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Lysosomal acid lipase activity and liver fibrosis in the clinical continuum of non-alcoholic fatty liver disease

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Abstract

Background and Aims: Recent evidence showed a reduced activity of the lysosomal acid lipase (LAL) in patients with non-alcoholic fatty liver disease (NAFLD) and cryptogenic cirrhosis (CC). However, the relationship between LAL activity and liver fibrosis has never been investigated.

Methods: Cross-sectional study including 575 outpatients referred for the management of cardio-metabolic and liver disease. The absence of liver fibrosis was defined by a FIB-4 < 1.30 and NAFLD fibrosis score (NFS) <−1.455. LAL activity was measured with dried blood spot technique.

Results: Overall, 515 patients had a diagnosis of NAFLD (454 NAFL and 61 biopsy-proven NASH) and 60 of CC. The value of LAL activity progressively decreased from healthy subjects to NAFL/NASH patients to CC (P < .001). LAL activity was reduced by 10% in patients with NAFL, by 20% in NASH and by 50% in CC. The prevalence of CC decreased across the tertiles of LAL activity: 22.2% in the lowest, 4.6% in the intermediate and 0.5% in the highest tertile. In NAFLD patients, 69.9% had a FIB4 < 1.30, and 43.1% a NFS <−1.455. Multivariate logistic regression analysis showed that Log (LAL activity) was associated with FIB-4 < 1.30 (Odds ratio [OR] 2.19 95% confidence interval [CI] 1.33-3.62, P = .002) and NFS < −1.455 (OR 2.43, 95% CI 1.51-3.91, P < .001) after adjustment for confounding factors.

Conclusions: We found a progressive reduction of LAL activity according to liver disease severity. LAL activity was inversely associated with markers of liver fibrosis in patients with NAFLD.

KEYWORDS
cryptogenic cirrhosis, liver fibrosis, lysosomal acid lipase, NAFLD

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adult treatment panel; CC, cryptogenic cirrhosis; CESD, cholesterol ester storage disease; CPT, Child-Pugh-Turcotte; DBS, dried blood spot; FIB-4, fibrosis-4; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; HS, healthy subjects; LAL, lysosomal acid lipase; LAL-D, lysosomal acid lipase deficiency; LIPA, lipase a, lysosomal acid; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score; OR, odds ratio; PNPLA3, patatin-like phospholipase domain-containing protein 3.

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1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) includes a broad spectrum of liver disorders, which may progress from simple steatosis (NAFL) to non-alcoholic steatohepatitis (NASH), NASH cirrhosis, hepatocellular carcinoma and liver-related death.

Non-alcoholic fatty liver disease represents the most common and emerging chronic liver disease worldwide with an estimated prevalence of 25%-30% in the general population rising to 50% in some clinical conditions such as diabetes, metabolic syndrome and obesity. Most patients develop a mild disease with minimal inflammation and no liver fibrosis, and about 25% of patients affected by NASH may develop significant fibrosis and eventually cirrhosis.

The pathogenesis of NAFLD appears to be multifactorial and many mechanisms promoting the excessive fat accumulation in the liver have been proposed, including patatin-like phospholipase domain-containing protein 3 (PNPLA3) genetic mutation and insulin resistance.[1,2] However, the pathogenic mechanisms that promote the development of significant liver inflammation and fibrosis are not yet fully elucidated. This is of concern as the presence of liver fibrosis has been shown to be the most important factor worsening the prognosis of patients with NAFLD.[3]

Lysosomal acid lipase (LAL) is a hydrolase that plays a key role in intracellular cholesterol trafficking. A reduced LAL (LAL-D) activity promotes increased lysosomal cholesterol ester storage, as observed in two recessive autosomal genetic diseases, namely Wolman disease and Cholesterol ester storage disease (CESD). LAL activity is absent in Wolman disease and greatly reduced (<10%) in the case of the CESD. The most common mutation is the E8SJM variant, a single mutation affecting the LIPA gene. However, more than 40 loss-of-function mutations have been reported and, at the moment, no data are present on the modulation of LAL activity in vivo, nor on the epigenetic and metabolic factors able to regulate its activity in subjects without mutations of the LIPA gene.

