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Relationship between gut microbiota dysbiosis and bile acid in patients with hepatitis B-induced cirrhosis

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Abstract

Background Dysbiosis of the gut microbiota is a significant factor influencing the progression of hepatitis B-related cirrhosis (HBC). Bile acid (BA) metabolism is increasingly recognized as a key participant in the liver–gut microbiota axis.

Methods A total of 46 patients with HBC and 33 healthy adults were enrolled in this study. The HBC patients were divided into a BA-normal group and a BA-high group. Fecal samples were collected from patients under the conditions of their daily diet, and the 16 S rRNA test was performed for each sample.

Results Compared with that in healthy adults, the alpha diversity of the gut microbiota in HBC patients significantly changed, with a decrease in beneficial microbiota and an increase in opportunistic pathogens. Notably, Bacilli, Enterobacteriales, Streptococcaceae, Veillonella and Lactobacillales were significantly increased in BA-high patients, whereas Clostridia and Clostridiales were significantly decreased. Akkermansiaceae abundance was reduced in the HBC group, and Lactobacillales was markedly enriched in HBC patients, with its abundance proportionally increasing with increasing BA.

Conclusion These findings provide critical insights for investigating the gut microbiota–BA crosstalk in HBC, facilitating the discovery of novel biomarkers for disease monitoring and the development of microbiota-targeted therapeutic strategies modulating BA metabolism to intervene in HBC progression.

Trial registration This study was registered in the Chinese Clinical Trial Registry (ChiCTR2400090990) on October 17, 2024 (retrospectively registered).

Keywords Cirrhosis, Hepatitis B, Bile acid, Gut microbiota

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Background

The term "gut microbiota" typically refers to the bacterial communities residing in the intestinal tract. In adults, the dominant bacterial phyla are Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria, with Bacteroidetes and Firmicutes being the most predominant [1]. The composition of this microbiota is influenced by numerous factors, including age, ethnicity, nutrition, diet, immune status, disease state, and medication use [2]. Furthermore, recent studies have shown that the utilization of probiotics, fecal microbiota transplantation (FMT), and nutrition in patient care has yielded unanticipated positive clinical outcomes [3].

Cirrhosis is a major cause of mortality worldwide. In China, hepatitis B virus (HBV) is the predominant etiological factor for liver cirrhosis [4]. Hepatitis B-related cirrhosis (HBC) is widely regarded as irreversible, and the main goal of current treatment is to slow disease progression and control complications. The gut contains a large and complex microbial community, and the gut microbiota and its metabolites maintain homeostasis through complex processes. A growing number of experiments have shown a strong association between the gut microbiota and HBC progression through 16 S rRNA sequencing of fecal samples, combined microbiomics, and metabolomic analyses [5].

Microbial dysbiosis has been implicated in impaired intestinal barrier function, exacerbated inflammatory responses, and the progression of liver pathology. Bile acid (BA) metabolism is also a key mediator of the liver–gut axis [6, 7]. Cholesterol is enzymatically converted into primary bile acid through classical (CYP7A1-mediated) and alternative (CYP27A1-mediated) pathways in the liver [8], and these primary BAs undergo further transformation into secondary BAs by the gut microbiota, including genera such as Bifidobacterium, Lactobacillus, Clostridium, Enterococcus, Ruminococcus, Xanthomonas and Eubacterium [9].

Given the bidirectional interactions between BAs and the gut microbiota and their collective influence on HBC progression, this study aimed to perform a comprehensive analysis of the gut microbiota composition and BA profiles in patients with HBC. By elucidating their interplay, this study seeks to provide new ideas for the development of dynamic noninvasive metrics and therapeutic strategies.

