

Blood Metabolomic Signatures to Identify Bacterial Infection in Patients with Decompensated Cirrhosis

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ABSTRACT

Background & Aims: Bacterial infections are associated with high mortality rates in patients with decompensated cirrhosis. Early diagnosis with the available diagnostic tools is challenging. Metabolomics is a novel technique with a widespread application in hepatology. The aims of our study were to find new biomarkers for decompensated cirrhosis and for those with overlapping bacterial infections.

Methods: 43 patients with compensated and 54 patients with decompensated cirrhosis were enrolled in the study. In patients with decompensation, a complete infectious workup was performed at admission. Blood and ascitic fluid were collected and stored at -80° C until performing the metabolomic analysis. Statistical analysis was performed using the Metaboanalyst 4.0 software.

Results: 36 patients (66%) in the decompensated group were infected. Among them, 15 had multiple infections; thus, finally, 52 infections were diagnosed. The main metabolic pathways affected in patients with decompensated cirrhosis were those related to lipid metabolism, involving acylcarnitines, stearic acid derivatives, and 12/15 HETE-GABA. N-oleoyl ethanolamine was the most promising biomarker for bacterial infection diagnosis. Moreover, prostaglandin E2/D2/H2 and N-oleoyl alanine levels were higher in Gram-positive infections and ceramides (d16:2/18:0), in Gram-negative infections, respectively. L-phenylalanine (m/z=166.09) and lysophosphatidylethanolamine (18:3/0:0) were the two most relevant identified ascitic biomarkers for spontaneous bacterial peritonitis diagnosis.

Conclusions: The lipid and energetic metabolic pathways were the most affected in patients with decompensated cirrhosis and those with overlapping infections.

Key words: lipid metabolism – peritonitis – bacterial infection – biomarker – early diagnosis – metabolomic – cirrhosis – spontaneous bacterial peritonitis.

Abbreviations: ACLF: acute-on-chronic liver failure; CRP: C-reactive protein; HBV: hepatitis B virus; HCV: hepatitis C virus; LPC: lysophosphatidylcholine; MDA: Mean Decrease Accuracy; MS: mass spectrometry; PCA: Principal Component Analysis; PLSDA: Partial Least-Squares Discriminant Analysis; PCT: procalcitonin; SBP: spontaneous bacterial peritonitis; UHPLC: ultrahigh-performance liquid chromatography; UTI: urinary tract infection; VIP: Variable Importance in the Projection; WBC: white blood cell count; 15-HETE-GABA: N-(15S-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoyl)-gamma-aminobutyric acid.

INTRODUCTION

Bacterial infections are frequent and cause severe complications in patients with cirrhosis, especially in the more advanced stages [1]. Spontaneous bacterial peritonitis (SBP) is the most specific infection in patients with decompensated liver cirrhosis. This leads to prolonged hospitalization, higher healthcare costs, and significant

mortality rates [2]. Within one year of the infectious episode, the overall mortality rate reaches 60% [3]. Early initiation of proper antibiotic therapy is crucial, as each hour of delay could decrease survival rates by almost 10% [4]. Since nearly half of infections can be asymptomatic [5], the diagnosis with the available tests is challenging.

The classic infection markers, such as leucocyte count, C-reactive protein (CRP), procalcitonin (PCT), and presepsin, have low sensitivity and specificity rates in patients with liver cirrhosis [6-8]. In addition, the traditional culture methods are time-consuming and underdiagnose sepsis in these patients, being positive in 50-70% of cases [9]. Therefore, new diagnostic methods are required.

Metabolomics allows the identification and quantification of all metabolites in a biological sample, with proven widespread applications in hepatology [10-12]. The metabolome, defined as the totality of small molecules produced by cells, offers a window to understand how biochemistry is related to the cellular phenotype [13]. Recent developments in mass spectrometry (MS) coupled with ultrahigh-performance liquid chromatography (UHPLC) allow us to rapidly identify thousands of metabolites simultaneously from minimal sample amounts [14].

Recent studies evaluated metabolomics' role as a diagnostic tool for infection and sepsis in the noncirrhotic population [15-17].

