CVEs) in 660 patients with NAFLD.

Model A.	Hazard Ratio	95% Confidence Interval		P value
Male sex	2.46	1.15	5.29	0.021
Previous cardiovascular and cerebrovascular events	2.12	1.02	4.39	0.043
Statin Use	1.86	1.01	3.41	0.044
Smoking	-	-	-	-
Hamaguchi score	-	-	-	-
High blood pressure (≥ 130 / ≥85 mm/Hg or use of antihypertensive drugs)	-	-	-	-
NAFLD Fibrosis Score > 0.676	2.29	1.17	4.47	0.016

Model B.	Hazard Ratio	95% Confidence Interval		P value
Male sex	2.58	1.20	5.55	0.015
Previous cardiovascular and cerebrovascular events	2.95	1.49	5.84	0.002
Statin use	-	-	-	-
Smoking	-	-	-	-
Hamaguchi score	-	-	-	-
Metabolic Syndrome	2.30	1.14	4.64	0.019
FIB-4 > 2.67	4.57	1.61	12.98	0.004

Figure legends

Figure 1. Cumulative risk of cardiovascular events according to FIB-4 ≤ 2.67 or > 2.67 (Panel A) and or NFS ≤ 0.676 or > 0.676 (Panel B).

Figure 2. Cumulative risk of cardiovascular events (excluding new-onset atrial fibrillation) according to FIB-4 ≤ 2.67 or > 2.67 (Panel A) and or NFS ≤ 0.676 or > 0.676 (Panel B).

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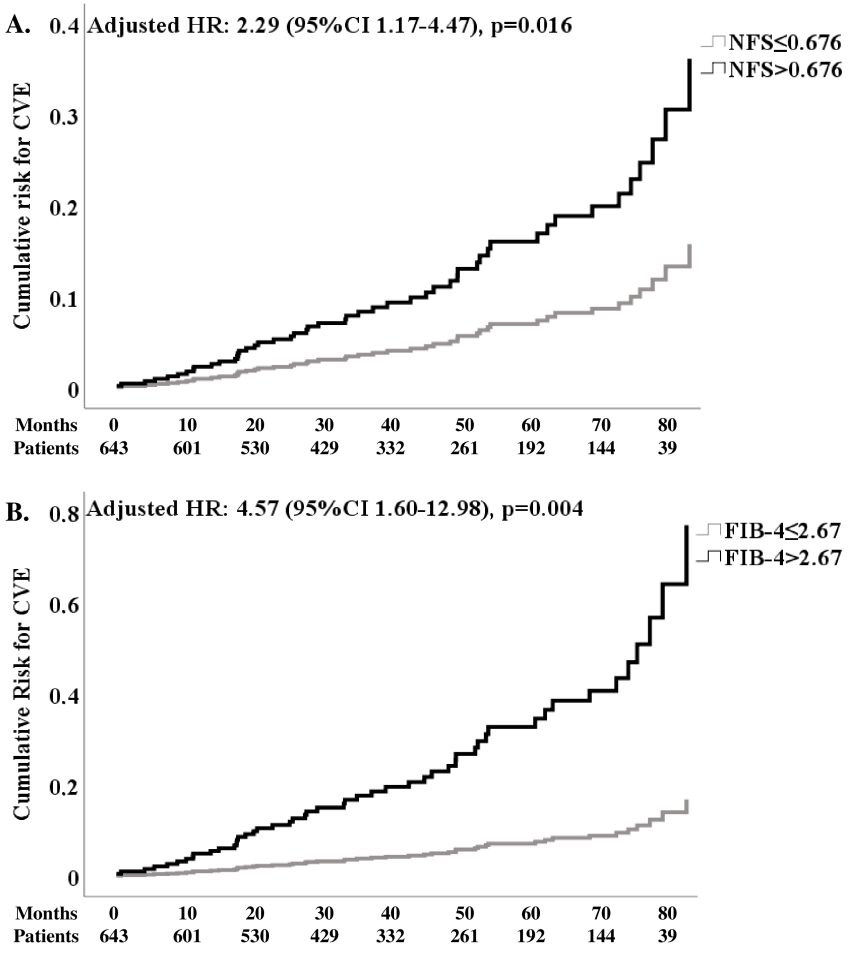
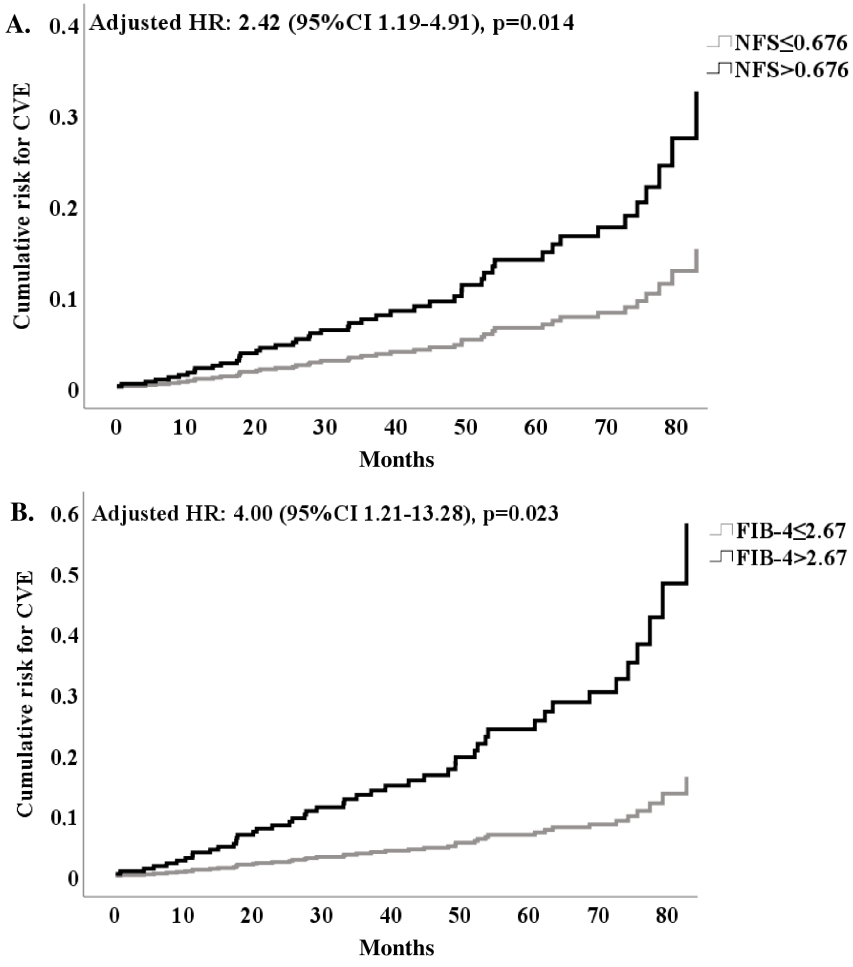

