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## **Bile acid homeostasis and intestinal dysbiosis in alcoholic hepatitis**

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3 1 **Title: Bile acid homeostasis and intestinal dysbiosis in alcoholic hepatitis**

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5 2 **Running Title: Bile acid, microbiota and alcoholic hepatitis**

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46  
47 23 **Authorship Statement**

48  
49 24 Gabriel Perlemuter is the submission's guarantor. Specific author contributions: DC  
50  
51 25 designed the project, performed the post-sequencing data processing and the

1  
2  
3 26 statistical analysis, and wrote the manuscript. CSV designed the project and included  
4  
5 27 patients. LW designed the project and performed presequencing preparation  
6  
7 28 procedures. CH performed presequencing preparation procedures. DR and LH  
8  
9 29 performed bile acids analyses. AMC designed, performed and supervised  
10  
11 30 experiments and wrote the manuscript. GP designed the project, included patients,  
12  
13 31 supervised experiments and wrote the paper. All authors discussed the results,  
14  
15 32 commented and approved the final version of the manuscript.  
16  
17

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## 35 **Summary**

36 **Background:** Intestinal microbiota plays an important role in bile acid homeostasis.

37 **Aim:** We aimed to study the structure of the intestinal microbiota and its function in  
38 bile acid homeostasis in alcoholic patients based on the severity of alcoholic liver  
39 disease.

40 **Methods:** In this prospective study, we included four groups of active alcoholic  
41 patients (N=108): two non-cirrhotic, with (noCir\_AH, n=13) or without alcoholic  
42 hepatitis (noCir\_noAH, n=61), and two cirrhotic, with (Cir\_sAH, n=17) or without  
43 severe alcoholic hepatitis (Cir\_noAH, n=17). Plasma and faecal bile acids profiles,  
44 and intestinal microbiota composition, were assessed.

45 **Results:** Plasma levels of total bile acids (84.6 vs. 6.8  $\mu\text{mol/l}$ ,  $p<0.001$ ) and total  
46 ursodeoxycholic acid (1.3 vs. 0.3  $\mu\text{mol/l}$ ,  $p=0.03$ ) were higher in Cir\_sAH than  
47 Cir\_noAH whereas faecal total (2.4 vs. 11.3,  $p=0.01$ ) and secondary bile acids (0.7  
48 vs. 10.7,  $p<0.01$ ) levels were lower. Cir\_sAH patients had a different microbiota than  
49 Cir\_noAH patients: at the phyla level, the abundance of Actinobacteria (9 vs 1%,  
50  $p=0.01$ ) was higher and that of Bacteroidetes was lower (25 vs 40%,  $p=0.04$ ).  
51 Moreover, the microbiota of Cir\_sAH patients showed changes in the abundance of  
52 genes involved in 15 metabolic pathways, including upregulation of glutathione  
53 metabolism, and downregulation of biotin metabolism.

54 **Conclusions:** Patients with Cir\_sAH show specific changes of the bile acid pool with  
55 a shift towards more hydrophobic and toxic species that may be responsible for the  
56 specific microbiota changes. Conversely, the microbiota may also alter the bile acid  
57 pool by transforming primary to secondary bile acids, leading to a vicious cycle.

58 **Keywords:** 16S sequencing, microbiota, UDCA, biotin, glutathione, Actinobacteria,  
59 Bacteroidetes

## 60 INTRODUCTION

61 Severe alcoholic hepatitis is a life-threatening complication seen in a subset of  
62 patients with alcoholic liver disease with a mortality rate of up to 25% and few  
63 therapeutic options (1,2). The causal role of the intestinal microbiota in the  
64 development and individual susceptibility to alcoholic hepatitis has only recently been  
65 shown (3–6). It has also been suggested that faecal microbiota transplantation may  
66 improve gut dysbiosis and clinical outcomes in patients with cortico-resistant severe  
67 alcoholic hepatitis in a recent pilot study (7). Nevertheless, the mechanisms related to  
68 the role of the intestinal microbiota in alcoholic liver disease are not fully understood.

69 The relationship between bile acids and the intestinal microbiota is complex. Bile  
70 acids have both direct antimicrobial effects on bacteria (8), and indirect effects  
71 through their signaling properties which allows them to induce antimicrobial peptides  
72 production (9). Detergent properties of bile acids, needed for fat digestion, influence  
73 the composition of the intestinal microbiota by acting on bacterial cell membranes (8).  
74 However, the diversity of the bile acids pool and enterohepatic circulation are  
75 dependent on the intestinal microbiota. Indeed, primary bile acids (cholic acid, CA,  
76 and chenodeoxycholic acid, CDCA) are synthesized in the liver, but secondary bile  
77 acids are produced in the digestive tract. The complex pool of bile acids is then  
78 reabsorbed in the portal circulation via a large panel of transporters. In addition, bile  
79 acids are signaling molecules involved in regulating hepatic metabolism,  
80 inflammation, and their own synthesis through the activation of various nuclear  
81 receptors, such as the farnesoid X receptor (FXR) (10).

82 Chronic alcohol consumption is associated with an impaired bile acids homeostasis  
83 (11–14). The level of plasma bile acids positively correlates with the histological  
84 severity of AH (11) and is predictive of poor patient survival (15). Moreover, FXR-

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85 specific agonists attenuate chronic alcohol-induced liver injury and steatosis in  
86 experimental alcoholic liver disease models (16,17). Conversely, FXR-deficient mice  
87 develop more severe liver injury (17). Overall, these results suggest that bile acids-  
88 dependent hepatotoxicity may be due, in part, to impaired FXR signaling.

89 We have shown in a recent work that patients with severe alcoholic hepatitis have a  
90 specific dysbiosis that renders their liver more susceptible to alcohol-induced injury  
91 (4). This sensitivity was transmissible from patients to mice by intestinal microbiota  
92 transplant. In these humanized mice, the bile acids pool was impaired in the feces.  
93 Moreover we also showed that alcoholic patients that develop severe alcoholic  
94 hepatitis have a different microbial composition as compared to patients that develop  
95 other types of complications such as alcoholic pancreatitis (6).

96 However, while both the bile acids and intestinal microbiota profiles were reported in  
97 alcoholic liver disease, these studies focused either only on the bile acids profile  
98 (11,18) or intestinal microbiota profile (4) and never on both, in the same cohort of  
99 patients or didn't included patients with severe alcoholic hepatitis (12,13). In order to  
100 study the relationships between intestinal microbiota modifications and bile acids  
101 metabolism, we investigated herein the interplay between bile acids and intestinal  
102 microbiota in well phenotyped patients at different stages of alcoholic liver disease by  
103 assessing and comparing plasma and faecal bile acids profiles and intestinal  
104 microbiota composition and functions in currently drinking alcoholic patients  
105 according to the severity of liver lesions.

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3 106 **METHODS**

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5 107 ***Study subjects***

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7 108 All patients included in this prospective study were admitted to the Hepato-  
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9 109 Gastroenterology and Nutrition Department of Antoine-Béclère University Hospital,  
10  
11 110 Clamart, France, for the management of excessive drinking.

12  
13 111 Alcoholic patients were eligible for inclusion if they were between 17 and 75 years old  
14  
15 112 and had been consuming at least 50 g of alcohol/day and were negative for hepatitis  
16  
17 113 B surface antigens and hepatitis C. The exclusion criteria were gastrointestinal  
18  
19 114 bleeding, bacterial infection, hepatocellular carcinoma, any other carcinoma, other  
20  
21 115 associated severe diseases, the presence of anti-HIV antibodies, antibiotic intake in  
22  
23 116 the last three months, probiotic drugs use, refusal to undergo a liver biopsy if required  
24  
25 117 (abnormal liver function), use of any hepatoprotective treatment (UDCA, TUDCA). A  
26  
27 118 standardized questionnaire was used to collect information about alcohol  
28  
29 119 consumption (19) and patients' families were also interviewed, when possible.

30  
31 120 General demographic and clinical characteristics were recorded for all patients at  
32  
33 121 inclusion. The study was carried out in accordance with the Helsinki Declaration and  
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35 122 was approved by the Ile de France VII ethics committee (Bicêtre Hospital, 94270 le  
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37 123 Kremlin-Bicêtre, France). All patients provided written informed consent for  
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39 124 participation in the study.

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41 125 Patients were classified into four groups:

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44 126 • patients with alcoholic cirrhosis and severe alcoholic hepatitis (Cir\_sAH, n =  
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46 127 17). Severe alcoholic hepatitis was suspected in patients with a Maddrey  
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48 128 score > 32 and was confirmed by a liver biopsy (histological score for AH ≥ 6  
49  
50 129 with neutrophilic infiltration) (4,20).

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3 130 • patients with alcoholic cirrhosis, but without severe alcoholic hepatitis  
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5 131 (Cir\_noAH, n = 17). As the impact of non-severe alcoholic hepatitis was limited  
6  
7 132 on the parameters that we studied in non-cirrhotic patients, we pooled the  
8  
9 133 patients with mild alcoholic hepatitis with patients with no alcoholic hepatitis in  
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11 134 the cirrhotic patients group.

13  
14 135 • patients without alcoholic cirrhosis or alcoholic hepatitis (noCir\_noAH, n = 61),  
15  
16 136 • patients without alcoholic cirrhosis, but with alcoholic hepatitis (noCir\_AH, n =  
17  
18 137 13). Alcoholic hepatitis was defined by aspartate aminotransferase > 50,  
19  
20 138 aspartate aminotransferase/alanine aminotransferase > 1.5, and both values <  
21  
22 139 400 IU/L (20,21) or, if a liver biopsy was available (12/13 patients), an AH  
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24 140 score between 3 and 5 (with neutrophilic infiltration) or  $\geq 6$  (with neutrophilic  
25  
26 141 infiltration and a Maddrey score < 32).

28  
29 142 Diagnosis of cirrhosis was made based on clinical examination, laboratory test,  
30  
31 143 imaging and endoscopy studies or by a liver biopsy, when available. As patients with  
32  
33 144 cirrhosis have a different intestinal microbiota profile than those without, and most  
34  
35 145 patients with severe alcoholic hepatitis exhibit histological evidence of micronodular  
36  
37 146 cirrhosis, we did not perform a global comparison between the four groups but  
38  
39 147 separately compared the patients with and without cirrhosis.

#### 41 148 ***Biochemical assays***

42  
43  
44 149 Bile acids measurements in plasma of 55 patients and feces of 73 patients were  
45  
46 150 performed using high-performance liquid chromatography-tandem mass  
47  
48 151 spectrometry as previously described (22). Serum fibroblast growth factor-19 (FGF-  
49  
50 152 19) was measured for 55 patients using a sandwich ELISA kit (R&D Systems)  
51  
52 153 according to the manufacturer's instructions.

