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Research paper

Semi-quantitative ultrasound assessment of nonalcoholic fatty liver disease highlightens early subclinical atherosclerotic vascular damage: From risk factors to vascular damage

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Abstract

Introduction: Nonalcoholic fatty liver disease (NAFLD) is an independent risk factor for coronary artery disease; moreover, it increases systemic atherosclerotic burden by inducing the overexpression of inflammatory mediators, promoting endothelial damage, and impairing blood pressure regulation.

Aim: Aim of this work was to evaluate whether a standardized evaluation of NAFLD improves cardiovascular risk assessment recognizing subclinical atherosclerosis in lower cardiovascular risk.

Material and methods: We investigated NAFLD occurrence and severity, carotid and femoral intima-media-thickness (IMT) and vascular stiffness by ultrasound technique, endothelial function by peripheral-arterial-tonometry, lipid profile and inflammatory markers in 220 subjects (100 men, 120 women; 45.42 ± 13.22 years old), without history of cardiovascular event, diabetes, liver infection, alcohol consumption, systemic diseases, and the use of drugs causing liver damage. NAFLD was evaluated, graded according to an eight-point scoring semi-quantitative severity score.

Results and discussion: At univariate logistic analysis, NAFLD ≥ 3 score was significantly associated with pathological IMT, augmentation index, pulse-wave-velocity at carotids and femoral arteries, and endothelial dysfunction, and this association was confirmed after adjustment for European Society of Cardiology Systematic Coronary Risk Evaluation (ESC SCORE) at multivariate analyses. Moreover, high sensitivity C-reactive protein levels were significantly higher in patients with at least 3 point steatosis, in comparison to the others. Receiver operating characteristic (ROC) curve analysis for NAFLD showed a significant higher area under curve for the detection of both early atherosclerotic burden and vascular stiffness, in comparison to ROC curve of ESC SCORE.

Conclusions: According to our findings a NAFLD ≥ 3 score was able to screen a subgroup with widespread morphological vascular damage and endothelial dysfunction in a primary prevention population.

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1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is considered as the accumulation of liver fat over 5% per-liver-weight, in the presence of less than 20 g/day for women and 30 g/day for men of daily alcohol intake; viral or other causes of liver disease must be excluded.[1–4]

NAFLD is an independent risk factor for coronary artery disease (CAD); moreover, it increases systemic atherosclerotic burden by inducing the overexpression of inflammatory mediators, promoting endothelial damage, and impairing blood pressure regulation.[5] According to above reported data, NAFLD should be considered not a local organ-specific but a multi-system disease.[5] Therefore, NAFLD is not merely a marker of cardiovascular disease (CVD), but rather concurs to its pathogenesis.[6] Indeed, NAFLD patients have a higher risk of death than the general population, mainly due to CVD.[7]

In these last years, NAFLD was reported as the hepatic manifestation of the metabolic syndrome (MS), with which shares several characteristics; however, recent data suggested that NAFLD increases cardiovascular risk independently of the other MS components.[8]

The high prevalence of NAFLD and its strong relationship with MS have stimulated interest for the possible role of the liver in the development of atherosclerosis. However, the close interrelationships between NAFLD, MS and atherosclerosis make it extremely difficult to assess the cause––effect relationship among these diseases.[8,9]

Nowadays ultrasound is the method of choice for screening patients for NAFLD. In patients with diabetes and histologically proven nonalcoholic steatohepatitis (NASH), abnormal liver enzymes may be seen in less than 20% of patients.[10,11]

2. AIM

Aim of this work was to evaluate whether a standardized evaluation of NAFLD improves cardiovascular risk assessment in lower cardiovascular risk subjects who underwent to a comprehensive vascular assessment.

3. MATERIAL AND METHODS

3.1. Study population

Study population consists of 220 (100 men, 120 women; age 45.42 ± 13.22) apparently healthy subjects, at low risk for CVD, according to European Society of Cardiology (ESC) guidelines,[12] referred to our Centre for primary prevention of CVDs.