Methods

Participant information

This study was approved by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center (KY2024009) and included 79 participants who visited the PLA Rocket Force Characteristic Medical Center between August 1, 2023, and August 30, 2024. All participants were required to provide informed consent prior to participation. This study was performed in accordance with the Declaration of Helsinki and is registered in the Chinese Clinical Trial Registry (ChiCTR2400090990). Among them, 33 participants with good health confirmed through health examinations were included in the control group. In addition, 46 participants with HBC were in the HBC group, which was divided into two subgroups (BA less than or equal to 15 μmol/L was considered normal, and BA greater than 15 μmol/L was considered high): 24 participants in the normal BA group (BA-N) and 22 participants in the high BA group (BA-H). The inclusion criteria were as follows: meeting the diagnostic criteria of the Guidelines for the Prevention and Treatment of Chronic Hepatitis B (version 2022) and infection with the hepatitis B virus for >6 months. The exclusion criteria for all groups were as follows: diarrhea, infection, or antibiotic and probiotic treatment within 3 months; coinfection with hepatitis A, hepatitis C, hepatitis D, human immunodeficiency, or other hepatitis viruses; and the coexistence of hypertension, diabetes, obesity, significant atherosclerosis, chronic kidney disease, a history of gastrointestinal surgery, irritable bowel syndrome, inflammatory bowel disease, malignant tumors, autoimmune diseases, Alzheimer's disease, Parkinson's disease, stroke, mental illness, pregnancy, or lactation.

Basic data

Demographic data, including age, sex, weight, height, body mass index (BMI), dietary habits (carbohydrate and protein intake, vegetarian diet, and balanced diet) and medication history, were collected from the enrolled patients. Blood biochemical values included complete blood count (white blood cell counts [WBC], red blood cell counts [RBC], platelet counts [PLT] and hemoglobin levels [HGB]), liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyl transferase [GGT], total bilirubin [TBIL], direct bilirubin [DBIL], alkaline phosphatase [ALP], total serum protein [TP], serum albumin [ALB], bile acid [BA], choline esterase [CHE]), kidney function tests (blood urea nitrogen [BUN] and serum creatinine [Scr]), coagulation function tests (prothrombin time [PT], prothrombin activity [PT%], activated partial thromboplastin time [APTT], international normalized ratio [INR], D-dimer level [D-DI], fibrinogen level [FIB], and thrombin time [TT]), and hepatitis B panel, including tests for hepatitis B surface antigen, e-antigen, surface antibody, e-antibody, core antibody, hepatitis B DNA and Child–Pugh score.

Child–Pugh scoring system

Clinical indices	1	2	3
Hepatic encephalopathy (grade)	None	1–2	3–4
Ascites	None	Mild	Moderate, severe
TBIL(μmol/L)	< 34	34–51	> 51
Albumin (g/L)	> 35	28–35	< 28
Prothrombin time extension (s)	< 4	4–6	> 6

Fecal microbiota analysis

Fasting fecal samples were collected in the morning (empty stomach for 8 hours) under daily diet, placed in sterile plastic tubes and immediately stored at − 80°C. Microbial genomic DNA was subsequently extracted from the samples via a fecal DNA isolation kit. Each fecal sample was subjected to 16S rRNA sequencing. The V4-V5 region of the 16S rRNA gene was amplified via PCR via the forward primer 515FB (5'-GTGYCAGC-MGCCGCGGTAA-3') and the reverse primer 926R (5'-CCGYCAATTYMTTTRAGTTT-3') [10]. The quality and size distribution of the PCR-amplified fragments were verified using via an Agilent 2100 Bioanalyzer. The sequences were compared with the Silva database.