Our study's primary objectives were to identify a metabolomic fingerprint for decompensated cirrhosis, comparing the serum metabolic profiles of patients with decompensated and compensated cirrhosis and to find new serum biomarkers of bacterial infections in patients with decompensated cirrhosis. The secondary objective was to find an ascitic biomarker for the diagnosis of SBP.

METHODS

Study Population

The study was conducted with respect to the ethical guidelines issued by the 2000 revision (Edinburgh) of the 1975 Declaration of Helsinki and was approved by the Ethical Committee of our Institution (29/2016).

Between January 2016 and December 2017, consecutive patients with cirrhosis admitted to a tertiary referral hospital in Cluj-Napoca, Romania, were screened for enrolment. The diagnosis of cirrhosis was based on specific biological, elastographic, ultrasonic, and histopathological criteria. The exclusion criteria included refusal to sign the informed consent, advanced hepatocellular carcinoma or other malignancies, severe alcoholic hepatitis proven by transjugular liver biopsy, or ascites of different etiology.

Decompensation was defined as: *de novo* or worsening ascites, portal hypertension-related bleeding, overt hepatic encephalopathy (grade 2 to 4 on West Haven scale), or jaundice (total serum bilirubin > 3mg/dl) [18].

Child-Pugh and MELD scores were calculated within the first 24 hours of admission in decompensated patients. Data regarding three-month survival was also registered.

In patients with decompensated cirrhosis, a complete screening for infection was done in the first 24 hours after admission as follows: total white blood cell count (WBC), CRP, PCT, plain chest radiograph, blood cultures, urine sediment and culture, ascitic/pleural fluid neutrophil count, and ascitic/pleural fluid culture. Apart from a particular epidemiological and clinical scenario, we did not perform routine screening for acute viral infections.

The diagnosis of infections were the following: i) SBP and spontaneous empyema: polymorphonuclear cell count in ascitic and pleural fluid > 250/mm³; ii) spontaneous bacteremia: positive blood cultures without overt cause of bacteremia; iii) urinary tract infections (UTI): more than 10 leukocytes per high-power field in urine and positive urine culture or uncountable (> 500) leukocytes per field even

without positive cultures; iv) respiratory and other types of infections were defined according to conventional criteria [19].

All patients with decompensated cirrhosis were treated according to the existing guidelines [18].

The antibiotic was chosen according to the type of infection, either empirically or specifically for any identified bacteria.

Metabolomic Analysis

1. Biofluids and extraction protocol

Ten mL of venous blood were drawn after overnight fasting in the first 24-48 hours after admission and centrifuged for 15 minutes at 5,000 rotations per minute (rpm). The serum obtained after centrifugation was stored at -80°C in cryotubes until extraction and analysis.

Ascitic fluid was drawn from 54 patients with decompensated cirrhosis. For the metabolomic analysis of ascitic fluid, 10 ml of it were centrifuged at 2500 rpm for 20 minutes. The supernatant obtained was mixed with 500 µl of Natrium azide 1% and stored in Eppendorf tubes at -80 °C until further analysis.

Before analysis, the serum and ascitic fluid samples were thawed at room temperature and vortexed for 20 seconds. Aliquots of 200 µl were mixed with 800 µl MeOH and vortexed for 1 minute to precipitate proteins. Afterward, they were ultrasonicated in a water bath for 5 minutes, then kept at -20°C for at least 30 minutes. After centrifugation at 12,500 rpm for 10 minutes, the supernatants from each sample were collected and filtered using a Nylon filter (0.2 µm), being placed into autosampler vials and injected into the ultrahigh-performance liquid chromatography (UHPLC) separation device.