#### 54 154 ***Analysis of the intestinal microbiota by 16S ribosomal RNA sequencing***



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3 155 Faecal samples were available for 96 patients. The composition of the faecal  
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5 156 microbiota was analyzed by high-throughput sequencing with Illumina MiSeq  
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7 157 technology, targeting the 16S ribosomal DNA V3-V4 region in the paired-end mode  
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9 158 (2 x 300 base pairs) (GenoToul, Toulouse), as previously described (23). Data were  
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11 159 processed with the quantitative insights into microbial ecology (QIIME v1.9.0)  
12  
13 160 pipeline, using its default parameters. Closed reference operation taxonomic  
14  
15 161 mapping was performed using the Greengenes database (v13.8, 97% sequence  
16  
17 162 similarity).

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19  
20 163 The mean number of quality-controlled reads was  $26535 \pm 7840$  (mean  $\pm$  SD) per  
21  
22 164 sample (minimum count: 9833, maximum count: 56968). After rarefaction at 9,000  
23  
24 165 reads per sample, bacterial alpha diversity was estimated on the basis of the  
25  
26 166 Shannon's index. OTUs with a prevalence  $< 5\%$  were removed from the analysis.

27  
28 167 Functional composition of the intestinal metagenome was predicted using  
29  
30 168 Phylogenetic Investigation of Communities by Reconstruction of Unobserved States  
31  
32 169 (PICRUST) (24). This is a computational approach that accurately predicts the  
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34 170 abundance of gene families in the microbiota and thus provides information about the  
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36 171 functional composition of the microbial community. Linear discriminant analysis (LDA)  
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38 172 effect size (LEfSe) analysis was performed to identify the taxa and functions  
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40 173 displaying the largest differences in abundance in the microbiota between groups  
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42 174 (25). Only taxa and functions with an LDA score  $> 2$  and a significance of  $< 0.05$ , as  
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44 175 determined by Wilcoxon signed-rank tests, are shown. LEfSe and PICRUST were  
45  
46 176 accessed online (<http://huttenhower.sph.harvard.edu/galaxy/>).

### 47 48 49 50 177 ***Statistical analysis***

51  
52 178 The results are expressed as the means  $\pm$  SD for normally distributed data or median  
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54 179 [min, max] for non-normally distributed data. Data normality was tested for each  
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3 180 parameter using the Shapiro-Wilk test ( $p > 0.05$ ). Unpaired t-tests or Mann–Whitney  
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5 181 U-tests were used to compare continuous data between groups, depending on the  
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7 182 data distribution. Chi<sup>2</sup> or Fisher’s exact tests were used to compare discrete  
8  
9 183 parameters between groups. The Spearman correlation test was used to find  
10  
11 184 correlations between bile acids and intestinal microbiota. Benjamini–Hochberg false  
12  
13 185 discovery rate (FDR) correction was used to correct for multiple hypothesis testing,  
14  
15 186 when applicable. A p-value  $< 0.05$  was considered to be statistically significant. The  
16  
17 187 comparisons were performed with R software v2.14.1 unless stated otherwise.  
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19  
20 188 Bile acids data were processed and analyzed in MetaboAnalyst  
21  
22 189 (<http://www.metaboanalyst.ca>) (26) using supervised and unsupervised methods:  
23  
24 190 Principal Component Analysis (PCA) and Partial Least Squares Discriminant  
25  
26 191 Analysis (PLS-DA). Data were log transformed and pareto-scaled and the results  
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28 192 validated using leave-one-out cross-validation procedures.  
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## 193 **RESULTS**

### 194 ***Demographic and laboratory data***

195 A total of 108 patients were included in the study. We classified patients into four  
196 groups: patients with alcoholic cirrhosis and severe alcoholic hepatitis (Cir\_sAH, n =  
197 17); patients with alcoholic cirrhosis, but without severe alcoholic hepatitis (Cir\_noAH,  
198 n = 17); patients without alcoholic cirrhosis or alcoholic hepatitis (noCir\_noAH, n =  
199 61); and patients without alcoholic cirrhosis, but with alcoholic hepatitis (noCir\_AH, n  
200 = 13).

201 The demographic and laboratory data are summarized in Table 1. There was no  
202 difference in age, sex, body mass index (BMI), or duration of alcohol intake between  
203 the groups. As expected, patients with noCir\_AH had higher aspartate  
204 aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, gamma-  
205 glutamyl transferase (GGT), and C-reactive protein levels than noCir\_noAH patients.  
206 Cir\_sAH patients had lower alcohol consumption than the Cir\_noAH patients and  
207 higher total bilirubin and C-reactive protein levels, a higher MELD score, and lower  
208 albumin levels.

### 209 ***Intestinal microbiota profiles***

210 We first studied interindividual bacterial diversity (beta diversity). Cir\_sAH patients  
211 had a different intestinal microbiota structure (proportion of bacteria) than Cir\_noAH  
212 patients (weighted UNIFRAC distances,  $R = 0.09$ ,  $p = 0.04$ ) (Figure 1A). There was  
213 no difference in the overall bacterial composition of the intestinal microbiota between  
214 these two groups (unweighted UNIFRAC distances,  $R = 0.05$ ,  $p = 0.1$ ) (Figure 1B).  
215 This result suggests that the two groups have an intestinal microbiota with similar  
216 bacterial species, but with different relative abundances. There was no difference in  
217 the beta diversity between noCir\_noAH and noCir\_AH patients (data not shown).

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3 218 There was also no difference in the intra-individual bacterial diversity (alpha diversity)  
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5 219 either between the noCir\_noAH and noCir\_AH groups, nor the Cir\_sAH and  
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7 220 Cir\_noAH groups, measured by various indices (observed OTUs, Shannon index,  
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9 221 Chao index, and PD whole tree index, data not shown).

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12 222 At the phyla level, Cir\_sAH patients had a higher abundance of Actinobacteria and  
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14 223 lower abundance of Bacteroidetes than Cir\_noAH patients. Among Actinobacteria,  
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16 224 Cir\_sAH patients had a higher abundance of *Actinomyces*, *Rothia*, and  
17  
18 225 *Bifidobacterium* than Cir\_noAH patients. Among Proteobacteria, Cir\_sAH patients  
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20 226 had a higher abundance of *Haemophilus* and *Enterobacteriaceae* and a lower  
21  
22 227 abundance of *Bilophila* than Cir\_noAH patients. Cir\_sAH patients also had a lower  
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24 228 relative abundance of *Parabacteroides* (Bacteroidetes phylum), *Oscillospira*, and  
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26 229 *Christensenellaceae* families (Firmicutes phylum) and a higher relative abundance of  
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28 230 *Lactobacillus* and *Lactococcus* (Firmicutes phylum) than Cir\_noAH patients (Figure  
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30 231 1C and Supplemental Table 1).

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35 232 Although there was no difference in the overall composition of the intestinal  
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37 233 microbiota between noCir\_AH and noCir\_noAH patients, LEFsE analysis showed  
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39 234 that noCir\_AH patients had a higher abundance of *Dorea* (Firmicutes phylum),  
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41 235 *Wolbachia* (Proteobacteria phylum) and *Rothia* (Actinobacteria phylum) than  
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43 236 noCir\_noAH patients (Figure 1D). These results suggest that a specific dysbiosis is  
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45 237 associated with hepatic inflammation in AH in both patients with and without cirrhosis  
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47 238 and independently of alcohol consumption.

### 50 51 239 **Functional Intestinal Metagenome Prediction in Alcoholic Hepatitis:**

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53 240 The dysbiosis identified in Cir\_sAH patients prompted us to also examine the  
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55 241 metabolic pathways associated with this specific intestinal microbiota. The intestinal

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3 242 microbiota of the Cir\_sAH group had a higher proportion of metabolic pathways  
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5 243 containing gene functions, such as glutathione metabolism, membrane transport  
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7 244 (phosphotransferase system), and nucleotide metabolism than that of the Cir\_noAH  
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9 245 group. The intestinal microbiota of Cir\_sAH patients also had a lower proportion of  
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11 246 genes for energy metabolism (methane metabolism and carbon fixation pathways in  
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13 247 prokaryotes), amino acid metabolism (arginine, proline and histidine metabolism),  
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15 248 lipid metabolism (lipid biosynthesis proteins), glycan biosynthesis and metabolism,  
16  
17 249 metabolism of cofactors and vitamins (biotin metabolism), metabolism of terpenoids  
18  
19 250 and polyketides (polyketide sugar unit biosynthesis), biosynthesis of other secondary  
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21 251 metabolites (streptomycin biosynthesis), and of the transcription machinery than that  
22  
23 252 of Cir\_noAH patients (Figure 1E). There was no difference between the two groups in  
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25 253 the secondary bile acids biosynthesis pathway.

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29 254 These results indicate that the dysbiosis observed in patients with severe alcoholic  
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31 255 hepatitis is also associated with a shift in the bacterial metabolic pathways.

### 32 33 34 35 256 ***Bile acids***

36  
37 257 Bile acids can shape the intestinal microbiota, and in turn, the intestinal microbiota  
38  
39 258 alters the bile acids pool. Thus, we studied plasma bile acids and faecal bile acids  
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41 259 profiles and their relationship with the dysbiosis observed in alcoholic patients with  
42  
43 260 and without alcoholic cirrhosis.

### 44 45 46 47 261 ***Plasma bile acid profile***

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49 262 We first studied the plasma bile acids profile associated with liver inflammation in  
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51 263 alcoholic patients. Cir\_sAH patients had a different plasma bile acids profile than  
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53 264 Cir\_noAH patients, as shown by PCA (Figure 2A) and on a heatmap (Supplementary  
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55 265 figure 1A). Cir\_sAH patients had higher levels of total bile acids, total primary bile