Table 1 Baseline characteristics of the groups

	Control ($n = 33$)	HBC($n = 46$)	*p*-value
Age (yr)	52.364 ± 1.762	52.152 ± 1.183	0.980
BMI (kg/m^2)	23.664 ± 0.601	24.477 ± 0.493	0.279
WBC (10^9/L)	5.645 ± 0.240	3.88 ± 0.218	0.000
RBC (10^{12}/L)	4.493 ± 0.086	4.247 ± 0.098	0.095
HGB (g/L)	138.394 ± 2.564	121.589 ± 4.866	0.022
PLT (10^9/L)	225.364 ± 9.697	118.413 ± 11.284	0.000
ALT (U/L)	17.588 ± 2.345	25.448 ± 2.793	0.005
AST (U/L)	17.942 ± 1.438	29.559 ± 3.306	0.000
GGT (U/L)	25.194 ± 3.211	58.302 ± 12.185	0.004
ALP (U/L)	67.033 ± 4.445	90.87 ± 5.922	0.003
TBIL (umol/L)	11.761 ± 0.751	16.723 ± 1.023	0.001
DBIL (umol/L)	4.044 ± 0.282	6.546 ± 0.435	0.000
BA (umol/L)	2.408 ± 0.270	21.576 ± 3.41	0.000
TP (g/L)	71.476 ± 0.971	69.387 ± 1.052	0.093
ALB (g/L)	43.082 ± 0.548	39.657 ± 0.872	0.002
CHE (U/L)	9018.212 ± 259.891	5838.935 ± 301.847	0.000
PT (S)	11.182 ± 0.100	13.037 ± 0.214	0.000
PT%	102.13 ± 1.714	83.252 ± 2.253	0.000
APTT	32.388 ± 0.694	32.283 ± 0.706	0.616
TT (S)	15.121 ± 0.235	18.054 ± 0.38	0.000
FIB (g/L)	2.699 ± 0.091	2.563 ± 0.149	0.037
D-DI (mg/L)	0.114 ± 0.014	0.429 ± 0.085	0.000
INR	0.987 ± 0.009	1.148 ± 0.021	0.000
BUN (mmol/L)	4.756 ± 0.151	6.429 ± 1.448	0.626
Scr (umol/L)	66.352 ± 2.350	69.107 ± 2.331	0.281

Continuous variables are expressed as the means ± standard errors. Letters indicate a significant difference ($p < 0.05$).

Statistical analysis

The data are reported as the means ± standard errors. Data analyses and sample size calculations were performed via IBM SPSS Statistics (version 27; IBM Corp., Armonk, NY, USA). Differences between independent groups for normally distributed clinical data were compared via Student's t test, and nonparametric tests were used for data that were not normally distributed. Categorical variables were compared via the chi-square ($\chi 2$) test with a confidence interval of 95%. All the statistical analyses of the 16 S rRNA sequencing data were performed in QIIME 2.0 and R. Differences between independent groups with normally distributed data were assessed via Student's t test, whereas nonparametric tests were applied to non-normally distributed variables. A p value < 0.05 was considered significant.

Results

Comparative analysis of basic data between the HBC and control groups

The clinical characteristics of the patients are presented in Table 1. Comparisons were made between 33 patients in the control group and 46 patients with HBC. Compared with patients in the control group, patients in the HBC group presented significantly lower white blood cell counts; hemoglobin, platelet, and albumin levels; and cholinesterase and prothrombin times. Conversely, thrombin times; international normalized ratios; and glutamyl transpeptidase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, indirect bilirubin, bile acid, and D-dimer levels were significantly elevated in the HBC group.

Comparative analysis of the gut microbiota between the HBC and control groups

The total length of the sequences read by both forward and reverse double-end sequencing was 500 bp, after which 24 bp molecular markers were subtracted, for a total of 476 bp. Sequences with double-end reads that were correctly spliced and between 382 bp and 442 bp in length were determined to be valid sequences. The average fragment length of all the samples was 410.68 bp.

The species accumulation curves flattened or reached a plateau, suggesting the sequencing depth basically covered all the species in the sample, and no additional OTUs could be identified by increasing the sequencing data (Fig. 1A). The Shannon–Wiener curves appeared to plateau, suggesting that the amount of sequencing data was large enough to reflect information about the vast majority of the microbial species in the sample (Fig. 1B).

In total, 17 phyla, 24 classes, 46 orders, 85 families, and 315 genera were identified. The entire sample contained 2,842 operational taxonomic units (OTUs), of which 1,892 were shared between the two groups. A total of

