Severe reduction of blood lysosomal acid lipase activity in cryptogenic cirrhosis: A nationwide multicentre cohort study

Francesco Angelico a, b, *, Stefano Ginanni Corradini c, Daniele Pastori a, d, Silvia Fargion e, Anna Ludovica Fracanzani e, Mario Angelico f, Luigi Bolondi g, Giulia Tozzi h, Pietro Luigi Pujatti i, Giancarlo Labbadia a, Gino Roberto Corazza i, Maurizio Averna k, Francesco Perticone i, Giuseppe Croce m, Marcello Persico n, Tommaso Bucci n, Francesco Baratta a, d, Licia Polimeni a, Maria Del Ben a, Francesco Viol i a, LAL-Cirrhosis Collaborative Research Group1

a I Clinica Medica, Atherothrombosis Center, Department of Internal Medicine and Medical Specialties, Sapienza University of Rome, Italy
b Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy
c Gastroenterology Division, Department of Clinical Medicine, Sapienza University of Rome, Italy
d Department of Anatomical, Histological, Forensic Medicine and Orthopedics Sciences – Sapienza University of Rome, Italy
e Department of Pathophysiology and Transplantation, Ca’Granda Foundation IRCCS Maggiore Policlinico Hospital, University of Milan, Milan, Italy
f Hepatology Unit, Tor Vergata University, Rome, Italy
g Department of Medical and Surgical Sciences, University of Bologna, Italy
h Unit for Neuromuscular and Neurodegenerative Diseases, Children’s Hospital and Research Institute “Bambino Gesù”, Rome, Italy
i First Department of Internal Medicine, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy
j Department of Medical and Surgical Sciences, University Magna Græcia of Catanzaro, Italy
k Department of Internal Medicine and Medical Specialties – DIBIMIS, School of Medicine, University of Palermo, Palermo, Italy
l Department of Medical and Surgical Sciences, University Magna Græcia of Catanzaro, Italy
m Internal Medicine Unit, Giuseppe Mazzini Hospital, Teramo, Italy
n Internal Medicine and Hepatology Unit, Salerno University of Medicine, Salerno, Italy

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ABSTRACT

Background and aims: Blood lysosomal acid lipase (LAL) is reduced in non-alcoholic steatohepatitis, which is the major cause of cryptogenic cirrhosis (CC); few data on LAL activity in CC do exist. We investigated LAL activity in a cohort of patients with liver cirrhosis.

Methods: This is a multicentre cohort study including 274 patients with liver cirrhosis of different aetiology from 19 centres of Internal Medicine, Gastroenterology and Hepatology distributed throughout Italy. Blood LAL activity (nmol/spot/h) was measured with dried blood spot extracts using Lalistat 2.

Results: Overall, 133 patients had CC, and 141 patients had cirrhosis by other causes (61 viral, 53 alcoholic, 20 alcoholic + viral, 7 autoimmune). Mean age was 64.2 ± 13.4 years, and 28.5% were women. Patients with CC were older compared to other aetiology-cirrhosis, with a lower Child-Turcotte-Pugh (CTP) score and a higher prevalence of cardio-metabolic risk factors and previous ischemic events. In the whole cohort, median LAL activity value was 0.58 nmol/spot/h, 0.49 and 0.65 in the groups of CC and known-aetiology cirrhosis, respectively (p=0.002). The difference remained significant after adjustment for white blood cells count (p=0.001). Multivariable linear regression analysis showed that CC (β/C0=0.144, p=0.001), platelet count (β=0.398, p<0.001) and CTP score (β=−0.133, p=0.022) were associated with log-LAL activity. Similar results were found using MELD as covariate.

Conclusions: We found a marked reduction of LAL activity in patients with cryptogenic cirrhosis compared to the other known aetiologies. A prospective study will clarify the role of LAL in chronic liver diseases.

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Abbreviations: CESD, cholesterol ester storage disease; CTP, Child-Turcotte-Pugh; LAL, lysosomal acid lipase; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; MACCE, major cardiovascular and cerebrovascular events; MELD, Model for End-stage Liver Disease.

* Corresponding author. Department of Public Health and Infectious Disease, Sapienza University of Rome, Italy, I Clinica Medica — Policlinico Umberto I, Viale del Policlinico 155, 00161 Rome, Italy.
E-mail address: francesco.angelico@uniroma1.it (F. Angelico).