Evaluation of traditional cardiovascular risk factors was made accordingly to current guidelines.

In particular, we considered risk factors: male sex; hypertension;[13] hyperlipidaemia;[14,15] diabetes mellitus;[16] family history of CAD (having first- or second-degree relatives with premature CVD); post-menopause; and smoking habit.

No subject reported a positive history for cardiovascular events or diabetes; subjects with positive history for cancer, autoimmune disease, or chronic pulmonary disturbances were excluded from the analysis.

Exclusion criteria included history for hepatitis (HBV, HCV, autoimmune hepatitis) or any hepatic disorder. Cardiovascular profile was assessed according to Systematic Coronary Risk Evaluation (SCORE), provided by ESC guidelines.[12]

Because findings described in the study were part of standard outpatient activity, it was not necessary to obtain Institutional Review Board approval. All subjects gave written informed consent, and the investigation was performed in accordance with the Declaration of Helsinki.[17]

3.2. Study protocol

All patients underwent to an outpatient evaluation, including medical questionnaire, physical examination, blood sampling, ultrasound assessment, and endothelial function assessment by peripheral arterial tonometry.

Clinical assessment was performed in the morning, in a quiet room. Resting heart rate (HR) and blood pressure, height, weight, body mass index (BMI) and waist circumference were measured. A 12-lead electrocardiogram (ECG) at rest was collected; and blood venous sampling (complete blood count, fasting glucose, lipid profile, kidney and liver function markers).

In the same day, we performed ultrasound assessment (intima media thickness – IMT, pulse-wave velocity – PWv, and augmentation index – AIX; NAFLD), endothelial function evaluation (flow mediated dilation) and ankle-brachial index (ABI). The same operator, who was blinded for the study population, performed the assessment.

3.3. Echographical examination

All patients underwent ultrasound assessment on a MyLab 70 XVisionEsaote Machine equipped of a 7.5 MHz linear and a 3.75 MHz convex transducers machine (Esaote Medical Systems, Rome, Italy). The system used a dedicated software: RF-data technology involving RF Quality Intima-media Thickness (^{RF}QIMT) and RF Quality Arterial Stiffness (^{RF}QAS); Esaote Medical Systems.

IMT of right and left common carotid arteries was measured at the 1-cm segment proximally to the carotid dilation with B-mode ultrasonography, by using the 7.5 MHz linear transducer.

Femoral (f-) IMT was measured in the far wall of a 1-cm long arterial segment proximal to the femoral bifurcation. For each subject the maximum carotid (c-) IMT value among at least 3 assays for each side was used for statistical analysis. According to the European Society of Hypertension guidelines[13] over 0.9 mm and 1.2 mm were used as cut-off values for c-IMT and f-IMT.

Arterial stiffness was investigated as local PWv and distensibility of common carotid arteries. It was bilaterally measured at the far wall, at the 1-cm segment proximal to the carotid dilation. f-PWv was measured at the far wall,

1-cm segment proximal to the femoral bifurcation. Cut-off value for both c-PWVs and f-PWVs was considered 12 m/s, according to current literature.[13]

The evaluation of AIX was performed at the common carotid and femoral arteries, simultaneous to the ultrasound investigation, in order to obtain local AIX values.[18] MyLab 70 XVisionEsaote software automatically executed the AIX analysis; algorithm and analysis were performed according to a previously described method.[19] For statistical analysis, we used the maximum c-AIX and f-AIX values for each subject.

The presence and the severity of NAFLD, was assayed by using the real-time electronic 3.75 MHz convex-type probe. The same operator (MB), who was unaware of the subjects' medical history and/or laboratory findings, performed all the exams.