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3 266 acids, total conjugated bile acids (total glyco- and tauroconjugated bile acids),  
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5 267 primary glyco- and tauroconjugated bile acids, total CA (glycocholate (GCA) and  
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7 268 taurocholate (TCA)), total CDCA (glycochenodeoxycholate (GCDCA) and  
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9 269 taurochenodeoxycholate (TCDCA)), and total UDCA (tauroursodeoxycholate  
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11 270 (TUDCA)) than Cir\_noAH patients (Figures 2 B-D and Supplementary Table 2).  
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13 271 Because of the higher total bile acids levels in Cir\_sAH patients, we also studied the  
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15 272 relative proportion of each bile acids (bile acids concentration/total bile acids  
16  
17 273 concentration) between the two groups. Cir\_sAH patients had a higher relative  
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19 274 proportion of total primary bile acids, total CDCA (TCDCA), total primary conjugated  
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21 275 bile acids, primary tauroconjugated bile acids, and TUDCA than Cir\_noAH patients.  
22  
23 276 They also had a lower relative proportion of total sulphoconjugated bile acids, total  
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25 277 secondary bile acids, total secondary conjugated bile acids, secondary  
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27 278 glycoconjugated bile acids, total lithocholate (LCA) (LCA, glycolithocholate (GLCA),  
28  
29 279 lithocholate-3-sulfate (LCA3s), tauroolithocholate-3-sulfate (TLCA3s), and  
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31 280 glycolithocholate-3-sulfate (GLCA3s)), total deoxycholate (DCA) (DCA;  
32  
33 281 glycodeoxycholate (GDCA), and taurodeoxycholate (TDCA)), chenodeoxycholic acid  
34  
35 282 3-sulfate (CDCA3s), and glyoursodeoxycholate-3-sulfate (GUDCA3s) than  
36  
37 283 Cir\_noAH patients (Figures 2 B, C, E).  
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41  
42 284 We then used PLS-DA to find the plasma bile acids that best discriminate between  
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44 285 the two groups. The model showed a significant distinction ( $R^2 = 0.6$ ,  $Q^2 = 0.4$ ,  
45  
46 286 prediction accuracy during training:  $p = 0.001$ , 1000 permutations) between the  
47  
48 287 Cir\_sAH and Cir\_noAH groups (Figure 3A). The average AUROC confirmed that the  
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50 288 model was able to discriminate Cir\_noAH from Cir\_sAH patients (0.955, 95% CI:  
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52 289 0.763-1, Supplementary Figure 2A). TUDCA was the most discriminant bile acids  
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3 290 between the two groups according to the PLS-DA (variable importance in projection,  
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5 291 VIP = 2.1, Figure 3B).  
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8 292 Comparison of the bile acids profile of noncirrhotic patients showed higher levels of  
9  
10 293 TUDCA in noCir\_AH patients than noCir\_noAH patients. Moreover, noCir\_AH  
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12 294 patients had higher proportions of TUDCA and total conjugated bile acids and a  
13  
14 295 lower proportion of DCA than noCir\_AH patients (data not show). However, these  
15  
16 296 changes were no longer significant after correction for multiple comparisons.  
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20 297 These results indicate an increase in the pool of bile acids in Cir\_sAH patients  
21  
22 298 Moreover, the increase in TUDCA levels, a bile acids produced exclusively by the  
23  
24 299 intestinal microbiota from CDCA, and that of primary bile acids s are consistent with a  
25  
26 300 shift in bile acids transformation in the gut.  
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### 29 301 ***Faecal bile acids***

30  
31 302 We further studied the bile acids profile in Cir\_sAH patients. Cir\_sAH patients had a  
32  
33 303 different faecal bile acids profile than Cir\_noAH patients (Figure 4A and  
34  
35 304 Supplementary Figure 1B). Cir\_sAH patients had lower total faecal bile acids, total  
36  
37 305 unconjugated bile acids, total glycoconjugated bile acids, total secondary bile acids,  
38  
39 306 secondary unconjugated bile acids, secondary glyco- and tauroconjugated bile acids,  
40  
41 307 total LCA (LCA), and total DCA (DCA, GDCA, TDCA, deoxycholate 3-sulfate:  
42  
43 308 DCA3s) than Cir\_noAH patients (Figures 4B-D and Supplementary Table 3). We also  
44  
45 309 examined the relative amount of each bile acids (faecal bile acids/total faecal bile  
46  
47 310 acids). Cir\_sAH patients had a higher percentage of total primary bile acids, total  
48  
49 311 CDCA (CDCA, TCDCA), primary unconjugated bile acids, and CA than Cir\_noAH  
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51 312 patients and a lower percentage of total secondary bile acids, total secondary  
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3 313 unconjugated bile acids, secondary glycoconjugated bile acids, secondary  
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5 314 tauroconjugated bile acids, and total DCA (DCA, GDCA, TDCA) (Figures 4 B, C, E).

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8 315 PLS-DA showed a significant distinction ( $R^2 = 0.8$ ,  $Q^2 = 0.6$ , prediction accuracy  
9  
10 316 during training:  $p = 0.001$ , 1000 permutations) between the Cir\_sAH and Cir\_noAH  
11  
12 317 groups (Figure 5A). The average AUROC confirmed that the model was able to  
13  
14 318 discriminate Cir\_noAH from Cir\_sAH patients (0.977, 95% CI: 0.769-1)  
15  
16 319 (Supplementary Figure 2B). Secondary glycoconjugated bile acids and GDCA were  
17  
18 320 the most discriminant bile acids between the two groups according to the PLS-DA  
19  
20 321 (variable importance in projection, VIP = 2.1 and 2.1, respectively, Figure 5B).

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23  
24 322 Comparison of the faecal bile acids profiles between noncirrhotic patients showed the  
25  
26 323 observed changes (higher tauroolithocholate, TLCA, and GLCA ratios in noCir\_AH  
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28 324 than noCir\_noAH patients) to no longer be significant after correction for multiple  
29  
30 325 comparisons (data not show), as observed in plasma.

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33 326 These results confirm the decrease in bile acids excretion and impaired bile acids  
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35 327 transformation in the gut by the intestinal microbiota of Cir\_sAH patients relative to  
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37 328 that of Cir\_noAH patients.

### 329 ***The relationship between intestinal microbiota and bile acids homeostasis***

330 We assessed the correlation between the bile acids profiles with the bacteria species  
331 identified in the feces, as the composition and quantity of the bile acids pool influence  
332 the intestinal microbiota, and conversely, the metabolism of bile acids is dependent  
333 on intestinal microbiota composition. Primary and secondary plasma bile acids levels  
334 positively correlated with most of the taxa in Cir\_noAH patients (Figure 6A) while  
335 primary plasma bile acids negatively correlated with taxa from Bacteroidetes and  
336 Firmicutes phyla in Cir\_sAH patients (Figure 6B). Total UDCA, TUDCA, and



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3 337 glyoursodeoxycholate (GUDCA) positively correlated with most of the taxa in  
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5 338 Cir\_noAH patients while it was negatively correlated with taxa from the Cir\_sAH  
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7 339 patients (Figure 6A and 6B).  
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10 340 Faecal primary bile acids were mostly negatively correlated with most of the taxa in  
11  
12 341 Cir\_noAH patients (Figure 6C) while in Cir\_sAH patients, primary faecal bile acids  
13  
14 342 were negatively correlated and secondary faecal bile acids were positively correlated  
15  
16 343 with most of the taxa (Figure 6D)  
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19 344 These correlations suggest that the abundance of bacteria carrying the enzymes  
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21 345 needed for bile acids deconjugation and transformation into secondary bile acids and  
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23 346 UDCA is reduced in the intestinal microbiota of Cir\_sAH patients.  
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#### 27 347 **FXR-FGF-19 in severe alcoholic hepatitis**

  
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29 348 As signaling molecules, bile acids activate ileal FXR and induce the production of  
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31 349 FGF19. Cir\_sAH patients had higher plasma levels of FGF19 than Cir\_noAH patients  
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33 350 ( $282 \pm 431$  vs.  $55 \pm 75$  pg/mL,  $p = 0.03$ , Table 1). In patients without cirrhosis,  
34  
35 351 plasma levels of FGF19 were higher in patients with AH patients than those without,  
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37 352 but did not reach statistical significance ( $154 \pm 368$  vs.  $69 \pm 74$  pg/mL,  $p = 0.5$ ).  
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39 353 However, FGF-19 positively correlated with the MELD score ( $r = 0.49$ ,  $p = 0.04$ ), but  
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41 354 not the Maddrey or AH histological scores.  
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45 355 These results suggest that FXR is activated in Cir\_sAH patients independently of the  
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47 356 faecal bile acids concentration in the gut.  
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3 357 **DISCUSSION**

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5 358 In this study, we characterized the intestinal microbiota, its functions, and its  
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7 359 relationship with bile acids homeostasis in well phenotyped alcoholic liver disease  
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9 360 patients, in order to overcome potential confounders such as alcoholic liver disease  
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11 361 stage and previous or concomitant treatments. Moreover, as alcohol induces a  
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13 362 specific dysbiosis in both animal models of alcoholic liver disease (4,23,27) and  
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15 363 humans (12,13,28), including higher levels of some members of Proteobacteria and  
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17 364 lower Bacteroidaceae, Lachnospiraceae, and Prevotellaceae, we only compared  
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19 365 patients with ongoing alcohol consumption. This allowed us to identify specific  
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21 366 changes related only to the liver disease, independently on the amount and duration  
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23 367 of alcohol consumption.

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27 368 Alcoholic hepatitis did not modify the overall composition of the intestinal microbiota  
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29 369 in patients without cirrhosis. A similar result has been recently reported in a mouse  
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31 370 model of acute-on-chronic alcohol feeding (29). However, we observed an increase  
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33 371 in the abundance of *Dorea*, *Wolbachia* and *Rothia* in noCir\_AH patients. Among  
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35 372 patients with alcoholic cirrhosis, severe alcoholic hepatitis patients had higher  
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37 373 abundance of bacteria of the Actinobacteria phylum including the *Actinomyces*,  
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39 374 *Rothia*, and *Bifidobacterium* genus. The abundance of *Lactobacillus* (Firmicutes  
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41 375 phylum), *Haemophilus* (from the Pasteurellaceae family, Proteobacteria phylum),  
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43 376 and an unidentified member of the Enterobacteriaceae family (Proteobacteria  
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45 377 phylum) was also higher. Conversely, the abundance of bacteria of the Bacteroidetes  
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47 378 phylum was lower. Interestingly, these changes are consistent with data from other  
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49 379 studies that investigated the intestinal microbiota in alcoholic liver disease and in  
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51 380 other liver diseases such as NAFLD (28–32), suggesting that these changes may be  
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53 381 related to cirrhosis and impaired liver function rather than to the cause of liver  
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3 382 disease. These results also confirms the increase seen in *Bifidobacterium* genus in  
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5 383 severe alcoholic hepatitis patients that we previously reported in a smaller sample of  
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7 384 severe alcoholic hepatitis patients (4). Furthermore, by increasing the number of  
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9 385 patients included in the present study we also identifies new taxa associated with  
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11 386 severe alcoholic hepatitis as compared to our previous study.

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14 387 We further explored the role of the intestinal microbiota in Cir\_sAH using PICRUST to  
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16 388 predict the metagenomic profile of the intestinal microbiota. We observed a switch in  
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18 389 the functions of the intestinal microbiota in Cir\_sAH patients, including a decrease in  
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20 390 the biotin metabolic pathway. Biotin is a member of the vitamin-B family of vitamins  
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22 391 and acts as a cofactor for several carboxylases in mitochondria. Exogenous biotin is  
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24 392 obtained from dietary sources or intestinal biotin-producing bacteria (33). Plasma  
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26 393 biotin levels in chronic alcohol patients are reduced due to inhibition of carrier-  
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28 394 mediated biotin transport in the jejunum and colon (34). Thus, reduced production in  
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30 395 the gut and decreased absorption in alcoholic patients could lead to the dysfunction  
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32 396 of mitochondria, which could impair the hepatic response to inflammation in severe  
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34 397 alcoholic hepatitis.