2. Ultra-high performance liquid chromatography coupled with quadrupole time-of-flight electrospray ionization mass spectrometry (UHPLC-QTOF-ESI+-MS) analysis

Aliquots of 5 µl serum were subjected to ultrahigh-pressure chromatography on a Thermo Scientific HPLC UltiMate 3000 system equipped with a DionexUltiMate 3000 (UHPLC) quaternary pump system, a DionexUltimate 3000 photodiode array detector, a column oven, and an autosampler. Serum metabolites were separated using a Thermo Scientific Acclaim C-18 column (3µm, 2.1 X 50 mm) at 40°C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The flow rate was set at 0.5 mL min⁻¹. The gradient elution initial conditions were 1% B with a linear gradient to 15% B from 0 to 3 min, followed by a linear gradient to 50% B at 6 min, linear gradient to 95% B at 9 min, isocratic on 95% B for 6 min and then returned to initial conditions at 15 min and kept isocratic at 1% B for 5 min. The PDA detector was set at 270 nm. The mass spectrometry was performed on a Bruker Daltonics MaXis Impact QTOF instrument, operating in positive ion mode (ESI⁺), and the mass range was set between 50-1000 m/z. The nebulizing gas pressure was set at 2.8 bar for measurements, the drying gas flow at 12 L/min, the drying gas temperature at 300°C. Before each chromatographic run, a calibrant solution of sodium formate was injected. The instrument control, acquisition, and data processing were performed using Chromeleon, Hystar 3.2, TofControl 3.2, and Data Analysis 4.2 (Bruker Daltonics).

Statistical Analysis

Data Analysis 4.2 (Bruker GmbH) was used to process the acquired data. Firstly, using specific algorithms, base peak chromatograms (BPC) were obtained from the total ion chromatogram (TIC). Secondly, an advanced bucket matrix was generated using Find Molecular Features (FMF). After time alignment and normalization, metabolites detected in more than 60% of the samples were included in the bucketing and statistical analysis. Multivariate and univariate statistical analysis was performed using Metaboanalyst 4.0 (<http://www.metaboanalyst.ca>). The most relevant statistical parameters to reflect the discrimination between groups, the prediction, and the correlation maps were tested from the specific matrices representing the m/z values versus peak intensity for all samples and subgroups of samples. Therefore, the Volcano test, the Variable Importance in the Projection (VIP) scores, principal component analysis (PCA) and Partial least Square Discriminant Analysis (PLS-DA), cross-validation parameters, Random Forest-based prediction, and calculations of p-values using T-test and ANOVA comparative statistics were used. Two cross-validation parameters were considered (R²-goodness of fit and Q²-model's predictive power). The Random Forest algorithm using the Mean Decrease Accuracy (MDA) values was applied to show which molecules could be considered the most relevant for discrimination between different groups of samples. According to statistical analysis, the relevant molecules were identified and confirmed using specialized databases such as Lipid Maps (<http://www.lipidmaps.org>) and Human Metabolome Database (<http://www.hmdb.ca>).

RESULTS

1. Patients' Characteristics

Table I shows the clinical and biochemical parameters of the patients from the study group. One hundred forty-three patients were screened for enrolment: 100 patients with decompensated cirrhosis were admitted through the emergency department, and 43 with hepatitis C virus (HCV)-related compensated cirrhosis. Forty-six patients were excluded from the study because of refusal to sign the informed consent (n=5), advanced hepatocellular carcinoma (n=17), or other malignancies (n=2), severe alcoholic hepatitis proven by transjugular liver biopsy (n=21), or ascites of different etiology (n=1). Finally, we enrolled 54 patients with decompensated cirrhosis as the study group and 43 patients with compensated HCV-related cirrhosis as the control group. All patients in the control group had Child-Pugh class A cirrhosis without prior decompensation and were evaluated before initiating direct-acting antiviral treatment. No infections were reported in the compensated cirrhosis group. The primary etiology in the decompensated cirrhosis group was alcohol (66% of cases), followed by hepatitis B virus (HBV)/HCV infection (29.6%) and other etiologies (4.4%). In this group, 36 patients (66%) were infected, of whom 15 had multiple infections. Thus, a total of 52 infections were diagnosed, as follows: UTI: 16 (31%), SBP: 10 (19%), soft tissue infection: 9 (17%), spontaneous bacteremia: 9 (17%), respiratory infection: 3 (6%), and other infections: 5 (10%). There were 39 culture-positive infections, and among

