FibroScan Detects Cardiovascular Damage in Patients With NAFLD

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Patients with nonalcoholic fatty liver disease (NAFLD), particularly in the presence of nonalcoholic steatohepatitis or fibrosis, are at high cardiac and cerebrovascular risk.[1]

Over the last years, FibroScan (Echosens, Paris, France) has been used to identify noninvasive fibrosis defining liver stiffness measurement (LSM).[2] LSM has been demonstrated to predict both liver-related complications and all-cause mortality,[3] but scarce attention has been addressed to cardiovascular (CV) damage.

The widely described patatin-like phospholipase domain-containing 3 (PNPLA3) polymorphism confers an increased risk of progressive liver disease,[4] but its possible effect on CV risk has not yet been defined.

The aim of this study was to evaluate whether 1) LSM detects CV damage and 2) PNPLA3 polymorphisms influence CV risk.

Methods

Patients

Four hundred seventy-two consecutive NAFLD patients in whom other causes of liver disease were ruled out were recruited at 3 Italian centers. Patients underwent liver biopsy within 6 months from CV and FibroScan assessment. The study project was approved by the Institutional Review Board. All patients provided informed consent to participate to the study according to the ethical guidelines of the 1975 Declaration of Helsinki.

Clinical, anthropometric, and laboratory data were collected at the time of biopsy.

Genetic Analysis

The rs738409 C>G (I148M PNPLA3) single nucleotide polymorphisms were assessed in duplicate by TaqMan 5′-nuclease assays (Life Technologies, Carlsbad, CA) in 426 patients, as previously described.[4]

Transient Elastography Assessment

FibroScan using LSM cutoffs 8.7 kPa and 7.2 kPa (M and XL probes)[2] was performed by 1 expert physician for each center.

CV Risk Profile Assessment

History of past or current CV events, including myocardial infarction and ischemic or hemorrhagic stroke, was recorded.

Carotid Atherosclerosis

Mean carotid intima-media thickness (cIMT) and presence of carotid plaques were recorded. We arbitrarily considered cIMT values <0.64 mm as normal. Subclinical atherosclerosis was defined as a cIMT value >0.9 mm and carotid plaque as a focal thickening >1.2 mm of the carotid artery.[5] In 103 patients, carotid arterial stiffness (pulse wave velocity [PWV]) was measured by radiofrequency ultrasonography.[6]

Transthoracic Echocardiography

Conventional echocardiographic parameters such as ejection fraction, left ventricular mass diastolic dysfunction (E/A ratio <1),[7] and epicardial adipose tissue[8] were measured.

Statistical Analysis

Multiple linear or logistic regression analyses were used employing a model fully adjusted for all the variables of important clinical relevance or highly

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Table 1. Association of LSM by FibroScan and PNPLA3 Genotype With Cardiovascular Parameters in Patients With Nonalcoholic Fatty Liver Disease

Parameter	Multivariate Analysis					Multivariate Analysis				
	LSM <8.7/7.2 kPa M/XL (n = 274)	LSM ≥8.7/7.2 kPa M/XL (n = 198)	*P* Value	OR/Beta Coefficient (95% CI)	*P* Value	PNPLA3 CC (n = 134)	PNPLA3 CG/GG (n = 292)	*P* Value	OR/Beta Coefficient (95% CI)	*P* Value
Overall series										
CV events	14 (5)	21 (11)	.031	1.11 (0.47 to 2.63)	.805	6 (5)	13 (4)	.9	1.73 (0.54 to 5.53)	.360
Carotid parameters										
cIMT >0.9 mm	82 (30)	83 (42)	.016	1.32 (0.8 to 2.2)	.272	41 (31)	102 (35)	.499	1.23 (0.67 to 2.29)	.500
cIMT >0.64 mm	204 (74)	169 (85)	.005	1.17 (0.61 to 2.2)	.625	104 (78)	225 (77)	.800	0.68 (0.35 to 1.25)	.250
Carotid plaques	100 (36)	112 (56)	<.001	1.83 (1.08 to 3.11)	.025	59 (44)	134 (46)	.831	1.09 (0.62 to 1.9)	.770
PWV, m/s	7.2 ± 2.2	9 ± 1.8	.012	0.18 (–0.09 to 2.67)	.307	7.2 ± 2.5	8.1 ± 2.1	.184	0.156 (–0.63 to 2.08)	.280
	LSM <8.7/7.2 kPa M/XL (n = 164)	LSM ≥8.7/7.2 kPa M/XL (n = 135)	*P* Value	OR/Beta Coefficient (95% CI)	*P* Value	PNPLA3 CC (n = 98)	PNPLA3 CG/GG (n = 169)	*P* Value	OR/Beta Coefficient (95% CI)	*P* Value
Echocardiographic parameters										
E/A ratio <1	71 (43)	81 (60)	.005	1.02 (0.57 to 1.82)	.942	45 (46)	101 (60)	.039	1.5 (0.77 to 2.92)	.234
EAT thickness >9.5/7.5 mm[a]	48 (29)	59 (44)	.063	1.26 (0.52 to 3.06)	.603	29 (30)	62 (37)	.386	1.02 (0.43 to 2.43)	.963
	LSM <8.7/7.2 kPa M/XL (n = 167)	LSM ≥8.7/7.2 kPa M/XL (n = 63)	*P* Value	OR/Beta Coefficient (95% CI)	*P* Value	PNPLA3 CC (n = 73)	PNPLA3 CG/GG (n = 136)	*P* Value	OR/Beta Coefficient (95% CI)	*P* Value
Patients <50 years of age										
Carotid parameters										
cIMT >0.9 mm	35 (21)	17 (27)	.377	1.07 (0.45 to 2.55)	.870	17 (23)	28 (21)	.723	1.08 (0.47 to 2.5)	.857
cIMT >0.64 mm	109 (65)	50 (79)	.053	2.28 (0.81 to 6.4)	.117	51 (70)	83 (61)	.210	0.72 (0.33 to 1.59)	.425
Carotid plaques	40 (24)	23 (36)	.094	1.25 (0.52 to 3.01)	.610	18 (25)	35 (26)	1.000	1.09 (0.47 to 2.05)	.840
PWV, m/s	6.2 ± 2.9	10.2 ± 1.1	.009	0.49 (0.51 to 6.42)	.026	6.09 ± 1.82	8.8 ± 2.4	.022	0.475 (0.31 to 3.81)	.025
	LSM <8.7/7.2 kPa M/XL (n = 98)	LSM ≥8.7/7.2 kPa M/XL (n = 42)	*P* Value	OR/Beta Coefficient (95% CI)	*P* Value	PNPLA3 CC (n = 51)	PNPLA3 CG/GG (n = 68)	*P* Value	OR/Beta Coefficient (95% CI)	*P* Value
Echocardiographic parameters										
E/A ratio <1	26 (26)	18 (42)	.070	1.12 (0.4 to 3.1)	.819	15 (29)	21 (31)	1.000	1.32 (0.47 to 3.66)	.590
EAT thickness >9.5/7.5 mm[a]	20 (20)	15 (36)	.206	1.31 (0.29 to 5.78)	.720	10 (20)	16 (23)	.788	1.73 (0.46 to 6.53)	.420