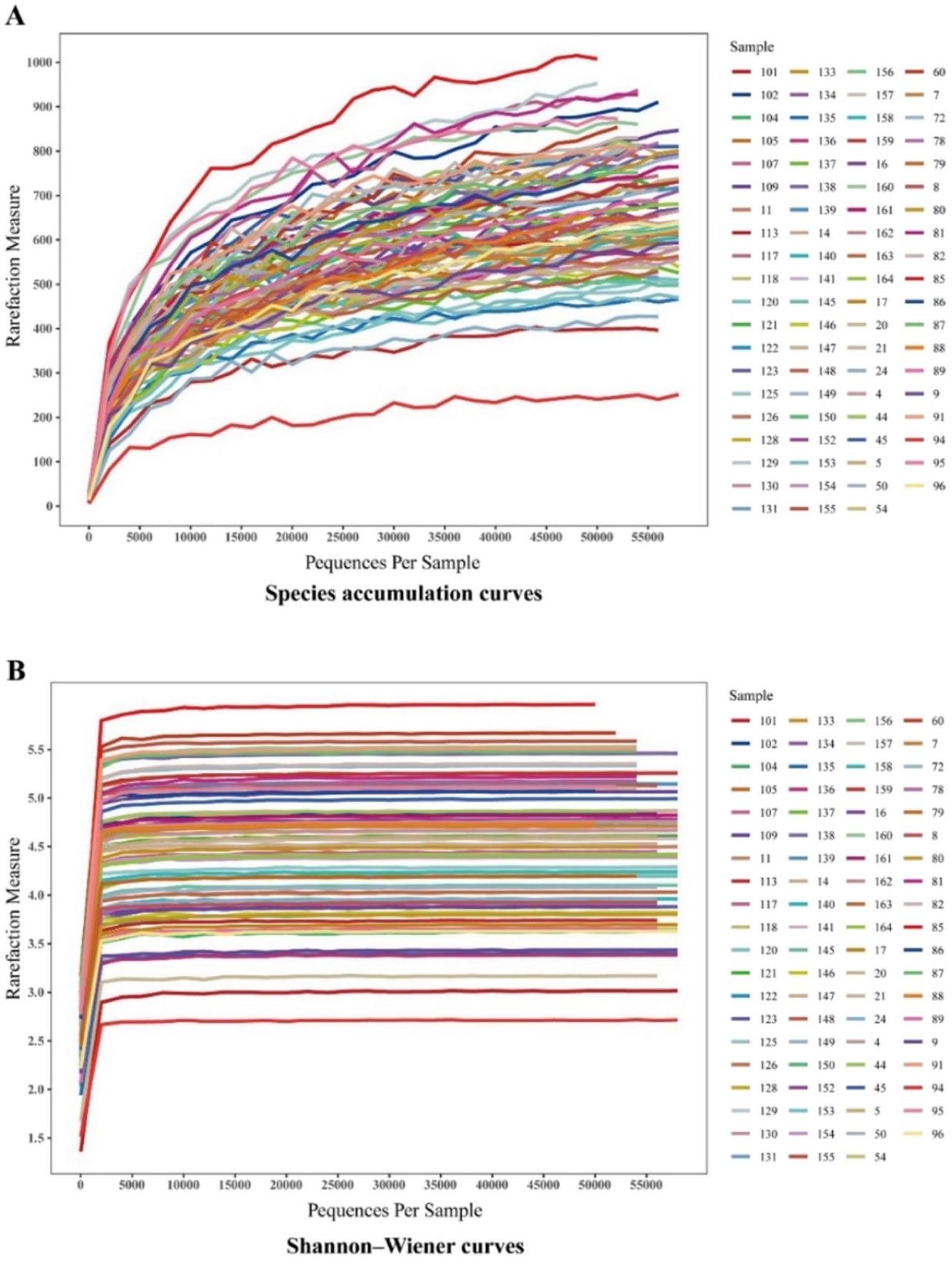

Fig. 1 Species accumulation curves (**A**) and Shannon–Wiener curves (**B**)