See Appendix for the complete list of authors.
1. Introduction

Cryptogenic cirrhosis accounts for about 5–30% of liver cirrhosis [1]. The exact pathophysiologic cause leading to cryptogenic cirrhosis is still a matter of debate; however, current knowledge strongly indicates that cryptogenic cirrhosis is, in truth, the evolution of non-alcoholic steatohepatitis (NASH) [2]. In fact, 25% of patients affected by NASH can develop cirrhosis with the subsequent risk of hepatocellular carcinoma. So far, the mechanisms underlying disease progression remain poorly understood. The pathogenesis appears multifactorial and many mechanisms have been proposed as possible causes of fatty liver infiltration. Insulin resistance, oxidative stress, chronic low-grade inflammation and intestinal microbiota have been considered to play a central role in the first stages of fatty liver infiltration as well as in liver disease progression [3]. Several lines of evidence indicate that also genetic factors might predispose to non-alcoholic fatty liver disease (NAFLD), and a polymorphism in the patatin-like phospholipase domain-containing protein 3 gene is the most widely studied in this setting [4].

Lysosomal acid lipase (LAL) is a key enzyme involved in lipid metabolism, responsible for the hydrolysis of cholesteryl esters and triglycerides within low-density lipoprotein particles into free cholesterol and free fatty acids. A reduced LAL activity promotes an increased cholesterol esters storage, as observed in two genetic diseases, namely Wolman and Cholesterol Ester Storage Disease (CESD), which are characterized by total or sub-total LAL deficiency [5,6]. These conditions are associated with severe liver steatosis and rapid liver failure. LAL deficiency has been suggested as an under-recognized cause of dyslipidemia and liver dysfunction [7]. Recently, reduced LAL activity was reported in patients with NAFLD and a progressive decrease in LAL activity was observed from subjects with simple steatosis to those with biopsy-proven NASH, suggesting a possible additional reduction in patients with cryptogenic cirrhosis [8]. Therefore, we hypothesized that epigenetic and/or environmental modulation of LAL activity could be an unrecognized contributing factor in the development and progression of NAFLD to cryptogenic cirrhosis [9,10]. The aim of the study was to assess LAL activity in patients with cryptogenic cirrhosis and to evaluate its possible pathophysiological role.

2. Materials and methods

This was a multicentre observational cohort study including 274 patients with clinical and/or histological diagnosis of liver cirrhosis of different aetiologies. The coordinator centre was the Department of Internal Medicine and Medical Specialties of the Sapienza University of Rome: 19 centres of Internal Medicine, Gastroenterology and Hepatology distributed throughout Italy participated in the study (see the Supplementary Data for the complete list of centres).

All patients with diagnosis of cryptogenic cirrhosis were included as experimental group; a similar number of patients with known-aetiology cirrhosis were included as control group with a random sampling procedure. We excluded patients with systemic autoimmune diseases and metastatic neoplastic disease. Subjects underwent routine biochemical evaluation including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), fasting blood glucose, total cholesterol, triglycerides, creatinine, albumin, total and direct bilirubin and INR. A complete blood count was obtained in a subgroup of 184 patients. Anthropometric data, as well as data about comorbidities (i.e. arterial hypertension [11], diabetes [12], metabolic syndrome [13] and dyslipidaemia [14]), concomitant drugs and previous major cardiovascular and cerebrovascular events (MACCE) were collected. MACCE included the following vascular events: nonfatal myocardial infarction and stroke, cardiac revascularization.

The diagnoses of alcoholic, viral and other cause cirrhosis were based on standard clinical, laboratory and histologic findings. Detailed quantitative information was obtained from the patients regarding current and past alcohol consumption. Routine laboratory tests for the diagnosis of virus B and C infection and for autoimmune cirrhosis were performed. Primary biliary cholangitis and haemochromatosis were also excluded. For the purpose of this study, cryptogenic cirrhosis was defined as cirrhosis of unknown aetiology, with no history of alcoholism or alcohol consumption higher than 20 g/day in men and 10 g/day in women, or previous acute or chronic viral hepatitis [15]. The severity and prognostic value of cirrhosis were evaluated by means of the Child-Turcotte-Pugh (CTP) score and the Model for End-stage Liver Disease (MELD).

All patients provided signed informed consent before the study. The study was approved by the ethical board of the Sapienza University of Rome (ref. n° 2277/2011), and by the ethical committees of each centre involved in the study, which was conducted according to the ethical principles embodied in the Declaration of Helsinki.