The presence and severity of NAFLD was graded semiquantitatively according to validated scoring systems, with minor modifications,[20] according to a scale ranging from 0 to 8 points, on the basis of liver–kidney differences (0–3 points), deep attenuation (0–1), blurring of diaphragm (0–1) and/or of the hepatic vein (0–1) and/or of gallbladder wall (0–1), and the presence of focal sparing (0–1). NAFLD was diagnosed when the liver–kidney difference was more than 0. According to NAFLD scoring subjects were divided into two groups:

(1) mild NAFLD with less than 3 total score built up by liver–kidney difference and/or deep attenuation, named mild fatty liver (MFLD) and
(2) moderate–severe fatty liver disease (SFLD) with at least 3 total score built up by at least three positive points (liver–kidney difference, deep attenuation and hepatic vein blurring).

Instrumental assessments were performed, following recommendations for standardization of subject conditions, as described elsewere.[18,19]

3.4. Blood samples

Venous blood samples were taken from each patient in the morning, after an overnight fasting and collected from the antecubital vein into evacuated plastic tubes (Vacutainer).

3.5. Biochemical parameters

Lipid profile and other biochemical parameters were evaluated by standard methods, immediately after extraction. High sensitivity C-reactive protein (HS-CRP) was assessed by an enzyme linked immunosorbent assay (ELISA) kit.

3.6. Surrogate measure of fatty liver disease

The fatty liver index (FLI) that indirectly estimates fatty liver disease was calculated by an algorithm based on triglycerides, BMI, γ-glutamyl-transpeptidase (γ-GT) and waist circumference; the FLI score ranges 0–100 and was validated versus ultrasound-detected NAFLD with a sensitivity of 0.61 and a specificity of 0.86 for a cut-off of FLI ≥ 60.[21] FLI < 30 rules out NAFLD that is diagnosed when FLI ≥ 60.

3.7. Micro vascular endothelial function assessment

Endothelial function was measured by peripheral arterial tonometry using the EndoPAT 2000 device (Itamar Medical LTD Caesarea, Israel), according to the method detailed in previous work.[22]

Table 1. Sudy population characteristics (n = 220) at the visit time.

Clinical characteristics	Values
Male, *n*(%)	100(45.5)
Age, mean ± SD, y [a,b]	45.42 ± 13.22
Smoking habit, *n*(%) [b]	20(0.9)
SBP, mean ± SD, mmHg	119.84 ± 14.45
DBP, mean ± SD, mmHg	74.95 ± 8.24
BP values over 140/90, *n*(%), mmHg [b]	38(17.3)
BMI, mean ± SD, kg/m^2	24.58 ± 3.27
BMI ≥ 25 kg/m^2, *n*(%) [b,c]	92(41.8)
Diabetes, *n*(%) [b]	0(0%)
Hypertension, *n*(%)	38(17.3)
Dyslipidemia, *n*(%)	92(41.8)
Family history for CAD	92(41.8)
Pharmacological therapies	
Anti-hypertensive therapy, *n*(%)	38(17.3)
Ace-inhibitors	20(9.1)
ATII blockers	10(4.5)
β-blockers	5(2.3)
Ca^{2+}-antagonists	3(1.4)
Diuretics	0(0)
Statins, *n*(%)	78(35.5)
Biochemical parameters	
RBC (normal: 4.2–5.4), mean ± SD, ×10^{12}/L	4.42 ± 1.23
WBC (normal: 4.8–10.8), mean ± SD, ×10^9/L	5.22 ± 1.45
Platelets (normal: 130–400), ×10^9/L	214 ± 117
Hb (normal: 12–16), mean ± SD, g/dL	14.32 ± 1.51
Hct (normal: 37–47), mean ± SD, %	38.9 ± 6.2
LDL-c (normal: <100), mean ± SD, mg/dL	96.77 ± 20.63
HDL-c (normal: >45), mean ± SD, mg/dl	67.91 ± 19.23
Glycaemia (normal: 60–99), mean ± SD, g/L	0.82 ± 0.23
Triglycerides (normal: <150), mean ± SD, mg/dL	73.25 ± 32.63
Creatinine (normal: 0.5–1.1), mean ± SD, mg/dL	0.73 ± 0.12
Urea (normal: 10–50), mean ± SD, mg/dL	0.43 ± 0.15
AST (normal: 0–40), mean ± SD, U/L	21.28 ± 8.68
ALT (normal: 5–35), mean ± SD, U/L	20.52 ± 9.61
GGT (normal: 8–35), mean ± SD, U/L	16.27 ± 11.07
CPK (normal: 10–165), mean ± SD, U/L	65 ± 12
FLI, mean ± SD	32.42 ± 23.91
HS-CRP (normal: 0.01–0.75), mean ± SD, μg/mL	2.6 ± 0.54