Previous studies showed a progressive decrease in LAL activity from healthy subjects (HS) to subjects with NAFL and those with biopsy-proven NASH.[4,5] More recently, in patients with cryptogenic cirrhosis (CC), a further reduction in the activity of LAL has been described.[6,7] These results were similar to those obtained in children with biopsy-proven NASH.[8]

However, the relationship between LAL activity and liver fibrosis has not been investigated in adult patients with NAFLD.

The aims of the study were to evaluate LAL activity across the wide clinical continuum of NAFLD and to assess the relationship between LAL activity and surrogate markers of liver fibrosis in this clinical setting.

Key points

- Lysosomal acid lipase (LAL) activity progressively reduces with liver disease severity by 10% in patients with NAFL, by 20% in NASH and by 50% in cryptogenic cirrhosis (CC).
- In patients with non-alcoholic fatty liver disease (NAFLD), LAL activity is inversely associated with non-invasive markers of liver fibrosis.

2 | MATERIALS AND METHODS

We performed a cross-sectional study which included 583 consecutive outpatients referring to the Day Service of Internal Medicine and Metabolic Diseases of the Department of Internal Medicine of Sapienza University of Rome for the management of cardio-metabolic risk factors, such as metabolic syndrome, dyslipidemia, arterial hypertension, diabetes and liver diseases. Inclusion criteria for the study were: no history of alcohol consumption above 20 gr/daily; no evidence of hepatitis C-B virus infections or autoimmune or drug-induced hepatitis. In addition, the study also included a cohort of 60 patients with cryptogenic cirrhosis (CC) followed up at the liver disease outpatient clinic.

Lysosomal acid lipase activity values from 100 voluntary HS, defined as subjects without evidence of any diseases, not taking drugs and without fatty liver at liver ultrasonography scanning (US) are reported in Figure 1 as reference of normal value. The reduction in LAL activity was defined by a value below the 2.5th percentile (<0.8 nmol/spot/h) of LAL activity from HS, as previously described.[4]

At first visit, a liver US was performed and data on comorbidities and previous major adverse cardiovascular and cerebrovascular events (MACCE) were collected. Metabolic syndrome was diagnosed according to the ATP III-modified criteria.[9] The presence of diabetes and arterial hypertension was defined according to international guidelines.[10,11]

Moreover, patients underwent a complete clinical and biochemical diagnostic work-up including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), fasting total and HDL cholesterol, triglycerides, glucose, creatinine, albumin and a complete blood count.

2.1 | Definition of liver diseases

Liver steatosis was defined by ultrasonography (US) according to Hamaguchi criteria based on the presence of abnormally intense, high level echoes arising from the hepatic parenchyma, liver-kidney difference in echo amplitude, echo penetration into deep portion of the liver and clarity of liver blood vessel structure.[12] To include patients in the NAFLD group, the following were considered as exclusion criteria: average daily consumption of alcohol >20 g in women and >30 g in men (assessed by Alcohol Use Disorders Identification Test, AUDIT[13]; the presence of hepatitis B surface antigen and antibody to hepatitis C virus; positive tests for autoimmune hepatitis; cirrhosis and other chronic liver diseases; diagnosis of oncological diseases and concomitant therapy with