A.
Adjusted HR: 2.29 (95%CI 1.17-4.47), p=0.016
NFS≤0.676
NFS>0.676
Cumulative risk for CVE
0.4
0.3
0.2
0.1
0
Months 0 10 20 30 40 50 60 70 80
Patients 643 601 530 429 332 261 192 144 39
B.
Adjusted HR: 4.57 (95%CI 1.60-12.98), p=0.004
FIB-4≤2.67
FIB-4>2.67
Cumulative Risk for CVE
0.8
0.6
0.4
0.2
0
Months 0 10 20 30 40 50 60 70 80
Patients 643 601 530 429 332 261 192 144 39


A.
Adjusted HR: 2.42 (95%CI 1.19-4.91), p=0.014
NFS≤0.676
NFS>0.676
Cumulative risk for CVE
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
Months
B.
Adjusted HR: 4.00 (95%CI 1.21-13.28), p=0.023
FIB-4≤2.67
FIB-4>2.67
Cumulative risk for CVE
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
Months

Liver fibrosis and cardiovascular events in non-alcoholic fatty liver disease. The prospective cohort PLINIO study.

Supplementary data

Supplementary Methods

Definition of cardiovascular risk factors.

Arterial hypertension as repeated elevated blood pressure values (≥140/≥90 mmHg) or taking antihypertensive drugs[1];

Diabetes as a casual plasma glucose ≥200 mg/dl (11.1 mmol/l), or fasting plasma glucose ≥126 mg/dl (7.0 mmol/l), or presence of anti-diabetic treatment[2].