Comments: [a] % of total smokers at the event, n = 38; [b] traditional cardiovascular risk factors according to guidelines; [c] % of diabetics (n = 34).

3.8. Statistical analysis

We used SPSS for Windows (v. 19; SPSS Inc) for database construction and statistical analyses. We reported categorical variables as frequencies and percentages; we evaluated analysis of data distribution using the χ^2 test (statistical significance, $P < 0.05$). Continuous variables were expressed as mean ± standard deviation (SD).

According to the guidelines of the European Society of Hypertension,[13] normal values for ABI, IMT, and PWv were considered to be more than or equal to 0.9, c-IMT less than 0.9, f-IMT less than 1.2, and both c-PWv and f-PWv less than 12 m/s. Values for AIX were averaged through 6 consecutive heartbeats; a value of less than 0.9 was considered pathological for the presence of PAD.[21]

The non-parametric Mann–Whitney and Kruskall–Wallis tests were used for analysis of unpaired data.

The relationship between continuous variables (clinical and biochemical variables) was tested using the Spearman's correlation test.

Univariate and multivariate linear regression tests were used for analysing relationships between independent factors able to influence markers of vascular dysfunction.

In particular, we used multiple linear regression models to investigate the relative influence of relevant factors; univariate linear regression analyses included age, heart rate, systolic and diastolic blood pressure (SBP and DBP, respectively), BMI, height, weight, family history for CVD, biochemical parameters and vascular markers.

We used different multivariate linear regression models in order to determine the influence of various predictors on vascular function markers. In particular, we adjusted our models for BMI, total cholesterol, ESC SCORE.

The $P < 0.05$ was considered to indicate statistical significance.

4. RESULTS

Main characteristics of the study population are shown in Table 1.

According to the ESC SCORE classification, we identified two groups: the 1st group consisted of 43 (19.5%) subjects, presenting a medium or high cardiovascular risk (ESC SCORE < 2%), and the 2nd group of 177 (80.5%) subjects, presenting a low cardiovascular risk (ESC SCORE ≤ 1%). Differences between two groups are explained in detail in the following paragraphs.

The whole population was analysed on the basis of NAFLD severity; 87 (39.5%) subjects out of 220 had NAFLD > 3 score. The characteristics of subjects divided according to NAFLD severity, are shown in Table 2.

We investigated the steatosis pattern according to anthropometrical features. In particular, NAFLD score was significantly related with BMI ($r = 0.539, P < 0.0001$) and waist circumference ($r = 0.412, P = 0.001$). No subject had a diagnosis of hypertension, but the NAFLD ≥ 3 group showed significantly higher BP values when compared with the NAFLD < 3 group (for SBP 126 ± 12 mmHg vs. 108 ± 7 mmHg, $P < 0.0001$; for DBP 81 ± 11 mmHg vs. 68 ± 12 mmHg, $P < 0.0001$).

At univariate logistic analysis NAFLD ≥ 3 score was significantly associated to BMI ≥ 25 (OR 10.1, CI 95%: 4.9–20.9, $P < 0.0001$).

We investigated the pattern of liver steatosis according to SCORE values. In the NAFLD < 3 group, we found 122 (89.1%) subjects with a low cardiovascular risk and only 15 (10.9%) presented a high risk profile.

At univariate logistic analysis, a pathological NAFLD score was associated to a moderate-high SCORE (OR 6.1, CI 95%: 2.5–12.6, $P < 0.0001$).