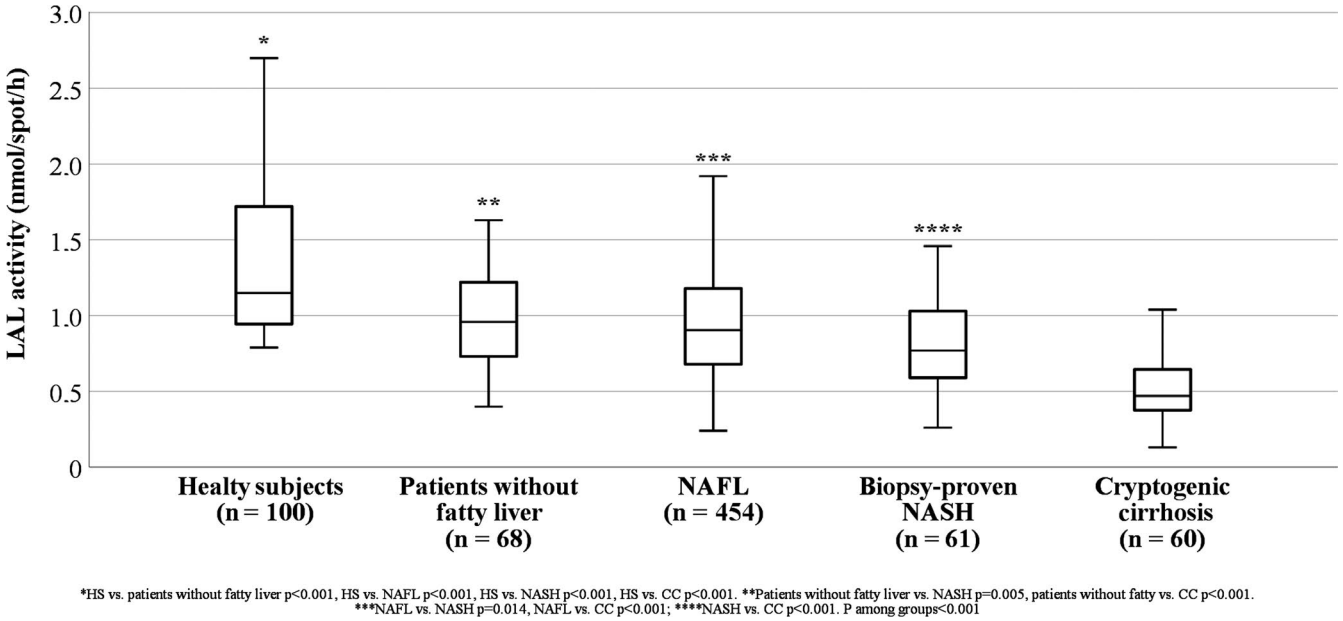

FIGURE 1 Lysosomal acid lipase (LAL) activity in the five study groups

drugs known to promote liver steatosis (eg amiodarone); other chronic infectious or autoimmune diseases; clinical, biochemical or US signs of cirrhosis or portal hypertension (nodular liver, ascites, portal flow mean velocity <14 cm/s, inversion of flow in the portal vein, portosystemic collaterals, portal vein diameter >13 mm, decreased or no respiratory variation in splenic and superior mesenteric vein diameter, portal/splenic/superior mesenteric vein thrombosis).[14,15]

Percutaneous ultrasonography-guided liver biopsy was performed in NAFLD patients with clinical suspicion of NASH and persistent elevation of liver enzymes (>6 months). NASH was diagnosed in 61 patients by means of standard criteria based on separate scores for steatosis, hepatocellular ballooning and inflammation.[16,17]

Cryptogenic cirrhosis was defined as cirrhosis of unknown aetiology in patients with clinical history of previous overweight/obesity, diabetes, insulin resistance and/or liver steatosis and no history of alcoholism or alcohol consumption higher than 20 g/d in men and 10 g/d in women, or previous acute or chronic viral and autoimmune hepatitis.[18] The severity of CC was assessed by the Child-Pugh-Turcotte (CPT) score.

2.2 | Non-invasive markers of liver fibrosis

Fibrosis-4 (FIB-4) index and the NAFLD fibrosis score (NFS) are two simple non-invasive, accurate and validated methods to identify a high probability of severe fibrosis in adult patients with NAFLD.[19,20] The use of these scores can accurately rule out the presence of liver fibrosis thus reducing the need for liver biopsy in most of the patients with NAFLD. Furthermore, these indexes can be easily calculated in large populations and repeated over time. Furthermore, high FIB-4 and NFS scores showed a prognostic value for liver disease mortality.[21]

2.3 | LAL activity assessment and LIPA gene sequencing

LAL activity was dosed with dried blood spot (DBS) technique using the inhibitor Lalistat 2 as previously reported,[6] and expressed as nmol/spot/h. DBS tests were performed in Bambino Gesù Hospital in Rome. Physicians analysing LAL activity were unaware of clinical and biochemical characteristics of any enrolled patient. Inter- and intra-assay variations were 2.4% and 2.3% respectively.

The LIPA gene was sequenced by the next-generation sequencing method as previously reported.[22] In addition, to definitely exclude the presence of CESD-causing mutations, the Sanger method was used to resequence the Exon 8 of LIPA gene in NAFLD patients with a LAL activity value of ≤0.6 mmol/spot/h. Standard PCR and Sanger sequencing were performed as reported elsewhere.[23] No CESD-causing mutations were found in all cases (data not shown).