Table I. General characteristics of the study population

Variable	Compensated group, n=43	Decompensated group, n=54
Gender (male), n %	21 (49)	30 (56%)
Age (years), mean±SD	58.2±9	59.6±10.4
Etiology		
Alcohol		36 (66)
Viral	43 (100)	16 (30)
Other		2 (4)
Decompensation type (number of events)		
Ascites	0 (0)	51 (94)
Hepatic encephalopathy	0 (0)	25 (46)
Variceal bleeding	0 (0)	11 (20)
Jaundice	0 (0)	22 (40)
ALT (U/l), mean±SD	69.2±32.9	31.48±35.6
AST (U/l), mean±SD	82.1±39.4	61.76±37.9
Platelets ×10 ³ , mean±SD	110.47±65.7	160.16±101.84
Bilirubin (mg/dl), mean±SD	1.16±0.48	3.13±3.21
Albumin (g/dl), mean±SD	4.02±0.45	2.79±0.44
Creatinine (mg/dl), mean±SD	0.7±0.17	1.18±0.88
Child-Pugh score, mean±SD	5	9.84±1.78
MELD score, mean±SD	6.18±0.85	17.55±5.55
CRP (mg/dl), mean±SD	2.47±1.89	3.34±4.08
PCT (ng/ml), mean±SD	0.35±0.51	0.82±2.01
Mortality at 3 months, n (%)	0 (0)	13 (24)

CRP-C reactive protein; MELD-model for end stage liver disease; PCT-procalcitonin; SD-standard deviation

them, there were 8 infections with more than one identified etiological agent. Thus, a total of 48 germs were identified: 23 (48%) Gram-negative bacteria, 20 (41%) Gram-positive bacteria, 4 (8%) fungi. There was also a single parasitic infection with *Toxocara canis* (Supplementary file).

Thirteen patients (24%) with decompensated cirrhosis died within three months from the first investigation. Sepsis with acute-on-chronic liver failure (ACLF) was the leading cause of death (54%), followed by liver failure (30%) and other causes (16%). There were no fatalities in the compensated group during the study.

2. Serum Metabolomic Markers for Liver Decompensation

2.1. Volcano Plot Analysis

The Volcano plot revealed the significantly increased or decreased molecules in the decompensated cirrhosis group vs. the compensated group. Table II shows the identification of molecules, their m/z values, the fold change (FC) values, log₂(FC), and significance (p-values) to illustrate their relevance as potential biomarkers for discrimination between the two groups.

2.2. Partial Least-Squares Discriminant Analysis

To highlight a difference between the metabolic signature of serum from patients with decompensated cirrhosis (D) against compensated ones (C), multivariate analysis was applied using PCA and PLSDA. The PCA scoring plot showed that the metabolomic profiles between the C and D groups had a covariance of 21.1%, with a slight differentiation between the two groups (data not shown). We further built a predictive model using PLSDA. PLSDA scores demonstrate obvious separation between the C and D groups (Fig. 1).

In this model, R² and Q² had the highest values of 0.8 and 0.6, respectively, demonstrating good model variance for the two groups. VIP scores were also calculated from the PLSDA model and reflected the influence of features (m/z values) in constructing the PLSDA model. We identified two metabolites with a VIP score greater than 2: 9-Hexadecenoylcarnitine and N-(15S-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoyl)-gamma-aminobutyric acid (15-HETE-GABA).

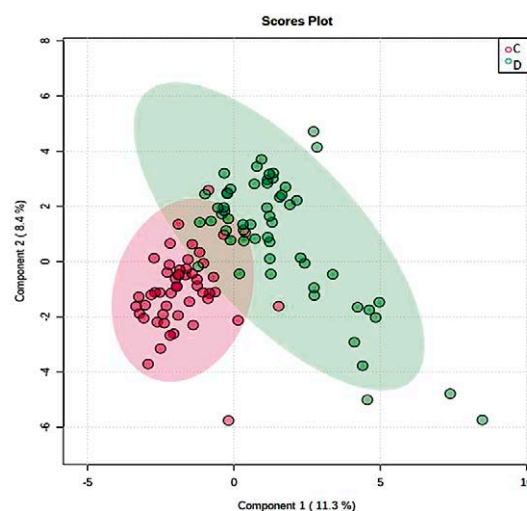


Fig. 1. PLSDA scores plot which discriminates the two groups, C (compensated) vs D (decompensated).