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39 398 We also observed altered glutathione metabolism in the intestinal microbiota of  
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41 399 Cir\_sAH patients. Glutathione is a powerful antioxidant and patients with alcoholic  
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43 400 liver disease have low hepatic and plasma glutathione levels (35). In this context, it  
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45 401 has been shown that the bile salt hydrolase (BSH) gene from *Bifidobacterium longum*  
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47 402 is co-transcribed with the gene encoding glutamine synthetase adenylyltransferase  
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49 403 (glnE), a component of the nitrogen regulation cascade (36). Thus, the increase in  
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51 404 the abundance of *Bifidobacterium* in Cir\_sAH patients could be responsible, at least  
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53 405 partially, for the increased proportion of primary unconjugated faecal bile acids, as a  
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3 406 result of BSH activity and increased glutathione metabolism of the intestinal  
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5 407 microbiota of these patients.  
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8 408 It has been suggested, in a previous work, that an increase in primary bile acids was  
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10 409 associated with the severity of histological lesions in AH (11). A similar result was  
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12 410 observed in our study. Of note, in the previous work (11), the authors provided an  
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14 411 overall plasma bile acids profile in patients with AH ranging from mild AH to severe  
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16 412 alcoholic hepatitis and irrespective of the presence of cirrhosis, that was present in  
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18 413 75 % of their patients. As alcoholic cirrhosis is associated with an impaired bile acids  
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20 414 profile (12,13), their results might be biased by the mix of cirrhotic and non-cirrhotic  
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22 415 patients. Moreover, according to the method used for bile acids assay (HPLC), they  
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24 416 could only detect primary and secondary bile acids but neither their conjugated forms  
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26 417 nor UDCA. Other studies have investigated plasma bile acids, faecal bile acids and  
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28 418 intestinal microbiota in alcoholic patients (12,13). They suggested, that alcohol intake  
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30 419 in both cirrhosis and non-cirrhotic patients is associated with a decrease in  
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32 420 conjugated CDCA in plasma (12). In our study, CDCA was increased in severe  
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34 421 alcoholic hepatitis, suggesting that this increase is due to liver inflammation (ie  
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36 422 severe alcoholic hepatitis) independently of the presence of cirrhosis of alcohol  
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38 423 intake. We also suggest that perturbation of intestinal microbiota is involved in the  
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40 424 specific modifications bile acids metabolism observed in patients with severe  
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42 425 alcoholic hepatitis.  
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48 426 More hydrophobic bile acids (CA, CDCA, DCA) rapidly induce apoptosis (37),  
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50 427 whereas less hydrophobic bile acids (UDCA) are less toxic (38). Moreover, total  
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52 428 plasma bile acids and primary plasma bile acids (CA and CDCA) levels have been  
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54 429 shown to positively correlated with the AH severity and steatosis (11,18,39). Several  
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56 430 mechanisms may explain the increase of the bile acids pool, including upregulation of  
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3 431 cholesterol 7 $\alpha$ -hydroxylase (Cyp7A1) induced by both chronic and acute alcohol  
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5 432 consumption (40) and/or a decrease in bile acids excretion in the bile and  
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7 433 subsequent release in the plasma in the context of AH. Moreover, it has also been  
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9 434 suggested that the intestinal microbiota can contribute to biliary inflammation (41),  
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11 435 which could impair bile acids circulation. These hypotheses are supported by the  
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13 436 increased levels of primary and conjugated plasma bile acids in the Cir\_sAH patients  
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15 437 and decrease in the proportion of secondary plasma bile acids and total faecal bile  
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17 438 acids. We can thus hypothesize that the excess plasma bile acids do not reach the  
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19 439 gut where they could be deconjugated and transformed into secondary bile acids.  
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22 440 Moreover, conjugation of either taurine or glycine to bile acids decreases their  
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24 441 hydrophobicity and thus their toxicity. There was also a trend towards switching the  
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26 442 plasma bile acids pool from glycoconjugated forms towards tauroconjugated forms,  
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28 443 which are less toxic.

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31 444 CDCA is the most potent FXR agonist capable of inducing FGF19 expression (42).  
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33 445 Here, plasma FGF-19 levels were higher in Cir\_sAH than Cir\_noAH patients. FGF-19  
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35 446 is produced in the ileum by FXR activation. Faecal bile acids activates FXR and acts  
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37 447 in a negative feedback loop by blocking CYP7A1 and bile acid synthesis (classical  
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39 448 pathway). FGF-19 is absent from primary, non-activated hepatocytes, but bile acids -  
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41 449 activated hepatic FXR can induce FGF19 secretion *in vitro* (43) and *in vivo* in patients  
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43 450 with cholestasis (44) by an autocrine/paracrine mechanism, independently of SHP  
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45 451 (43,44). However, a recent study found that FGF19 levels were significantly elevated  
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47 452 in patients with alcoholic hepatitis while serum 7-alpha-hydroxy-4-cholesten-3-one  
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49 453 (C4) levels, a bile acids synthesis marker for de novo synthesis was decreased  
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51 454 suggesting a prominent role of cholestasis (14). Moreover, the authors showed that in  
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53 455 alcoholic hepatitis FGF-19 originates in cholangiocytes and ductular cells from  
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3 456 smaller ductules (progenitor cells). Here, FGF-19 positively correlated with the MELD  
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5 457 score. This is consistent with other studies that reported a correlation between FGF-  
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7 458 19 levels and liver disease severity (14,44) and suggests that the increase in FGF-19  
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9 459 levels observed in our study has a double origin, hepatic (due to increased plasma  
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11 460 bile acids , dominated by CDCA) and intestinal. Indeed, there was a shift of the faecal  
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13 461 bile acids pool in the gut toward species with a higher affinity for FXR, as shown by  
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15 462 the increase in the proportion of CDCA, although there was an overall decrease in  
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17 463 total faecal bile acids. Thus, high levels of FGF-19 could increase CDCA synthesis by  
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19 464 directing bile acids synthesis from the classic (neutral) to the alternative (acidic)  
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21 465 pathway, due to the blockade of CYP7A1, but not of cholesterol 7 $\beta$ -hydroxylase  
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23 466 (CYP7B1). Therefore, high levels of FGF-19 are probably insufficient to counteract  
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25 467 the increased bile acids synthesis induced by alcohol in alcoholic liver disease.  
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27 468 Moreover, it promotes the shift of the bile acids pool towards more hydrophobic, toxic  
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29 469 species.  
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34 470 There was also an increase in total plasma UDCA and TUDCA levels in Cir\_sAH  
35  
36 471 patients relative to Cir\_noAH patients. These bile acids have hepatoprotective  
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38 472 effects. However, TUDCA exerts this effect by replenishing hepatic mitochondrial  
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40 473 glutathione (45). Thus, the increase in glutathione metabolism of the intestinal  
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42 474 microbiota, which may decrease its bioavailability to the mitochondria, combined with  
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44 475 an intestinal microbiota-associated decrease in the levels of biotin, an essential  
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46 476 cofactor of mitochondrial metabolism, could explain why the increased TUDCA levels  
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48 477 seen in Cir\_sAH patients does not have a hepatoprotective effect. This is supported  
49  
50 478 by the fact that UDCA showed hepatoprotective effects in *in vitro* studies and early  
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52 479 stages of alcoholic liver disease, but not in severe alcoholic hepatitis patients with  
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54 480 cholestasis (46).  
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3 481 Specific bacteria may be involved in the production of UDCA in severe alcoholic  
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5 482 hepatitis patients. Plasma UDCA, that was increased in severe alcoholic hepatitis  
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7 483 patients, and that is only produced by bacteria in the gut from CDCA, positively  
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9 484 correlated with the abundance of Actinobacteria and Proteobacteria phyla. The  
10  
11 485 abundance of these phyla was increased in severe alcoholic hepatitis patients, as  
12  
13 486 was the abundance of the *Bifidobacteria* and *Clostridium* genera. Administration of  
14  
15 487 *Bifidobacteria animalis*, as a bile salt-hydrolysing bacteria, and *Clostridium absonum*,  
16  
17 488 as a CDCA to UDCA epimerizing bacteria, result in increased levels of faecal UDCA  
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19 489 in pigs (47).

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23 490 An increased level of faecal bile acids was reported in cirrhotic patients with ongoing  
24  
25 491 alcohol consumption but, in these patients, the consequences of a potential liver  
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27 492 inflammatory process (ie alcoholic hepatitis) is unknown (12,13). We now show that  
28  
29 493 total faecal bile acids levels probably decreased in Cir\_sAH patients due to  
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31 494 decreased excretion of plasma bile acids in the bile, as discussed earlier. Faecal bile  
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33 495 acids shape the intestinal microbiota as deconjugation provides cellular carbon,  
34  
35 496 nitrogen, and sulfur for some bacterial species, especially *Bacteroides* and *Bilophila*  
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37 497 (48). Thus, the decrease in faecal bile acids levels in Cir\_sAH patients may be  
38  
39 498 responsible for the decrease in the abundance of Bacteroidetes and *Bilophila*.  
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41 499 Moreover, primary faecal bile acids levels have been shown to increase intestinal  
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43 500 permeability (49), which can increase PAMP release into the systemic circulation,  
44  
45 501 participating in the higher levels of endotoxemia observed in alcoholic liver disease  
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47 502 patients. Furthermore, bile acids bactericidal activity is related to their hydrophobicity,  
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49 503 which increases their affinity for the phospholipid bilayer of the bacterial cell  
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51 504 membrane, and unconjugated bile acids are weak acids with strong bactericidal  
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53 505 activities. Among the bile acids, DCA is extremely toxic and inhibits the growth of  
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506 many intestinal bacteria, including *Clostridium perfringens*, *Bacteroides fragilis*,  
507 *Lactobacilli*, and *Bifidobacteria* (50,51). In our study we observed a decrease in  
508 unconjugated bile acids, total secondary bile acids and DCA in the feces, that are  
509 highly hydrophobic. This may be responsible for the increase levels of *Lactobacillus*  
510 and *Bifidobacterium* seen in the microbiota of these patients. It has also been  
511 suggested that a decreased level of faecal bile acids stimulated the growth of gram-  
512 negative and conversely decreases the growth gram-positive bacteria (52). Indeed,  
513 we observed in severe alcoholic hepatitis patients an increase in several taxa that are  
514 gram-negative (eg Gammaproteobacteria) that could be secondary to the decrease  
515 faecal bile acids level. Moreover, gram-negative bacteria produce LPS that was  
516 related to increases alcoholic liver necrosis and inflammation (53). We also observed  
517 in our study a decrease in several taxa from the gram-positive Firmicutes phylum  
518 (Christensenellaceae, Oscillospira) that 7 $\alpha$ -dehydroxylate primary bile acids to toxic  
519 secondary bile acids. Thus, the decrease and shift of the bile acids pool in the feces  
520 could be responsible for the increase in LPS-producing bacteria and for the decrease  
521 of gram-positive members of Firmicutes able to transform primary bile acids into  
522 secondary bile acids. This hypothesis may explain the decrease in secondary bile  
523 acids in severe alcoholic hepatitis patients observed in our study.