Informed written consent was obtained, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the local ethical board of Sapienza University of Rome (ref. n° 2277/2011).

2.4 | Statistical analysis

Continuous variables were expressed as mean and standard deviation or median and interquartile range (IQR) depending on their distribution. Group comparisons were performed by unpaired Student's *t* test and ANOVA, or by Mann-Whitney or Kruskal-Wallis test when appropriate. Proportions and categorical variables were tested by the χ^2 test. Correlation analysis was performed by Spearman rank correlation test (rS).

We performed a descriptive analysis of characteristics of patients according to liver disease groups: Patients without fatty liver

(n = 68); NAFL (n = 454); NASH (n = 61); CC (n = 60). A univariate and multivariate logistic regression analysis of factors associated with the clinical diagnosis of CC was performed to estimate the adjusted odds ratio (OR) with 95% confidence interval (95% CI) of each variable. In the multivariate model, variables presenting significant differences among groups, as reported in Table 1, were used as covariates. Then, factors associated with FIB-4 < 1.30 and NFS < −1.455 were investigated by a multivariable logistic regression analysis.

For the logistic regression analysis, variables with non-normal distribution were log-transformed (ie LAL and AST values) or categorized (ie platelet count and albuminemia) when possible.

Variables were categorized or dichotomized for the logistic regression analysis. All tests were two-tailed, a P value <.05 was considered statistically significant. Analyses were performed using computer software (SPSS 23).

3 | RESULTS

In the entire cohort, 515 patients had a diagnosis of NAFLD (454 had NAFL and 61 biopsy-proven NASH), 68 were without steatosis at liver US and 60 had CC. Values of clinical and biochemical parameters in subjects belonging to the four groups are reported in Table 1.

3.1 | Characteristics of patients with CC

In cirrhotic patients, 57.9% were CPT A, 35.1% were CTP B and 7.0% were CTP C. Patients with CC were older, more frequently diabetic and hypertensive and less frequently on statin therapy (Table 1). They presented worse liver function indexes, as shown by a lower mean albumin, cholesterol (total, HDL and LDL) and triglycerides, higher median liver function tests and lower platelet count (Table 1).

The value of LAL activity progressively decreased from HS to NAFL/NASH and CC (Table 1). In particular, a 10% reduction in LAL activity was present in patients with NAFL, while those with NASH had a 20% reduction and those with CC had a 50% reduction (Figure 1). Moreover, the proportion of LAL < 0.8 nmol/spot/h increased from NAFL to NASH and CC (Figure 2).

In particular, the prevalence of CC decreased across the tertiles of LAL activity, with a higher prevalence in the lowest tertile (22.2%)

TABLE 1 Clinical and biochemical characteristics in patients without steatosis and in those with NAFL, NASH and cryptogenic cirrhosis (CC)