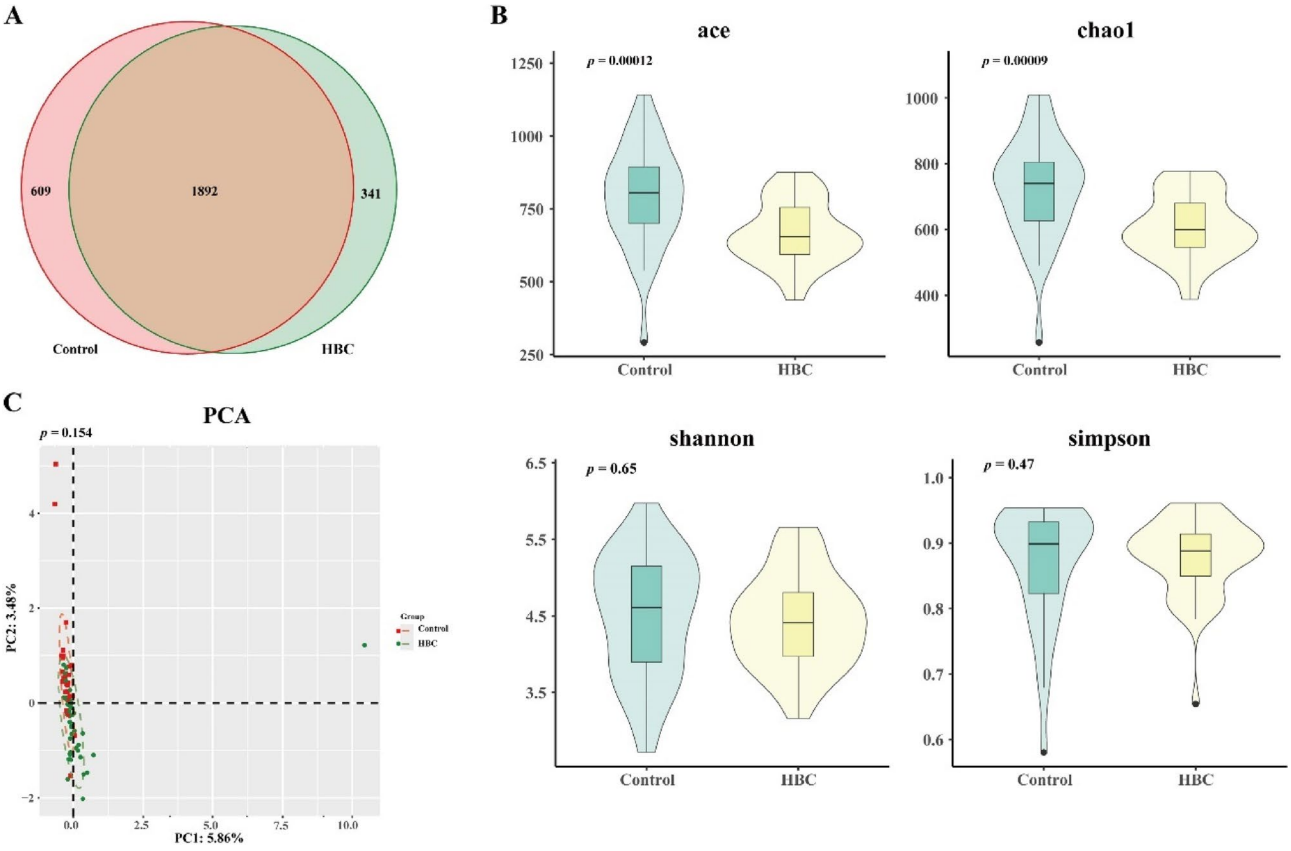

Fig. 2 Characteristics of the gut microbiota in the control and HBC groups. **A** Overall, 2842 OTUs were identified in both groups. The control group had 2501 OTUs, and the HBC group had 2233 OTUs. **B** α diversity index analysis of the ACE $p < 0.05$), Chao1 ($p < 0.05$), Shannon $p > 0.05$) and Simpson ($p > 0.05$) indices. **C**βdiversity analysis via PCA ($p > 0.05$)

Table 2 Differences in the relative abundance of the gut microbiota in the control and HBC groups

Gut microbiota	Control	HBC	*p*-value
Bacilli	1.387 ± 0.382	8.994 ± 2.369	0.009
Lactobacillales	1.382 ± 0.381	8.988 ± 2.368	0.009
Verrucomicrobiales	8.516 ± 3.191	2.573 ± 1.02	0.048
Prevotellaceae	1.619 ± 0.718	8.968 ± 2.369	0.012
Bacteroides	27.995 ± 3.331	19.708 ± 2.22	0.034
Akkermansia	8.516 ± 3.191	2.573 ± 1.02	0.048
Streptococcus	1.164 ± 0.366	7.366 ± 2.186	0.020

Continuous variables are expressed as the means ± standard errors. Letters indicate a significant difference ($p < 0.05$).