2.1. Lysosomal acid lipase activity

LAL-activity was dosed at Children’s Hospital “Bambino Gesù” in Rome on dried blood spot extracts using the inhibitors Lalistat 2 [16]. Ethylene-diamine-tetra acetic acid blood, obtained by venepuncture, was spotted on to filter paper (Whatman grade 903 Schleicher & Schuell) and allowed to dry overnight at room temperature. Samples were stored double-bagged with desiccant at −20 °C and analysed within 2 weeks of storage. Uninhibited and inhibited activities with lalistat 2 were determined. LAL activity was determined using the fluorimetric substrate 4-methylumbelliferyl palmitate and by subtracting the activity in the inhibited reaction from uninhibited reaction (total lipase) and expressed as nmol/spot/h of 4-methylumbellifereone, by someone unaware of clinical and biochemical characteristics of any enrolled patient. Inter and intra-assay variations were 2.4% and 2.3%, respectively.

2.2. Statistical analysis

Categorical variables were reported as counts (percentage). Independence of categorical variables was tested with the Chi-squared test. Distribution of continuous variables was tested with the Kolmogorov-Smirnov test and expressed as mean and standard deviation or median and interquartile range accordingly. Kruskall-Wallis test was used for comparisons among groups. Univariate correlation analysis was performed by the Spearman rank correlation test. For the analysis, the study population was divided in two groups, namely “cryptogenetic cirrhosis” and “known-aetiology cirrhosis”, the latter including alcoholic, viral and autoimmune cirrhosis. Multivariable linear regression analysis was performed to assess the independent predictors of LAL activity, after logarithmic transformation. After testing for collinearity, we built two different regression models, one including the CTP score and another with the MELD score as covariate for the severity of liver disease, in addition to female sex, age, cryptogenic cirrhosis (vs. known aetiology), previous MACCE, AST, platelet count,
hypertension, diabetes and statin use. All tests are two-tailed, and \( p \) values < 0.05 were considered as statistically significant. Statistical analysis was performed by using the SPSS statistical software version 20.0 for Windows (SPSS, Inc., Chicago, Illinois).

2.3. Sample size calculation

At the start of study, there were no data showing LAL activity in patients with liver cirrhosis, particularly in those with cryptogenic cirrhosis. Therefore, the sample size has been calculated hypothesizing a difference between patients with cryptogenic and those with other known aetiology similar to that observed between patients with fatty liver and those with biopsy-proven non-alcoholic steatohepatitis, which is a pre-cirrhotic condition. The number of patients to be included was calculated by a 2-tailed Student \( t \)-test for independent variables, considering a standard deviation between the two groups SD = 0.35, and a mean difference of 0.2. With a type 1 error \( \alpha = 0.05 \), the inclusion of 65 patients in each group guarantees a power of 90%.

Considering that white blood cells are the major source of circulating LAL, we performed a sub-analysis in the subgroup of 184 patients with available data on white blood cells. Based on sample size calculation for the primary endpoint, this subgroup was adequate to address this issue.

3. Results

In the entire cohort, 133 patients had cryptogenic cirrhosis, and 141 had cirrhosis by other causes (61 viral, 53 alcoholic, 20 alcoholic + viral, 7 autoimmune).

Values of clinical and biochemical parameters in cryptogenic and other-cirsehosis are reported in Table 1. Overall, patients with cryptogenic cirrhosis were older and had a less severe disease. In fact, as compared to patients with known-aetiology cirrhosis, those with cryptogenic cirrhosis had a statistically significant less severe CTP score (6.0 [5.0–8.0] vs. 7.0 [6.0–9.0], \( p = 0.003 \)) and MELD score (10.0 [8.0–14.0] vs. 12.0 [10.0–16.0], \( p = 0.009 \)). Moreover, patients with cryptogenic cirrhosis had significantly lower AST (\( p < 0.001 \)) and higher albumin (\( p < 0.001 \)) levels (Table 1).

The prevalence of cardiovascular risk factors and previous MACCE was higher in the group of patients with cryptogenic cirrhosis compared to other aetiologies (Table 1).

3.1. LAL activity

In the whole cohort, median LAL activity value was 0.58 [0.42–0.82] nmol/spot/h.