Regarding vascular echographic markers, assessed in whole population, 85 (38.6%) and 73 (33.2%) subjects showed pathological values of c-IMT or f-IMT. Moreover, 83 (37.7%) and 70 (31.8%) subjects showed pathological values of c-PWV and f-PWv. Relationships between NAFLD and vascular markers were expressed in the following paragraphs.

4.1. NAFLD and biochemical findings

Biochemical results were reported in Tables 1 and 2. Patients with at least 3 point steatosis presented higher total cholesterol, triglycerides and LDL-c levels, and slightly higher GGT values, compared to others. Moreover, we found a significant inverse relationship between NAFLD score and HDL-c levels ($r = –0.433, P = 0.001$); at the opposite, NAFLD score and triglycerides were positively related ($r = 0.491, P < 0.001$). At univariate logistic analysis, a pathological NAFLD score was associated to a high total cholesterol levels (OR 6.1, CI 95%: 3.5–17.6, $P = 0.01$).

HS-CRP levels were significantly higher in patients with at least 3 point steatosis, in comparison to the others. We found a significant positive correlation between HS-CRP levels and severity of steatosis ($r = 0.44, P = 0.01$) and FLI ($r = 0.41, P < 0.05$).

We did not detect differences regarding renal function and fasting glucose levels, according to NAFLD severity; moreover, all subjects had normal levels of transaminases, with no differences from two groups.

FLI values in the whole population were reported in Table 1. FLI was strongly related with NAFLD score ($r = 0.62$, $P < 0.001$) and with SCORE for cardiovascular risk ($r = 0.47$, $P = 0.01$).

4.2. NAFLD: Relationship with vascular echographic findings

As expressed in the Table 3, we found that the NAFLD ≥ 3 group had a worse morphological and functional vascular pattern, in comparison to the NAFLD < 3 group. In particular, NAFLD ≥ 3 subjects showed higher values of c-IMT and f-IMT and PWv.

At univariate logistic analysis, the NAFLD ≥ 3 score was significantly associated with pathological IMT at carotids (OR 10.5, CI 95%: 6.2–17.8, $P < 0.0001$), pathological IMT at femoral arteries (OR 12.3, CI 95%: 7.2–20.9, $P < 0.0001$), and with altered c-PWv and f-PWV values (OR 4.2, CI 95%: 1.7–10.6, $P = 0.002$ and OR 3.9, CI 95%: 1.9–8.3, $P < 0.0001$, respectively).

Table 2. Distribution of study population characteristics, accordingly to NAFLD cut-off.