MetS was diagnosed according to the modified criteria of the ATP III Expert Panel of the US-NCEP (see supplementary file)[3], as at least three of the following five clinical features: waist circumference (central obesity) > 102 cm in men and > 88 cm in women, fasting blood glucose > 100 mg/dl or antidiabetic drugs, triglycerides > 150 mg/dl or specific therapy, HDL-cholesterol < 40 mg/dl in men and < 50 mg/dl in women or specific therapy, arterial systolic/diastolic blood pressure > 130 / > 85 mm/Hg or antihypertensive drugs.

Supplementary Results

Supplementary table 1. Clinical and biochemical characteristics of subjects with and without NAFLD.

	Non-NAFLD n=255	***NAFLD n=643***	***p***
Age (years)	57.8 ± 13.73	55.94 ± 12.51	0.059
Men	60.2 %	62.3 %	0.568
Smoking	25.0 %	22.5 %	0.462
Diabetes	13.2 %	29.3 %	<0.001
Metabolic syndrome	23.6 %	56.5 %	<0.001
Previous coronary heart disease	8.3 %	5.4 %	0.137
Previous cerebrovascular disease	2.4 %	1.9 %	0.588
Atrial fibrillation	8.8 %	5.3 %	0.069
Statins	43.1 %	39.8 %	0.397
High blood pressure (≥130 / ≥85 mm/Hg or use of antihypertensive drugs)	65.4 %	72.3 %	0.165
Waist circumference (cm)	96.99 ± 9.43	107.432 ± 12	<0.001
Abdominal obesity (waist circumference ≥102 cm in men and ≥88 cm in women)	50.6 %	80.6 %	<0.001
Body mass index (Kg/m^2)	26.67 ± 3.83	30.58 ± 5.21	<0.001
Fasting blood glucose (mg/dl)	96.51 ± 20.57	105.42 ± 28.81	<0.001
Total cholesterol (mg/dl)	198.10 ± 43.62	196.78 ± 40.44	<0.692
HDL cholesterol (mg/dl)	55.55 ± 13.65	47.76 ± 13.50	<0.001
LDL cholesterol (mg/dl)	118.68 ± 38.32	117.76 ± 35.38	0.757
Triglycerides (mg/dl)	114.25 ± 60.83	160.21 ± 103.91	<0.001
AST (U/L)	19.62 ± 7.19	25.96 ± 16.395	<0.001
ALT (U/L)	20.85 ± 15.41	35.21 ± 26.91	<0.001

Supplementary table 2. Number of CV events in patients with NAFLD and in control subjects.

	Patients without NAFLD (n=255)	**Patients with NAFLD (n=643)**
Fatal/non-fatal myocardial infarction	3	26
Cardiovascular Death	0	1
Peripheral revascularization	0	12
Fatal/non-fatal ischemic stroke	2	3
Transitory Ischemic Attack	0	4
New-onset atrial fibrillation	2	5
Total Events	7	51

Supplementary table 3. Univariate and multivariate Cox proportional hazard regression analysis of factors associated with CVEs in the whole cohort.

	Univariate Hazard Ratio (95% Confidence Interval)	p **value**	**Multivariate Hazard Ratio (95% Confidence Interval)**	p **value**
Age	1.06 (1.03-1.08)	<0.001	1.07 (1.04-1.10)	<0.001
Male sex	2.44 (1.25-4.76)	0.009	3.20 (1.57-6.54)	0.001
Previous cardiovascular and cerebrovascular events	3.46 (1.88-6.33)	0.009	-	-
Metabolic Syndrome	2.35 (1.24-4.06)	0.007	-	-
Smoking	0.68 (0.34-1.34)	0.269	-	-
Statin use	2.12 (1.233.65-)	0.007	-	-
NAFLD	2.02 (0.92-4.46)	0.081	2.73 (1.22-6.12)	0.015