LAL activity was negatively correlated with MELD score (\( R_s = -0.182; p = 0.003 \)), CTP score (\( R_s = -0.179; p = 0.005 \)), total bilirubin (\( R_s = -0.133; p = 0.029 \)) and direct bilirubin (\( R_s = -0.128; p = 0.042 \)), and positively correlated with albumin (\( R_s = 0.166; p = 0.007 \)), platelet count (\( R_s = 0.420; p < 0.001 \)) and WBC (\( R_s = 0.407; p < 0.001 \)) (Table 2).

As reported in Table 1, median blood LAL activity was significantly lower in patients with cryptogenic cirrhosis as compared to those with viral and/or alcoholic cirrhosis (0.49 vs. 0.65 nmol/spot/h, respectively, \( p = 0.002 \)). Moreover, we found a significant higher proportion of patients with a moderate (i.e. <0.8 nmol/spot/h) or severe (i.e. <0.4 nmol/spot/h) reduction of LAL activity in the group of CC than other aetiology (Table 1).

Similar results were obtained after excluding patients treated with statins (CC patients 0.49 nmol/spot/h vs. other-aetiology cirrhosis 0.65 nmol/spot/h, \( p = 0.003 \)).

Patients with cryptogenic cirrhosis had lower median LAL activity compared to those with known aetiology cirrhosis in CTP class A (0.62 [0.41–0.81] vs. 0.71 [0.48–1.06] nmol/spot/h, respectively, \( p = 0.02 \)) and CTP class B (0.47 [0.37–0.67] vs. 0.59 [0.44–0.86] nmol/spot/h, respectively, \( p = 0.016 \)), and only a trend was found for patients in CTP class C (0.47 [0.38–0.62] vs. 0.62 [0.38–0.78] nmol/spot/h, respectively, \( p = 0.136 \)).

At multivariable linear regression, cryptogenic cirrhosis (vs. other aetiologies) was inversely associated with log-LAL activity after adjustment for liver disease severity, both using CTP (Table 3, model A) and MELD score (Table 3, model B) as covariate.

When we adjusted LAL values for white blood cells count, the difference between cryptogenic and other-aetiology cirrhosis remained significant (Table 1).

Moreover, when we investigated factors associated with the presence of cryptogenic cirrhosis, we found that AST, LAL/WBC ratio and diabetes were associated with cryptogenic cirrhosis (Table 4).

4. Discussion

In this multicentre cohort study, we found a marked reduction of LAL activity, as assessed by dried blood spot testing, in patients with cryptogenic cirrhosis. None of the patients had LAL activity indicative of the diagnosis of Wolman disease or CESD, but approximately 30% of patients with cryptogenic cirrhosis showed a severe reduction of LAL activity (i.e. <0.40 nmol/spot/h). That proportion is higher than that previously reported in control subjects and in those with simple steatosis and NASH, (none, 4.5% and 6% respectively) [8]. These findings confirm our previous observations of a decrease of LAL activity in a large cohort of subjects with NAFLD (0.78 nmol/spot/h), being the reduction more pronounced in a subgroup of patients with biopsy proven NASH (0.67 nmol/spot/h) [8].

In the present study, LAL activity was significantly reduced in patients with cryptogenic cirrhosis compared to those with known-aetiology cirrhosis, mostly represented by alcoholic and viral cause. Interestingly, LAL activity reduction was more evident in patients with cryptogenic cirrhosis despite having a less severe liver disease, as demonstrated by less pronounced alterations of liver function tests and by a lower CTP and MELD scores.

Our result is in keeping with findings from one previous study performed in cryptogenic cirrhosis showing a severe reduction of LAL activity in these patients with respect to healthy subjects [17]. However, in the study by Vespasiani et al., the two groups of patients with cryptogenic and other-cirsehosis disclosed similar LAL values (0.62 and 0.54 nmol/spot/h, respectively) [17].

Furthermore, we observed a strong association between LAL activity reduction and severity of liver disease. Thus, we found that LAL activity negatively correlated with CTP and MELD scores, but the difference between cryptogenic cirrhosis and other cause cirrhosis was evident only in CTP class A and B, while only a trend was found in class C, probably due to the limited number of patients. Our findings are in agreement with the results of a recent study where, in a group of 22 patients with cryptogenic cirrhosis or microvesicular steatosis, LAL activity inversely predicted liver disease severity, with a LAL level of 0.5 nmol/spot/h as the most sensitive cut-off for detecting both histologic and non-invasive markers of disease severity [18].