524 A limit of our study was a potential lack of power related to the small number of  
525 patients, which did not allow us to identify changes in taxa with low counts. However,  
526 the recruitment of severe alcoholic hepatitis patients for intestinal microbiota studies  
527 is challenging, as most are rapidly treated (often by antibiotics to prevent or treat  
528 complications). This bias did not occur in our patients as they were included before  
529 any specific treatment for severe alcoholic hepatitis . Moreover, we did not exclude  
530 patients with proton-pump inhibitors intake which was shown to alter the IM



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3 531 composition (54). However, there was no difference in the use of proton-pump  
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5 532 inhibitors between groups, suggesting that they are not responsible for the results  
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7 533 observed in our study.  
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10 534 In conclusion, severe alcoholic hepatitis is associated with specific alterations of the  
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12 535 bile acids homeostasis and of the intestinal microbiota. These changes are  
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14 536 characterized by an increased level of hydrophobic bile acids and of Actinobacteria  
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16 537 and a decrease of Bacteroidetes. The increase and shift in the bile acids pool  
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18 538 towards hydrophobic and toxic species could be responsible for the specific intestinal  
19  
20 539 microbiota changes, including an increase in the LPS-producing gram-negative  
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22 540 bacteria such as Gammaproteobacteria and a decrease in certain gram-positive  
23  
24 541 bacteria capable to transform primary into secondary bile acids. Furthermore, the  
25  
26 542 changes in the intestinal microbiota were associated with a shift in its functions,  
27  
28 543 especially decreased biotin metabolism and increased glutathione metabolism, which  
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30 544 could play a role in the initiation and progression of severe alcoholic hepatitis,  
31  
32 545 through impairment of the protective effects of UDCA on mitochondrial metabolism.  
33  
34 546 Our study provides a new hypothesis for future studies to address bile acids and the  
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36 547 intestinal microbiota as new therapeutic targets to improve the management of  
37  
38 548 alcoholic liver disease patients.  
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728 **Table 1: Patient's characteristics**

	Without alcoholic cirrhosis		Alcoholic cirrhosis	
	noCir_noAH	noCir_AH	Cir_noAH	Cir_sAH
	(n=61)	(n=13)	(n=17)	(n=17)
Age (years)	51±8.5	46±8.1	58±9.8	56±12.3
Sex: male/female (%)	50/10(83/17)	11/2(85/15)	15/3 (83/17)	14/4(78/22)
BMI (kg/m <sup>2</sup> )	23.4 [16.4-31.2]	23.9 [20-41.2]	23.7 [15.9-31.2]	24.2 [19.4-39]
Alcohol intake (g/day)	150 [50-500]	200 [60-400]	140 [50-360]	80 [50-240]*
Alcohol time (years)	15 [0.5-40]	10 [1-40]	22.5 [3-50]	20 [6-40]
Smoking (%)	48 (81)	9 (70)	13 (72)	13 (72)
PPIs use (%)	5 (8)	1 (8)	7 (41)	7 (41)
Diabetes	5 (8)	1 (8)	4 (22)	2 (11)
AST (U/L)	47 [15-240]	175 [58-511]***	64 [38-252]	88 [18-2657]*
ALT (U/L)	40.5 [8-224]	59 [32-217]*	40.5 [11-143]	41.5 [19-481]
Total bilirubin (µmol/L)	12 [6-34]	22 [10-186]**	34.5 [4-110]	143 [44-751]***
GGT (U/L)	141 [18-1641]	928 [79-3970]**	382 [58-3923]	185.5 [45-990]
Platelets (x10 <sup>9</sup> /L)	189.5 [36-455]	193 [71-459]	98.5 [53-378]	94 [33-475]
Prothrombine Time (%)	100 [67-100]	91 [79-100]	66 [25-100]	35 [25-64]***
Glucose (mmol/L)	4.9 [3.8-11.8]	4.8 [4.3-7.5]	5.2 [4.4-13.6]	5.2 [4.4-8.9]
Albumin (g/L)	37.8 [27.6-46.7]	39.5 [23.1-47.5]	36.1 [21.5-40.8]	30 [22.3-36]***
Creatinin (µmol/L)	74 [50-107]	73 [57-118]	71 [52-130]	73 [57-207]
CRP (mg/L)	5 [5-54]	8 [5-91]*	5 [5-34]	22 [5-127]**
Maddrey Score	1.15 [0.35-30.9]	5.5 [0.59-16.5]	19 [0.2-60]	48.9 [32-101]***
FGF-19 (pg/mL)	86 [0-600]	36.18 [4.8-1258]	66 [16-541]	166 [24-2450]
MELD Score			13.7±7.2	24.33±6.8***
Liver Biopsy (%)	24 (39)	11 (85)*	12 (71)	17 (100)*

The data are expressed as the mean ± SD for continuous variables with a normal distribution, median and min and max for data with a non-normal distribution, and *n* (%) for discrete variables. Comparisons between noCir and AH patients, and alcoholic cirrhosis (Cir) and severe alcoholic hepatitis (sAH) patients in Mann-Whitney tests or independent *t*-tests for continuous data and  $\chi^2$  tests or Fisher's exact tests for discrete data. \**p*<0.05; \*\**p*<0.01; \*\*\**p*<0.001. BMI, body mass index; PPIs, proton-pump inhibitors; AST, alanine aminotransferase; ALT, aspartate aminotransferase; GGT, gammaglutamyltransferase; CRP, C-reactive protein; MELD, Model for End-stage Liver Disease, FGF-19, fibroblast growth factor-19.

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3 730 Figure legends

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5 731 **Figure 1: Intestinal microbiota profiles and its metabolic functions. (A)**

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7 732 Weighted UniFrac distances (quantitative method reflecting the structure of the  
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9 733 intestinal microbiota) and **(B)** Unweighted UniFrac distances (qualitative method  
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11 734 reflecting the composition of the intestinal microbiota) showing a difference in the  
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13 735 structure of the intestinal microbiota only between Cir\_sAH patients (blue) and  
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15 736 Cir\_noAH patients (red,  $p < 0.05$  for Weighted UniFrac distances). Each point  
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17 737 represents a subject and the distance between the points is proportional to the  
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19 738 similarity of the intestinal microbiota. Cladogram showing the taxa with the largest  
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21 739 differences in abundance between **(C)** Cir\_sAH patients (green) and Cir\_noAH  
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23 740 patients (red) and **(D)** noCir\_AH patients (red) and noCir\_noAH patients. The size of  
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25 741 the circle in the cladogram plot is proportional to bacterial abundance. From inside to  
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27 742 outside, the circles represent phylum, class, order, family, and genus. Only taxa with  
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29 743 a LDA score  $> 2$  and  $p < 0.05$ , determined by the Wilcoxon signed rank test, are  
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31 744 shown. **(E)** LDA Effect Size (LEfSe) for the predicted metagenome metabolic  
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33 745 pathways (KEGG modules) increased in Cir\_sAH (green) and Cir\_noAH patients  
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35 746 (red) (LDA score  $> 2.0$ ,  $p < 0.05$  determined by the Wilcoxon signed rank test).

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40 747 **Figure 2: Plasma bile acids profiles in patients with alcoholic liver disease. (A)**

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42 748 PCA ordination plot with 95% confidence ellipse for all plasma bile acids in cir\_sAH  
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44 749 and cir\_noAH patients showing clustering of patients according to the liver  
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46 750 complication. The first two components of the PCA explained 64% of the total  
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48 751 variance (component 1 = 42.4%; component 2 = 21.5%). **(B)** Total plasma bile acids  
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50 752 , primary, total conjugated, glyco-conjugated and tauro-conjugated levels of plasma  
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52 753 bile acids . **(C)** and **(D)** plasma bile acids composition (% of total plasma bile acids  
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54 754 ). **(E)** Individual plasma bile acids levels.\* $p < 0.05$ , \*\* $p < 0.01$ . CA: cholic acid;

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755 CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; GCA,  
 756 glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic  
 757 acid; GLCA: glycolithocholic acid; GUDCA: glycoursodeoxycholic acid; sAH: severe  
 758 alcoholic hepatitis; TCA: taurocholic acid; TCDCA: taurochenodeoxycholic acid;  
 759 TDCA: taurodeoxycholic acid; TLCA: tauroolithocholic acid; TUDCA:  
 760 tauroursodeoxycholic acid; UDCA: ursodeoxycholic acid; \_3s: sulfated forms.

761 **Figure 3: Specificity of the plasma bile acids profile depending on alcoholic-**  
 762 **induced liver inflammation. (A)** PLS-DA score plot of plasma bile acids  
 763 concentrations in Cir\_sAH vs. Cir\_noAH patients with the 95% confidence ellipse  
 764 showing a significant difference between the two groups ( $R^2 = 0.6$ ,  $Q^2 = 0.4$ ,  $p =$   
 765  $0.001$ ). **(B)** Variable importance in projection (VIP) of PLS-DA showing the plasma  
 766 bile acids that discriminate Cir\_sAH from Cir\_noAH patients (VIP score  $>1$ ). The  
 767 colored boxes on the right indicate the relative concentrations of the corresponding  
 768 plasma bile acids in each group.