NOTE. Values are n (%) or mean ± SD, unless otherwise indicated. There were 472 consecutive patients with nonalcoholic fatty liver disease, 439 biopsy-proven and 33 with a clinical diagnosis of metabolic cirrhosis. Multivariate analysis was adjusted for age, sex, waist circumference, current smoking, type 2 diabetes, hypertension, statins use, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker use; parameters not associated with liver stiffness measurement (LSM): ejection fraction, left ventricular mass.

CV, cardiovascular; cIMT, carotid intima-media thickness; E/A ratio, peak early diastolic and peak late diastolic ratio; EAT, epicardial adipose tissue; LSM, liver stiffness measurement; OR, odds ratio; PNPLA3, patatin-like phospholipase domain-containing 3; PWV, pulse wave velocity.

[a]EAT >9.5/7.5 mm is the referred normal value for men and women in general population.

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statistical significance at univariate analyses ($P < .05$). Bonferroni correction for 12 tests was also done.

Results

CV Risk Profile

Previous CV events occurred in 35 (8%) patients. Increased cIMT (>0.64) was present in 373 (79%) patients, subclinical atherosclerosis (cIMT >0.9 mm) in 165 (35%), and carotid plaques in 212 (45%). Mean PWV was 7.75 ± 2.27 m/s.

Liver Damage and CV Parameters

FibroScan mean values were 10.8 ± 10 (2.2–75 kPa) and 11 ± 10 (2.9–45 kPa) for M and XL probes, respectively. High LSM values, confirmed by histology (≥F3) in 84% of cases, were found in 198 (42%) patients.

Carotid thickening and plaques, E/A ratio <1, increased PWV, and a past history of CV events were significantly more prevalent in patients with LSM ≥8.7/7.2 kPa.

When significant variables were analyzed in a multivariate model, LSM > 8.7/7.2 kPa was significantly associated with carotid plaques in the overall series (odds ratio, 1.83; 95% confidence interval, 1.08–3.1; $P = .025$). In patients <50 years of age, LSM values ≥8.7/7.2 kPa were also independently associated with increased PWV values (beta coefficient, 0.49; 95% confidence interval, 0.51-6.45; $P = .026$) (Table 1).

Influence of PNPLA3 on CV Risk

PNPLA3 polymorphisms, evaluated in 426 patients, showed that the PNPLA3 G allele in both homozygosity and heterozygosity state compared with wild-type homozygosity (6.09 ± 1.82 m/s vs 8.8 ± 2.4 m/s; $P = .022$) was significantly associated with PWV in patients <50 years of age, despite that this significance was lost after Bonferroni adjustment for 12 tests. The significant association between the PNPLA3 G allele and PWV was demonstrated at multivariate analysis adjusted for age, sex, waist circumference, current smoking, diabetes mellitus, hypertension, statin use, and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use in patients <50 years of age (beta coefficient, 0.475; 95% confidence interval, 0.31–3.81; $P = .025$) (Table 1).

Discussion

Our results indicate that FibroScan can detect CV alterations in NAFLD patients and that PNPLA3 polymorphisms may help to identify early CV impairment in younger patients.

LSM detected the presence of carotid plaques in the overall series, identifying patients with a more advanced CV disease, while in patients <50 years of age, who have significantly lower prevalence of metabolic alterations, it resulted independently associated with carotid stiffness, a very early marker of CV damage, previously associated with increased incidence of CV events and all-cause mortality.[6] In these patients, a positive independent association between homozygosity for PNPLA3 mutated alleles and increased carotid stiffness was detected, possibly due to accumulation of lipids in both the liver and vessels mediated by PNPLA3.

In conclusion, the combined use of FibroScan and PNPLA3 polymorphism could be proposed for the detection of CV damage in NAFLD patients, especially if young.

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Reprint Requests
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Conflicts of interest
The authors disclose no conflicts. Q2

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