In our cohort of patients with cryptogenic cirrhosis, we observed a high prevalence (18%) of previous coronary and cerebrovascular events. Those patients had also a high prevalence of cardio-metabolic risk factors including type 2 diabetes, arterial hypertension, dyslipidaemia, and the metabolic syndrome. This is in agreement with previous studies [15,19,20] showing that both type 2 diabetes and obesity are more common in cryptogenic cirrhotic patients compared with the cirrhotic patients with known-aetiology, reinforcing the concept of a metabolic-based aetiology.
Table 1
Clinical and biochemical characteristics of the study cohort according to the aetiology of liver cirrhosis.

<table>
<thead>
<tr>
<th></th>
<th>All cohort (n = 274)</th>
<th>Cryptogenic cirrhosis (n = 133)</th>
<th>Known aetiology cirrhosis (n = 141)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.2 ± 13.4</td>
<td>66.3 ± 15.0</td>
<td>62.2 ± 11.3</td>
<td>0.011</td>
</tr>
<tr>
<td>Women (%)</td>
<td>28.5</td>
<td>33.8</td>
<td>23.4</td>
<td>0.062</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>37.6</td>
<td>49.6</td>
<td>26.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>35.8</td>
<td>45.1</td>
<td>27.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Dyslipidaemia (%)</td>
<td>16.1</td>
<td>24.8</td>
<td>7.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metabolic syndrome (%)</td>
<td>20.7</td>
<td>28.9</td>
<td>13.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Previous MACCE (%)</td>
<td>13.1</td>
<td>18.0</td>
<td>8.5</td>
<td>0.021</td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAL activity (nmol/spot/h)</td>
<td>0.58 (0.42–0.82)</td>
<td>0.49 [0.38–0.75]</td>
<td>0.65 [0.46–0.94]</td>
<td>0.002</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>54.5</td>
<td>143.8 ± 48.5</td>
<td>135.9 ± 44.9</td>
<td>0.196</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>40.7 ± 16.0</td>
<td>40.0 ± 15.8</td>
<td>41.6 ± 16.4</td>
<td>0.486</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9 [0.7–1.2]</td>
<td>0.9 [0.7–1.1]</td>
<td>0.9 [0.7–1.3]</td>
<td>0.349</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>96.3 ± 49.1</td>
<td>101.0 ± 53.4</td>
<td>91.9 ± 44.7</td>
<td>0.161</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>111.4 ± 41.3</td>
<td>111.7 ± 40.6</td>
<td>111.1 ± 42.4</td>
<td>0.911</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.4 ± 0.7</td>
<td>3.5 ± 0.7</td>
<td>3.2 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>54.5 ± 43.6</td>
<td>44.9 ± 30.2</td>
<td>63.4 ± 51.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>42.9 ± 39.8</td>
<td>39.4 ± 36.0</td>
<td>46.2 ± 42.8</td>
<td>0.164</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.4 [0.8–2.4]</td>
<td>1.3 [0.8–2.3]</td>
<td>1.5 [0.9–2.6]</td>
<td>0.095</td>
</tr>
<tr>
<td>Platelets (x10⁹/μl)</td>
<td>115.0 ± 81.2</td>
<td>112.5 ± 73.2</td>
<td>117.2 ± 88.1</td>
<td>0.637</td>
</tr>
<tr>
<td>White blood cells (n/mm³)</td>
<td>5.1 ± 2.2</td>
<td>4.7 ± 1.8</td>
<td>5.4 ± 2.5</td>
<td>0.033</td>
</tr>
<tr>
<td>Liver cirrhosis severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MELD score</td>
<td>11.0 [9.0–15.0]</td>
<td>10.0 [8.0–14.0]</td>
<td>12.0 [10.0–16.0]</td>
<td>0.009</td>
</tr>
<tr>
<td>CTP score</td>
<td>7.0 [5.0–8.0]</td>
<td>6.0 [5.0–8.0]</td>
<td>7.0 [6.0–9.0]</td>
<td>0.003</td>
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<tr>
<td>CTP classes (%)</td>
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<td></td>
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</tr>
<tr>
<td>A</td>
<td>47.1</td>
<td>56.9</td>
<td>37.6</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>39.9</td>
<td>33.8</td>
<td>45.9</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>12.9</td>
<td>9.2</td>
<td>16.5</td>
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<tr>
<td>Drug treatments</td>
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<td></td>
<td></td>
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<tr>
<td>Loop diuretics (%)</td>
<td>48.9</td>
<td>40.6</td>
<td>56.7</td>
<td>0.008</td>
</tr>
<tr>
<td>Antialdosterone diuretics (%)</td>
<td>49.6</td>
<td>44.4</td>
<td>54.6</td>
<td>0.093</td>
</tr>
<tr>
<td>Propranolol (%)</td>
<td>28.8</td>
<td>29.3</td>
<td>28.4</td>
<td>0.894</td>
</tr>
<tr>
<td>Osmotic laxatives (%)</td>
<td>31.0</td>
<td>27.8</td>
<td>34.0</td>
<td>0.297</td>
</tr>
<tr>
<td>Non-absorbable antibiotics (%)</td>
<td>23.4</td>
<td>19.5</td>
<td>27.0</td>
<td>0.156</td>
</tr>
<tr>
<td>Proton pump inhibitors (%)</td>
<td>50.4</td>
<td>49.6</td>
<td>51.1</td>
<td>0.904</td>
</tr>
<tr>
<td>Anticoagulants (%)</td>
<td>7.7</td>
<td>9.0</td>
<td>6.4</td>
<td>0.498</td>
</tr>
<tr>
<td>Antiplatelet drugs (%)</td>
<td>8.0</td>
<td>9.8</td>
<td>6.4</td>
<td>0.375</td>
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<tr>
<td>Statins (%)</td>
<td>5.1</td>
<td>7.5</td>
<td>2.8</td>
<td>0.101</td>
</tr>
<tr>
<td>Anti-diabetics (%)</td>
<td>27.0</td>
<td>33.1</td>
<td>21.3</td>
<td>0.030</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTP, Child-Turcotte-Pugh; GGT, gamma glutamyltransferase; MACCE, major adverse cardiovascular and cerebrovascular events; MELD, Model for End-stage Liver Disease.