769 **Figure 4: Faecal bile acids profiles in alcoholic liver disease. (A)** PCA ordination  
 770 plot with 95% confidence ellipse for all faecal bile acids showing clustering of patients  
 771 according to the liver complication. The first two components of the PCA explained  
 772 57% of the total variance (component 1 = 39.3%; component 2 = 17.9%). **(B)** Total  
 773 faecal bile acids, total unconjugated, secondary and secondary unconjugated levels  
 774 of faecal bile acids. **(C)** and **(D)** faecal bile acids composition (% of total faecal bile  
 775 acids). **(E)** Individual faecal bile acids levels. \* $p < 0.05$ , \*\* $p < 0.01$ . CA: cholic acid;  
 776 CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; GCA:  
 777 glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic  
 778 acid; GLCA: glycolithocholic acid; GUDCA: glycoursodeoxycholic acid; sAH: severe  
 779 alcoholic hepatitis; TCA: taurocholic acid; TCDCA: taurochenodeoxycholic acid;

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3 780 TDCA: taurodeoxycholic acid; TLCA: tauroolithocholic acid; TUDCA:  
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5 781 tauroursodeoxycholic acid; UDCA: ursodeoxycholic acid; \_3s: sulfated forms.  
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8 782 **Figure 5: Specificity of faecal bile acids profiles depending on alcoholic-**  
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10 783 **induced liver inflammation. (A)** PLS-DA score plot of faecal bile acids  
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12 784 concentrations of Cir\_sAH vs. Cir\_noAH patients with 95% confidence ellipse  
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14 785 showing a significant difference between the two groups ( $R^2 = 0.8$ ,  $Q^2 = 0.6$ ,  $p =$   
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16 786  $0.001$ ). **(B)** Variable importance in projection (VIP) of PLS-DA showing the faecal bile  
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18 787 acids that discriminate Cir\_sAH from Cir\_noAH patients (VIP score  $>1$ ). The colored  
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20 788 boxes on the right indicate the relative concentrations of the corresponding faecal  
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22 789 bile acids in each group.  
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26 790 **Figure 6:** Heatmap representation of the Spearman's  $r$  correlation coefficient  
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28 791 between bacterial taxa (phylum and genus level) and bile acids profiles in plasma of  
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30 792 Cir\_noAH **(A)** and Cir\_sAH **(B)** patients and feces of Cir\_noAH **(C)** and Cir\_sAH **(D)**.  
31  
32 793 Only the bacteria for which at least one significant correlation with bile acids was  
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34 794 found are displayed (p, phyla; g, genus). CA: cholic acid; CDCA: chenodeoxycholic  
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36 795 acid; DCA: deoxycholic acid; LCA: lithocholic acid; GCA: glycocholic acid; GCDCA:  
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38 796 glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; GLCA: glycolithocholic  
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40 797 acid; GUDCA: glyoursodeoxycholic acid; TCA: taurocholic acid; TCDCA:  
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42 798 taurochenodeoxycholic acid; TDCA: taurodeoxycholic acid; TLCA: tauroolithocholic  
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44 799 acid; TUDCA: tauroursodeoxycholic acid; UDCA: ursodeoxycholic acid; A:  
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46 800 Actinobacteria, B: Bacteroidetes, F: Fusobacteria; P: Proteobacteria. \*Adjusted p  
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48 801 value  $< 0.05$ . Red: negative correlation, blue: positive correlation.  
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Figure 1

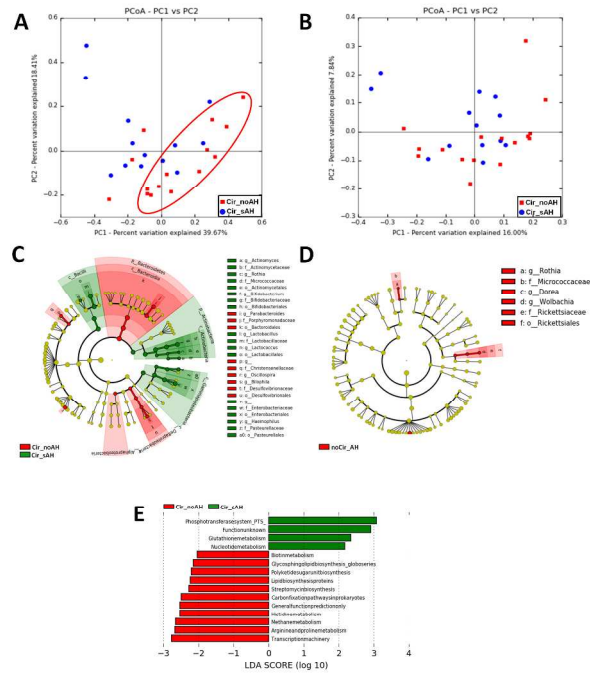


Figure 1: Intestinal microbiota profiles and its metabolic functions. (A) Weighted UniFrac distances (quantitative method reflecting the structure of the intestinal microbiota) and (B) Unweighted UniFrac distances (qualitative method reflecting the composition of the intestinal microbiota) showing a difference in the structure of the intestinal microbiota only between Cir\_sAH patients (blue) and Cir\_noAH patients (red,  $p < 0.05$  for Weighted UniFrac distances). Each point represents a subject and the distance between the points is proportional to the similarity of the intestinal microbiota. Cladogram showing the taxa with the largest differences in abundance between (C) Cir\_sAH patients (green) and Cir\_noAH patients (red) and (D) noCir\_AH patients (red) and noCir\_noAH patients (red). The size of the circle in the cladogram plot is proportional to bacterial abundance. From inside to outside, the circles represent phylum, class, order, family, and genus. Only taxa with a LDA score  $> 2$  and  $p < 0.05$ , determined by the Wilcoxon signed rank test, are shown. (E) LDA Effect Size (LefSe) for the predicted metagenome metabolic pathways (KEGG modules) increased in Cir\_sAH (green) and Cir\_noAH patients (red) (LDA score  $> 2.0$ ,  $p < 0.05$  determined by the Wilcoxon signed rank test).

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Figure 2

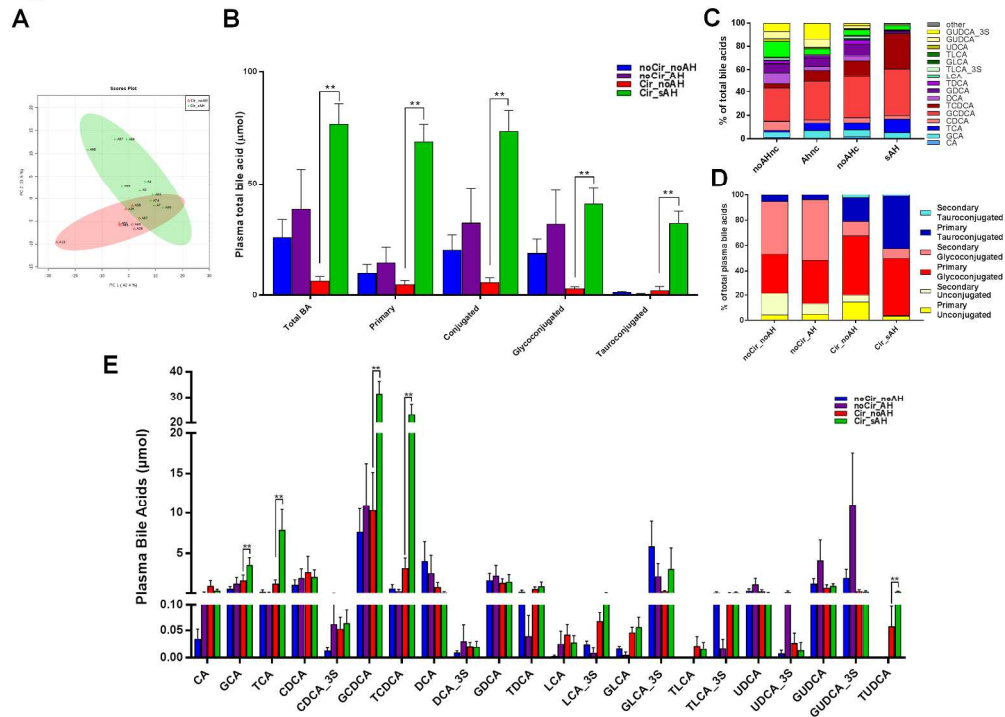


Figure 2: Plasma bile acids profiles in patients with alcoholic liver disease. (A) PCA ordination plot with 95% confidence ellipse for all plasma bile acids in cir\_sAH and cir\_noAH patients showing clustering of patients according to the liver complication. The first two components of the PCA explained 64% of the total variance (component 1 = 42.4%; component 2 = 21.5%). (B) Total plasma bile acids, primary, total conjugated, glyco-conjugated and tauro-conjugated levels of plasma bile acids. (C) and (D) plasma bile acids composition (% of total plasma bile acids). (E) Individual plasma bile acids levels. \*p < 0.05, \*\*p < 0.01. CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; GCA, glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; GLCA: glycolithocholic acid; GUDCA: glyoursodeoxycholic acid; sAH: severe alcoholic hepatitis; TCA: taurocholic acid; TCDCA: taurochenodeoxycholic acid; TDCA: taurodeoxycholic acid; TLCA: tauroolithocholic acid; TUDCA: tauroursodeoxycholic acid; UDCA: ursodeoxycholic acid; \_3s: sulfated forms.

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Figure 4

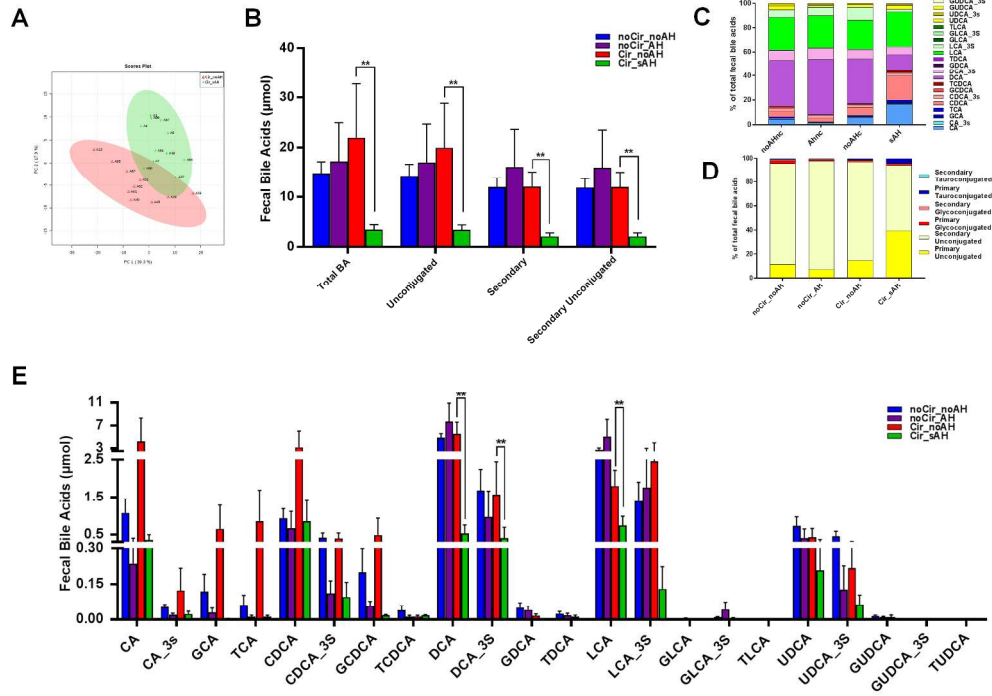


Figure 4: Faecal bile acids profiles in alcoholic liver disease. (A) PCA ordination plot with 95% confidence ellipse for all faecal bile acids showing clustering of patients according to the liver complication. The first two components of the PCA explained 57% of the total variance (component 1 = 39.3%; component 2 = 17.9%). (B) Total faecal bile acids, total unconjugated, secondary and secondary unconjugated levels of faecal bile acids. (C) and (D) faecal bile acids composition (% of total faecal bile acids). (E) Individual faecal bile acids levels. \* $p < 0.05$ , \*\* $p < 0.01$ . CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; GCA: glycocholic acid; GCDCa: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; GLCA: glycolithocholic acid; GUDCA: glyoursodeoxycholic acid; sAH: severe alcoholic hepatitis; TCA: taurocholic acid; TCDCA: taurochenodeoxycholic acid; TDCA: taurodeoxycholic acid; TLCA: tauroolithocholic acid; TUDCA: taoursodeoxycholic acid; UDCA: ursodeoxycholic acid; \_3s: sulfated forms.