2.3. Prediction of putative biomarkers using Random Forest algorithm

Using the Random Forest algorithm, from the separated molecules (n= 120), the first fifteen molecules with the highest MDA values were selected as possible differentiation biomarkers between the two groups (Fig. 2). Nine out of 15 molecules were decreased in the decompensated group, 9-hexadecenoylcarnitine, decadienoyl carnitine, choline-dependent glycerophospholipids, stearyl- and palmitoyl amides having the highest relevance. Meanwhile, increased cholic acids, pregnenolone, and oxilipins like 15-HETE-GABA were noted.

Thus, the central metabolic pathways affected in patients with decompensated liver cirrhosis compared to controls are related to lipid metabolism and involve phosphatidylcholines, acylcarnitines, stearic acid derivatives, and 15 HETE-GABA.

2.4. Metabolomic markers of prognosis in patients with decompensated cirrhosis

In patients with decompensated cirrhosis, the metabolomic profiles of those who died were compared to the serum profiles of those who survived at three months.

Table II. Molecules and m/z values identified by Volcano Plot Analysis, Fold change (FC), log₂(FC) and p values, which show their tendency (increase or decrease) in the decompensated (D) group relative to the compensated group (C).

Molecules	m/z	FC	log ₂ (FC)	p	Decompensated vs. Compensated
9-hexadecenoylcarnitine (palmitoleyl C16:1 carnitine)	398.76	4.24	2.08	0.026 x10 ⁻¹¹	Decreased
12/15 -HETE-GABA	406.33	0.25	-1.98	0.078x10 ⁻¹⁰	Increased
Stearic amide	284.2986	2.16	1.11	0.0001	Decreased
Butenyl carnitine	230.71	0.45	-1.14	0.003	Increased
PG (16:1/0:0)	483.3485	0.28	-1.86	0.023	Increased
Cer (d18:1/20:0(2OH)	610.1887	0.42	-1.24	0.024	Increased
PA(O-18:0/16:0)	663.4599	0.46	-1.13	0.041	Increased
Dinor-deoxycholic acid	365.1903	0.35	-1.53	0.044	Increased

Cer: ceramides; HETE-GABA: hidroxy-eicosa-tetra enoyl-gama aminobutyric acid; PG-phosphatidyl-glycerol; PA: phosphatidic acid

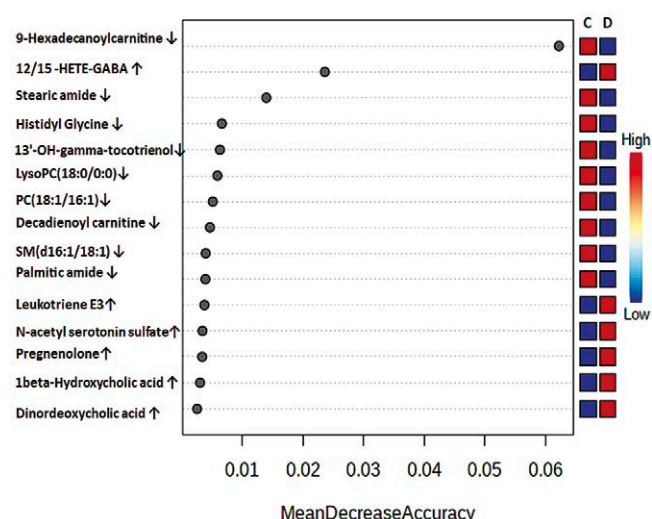


Fig. 2. Random forest classification (MDA values) for the first 15 molecules and their tendency in the decompensated group (D) compared to the compensated group (C).