a Available in 184 patients (90 with known-aetiology and 94 with cryptogenic cirrhosis).

Table 2
Correlations of LAL activity with clinical and biochemical variables.

<table>
<thead>
<tr>
<th></th>
<th>Rs</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>Model for End-stage Liver Disease score</td>
<td>−0.182</td>
<td>0.003</td>
</tr>
<tr>
<td>Child-Turcotte-Pugh score</td>
<td>−0.170</td>
<td>0.005</td>
</tr>
<tr>
<td>Age</td>
<td>−0.086</td>
<td>0.156</td>
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<tr>
<td>Total cholesterol</td>
<td>0.140</td>
<td>0.033</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.121</td>
<td>0.068</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.103</td>
<td>0.098</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.071</td>
<td>0.248</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>−0.133</td>
<td>0.029</td>
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<td>Direct bilirubin</td>
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<td>Aspartate aminotransferase</td>
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<td>Alanine aminotransferase</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>White blood cells</td>
<td>0.407</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.166</td>
<td>0.007</td>
</tr>
</tbody>
</table>

of cryptogenic cirrhosis.

This is in keeping with the results of a 10-year longitudinal study showing that cardiovascular mortality is greater in patients with cirrhosis due to NASH as compared to those with cirrhosis due to HCV infection [21]. Moreover, in a recent study, mortality rate was significantly higher for cryptogenic cirrhotic patients with metabolic syndrome, as compared to those without (42.5% and 33.3%, respectively) [20]. These findings support the hypothesis that metabolic liver disease, such as NASH, contributes to a higher risk of CVD also in cryptogenic cirrhosis.

However, mechanisms accounting for this apparent increased cardiovascular risk are not fully understood. Recently, a pro-coagulant imbalance and increased platelet activation has been described in cirrhotic patients [22,23]. Moreover, in patients with liver cirrhosis, important modifications of the lipid profile toward a general "hypolipidemia" have been described [24]. Of note, in addition to a low serum HDL concentration, liver cirrhosis patients may suffer from an impaired HDL cholesterol efflux capacity, similarly to patients with CESTD [25]. This aspect deserves further
The reduction of LAL activity and the related alterations in the intracellular cholesterol metabolism could play a role in this context. Thus, an intracellular accumulation of cholesterol esters secondary to an impaired LAL activity may alter the activation of sterol regulatory element-binding proteins (SREBPs), mostly SREBP-2, which is physiologically activated in the liver by cholesterol-derived products [26]. The SREBP-2 inhibition leads to a reduced LDL receptor transcription [27], impaired cholesterol efflux capacity and reduced HDL formation [28], all factors contributing to the dyslipidaemia observed in liver cirrhosis patients.