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Figure 5

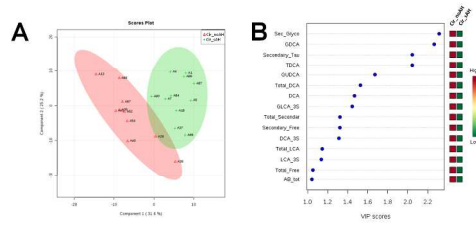


Figure 5: Specificity of faecal bile acids profiles depending on alcoholic-induced liver inflammation. (A) PLS-DA score plot of faecal bile acids concentrations of Cir\_sAH vs. Cir\_noAH patients with 95% confidence ellipse showing a significant difference between the two groups ( $R^2 = 0.8$ ,  $Q^2 = 0.6$ ,  $p = 0.001$ ). (B) Variable importance in projection (VIP) of PLS-DA showing the faecal bile acids that discriminate Cir\_sAH from Cir\_noAH patients (VIP score  $> 1$ ). The colored boxes on the right indicate the relative concentrations of the corresponding faecal bile acids in each group.

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Figure 6

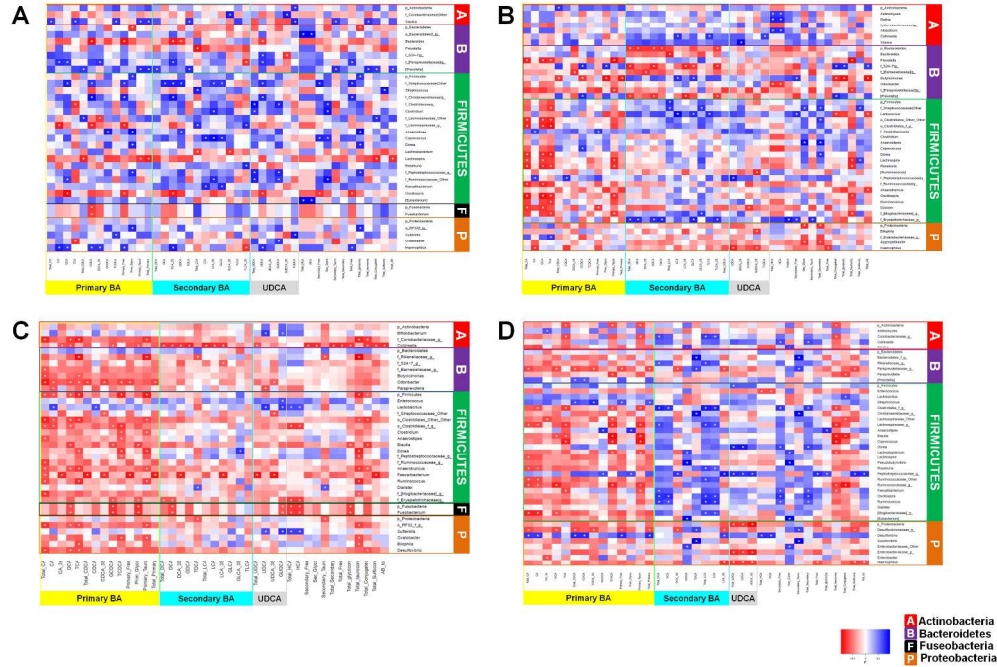


Figure 6: Heatmap representation of the Spearman's  $r$  correlation coefficient between bacterial taxa (phylum and genus level) and bile acids profiles in plasma of Cir\_noAH (A) and Cir\_sAH (B) patients and feces of Cir\_noAH (C) and Cir\_sAH (D). Only the bacteria for which at least one significant correlation with bile acids was found are displayed (p, phyla; g, genus). CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; GCA: glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; GLCA: glycolithocholic acid; GUDCA: glyoursodeoxycholic acid; TCA: taurocholic acid; TCDCA: taurochenodeoxycholic acid; TDCA: taurodeoxycholic acid; TLCA: tauroolithocholic acid; TUDCA: taoursodeoxycholic acid; UDCA: ursodeoxycholic acid; A: Actinobacteria, B: Bacteroidetes, F: Fusobacteria; P: Proteobacteria. \*Adjusted p value < 0.05. Red: negative correlation, blue: positive correlation.

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3 **Title: Bile acids homeostasis and intestinal dysbiosis in alcoholic hepatitis**

4 **Running Title: Bile acid, microbiota and alcoholic hepatitis**

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**Supplementary Table 1:** Differences in intestinal microbiota at the genus level between patients with alcoholic cirrhosis, with (Cir\_sAH ) and without severe alcoholic hepatitis (Cir\_noAH).

Phyla	Family	Genus	Increased in	Relative abundance		Mann Whitney		LEfSe	
				Cir_noAH	Cir_sAH	p	FDR	LDA score	p
Actinobacteria	Actinomycetaceae	Actinomyces	Cir_sAH	18*10 <sup>-5</sup>	79*10 <sup>-5</sup>	0.03	0.23	3.06	0.03
	Micrococcaceae	Rothia	Cir_sAH	1*10 <sup>-b</sup>	22*10 <sup>-b</sup>	0.01	0.20	3.29	0.01
	Bifidobacteriaceae	Bifidobacterium	Cir_sAH	1200*10 <sup>-b</sup>	8373*10 <sup>-b</sup>	0.02	0.23	3.84	0.02
Bacteroidetes	Porphyromonadaceae	Parabacteroides	Cir_noAH	1665*10 <sup>-5</sup>	555*10 <sup>-5</sup>	0.03	0.23	3.14	0.03
Firmicutes	Streptococcaceae	Lactococcus	Cir_sAH	68*10 <sup>-5</sup>	79*10 <sup>-5</sup>	0.04	0.27	2.87	0.04
	Christensenellaceae	g_	Cir_noAH	172*10 <sup>-b</sup>	3*10 <sup>-b</sup>	0.03	0.23	2.73	0.03
	Ruminococcaceae	Oscillospira	Cir_noAH	1247*10 <sup>-b</sup>	421*10 <sup>-b</sup>	0.00	0.14	3.00	<0.01
	Lactobacillaceae	Lactobacillus	Cir_sAH	1294*10 <sup>-b</sup>	12397*10 <sup>-b</sup>	0.00	0.14	4.09	<0.01
Proteobacteria	Pasteurellaceae	Haemophilus	Cir_sAH	165*10 <sup>-b</sup>	503*10 <sup>-b</sup>	0.01	0.22	2.88	0.01
	Desulfovibrionaceae	Bilophila	Cir_noAH	217*10 <sup>-5</sup>	26*10 <sup>-5</sup>	0.01	0.20	2.97	0.01
	Enterobacteriaceae	g_	Cir_sAH	3601*10 <sup>-b</sup>	8156*10 <sup>-b</sup>	0.02	0.23	3.79	0.02

Supplementary Table 2: Plasma bile acids concentrations between groups.

Plasma Bile Acids (n=56)	Without cirrhosis						Cirrhosis					
	noCir_noAH (n=29)			noCir_AH (n=8)			Cir_noAH (n=8)			Cir_sAH (n=10)		
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
CA	0	0	0.51	0.00	0	0.90	0	0	0.33	0.19	0	1.38
GCA	0.07	0	7.10	0.26	0.002	5.36	<b>0.36</b>	<b>0</b>	<b>0.54</b>	<b>3.11</b>	<b>0</b>	<b>9.30</b>
TCA	0.03	0	6.44	0.01	0	0.74	<b>0.16</b>	<b>0</b>	<b>2.78</b>	<b>4.48</b>	<b>0</b>	<b>25.46</b>
Total CA	0.16	0	13.54	0.32	0.003	6.25	<b>0.59</b>	<b>0</b>	<b>3.05</b>	<b>8.46</b>	<b>0</b>	<b>34.82</b>
CDCA	0.07	0	17.95	0.03	0	8.37	0.02	0	0.63	1.49	0	9.15
CDCA_3S	0	0	0.16	0.00	0	0.28	0.02	0	0.07	0.02	0	0.22
GCDCA	1.05	0	56.08	1.49	0.01	32.55	<b>2.49</b>	<b>0.002</b>	<b>4.10</b>	<b>34.89</b>	<b>7.36</b>	<b>57.37</b>
TCDCA	0.06	0	12.81	0.05	0	1.83	<b>0.48</b>	<b>0</b>	<b>9.05</b>	<b>21.97</b>	<b>5.02</b>	<b>45.01</b>
Total CDCA	1.73	0.001	68.89	1.85	0.01	42.94	<b>4.00</b>	<b>0.002</b>	<b>11.39</b>	<b>61.35</b>	<b>12.38</b>	<b>81.23</b>
Total Primary	1.90	0.002	82.43	2.30	0.01	43.24	4.74	0.002	14.44	70.67	16.57	99.42
DCA	0.11	0	67.94	0.24	0	17.99	0.16	0.001	0.55	0.01	0	0.88
DCA_3S	0	0	0.07	0	0	0.24	0	0	0.05	0	0	0.10
GDCA	0.21	0	23.50	0.75	0	10.59	0.50	0.002	1.19	0.16	0	6.98
TDCA	0.02	0	6.30	0.00	0	0.31	0.14	0	2.01	0.17	0	4.51
Total DCA	0.47	0	70.94	1.15	0	28.58	1.02	0.002	2.51	0.36	0	12.19
LCA	0	0	0.03	0	0	0.19	0.02	0	0.08	0.01	0	0.11
LCA_3S	0	0	0.11	0	0	0.07	0.06	0	0.23	0.04	0	0.48
GLCA	0	0	0.07	0	0	0.04	0.05	0	0.10	0.04	0	0.17
GLCA_3S	0.18	0	74.05	0.007	0	12.83	0.27	0	0.68	0.19	0	26.48
TLCA	0	0	0	0	0	0	0	0	0.20	0	0	0.10
TLCA_3S	0	0	3.49	0	0	0.13	0.09	0	0.66	0.11	0	0.76
Total LCA	0.29	0	74.05	0.01	0	13.02	0.66	0.001	1.25	0.47	0	26.48
UDCA	0.03	0	7.97	0.00	0	5.94	0.01	0	0.06	0.05	0	0.73
UDCA_3S	0	0	0.13	0	0	1.01	0	0	0.13	0	0	0.14
GUDCA	0.17	0	13.26	0.32	0.000	20.54	0.13	0	0.27	0.76	0	2.49
GUDCA_3S	0.08	0	22.34	0.24	0	49.30	0.09	0	0.15	0.13	0	1.16
TUDCA	0	0	0.01	0	0	0.00	<b>0</b>	<b>0</b>	<b>0.02</b>	<b>0.26</b>	<b>0</b>	<b>0.66</b>
Total UDCA	0.37	0	42.49	0.56	0.001	56.73	<b>0.28</b>	<b>0</b>	<b>0.44</b>	<b>1.30</b>	<b>0</b>	<b>4.09</b>
Total Secondary	1.68	0.002	96.84	2.32	0.00	66.00	2.12	0.004	3.93	2.47	0.58	26.48
Total Unconjugated	0.64	0	85.89	0.37	0	26.49	0.27	0.001	1.74	1.89	0	12.02
Total Glycoconjugated	2.32	0.002	96.49	3.56	0.01	93.90	<b>3.90</b>	<b>0.005</b>	<b>6.58</b>	<b>39.98</b>	<b>9.11</b>	<b>83.85</b>
Total Tauroconjugated	0.16	0	19.25	0.36	0.003	1.83	<b>0.90</b>	<b>0</b>	<b>14.71</b>	<b>32.17</b>	<b>8.19</b>	<b>60.33</b>
Total Sulfoconjugated	0.50	0	74.05	0.84	0.001	54.38	0.79	0.001	1.06	0.65	0.06	26.48
Total Conjugated	2.60	0.002	100	4.26	0.01	93.98	<b>5.89</b>	<b>0.005</b>	<b>18.19</b>	<b>81.77</b>	<b>17.30</b>	<b>100</b>
Total Bile Acids	3.60	0.003	100	4.62	0.01	100	<b>6.84</b>	<b>0.01</b>	<b>18.36</b>	<b>84.57</b>	<b>17.37</b>	<b>100</b>