	Patients without fatty liver (n = 68)	NAFL (n = 454)	NASH (n = 61)	Cryptogenic cirrhosis (n = 60)	*P* (among groups)	*P* (CC vs NAFLD)
Age (y)	59.3 ± 13.9	57.4 ± 11.3	49.5 ± 12.9	68.6 ± 10.6	<.001[a]	<.001
Women (%)	50.0	39.6	37.7	28.3	.096[b]	.122
Diabetes (%)	9.0	30.1	29.5	58.3	<.001[b]	<.001
Arterial hypertension (%)	58.5	74.5	55.9	50.0	<.001[b]	.001
Previous MACCE (%)	7.5	6.0	1.6	16.1	.013	.007
Blood glucose (mg/dL)	95.2 ± 19.2	105.9 ± 26.6	100.0 ± 18.0	118.9 ± 40.5	<.001[a]	.018
Total cholesterol (mg/dL)	194.6 ± 41.0	197.9 ± 39.8	190.7 ± 38.0	153.1 ± 41.7	<.001[a]	<.001
HDL (mg/dL)	58.2 ± 12.2	48.3 ± 12.9	49.4 ± 19.8	45.7 ± 15.6	<.001[a]	.214
LDL (mg/dL)	117.3 ± 38.7	118.2 ± 34.7	112.9 ± 32.3	85.4 ± 32.7	<.001[a]	<.001
Triglycerides (mg/dL)	95.5 [78.5-113.7]	138.0 [104.0-182.7]	124.5 [88.0-165.5]	94.0 [73.0-120.0]	<.001[d]	<.001
ALT (UI/l)[c]	17.0 [13.0-22.0]	25.0 [18.0-37.0]	55.0 [41.5-104.5]	32.0 [22.0-43.0]	<.001[d]	.090
AST (UI/l)[c]	17.0 [16.0-21.0]	20.5 [17.0-26.0]	35 [27.0-51.5]	38 [25.2-55.2]	<.001[d]	<.001
GGT (UI/l)[c]	17.0 [12.0-25.5]	25.0 [16.0-37.0]	38.0 [28.0-86.0]	85.5 [38.0-173.0]	<.001[d]	>.001
Platelets (×10^9/L)	230.6 ± 57.7	239.4 ± 60.5	228.6 ± 54.4	107.7 ± 55.5	<.001[b]	<.001
Albumin (g/dL)	4.5 ± 0.4	4.4 ± 0.3	4.4 ± 0.3	3.5 ± 0.7	<.001[b]	<.001
Serum creatinine (mg/dL)[c]	0.9 [0.7-0.9]	0.9 [0.7-1.0]	0.9 [0.8-1.0]	0.9 [0.7-1.2]	.492[d]	.432
LAL activity (nmol/spot/h)[c]	1.0 [0.7-1.2]	0.9 [0.7-1.2]	0.8 [0.6-1.0]	0.5 [0.4-0.6]	<.001[d]	<.001
Statin (%)	47.7	43.2	21.7	7.0	<.001[c]	<.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CC, cryptogenic cirrhosis; GGT, γ-glutamil transferase; HDL, high-density lipoprotein; LAL, lysosomal acid lipase; LDL, low-density lipoprotein; MACCE, major adverse cardiovascular and cerebrovascular events; NAFL, non-alcoholic fatty liver (ie 'simple steatosis'); NASH, non-alcoholic steatohepatitis.

[a]ANOVA test.

[b]χ^2 test.

[c]Data reported as median and interquartile range.

[d]Kruskal-Wallis test.

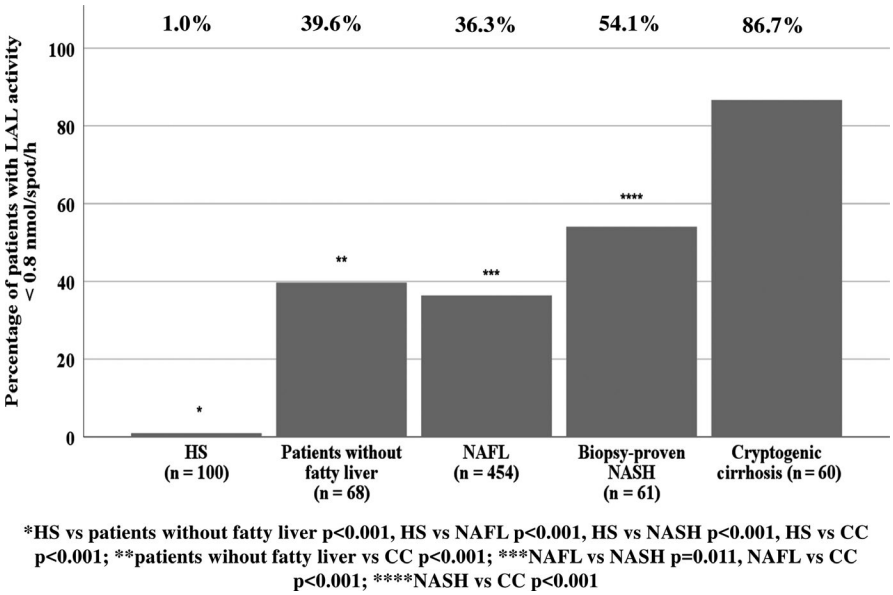

FIGURE 2 Percentage of LAL < 0.8 nmol/spot/h across liver disease groups

*HS vs patients without fatty liver p<0.001, HS vs NAFL p<0.001, HS vs NASH p<0.001, HS vs CC p<0.001; **patients wihout fatty liver vs CC p<0.001; ***NAFL vs NASH p=0.011, NAFL vs CC p<0.001; ****NASH vs CC p<0.001

as compared to 4.6% in the intermediate and 0.5% in the highest tertile (Figure 3).