Variable	NAFLD < 3 $n = 133$	NAFLD > 3 $n = 87$	P value
Age, mean ± SD, y	39.6 ± 15.7	42.2 ± 20.0	0.1
Male, *n*(%)	59(44.4)	42(48.3)	0.1
Current smokers, *n*(%)	11(8.3)	9(10.3)	0.1
Anthropometric parameters			
BMI, mean ± SD, kg/m^2	23.62 ± 3.17	26.30 ± 2.73	0.01
BMI > 25, *n*(%)	34(25.6)	58(66.7)	0.01
Waist circumference, mean ± SD, cm	91.3 ± 15.7	98.4 ± 15.7	0.01
SBP, mean ± SD, mmHg	117.13 ± 13.26	124.22 ± 15.25	<0.0001
DBP, mean ± SD, mmHg	73.17 ± 7.27	77.81 ± 8.93	<0.0001
Cardiovascular diseases			
Hypertension, *n*(%)	23(17.3)	15(17.3)	0.4
ACEi/ARBs, *n*(%)	19(14.3)	11(12.6)	0.1
Ca^{2+} antagonists, *n*(%)	2(1.5)	1(1.2)	0.1
β-blockers, *n*(%)	2(1.5)	3(3.4)	0.2
Dyslipidaemia, *n*(%)	33(24.8)	36(41.4)	0.049
Statin therapy, *n*(%)	40(30.1)	38(43.7)	0.08
Biochemical parameters			
LDL-c, mean ± SD, mg/dL	96.12 ± 21.05	104.43 ± 13.5	0.09
Total cholesterol, mean ± SD, mg/dL	185.96 ± 31.28	198.25 ± 32.26	0.001
HDL-c, mean ± SD, mg/dL	77.12 ± 33.02	51.33 ± 2.89	0.3
Triglycerides, mean ± SD, mg/dL	110.33 ± 27.18	121.33 ± 41.18	0.09
AST, mean ± SD, U/L	20.97 ± 8.27	20.78 ± 8.86	0.4
ALT, mean ± SD, U/L	20.44 ± 10.13	20.78 ± 8.86	0.5
GGT, mean ± SD, U/L	15.54 ± 10.72	17.48 ± 11.68	0.02
CPK, mean ± SD, U/L	60.27 ± 21.32	59.33 ± 21.36	0.4
Creatinine, mean ± SD, mg/dL	0.67 ± 0.11	0.76 ± 0.14	0.3
Urea, mean ± SD, mg/dL	0.38 ± 0.12	0.46 ± 0.18	0.5
Fasting glucose, mean ± SD	0.86 ± 0.08	0.88 ± 0.09	0.3
FLI, mean ± SD	28.23 ± 18.43	75.37 ± 14.55	<0.0001
HS-CRP, mean ± SD, μg/mL	1.23 ± 0.24	4.72 ± 2.61	<0.0001

Comments: ACEi/ARBs –ACE inhibitors/angiotensin – renin blockers.

At multivariate logistic analysis, these associations maintained their statistical power also after adjustment for other variables, such as BMI (OR 9.7, CI 95%: 5.1–22.8 vs. OR 2.9, CI 95%: 1.2–9.3, $P = 0.01$), total cholesterol (OR 6.7, CI 95%: 3.3–19.4 vs. OR 3.1, CI 95%: 2.4–13.1, $P < 0.05$), and ESC SCORE (OR 11.7, CI 95%: 5.0–27.2 vs. OR 2.9, CI 95%: 1.1–12.1, $P = 0.04$).

The results of receiver operating characteristic (ROC) analysis are reported in Table 4. We tested the accuracy of ESC SCORE or NAFLD score in identifying an early atherosclerotic damage or functional vascular alterations at carotid and femoral arteries.

The ESC SCORE was significantly related with c-IMT and f-IMT, but not with PWv values (Table 4). ROC curve analysis for NAFLD showed a significant higher area under ROC curve (AUC) for the detection of both early atherosclerotic burden and vascular stiffness.

Moreover, we compared ROC curves of ESC SCORE and NAFLD pattern for atherosclerotic burden, finding that ROC for NAFLD were more accurate in discriminating vascular alterations.

4.3. Reactive hyperaemia index

Peripheral arterial tonometry (PAT) analysis was performed in a subgroup of 50 subjects who had been randomly assigned. PAT values are described in details in Table 3.

Only 7 (8.8%) subjects showed pathological natural logarithm of reactive hyperemia index (LnRHI) values (<0.4), according to the standardised cut-off.[22] LnRHI was significantly related with ESC SCORE ($r = –0.464$, $P = 0.001$), and NAFLD score ($r = –0.569$, $P < 0.0001$).

LnRHI values of the NAFLD ≥ 3 group (0.49 ± 0.14) were significantly lower in comparison to NAFLD < 3 subjects (0.44 ± 0.23 vs. 0.72 ± 0.22, $P < 0.0001$). Endothelial

Table 3. Distribution of echographic and endothelial markers, accordingly to NAFLD cut-off.