In bold bile acids that were different between the groups ( $p < 0.05$  using a Mann Whitney test with a FDR correction for multiple comparissons).

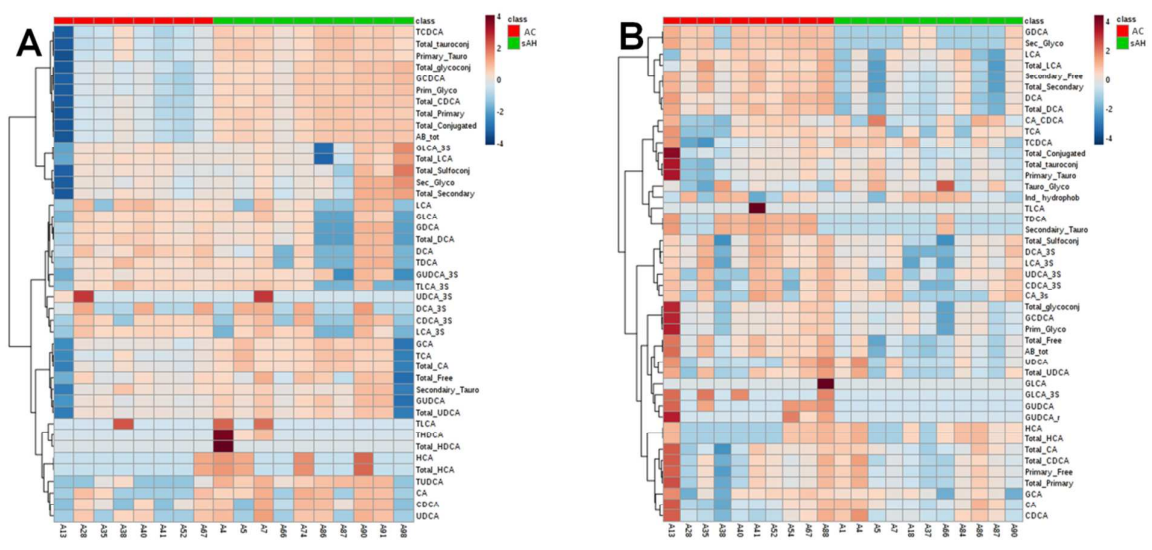
Supplementary Table 3: Fecal bile acids concentrations between groups.

Fecal Bile Acids (n=73)	Without cirrhosis						Cirrhosis					
	noCir_noAH (n=46)			noCir_AH (n=6)			Cir_noAH (n=10)			Cir_sAH (n=11)		
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
CA	0.07	0	11.66	0.10	0	1.02	0.03	0	49.09	0.09	0.01	1.56
CA_3s	0	0	0.32	0.02	0.001	0.05	0.02	0	1.14	0.001	0	0.08
GCA	0.01	0.001	3.17	0.01	0.01	0.13	0.01	0	7.62	0	0	0.01
TCA	0.01	0	1.90	0	0	0.04	0.01	0	10.07	0.01	0	0.08
Total CA	0.17	0.003	12.13	0.14	0.02	1.07	0.23	0	66.82	0.10	0.01	1.58
CDCA	0.16	0.01	6.82	0.08	0.01	2.73	0.05	0.005	34.24	0.11	0.04	6.77
CDCA_3S	0.02	0	4.16	0.07	0.003	0.36	0.32	0	1.30	0.01	0	0.18
GCDCA	0.05	0	4.35	0.05	0.01	0.13	0.02	0.002	5.44	0.01	0.001	0.04
TCDCa	0.01	0	0.94	0.01	0	0.03	0.01	0.001	0.05	0.02	0.004	0.03
Total CDCA	0.45	0.05	9.61	0.33	0.11	2.93	0.64	0.01	40.34	0.31	0.05	6.84
Total Primary	0.78	0.07	20.72	0.53	0.13	4.00	0.89	0.01	107.16	0.38	0.08	7.75
DCA	3.78	0.01	24.12	5.61	0.28	22.36	<b>3.76</b>	<b>0.62</b>	<b>25.27</b>	<b>0.13</b>	<b>0.01</b>	<b>2.61</b>
DCA_3S	0.14	0.002	22.06	0.30	0.01	4.45	<b>0.07</b>	<b>0</b>	<b>9.86</b>	<b>0</b>	<b>0</b>	<b>1.24</b>
GDCA	0.02	0	1.05	0.03	0	0.12	<b>0.01</b>	<b>0</b>	<b>0.10</b>	<b>0</b>	<b>0</b>	<b>0.003</b>
TDCA	0.01	0	0.41	0.01	0	0.04	<b>0.01</b>	<b>0</b>	<b>0.08</b>	<b>0</b>	<b>0</b>	<b>0.002</b>
Total DCA	4.54	0.05	46.49	7.43	0.36	22.74	<b>6.49</b>	<b>0.63</b>	<b>25.55</b>	<b>0.13</b>	<b>0.01</b>	<b>2.61</b>
LCA	1.96	0.003	8.74	1.96	0.38	20.00	<b>1.95</b>	<b>0.02</b>	<b>5.43</b>	<b>0.58</b>	<b>0.005</b>	<b>2.93</b>
LCA_3S	0.17	0	18.64	0.60	0.004	7.82	0.21	0	18.31	0	0	0.25
GLCA	0	0	0.003	0	0	0.02	0.00	0	0	0	0	0
GLCA_3S	0	0	0.06	0.01	0	0.21	0.00	0	0.03	0	0	0
TLCA	0	0	0	0.00	0	0.001	0.00	0	0	0	0	0
Total LCA	2.53	0.05	22.91	2.93	0.44	28.04	<b>2.69</b>	<b>0.38</b>	<b>20.15</b>	<b>0.58</b>	<b>0.01</b>	<b>2.93</b>
UDCA	0.10	0	7.18	0.06	0	1.47	0.01	0.002	2.38	0.01	0.003	1.94
UDCA_3S	0.04	0	4.09	0.04	0	0.63	0.09	0	0.90	0	0	0.23
GUDCA	0	0	0.15	0.01	0	0.02	0	0	0.11	0	0	0
GUDCA_3S	0	0	0.08	0	0	0	0	0	0	0	0	0
TUDCA	0	0	0.04	0	0	0.001	0	0	0	0	0	0
Total UDCA	0.30	0.01	11.31	0.39	0.02	1.54	0.41	0.002	3.11	0.02	0.003	1.94
Total Secondary	8.40	0.21	63.85	10.24	0.83	52.40	<b>10.68</b>	<b>1.80</b>	<b>27.92</b>	<b>0.72</b>	<b>0.02</b>	<b>5.68</b>
Total Unconjugated	9.33	0.40	83.97	10.54	0.93	53.27	<b>11.17</b>	<b>1.80</b>	<b>111.58</b>	<b>2.30</b>	<b>0.19</b>	<b>10.97</b>
Total Glycoconjugated	0.10	0.002	7.67	0.10	0.03	0.51	<b>0.05</b>	<b>0.002</b>	<b>13.30</b>	<b>0.02</b>	<b>0.001</b>	<b>0.05</b>
Total Tauroconjugated	0.02	0	2.86	0.02	0	0.11	0.02	0.001	10.20	0.02	0.01	0.10
Total Sulfoconjugated	0.46	0.01	31.88	1.23	0.05	8.36	0.71	0	25.13	0.04	0	1.99
Total Conjugated	0.12	0.002	10.54	0.16	0.03	0.53	0.08	0.02	23.50	0.03	0.02	0.12
Total BA	9.87	1.21	84.57	10.77	0.96	53.80	<b>11.32</b>	<b>1.82</b>	<b>135.08</b>	<b>2.36</b>	<b>0.31</b>	<b>11.03</b>

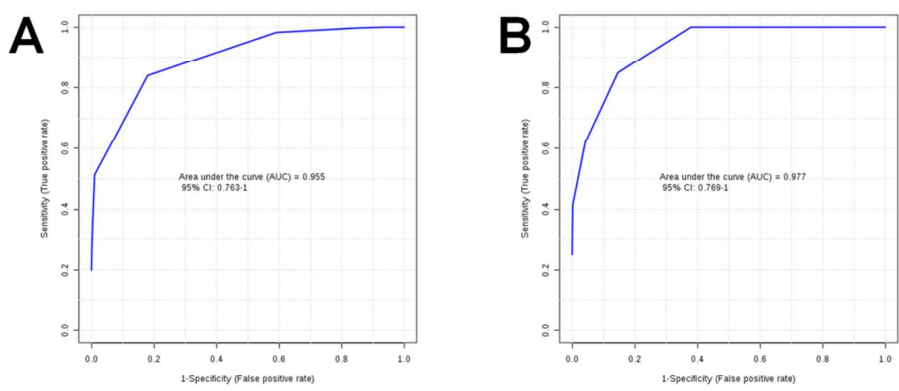
In bold bile acids were different between the groups ( $p < 0.05$  using a Mann Whitney test with a FDR correction for multiple comparisons).

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### Supplemental Figures



**Supplemental Figure 1:** Bile acids profiles in plasma (A) and feces (B). The relative amounts of bile acids are displayed as a heatmap (values are pareto and log2 scaled).



**Supplemental figure 2:** ROC curves showing that plasma (A) and feces (B) bile acids are able to discriminate Cir\_sAH patients from Cir\_noAH patients (average AUROC = 0.955 and 0.977 respectively).