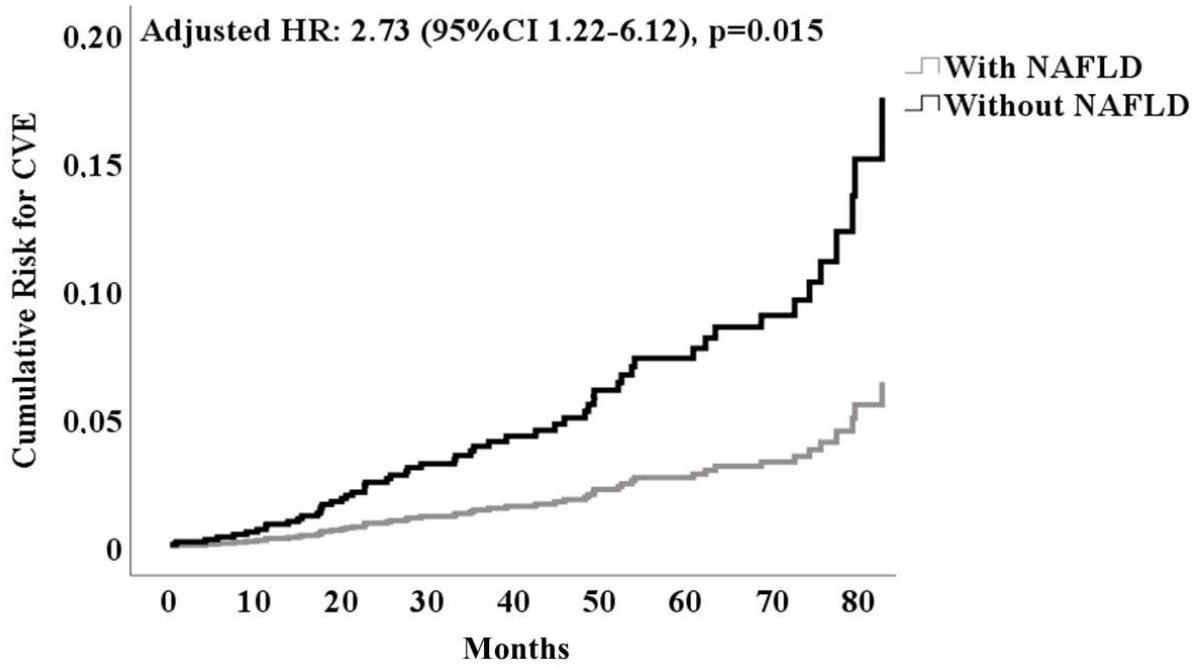
Supplementary table 4. Number of cardiovascular events according to NAFLD fibrosis score (NFS) (Panel A) and FIB-4 (Panel B).

Panel A	**Patient with negative NFS[#] (n=320)**	**Patients with indeterminate NFS[##] (n=230)**	**Patients with positive NFS[###] (n=93)**
Fatal/non-fatal myocardial infarction	8	11	7
Cardiovascular Death	1	0	0
Peripheral revascularization	6	3	3
Fatal/non-fatal ischemic stroke	1	1	1
Transitory Ischemic Attack	3	0	1
New-onset atrial fibrillation	2	2	1
Total Events	21	17	13
Panel B	**Patients with negative FIB-4* (n=487)**	**Patients with indeterminate FIB-4** (n=138)**	**Patients with positive FIB-4*** (n=18)**
Fatal/non-fatal myocardial infarction	15	10	1
Cardiovascular Death	1	0	0
Peripheral revascularization	10	0	2
Fatal/non-fatal ischemic stroke	2	1	0
Transitory Ischemic Attack	4	0	0
New-onset atrial fibrillation	4	0	1
Total Events	36	11	4