609 and 341 unique OTUs were identified in the control and HBC groups, respectively (Fig. 2A). The α diversity indices included the Ace, Chao1, Shannon and Simpson indices. The ACE and Chao1 indices are commonly used to assess species richness, and the Shannon and Simpson indices are used to measure species diversity. Analysis of α diversity revealed significantly reduced species richness in the experimental group, evidenced by decreased ACE ($p < 0.05$), and Chao1 ($p < 0.05$), indices compared with those in the control group. In contrast, the Simpson and Shannon diversity indices showed no statistically significant intergroup differences ($p > 0.05$) (Fig. 2B). Principal component analysis (PCA) of the β diversity (Fig. 2C) revealed differences in the composition of the gut microbiota between the two groups ($p > 0.05$).

Compared with the HBC group, the HBC group presented significantly more Bacilli Lactobacillus, Prevotellaceae and Streptococcus and fewer Verrucomicrobiales, Bacteroides and Akkermansia (Table 2).

We used the linear discriminant analysis effect size (LEfSe) model to identify microbiota with differential abundances between the groups (Fig. 3). In the control group, Eggerthellaceae, Aeromonadaceae, Bacillaceae, Acidaminococcaceae and Bacteroidaceae were more abundant. Conversely, in the HBC group, Bacilli, Lactobacillales, Streptococcaceae, Veillonellaceae, Micrococcales, Enterobacteriales, Enterobacteriaceae, Micrococcaceae, Gammaproteobacteria and Aeromonadales presented relatively high relative abundances.

Comparative analysis of basic data between the BA-N and BA-H groups

Bile acid (BA) metabolism is also a key mediator of the liver–gut axis [6]. We further divided the HBC group