Variable	NAFLD < 3 $n = 133$	NAFLD > 3 $n = 87$	P value
IMT			
c-IMT, mean ± SD, m	816.9 ± 668.3	1428.94 ± 739.5	<0.0001
c-IMT ≥ 0.9, n(%)	25(18.7)	60(69.0)	<0.0001
f-IMT, mean ± SD, m	805.8 ± 630.6	1643.6 ± 931.7	<0.0001
f-IMT ≥ 1.2, n(%)	15(11.3)	57(65.5)	<0.0001
PWv			
c-PWv, mean ± SD, m/s	7.07 ± 2.1	9.04 ± 2.9	<0.0001
c-PWv ≥ 0.9, n(%)	2(1.5)	22(25.3)	<0.0001
f-PWv, mean±SD, m/s	7.80 ± 2.3	9.78 ± 3.3	<0.0001
f-PWv ≥ 1.2, n(%)	8(6.0)	22(25.3)	<0.0001
ABIx			
ABI, mean ± SD	1.04 ± 0.1	1.01 ± 0.2	0.2
ABI < 0.9, n(%)	8(6.0)	13(14.9)	0.08
PAT* ($n = 50$)			
LnRHI, mean ± SD	0.72 ± 0.22	0.49 ± 0.14	0.01

Comments: * Subgroup analyses on 50 randomly assigned subjects.

Table 4. ROC curve analyses and differences among ROC curves.

Variable	ROC curve	AUC difference	95% CI	Difference P value
c-IMT				
NAFLD ≥ 3 vs.	AUC 0.76, 95% CI: 0.68–0.82	–0.03	–0.09 to –0.03	<0.0001
ESC	AUC 0.73, 95% IC: 0.64–0.83		–0.08 to –0.03	
f-IMT				
NAFLD ≥ 3 vs.	AUC 0.76, 95% CI: 0.69–0.81	–0.04	–0.08 to –0.03	<0.0001
ESC Atherosclerosis	AUC 0.72, 95% CI: 0.63–0.80		–0.09 to –0.03	
NAFLD ≥ 3 vs.	AUC 0.77, 95% CI: 0.68–0.82	–0.07	–0.09 to –0.03	<0.0001
ESC	AUC 0.69, 95% CI: 0.61–0.78		–0.07 to –0.03	
c-PWv				
NAFLD ≥ 3 vs.	AUC 0.72, 95% CI: 0.67–0.80	–0.04	–0.08 to –0.03	0.01
ESC	AUC 0.68, 95% CI: 0.62–0.74		–0.08 to –0.04	
f-PWv				
NAFLD ≥ 3 vs.	AUC 0.75, 95% CI: 0.67–0.81	–0.05	–0.09 to –0.03	0.01
ESC Vascular Compliance	AUC 0.70, 95% CI: 0.63–0.77		–0.07 to –0.03	
NAFLD ≥ 3 vs.	AUC 0.74, 95% CI: 0.68–0.82	–0.05	–0.09 to –0.03	0.01
ESC	AUC 0.69, 95% CI: 0.65–0.79		–0.07 to –0.03	

dysfunction was associated with higher HS-CRP levels (4.7 ± 0.48 μg/mL vs. 1.4 ± 0.62 μg/mL, $P = 0.001$).

At univariate logistic analysis, a NAFLD ≥ 3 score was significantly associated to endothelial dysfunction (OR 19.1, CI 95%: 5.3–68.5; $P < 0.0001$); this statistical relevance was confirmed even after adjustment for ESC SCORE at multivariate logistic analyses (OR 7.7, CI 95%: 1.3–44.2; $P = 0.02$).

At ROC analyses, NAFLD score showed higher accuracy in detecting endothelial dysfunction (AUC 0.80, 95% CI: 0.72–0.87, $P < 0.0001$), in comparison to ESC SCORE (AUC 0.74, 95% CI: 0.65–0.85, $P = 0.0001$).

5. DISCUSSION

In this work we evaluated the pattern of ultrasound-detected NAFLD occurrence and severity in a primary prevention non-diabetic population, stratified according to ESC guidelines and extensively investigated for peripheral vascular function.