Metabolites associated with three-month survival rates in patients with decompensated cirrhosis were related to higher levels of unsaturated polar lipids such as lysophosphatidylcholine (LPC), ceramides of linoleic (C18:2), and linolenic acids (C18:3), phosphatidic acid, and lower levels of free fatty acids, cholesteryl esters or saturated stearic acid-related ceramides (Supplementary file).

3. Metabolomic Markers of Bacterial Infection in Decompensated Cirrhosis

In the decompensated cirrhosis group, those with bacterial infections ($n=36$) were compared with those without infection ($n=18$).

3.1. Volcano plot analysis

Applying a similar algorithm as before, four molecules were significantly different in the infected subgroup, with FC values higher than 2 and increased levels compared to the non-infected subgroup (Table III). These data have been completed using PLSDA analysis (Fig. 3).

3.2. Partial Least-Squares Discriminant Analysis and Random Forest analysis

Figure 3 shows the PLSDA scores plot for the infected (I) vs. non-infected (N) subgroups.

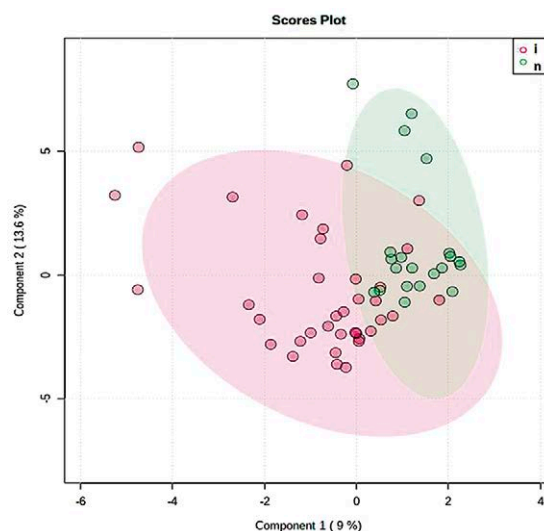


Fig. 3. The PLSDA scores plot corresponding to the infected (i) versus non-infected (n) subgroups of patients with decompensated cirrhosis.

The variance of 9.0% (PC1) and 13.6% (PC2) covered a total co-variance of 22.6% and reflected weak discrimination between the two groups. Moreover, the PLSDA regression model cross-validation showed that this model could not be considered significantly valid.

The first 15 molecules with the highest MDA values were selected using the Random Forest algorithm. These may be considered responsible for the differentiation between the two groups (Fig. 4).

The best markers of infection validated using both methods (Volcano plot analysis and Random Forest classification) proved to be N-oleoylethanolamine, 2-hydroxyglutarate, fatty acid derivatives, and glutathione, which had an increasing trend, while ceramides (d18:1/20:0(2OH), LPC (18:2/0:0) had a decreasing trend in the presence of bacterial infection.

This data suggests that the affected metabolic pathways in the infected subgroup include the metabolism of lipids, those of endocannabinoids, some known oncometabolites (hydroxyglutarate), and glutathione. The latter is involved in the energetic metabolism and antioxidant protection against fatty acid beta-oxidation.

There were six Gram-positive and five Gram-negative monobacterial infections in the infected subgroup. Multivariate and univariate analysis showed that 15 possible biomarkers of differentiation could be used to distinguish between Gram-

Table III. Volcano plot analysis: Fold change (FC), $\log_2(\text{FC})$, and the comparative tendency (increase or decrease) in the bacterial infection group relative to those without infections

Molecules	m/z	Fold Change	$\log_2(\text{FC})$	Infected vs Non-infected
2-Hydroxyglutarate	149.0348	3.46	1.79	Increased
26-OH-cholesterol 3-sulfate	483.3485	2.68	1.42	Increased
N-oleoylethanolamine	326.3584	2.53	1.34	Increased
9-Hexadecenoylcarnitine	398.76	0.45	-1.15	Decreased
Cer (d18:1/20:0(2OH)	610.1887	0.45	-1.14	Decreased
PA(O-18:0/16:0)	663.4599	0.46	-1.11	Decreased
Oleic acid	283.1965	2.1	1.09	Increased