This study has some strengths. Unlike previous studies, this is a multicenter collaborative cohort study of a representative sample of cirrhotic patients from 19 centres well distributed throughout Italy. Moreover, in this study, cirrhotic patients were well characterized, not only in terms of liver disease severity, but also for current pharmacological treatments, cardio-metabolic risk and previous cardiovascular events.

The study has also limitations. It has a cross-sectional design, which does not make it possible to establish any cause-effect relationship in the pathogenesis of cryptogenic cirrhosis. Moreover, the significant overlap between LAL activity levels in the two cirrhotic groups does not clarify whether LAL may behave differently according to cirrhosis aetiology. Prospective, follow-up studies of cirrhotic patients are needed to clarify these aspects.

In conclusion, patients with cryptogenic cirrhosis had a marked reduction of LAL activity as compared to those with known-aetiology cirrhosis, despite a more severe liver disease in the latter. These findings suggest the need for a prospective study to better investigate the possible causal role of LAL in the setting of chronic liver disease.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

F.A., F.V.: study concept and design; study supervision; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content.

D.P., F.B.: study concept and design; acquisition of data; analysis and interpretation of data; statistical analysis; drafting of the manuscript; critical revision of the manuscript for important intellectual content.

S.G.C., M.D.B.: analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content.

G.T.: laboratory analysis; drafting of the manuscript; critical revision of the manuscript for important intellectual content.

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Appendix

Members of the LAL-Cirrhosis collaborative research group

Francesco Violi, Francesco Angelico, Daniele Pastori, Francesco Baratta, Maria Del Ben, Licia Polimeni, Giancarlo Labbadia, Stefania Basili, Valeria Raparelli, Laura Napoleon (Sapienza - University of Rome Department of Internal Medicine and Medical Specialties).

Stefano Giovanni Corradi, Flaminia Ferri, Monica Pellone, Monica Mischitelli, Lucia Parlati (Gastroenterology Division, Department of Internal Medicine and Medical Specialties, Sapienza University of Rome).

Schi

Institute Neurodegenerative Diseases, Children's Hospital and Research Department of Clinical Medicine, Sapienza University of Rome).

Monica Mischitelli, Lucia Parlati (Sapienza - University of Medicine and Medical Specialties).

Basili, Valeria Raparelli, Laura Napoleone (Sapienza - University of Rome Department of Medical and Surgical Sciences, University Magna Graecia of Catanzaro, Catanzaro, Italy).

Gino Roberto Corazza, Michela Masotti, Gaetano Bergamaschi (First Department of Internal Medicine, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Italy).

David Sacerdoti, Silvia Brocco (Department of Internal Medicine (DIMED), University of Padova, Padua, Italy).

Maria Angelico, Francesco Santopaulo, Simona Franciosi (Hepatology Unit, Tor Vergata University, Rome, Italy).

Pietro Luigi Pujatti, Alessandra Faedo (Department of Internal Medicine - Ospedale di Arzignano - ULSS n. 5 “Ovest Vicentino”).

Angelo Andriulli, Antonio M. Ippolito (Division of Gastroenterology, Casa Sollievo Sofferenza Hospital, IRCCS, San Giovanni Rotondo, Italy).

Luigi Bolondi, Francesco Tovoli (Department of Medical and Surgical Sciences, University of Bologna, Italy).

Silvia Fargion, Anna Ludovica Fracanzani (Department of Pathophysiology and Transplantation, Ca' Granda Foundation IRCCS Maggiore Policlinico Hospital, University of Milan, Milan, Italy).

Giovanni Davi (Department of Medicine and Aging, University of Chieti “G. d’Annunzio” School of Medicine, Chieti, Italy).

Dario Di Michele, Giuseppe Croce (Internal Medicine Unit – Giuseppe Mazzini Hospital – Teramo).

Maurizio Averna, Antonina Gimmanco (Department of Internal Medicine and Medical Specialties - DIBIMIS, School of Medicine, University of Palermo, Palermo, Italy).

Marcello Persico, Tommaso Bucco (Internal Medicine and Hepatology Unit, Salerno University of Medicine, Salerno, Italy).

Luigi Iuliano, Marco Ciaciarelli (Department of Medico-Surgical Sciences and Biotechnology, Vascular Biology and Mass Spectrometry Laboratory, Sapienza University of Rome, Latina, Italy).

References