NAFLD score was significantly associated to anthropometrical features (as BMI and waist circumference) and lipid profile. The association between NAFLD and features of metabolic syndrome is widely reported in literature;[23,24] in particular, it is well known that NAFLD is strongly associated with obesity, insulin resistance, hypertension, and

dyslipidemia and these cardiovascular risk factors are active in atherosclerotic trigger and progression.[25,26] However, despite the strong association between NAFLD and metabolic syndrome, recent studies suggest that NAFLD is not only a marker of cardiovascular risk, but a trigger of systemic atherosclerosis, involved in pathogenesis independently of known risk factors of metabolic syndrome.[9]

Despite the low cardiovascular risk profile according to the ESC SCORE, and the absolutely silent clinical presentation, more than the 30% of enrolled subjects showed c-IMT more than or equal to and f-IMT above the normal cut-off values and/or increased carotid or femoral vascular stiffness, evaluated by c-PWv and f-PWv more than 12 s/m. As suggested in a different study, vascular stiffness and intima media thickening could express different aspects of atherosclerosis, and they are both significantly associated to increased risk of cardiovascular events.[27] ESC SCORE succeeded in selecting subjects with pathological c-IMT and f-IMT, but not with increased PWVs. However, when subjects were divided according to steatosis scoring, NAFLD ≥ 3 group showed the worst carotid and femoral atherosclerotic burden. In particular, a NAFLD ≥ 3 score was able to identify subjects with pathological c-IMT and f-IMT, and/or PWV more than 12 s. Our data showed that the assessment of degree of liver steatosis could provide a global evaluation of vascular risk profile; when compared with ESC SCORE stratification, NAFLD scoring was the strongest independent risk factor for subclinical atherosclerosis (c-IMT and/or f-IMT) and functional vascular damage (c-PWv and/or f-PWv), even after adjustments at multivariate analysis.

Several works pointed out the relationship between the occurrence of NAFLD and the development of early atherosclerotic damage at different vascular districts. Patients suffering from NAFLD have a higher prevalence of increased carotid wall intimal thickness, atherosclerotic plaques, and elevated circulating levels of markers of endothelial dysfunction.[28–30] Several recent researches reported a strong association between NAFLD and impaired arterial compliance; in particular, in a case-control study the presence and the severity of NAFLD was associated with an impaired vascular compliance, assessed by brachial-ankle PWv, in non-hypertensive, non-diabetic patients,[31] suggesting an independent role in damaging vascular properties.

Moreover, we provided a detailed district-specific assessment of vascular alterations; accordingly to our findings, the contemporaneous involvement of different artery sides confirmed the presence of a global atherosclerotic pattern, secondary to a systemic trigger, like a pro-inflammatory and pro-thrombotic burden.

Moreover, our data collected on a subgroup of 50 individuals showed that NAFLD was significantly associated to endothelial dysfunction.

As suggested in current literature, the link between NAFLD and cardiovascular adverse events could be researched in a damaged endothelium, secondary to a chronic activated inflammatory burden and to alterations of hepatic function,[32] independently of other traditional cardiovascular risk factors. Moreover, endothelial dysfunction and NAFLD were extensively associated to an increased inflammatory burden and a pro-thrombotic pattern,[33] typical of atherosclerosis, and both NAFLD and endothelial dysfunction were extensively associated to atherosclerotic vascular lesions and their progression.[34]

The main limit of the study was that we did not provide any invasive method to assess severity of NAFLD, in particular liver biopsy. We provided only noninvasive methods, which are not adequately validated in comparison to liver biopsy. Moreover, we used a semiquantitative scale for NAFLD basing on USG imaging, which has a limited value and is not commonly used in the European population.

CRP is a weak marker of systemic inflammation.

6. CONCLUSIONS

The screening of ultrasound-detected NAFLD severity could optimize the assessment of cardiovascular risk in primary prevention.

Namely NAFLD scoring could identify those subjects who have subclinical early vascular damage.

A NAFLD ≥ 3 score was able to screen a subgroup of patients with widespread morphological vascular damage and endothelial dysfunction in a primary prevention population.

Conflict of interest

Authors declare no conflict of interest.

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