In Table 2, the multivariate logistic regression analysis showed that age (OR = 1.09, *P* < .001), diabetes (OR = 9.24, *P* = .006), log (AST) (OR = 7.74, *P* = .003), thrombocytopenia (OR = 16.03, <0.001), log (LAL) (OR = 0.12, *P* = .010) and statin use (OR = 0.13, *P* = .019) were independently associated with CC.

3.2 | Determinants of liver fibrosis in patients with NAFLD

In NAFLD patients, 69.9% had a FIB4 < 1.30, and 43.1% a NFS <−1.455. Patients with negative FIB-4 were younger and less likely to have arterial hypertension and previous MACCE than patients with FIB4 ≥ 1.30 (Table 3, panel A). They also had higher LDL cholesterol and platelets and lower AST and GGT values. LAL activity was higher in patients with negative FIB-4 (Table 3, panel A). Similar results were obtained using NFS (Table 3, panel B).

Multivariate logistic regression analysis showed that log (LAL activity) and previous MACCE were associated with FIB-4 < 1.30 (Table 4, model A). Using NFS as a dependent variable, we found that female sex, arterial hypertension, log (LAL activity) and previous MACCE were associated with NFS < −1.455 (Table 4, model B).

3.3 | LAL activity and white blood cells

In a subgroup of 330 NAFLD patients, we investigated the relationship between LAL activity and white blood cells count (WBC). We found a mean value of WBC of 6.65 ± 1.77 x 10^3/μl; WBC and LAL activity were weakly correlated (rS = 0.215, *P* < .001). After adjustment for WBC, log (LAL activity)/log (WBC) remained significantly associated with Fib4 < 1.30 (OR = 4.74; 95% CI 1.69-13.35, *P* = .003) and with NFS <−1.455 (OR = 4.20; 95% CI 1.61-10.96, *P* = .003) at univariable logistic regression analysis.

4 | DISCUSSION

Our cross-sectional study confirmed a progressive reduction in LAL activity with the severity of NAFLD, and we found a correlation between LAL activity and two validated non-invasive markers of liver fibrosis, namely FIB-4 and NFS.

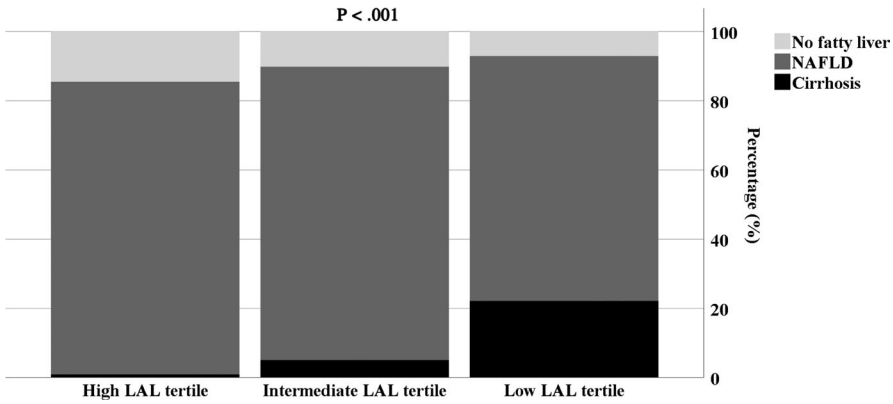

FIGURE 3 Proportion of liver diseases across tertiles of LAL activity

TABLE 2 Univariate and multivariate logistic regression analysis of factors associated with cryptogenic cirrhosis

	Univariate analysis OR (95% CI)	*P*	Multivariate analysis OR (95% CI)	*P*
Age	1.11 (1.07-1.14)	<.001	1.09 (1.02-1.17)	.011
Female sex	0.57 (0.32-1.02)	.058	0.28 (0.06-1.44)	.129
Diabetes	3.68 (2.13-6.35)	<.001	9.24 (1.90-44.9)	.006
Arterial hypertension	0.41 (0.24-0.71)	.001	0.29 (0.06-1.38)	.117
Total cholesterol	0.97 (0.96-0.98)	<.001	0.99 (0.97-1.00)	.114
Log (AST)	6.94 (4.06-11.85)	<.001	7.74 (2.05-29.17)	.003
Thrombocytopenia (platelets <150 × 10^9/l)	59.34 (29.10-121.04)	<.001	16.03 (3.68-82.62)	<.001
Hypoalbumineamia (albumin <3.5 g/dL)	328.70 (43.00-2512.49)	<.001	24.88 (0.84-739.53)	.063
Log (LAL activity)	0.03 (0.01-0.06)	<.001	0.12 (0.02-0.60)	.010
Statin use	0.11 (0.04-0.30)	<.001	0.13 (0.02-0.71)	.019

Abbreviations: AST, aspartate aminotransferase; CI, confidence interval; LAL, lysosomal acid lipase; OR, odds ratio.