Cer-ceramides; PA-phosphatidic acid

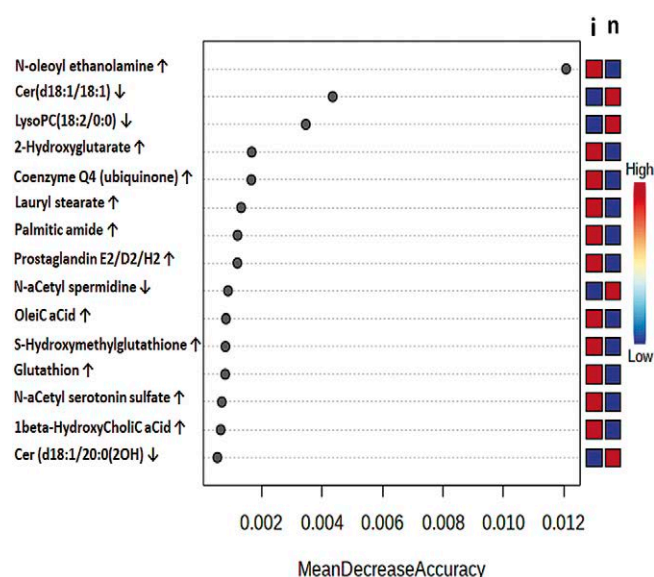


Fig. 4. Random forest classification (MDA values) for the first 15 molecules and their tendency in the infected subgroup (i) compared to the non-infected group (n).

positive and Gram-negative infections (Supplementary file). The most relevant ones were prostaglandin E2/D2/H2 and N-oleoyl alanine which had higher levels in Gram-positive infections, while ceramides (d16:2/18:0) had lower levels in Gram-negative infections.

4. Ascites Metabolomic Analysis

In our study, we also included nine patients with SBP. For their diagnosis, ascites metabolomic analysis was performed to differentiate between infected ($n=9$) and non-infected ascites ($n=41$). Using univariate and multivariate analysis, we identified 25 ascitic metabolites as possible biomarkers (Supplementary file). The highest VIP and MDA values were observed for L-phenylalanine ($m/z=166.09$) and lysophosphatidylethanolamine (18:3/0:0) ($m/z=476.31$), both with an increasing tendency in patients with SBP.

DISCUSSIONS

The diagnosis of decompensation in patients with cirrhosis is simple. The challenge is to understand the causes of its appearance. For sure, the progression of portal hypertension plays a critical role [20]. However, our cellular and molecular level understanding is much more limited.

Our study highlighted a significant disturbance in lipid metabolism (especially phospholipids, fatty acid derivatives, bile acids, carnitines) and neurotransmitters concentration (pregnenolone, 15-HETE-GABA) in patients with decompensated cirrhosis. Furthermore, the affected metabolic pathways in patients with decompensation and the overlapping infection seem to include the metabolism of lipids (phosphatidylcholines and ceramides), those of endocannabinoids (N-oleoyl ethanolamine), some known oncometabolites (hydroxyglutarate), and glutathione.

The liver plays a central role in lipid metabolism, comprising mainly two metabolic routes. First, hepatic lipogenesis implies the esterification of free fatty acids into

triglycerides and secretion as VLDL particles. Second, hepatic lipolysis is a crucial mechanism in the beta-oxidation of free fatty acids [21]. Thus, advanced liver disease is characterized by altered circulating lipoprotein levels. Moreover, lipoprotein abnormalities seem to play a critical pathogenic role in several clinical manifestations of cirrhosis, including bacterial infections, hematological complications, malnourishment, and adrenal dysfunction [22].

A core metabolic phenotype in chronic liver diseases is characterized by decreased phosphatidylcholines and increased serum bile acids [23]. The phosphatidylcholines resonances seem to be significantly lower with the progression of liver failure [24].

This trend seems to be validated in our study as well: the resonances of phosphatidylcholines decrease while bile acid derivatives (dinordeoxycholic acid, 1βeta-hydroxycholic acid) increase in patients with decompensated disease.