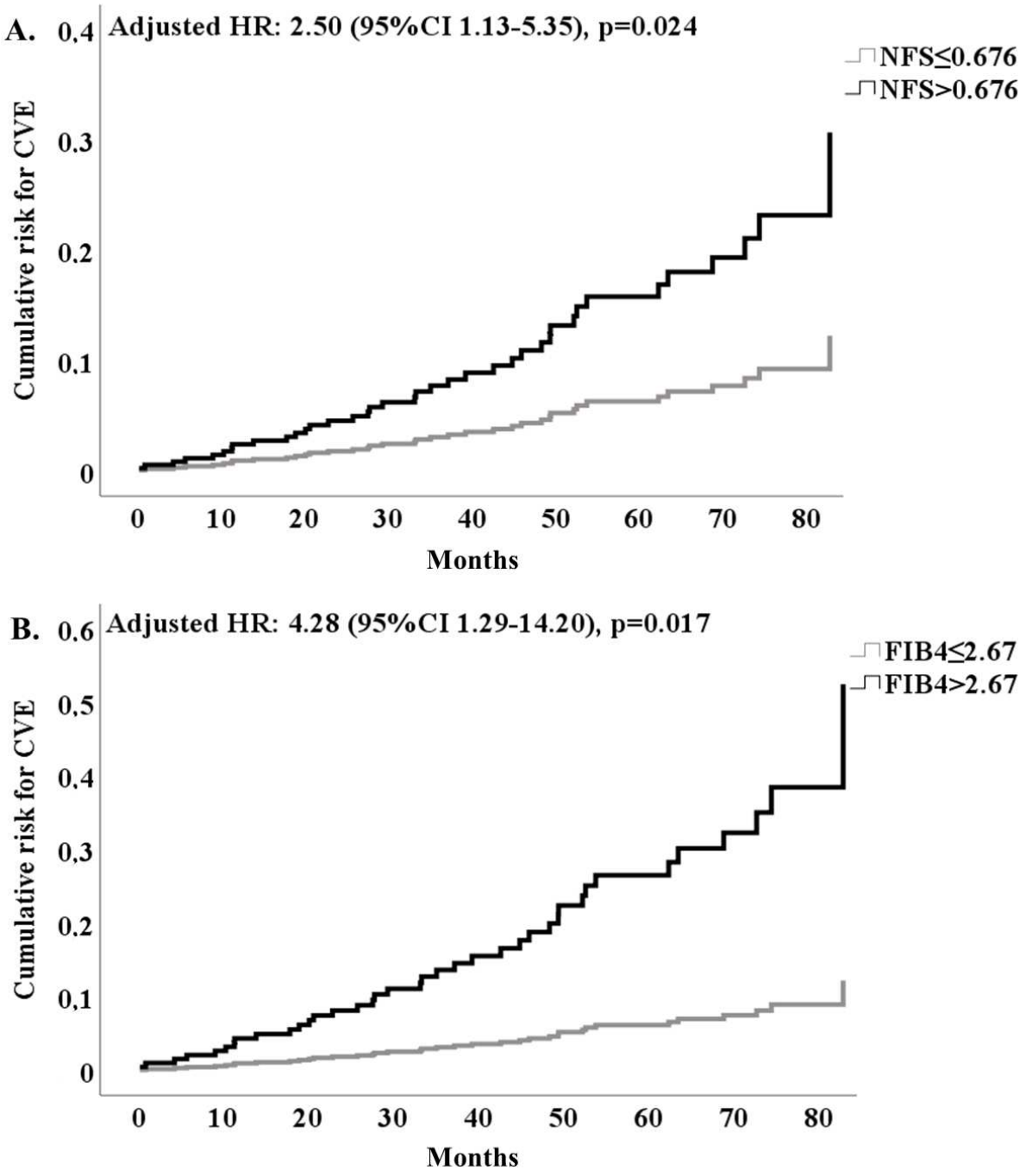
[#]NFS < -1.455 (less 65 years)/NFS <0.120 (over 65 years); [##]NFS ≥ -1.455 (less 65 years)/NFS ≥ 0.120 (over 65 years) and NFS ≤0.676; [###]NFS>0.676.

* FIB-4 < 1.30 (less 65 years)/FIB-4 <2.0 (over 65 years); ** FIB-4 between ≥ 1.30 (less 65 years)/FIB-4 ≥2.0 (over 65 years) and ≤2.67; *** FIB-4>2.67.

Supplementary Figure 1. Rik of cardiovascular events in patients with and without NAFLD.



Supplementary figure 2. Cumulative risk factor for CVE in patients without prior CVE, according to FIB-4 (Panel A) and NFS (Panel B).



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WHAT YOU NEED TO KNOW:

- **Background**: Little is known about the association between liver fibrosis and cardiovascular events in patients with NAFLD.
- **Findings**: We found a significant association between non-invasive scores of liver fibrosis (FIB-4 score and nonalcoholic fatty liver disease score), and cardiovascular events.
- **Implication for patients care**: Liver fibrosis scores can identify patients with NAFLD who have an increased risk of cardiovascular disease.