TABLE 3 Characteristics of 515 NAFLD patients with and without liver fibrosis according to FIB-4 (Panel A) and NFS (Panel B) scores

	Panel A			Panel B		
	FIB-4 < 1.30 (n = 360)	FIB-4 ≥ 1.30 (n = 155)	*P*	NFS < −1.455 (n = 222)	NFS≥ −1.455 (n = 293)	*P*
Age (y)	50.7 ± 12.2	60.7 ± 9.4	<.001	50.7 ± 12.2	60.7 ± 9.4	<.001
Women (%)	40.3	39.7	.921	35.3	42.6	.101
Diabetes (%)	28.0	34.5	.489	13.3	42.6	<.001
Arterial hypertension (%)	59.1	69.6	.033	49.5	70.2	<.001
Previous MACCE (%)	3.2	11.5	.001	0.5	9.3	<.001
Blood glucose (mg/dL)	104.8 ± 27.9	105.6.4 ± 19.5	.753	96.0 ± 18.1	112.4 ± 28.6	<.001
Total cholesterol (mg/dL)	199.3 ± 38.1	192.2 ± 41.7	.065	203.8 ± 40.0	192.6 ± 38.3	.001
HDL (mg/dL)	47.8 ± 12.9	50.2 ± 16.1	.074	49.1 ± 15.8	48.0 ± 12.3	.399
LDL (mg/dL)	119.9 ± 33.5	112.0 ± 35.3	.019	121.7 ± 33.5	114.6 ± 34.9	.023
Triglycerides (mg/dL)	135.0 [104.0-185.0]	138.0 [96.0-173.0]	.247	136.0 [108.5-189.5]	138.0 [97.7-178.0]	.191
ALT (UI/l)	27.0 [19.0-40.2]	28.0 [18.0-47]	.596	29.0 [20.0-45.0]	25.0 [18.0-40.0]	.007
AST (UI/l)	20.0 [17.0-26.0]	25.0 [20.0-39.5]	<.001	22 [18.0-28.0]	21 [17.0-29.0]	.361
GGT (UI/l)	25.0 [17.0-37.0]	30.0 [17.5-48.5]	.027	27.0 [17.0-43.7]	26.0 [17.0-38.0]	.437
Platelets (x10^9/l)	256.8 ± 56.8	194.5 ± 42.1	<.001	269.0 ± 58.5	214.1 ± 48.8	<.001
Albumin (g/l)	4.4 ± 0.4	4.4 ± 0.3	.363	4.5 ± 0.4	4.3 ± 0.3	<.001
Serum creatinine (mg/dL)	0.9 [0.7-0.9]	0.9 [0.7-1.1]	.110	0.9 [0.8-1.0]	0.9 [0.7-1.0]	.712
LAL activity (nmol/spot/h)	0.9 [0.7-1.2]	0.8 [0.6-1.0]	.008	0.9 [0.7-1.2]	0.8 [0.6-1.1]	.001
Statin use (%)	38.8	48.3	.069	33.5	46.6	.04

Note: See Table 1 for abbreviation list.

These findings confirm the results from previous studies showing a significant reduction of LAL activity in NAFLD patients, compared to HS, with a further reduction in the subgroup of those with NASH.[4,5] In particular, patients with NAFL had a 27.0% reduction in LAL activity, which increased to 41.8% in the subgroup of patients with biopsy-proven NASH.

Despite we found a significant reduction of LAL activity in patients with NAFLD and CC, this was not compatible with a genetic deficiency of LAL in which the residual enzimatic activity is usually <15%.