Our study highlighted that one of the most important biomarkers of decompensation was GABA, hepatic encephalopathy of different grades being present in almost 50% of decompensated patients. Moreover, other neurotransmitters, such as pregnenolone, were also upregulated. Previous studies have shown similar results, as pregnenolone was found to be increased in cirrhotic patients with hepatic coma [25].

Our results showed that unsaturated acylarnitines were decreased in patients with decompensated cirrhosis while saturated acylcarnitines increased compared to controls. Recent studies showed that acylcarnitines increase according to liver fibrosis progression, promising biomarkers for nonalcoholic liver diseases/nonalcoholic steatohepatitis [26].

As far as we know, this study is the first that aims to identify a metabolomic fingerprint of infections in patients with decompensated liver cirrhosis.

The phosphatidylcholines levels were lower in infected, decompensated cirrhotic patients compared with the non-infected group. Moreover, low serum phosphatidylcholines levels seem to be associated with unfavorable outcomes in patients with sepsis [27].

N-oleoyl ethanolamine is part of the so-called endocannabinoids, which seem to be involved in fibrosis and portal hypertension progression in liver diseases [28]. In our study, N-oleoyl ethanolamine was higher in patients with bacterial infections and was the most promising identified biomarker for bacterial infection diagnosis.

2-hydroxyglutarate was upregulated in the presence of bacterial infection in our study. Accumulation of 2-hydroxyglutarate in different tissues can lead to mitochondrial dysfunction with severe consequences on energy metabolism at that level [29]. Glutathione is an essential antioxidant molecule that directly reacts with reactive oxygen species (ROS) generated during hepatic injury [30]. Glutathione signals in patients with bacterial infections were increased in our study, highlighting the intensification of inflammatory processes that characterize the advanced stages of cirrhosis.

We tried to refine the diagnosis of bacterial infections, performing a metabolomic analysis for Gram-positive and negative monobacterial infections, and we obtained good discrimination between groups; however, the sample size was very small. Prostaglandin E2 was increased in patients with Gram-positive bacterial infection in our study. During

inflammation, prostaglandin E2 synthesis is upregulated by various pro-inflammatory stimuli such as lipopolysaccharide, interleukin-1 β , and tumor necrosis factor- α [31]. On the other hand, ceramides (d16:2/18:0) were higher in patients with Gram-negative infections. Several studies have shown the involvement of ceramides in the host's response during bacterial infections [32]. Moreover, increased ceramides levels have been associated with high mortality rates in septic non-cirrhotic patients [27].

We analyzed the ascites of patients with SBP. Biomarkers previously identified for bacterial infection characterization have been found in the ascites of those with SBP, lipid, and protein resonances being the most involved. However, we only had nine patients with SBP, which decreases the statistical power of the results. This study has some limitations. It is a single-center study with a small sample size, and the decompensated cirrhosis group has high heterogeneity. Furthermore, targeted metabolomics was not performed.

On the other hand, as far as we know, this is the first study that has tried to identify a metabolic fingerprint for bacterial infections in patients with decompensated cirrhosis.

CONCLUSIONS

The altered lipid metabolism related to C18 and C16 fatty acid derivatives, glycerophospholipids, and acylcarnitines as important signaling molecules, especially at the mitochondrial level, confirm the perturbation of energetic processes which affect patients with decompensated cirrhosis compared to those with compensated disease. This trend is maintained in the more advanced stages of the disease, represented by the addition of a bacterial infection. Further studies of targeted metabolomics analysis are necessary to confirm our results.

Conflicts of interest: None to declare.

Authors' contribution: C.S. and B.P. conceived and designed the study. P.F. collected and analyzed the data, interpreted the results and drafted the manuscript. C.G., O.F. collected the data. C.S. and B.P. performed the statistical analyses and analyzed the results.. C.T.: English language editing. B.P. supervised the study. C.S., D.I., B.P., H.S., C.T., O.F.: critical revision of the manuscript for important intellectual content. All the authors approved the final version of the manuscript.

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