A new finding of the present study is the significant inverse association between LAL activity and liver fibrosis assessed

TABLE 4 Multivariate logistic regression analysis of factors associated with FIB-4 < 1.30 (model A) and NFS < −1.455 (model B), in 515 patients with NAFLD

	Model A		Model B	
	FIB-4 < 1.30 OR [95% CI]	*P*	NFS < −1.455 OR [95% CI]	*P*
Female sex	0.94 (0.62-1.45)	.794	0.67 (0.45-0.98)	.040
Diabetes	0.94 (0.59-1.50)	.805	—	
Arterial hypertension	0.78 (0.50-1.22)	.273	0.47 (0.32-0.69)	<.001
LDL cholesterol	1.00 (1.00-1.01)	.136	1.00 (1.00-1.01)	.216
Log (LAL activity)	2.19 (1.33-3.62)	.002	2.43 (1.51-3.91)	<.001
High waist circumference	0.85 (0.49-1.47)	.562	—	
Previous MACCE	0.34 (0.15-0.77)	.010	0.06 (0.01-0.49)	.008

Abbreviations: CI, confidence interval; LAL, lysosomal acid lipase; LDL, low density lipoprotein; MACCE, major advanced cardiovascular and cerebrovascular events; OR, odds ratio.

by FIB-4 score and NFS indexes, two well-validated surrogate markers of fibrosis. In particular, the FIB-4 index has been reported to be superior to seven other non-invasive markers of fibrosis in patients with NAFLD to identify a high probability of severe fibrosis.[20] Moreover, it has the advantage of having been validated for both HCV and NAFLD, two common chronic liver diseases.

To corroborate our results, we repeated our analysis using another validated index of fibrosis, the NFS, which is a more accurate scoring system, based on six routinely measured parameters, which stratifies fibrosis along the wide continuum of NAFLD. These findings are in agreement with Shteyer et al[24] who found that low LAL activity correlates with advanced liver disease and that LAL activity < 0.5 indicates severe liver injury in patients with fatty liver and cirrhosis. Finally, they are also in keeping with Selvakumar PK et al who reported a correlation between reduced blood LAL activity and the severity of liver fibrosis in children with NAFLD.[8]

Recently, the clinical outcomes of 18 patients undergoing liver transplantation with LAL deficiency have been reported. The study demonstrates that liver transplantation may be necessary for LAL-D-associated liver failure, but is neither sufficient to increase LAL activity, nor to prevent disease progression, or liver disease recurrence.[25] This is probably because of the fact that the pathophysiology of LAL-D is predominantly mediated by deficient enzyme activity in bone marrow-derived monocyte-macrophages.

In a Phase 3 interventional clinical trial, performed in LAL-D patients, sebelipase supplementation reduced the hepatic fat content compared with baseline (−32%, *P* = .001 vs placebo), and also caused a normalization of ALT and AST values, suggesting therefore a potential effect on reducing the risk of fibrosis.[26] Furthermore, sebelipase alfa supplementation seems to have a positive impact on surrogate markers of atherosclerosis,[27] a beneficial 'pleiotropic' effect which may turn useful in patients with NAFLD who experience a high rate of cardiovascular complications.[28,29] Moreover, in experimental models, supplementation of LAL resulted in the regression of coronary and aortic atheromatous lesions.[30] This could be of greater interest to patients with the more advanced liver disease, such as those with NASH or CC, for whom we do not currently have effective therapies.

Another interesting finding of our study relates to the use of statins. Thus, statin use was inversely associated with cirrhosis, confirming a potential efficacy and safety of statins in patients with NAFLD,[31] and their potential effectiveness in reducing progression to cirrhosis.[32,33]

The study has implications and limitations. Strength of the study is the very large series of patients studied across the wide clinical continuum of NAFLD from NAFL to CC. However, one major limitation is that it is a single-centre cross-sectional study, and we, therefore, cannot establish a cause-effect relationship between the reduction in LAL activity and the presence of liver damage. Thus, until new data become available, the reduction in LAL activity may be regarded to only as a marker of NAFLD severity.[34] Furthermore, liver fibrosis was estimated by non-invasive biomarkers, that despite being well validated, represent an indirect measure and do not provide neither a quantitative or qualitative evaluation of liver fibrosis.

In conclusion, our study showed a significant association between LAL activity and non-invasive markers of liver fibrosis. Longitudinal studies are needed to establish an association between LAL activity reduction and risk of fibrosis.

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