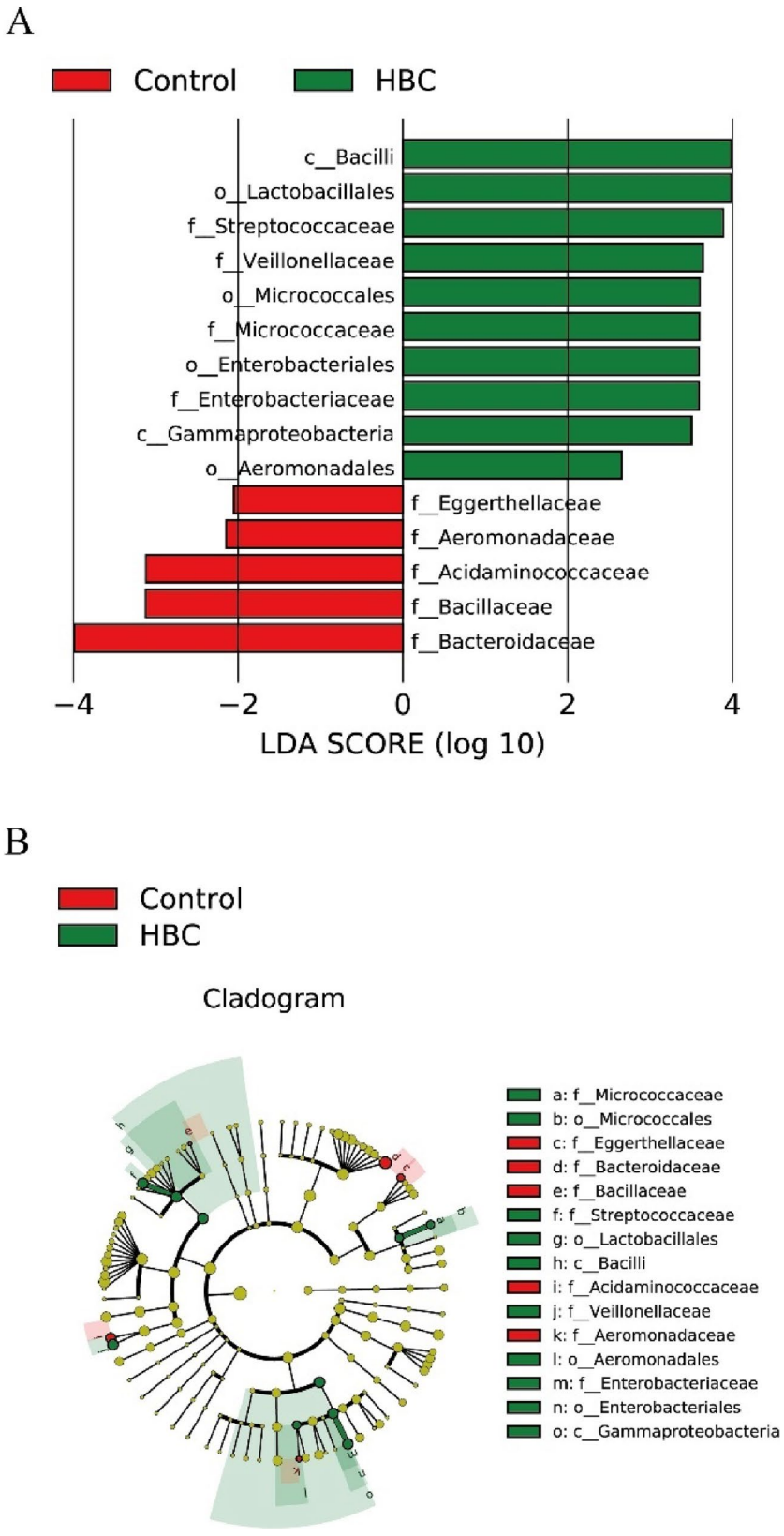

Fig. 3 Comparison of groups of microflora differences via the LEfSe online tool. **A** Histogram of the linear discriminant analysis (LDA) scores for differentially abundant genera between the control and HBC groups. **B** Taxonomic cladogram for significant differences between the control and HBC groups

into two subgroups on the basis of BA: the BA-N group ($n = 24$) and the BA-H group ($n = 22$). The results revealed that as BA levels increased, the Child–Pugh scores of patients also increased. The duration of antiviral therapy and viral load status were not significantly different between the two groups. Comparative analysis revealed that elevated BA levels were correlated with increased Child–Pugh scores. Furthermore, progressive BA elevation was associated with increasing levels of WBC, RBC, CHE, ALB and PT%, alongside decreasing levels of ALP, DBIL, BA, PT, APTT, D-DI and INR, indicating progressive disease severity in HBC with BA elevation (Table 3).

Comparative analysis of the gut microbiota between the BA-N and BA-H groups

In the HBC group, 2,233 OTUs were identified, of which 1,805 were shared between the two groups. A total of 260 and 168 unique OTUs were identified in the BA-N and BA-H groups, respectively (Fig. 4A). The α diversity indices ACE ($p > 0.05$), Chao1 ($p > 0.05$), Shannon ($p < 0.05$) and Simpson ($p < 0.05$) suggested significant differences in the microbial species diversity of the α diversity between the two groups (Fig. 4B). PCA-based β diversity analysis ($p < 0.05$) revealed significant differences in the microbial community composition (Fig. 4C).

Compared with the BA-N group, the BA-H group presented increased Bacilli, Lactobacillales, Streptococcus, Veillonella and Enterobacteriaceae and decreased Clostridiales. Akkermansiaceae had a decrease in mean relative abundance, although this reduction did not reach statistical significance ($p > 0.05$) (Table 4).

Using the LEfSe model, we identified microbiota with differential abundances between the BA-N and BA-H groups (Fig. 5). Clostridia and Clostridiales were more abundant in the BA-N group, whereas Aeromonadaceae, Enterobacteriales, Streptococcaceae, Bacilli and Lactobacillales were more abundant in the BA-H group.

Table 3 Baseline characteristics of the groups

	BA-N ($n = 24$)	BA-H ($n = 22$)	*p*-value
WBC (10^9/L)	4.356 ± 0.284	3.361 ± 0.303	0.021
RBC (10^{12}/L)	4.485 ± 0.106	3.988 ± 0.152	0.009
HGB (g/L)	130.304 ± 6.642	112.082 ± 6.720	0.061
PLT (10^9/L)	138.542 ± 16.479	96.455 ± 14.215	0.062
ALT (U/L)	24.017 ± 2.260	27.009 ± 5.350	0.598
AST (U/L)	24.217 ± 1.495	35.386 ± 6.573	0.111
GGT (U/L)	48.058 ± 14.063	69.477 ± 20.434	0.386
ALP (U/L)	79.696 ± 7.734	103.059 ± 8.491	0.048
TBIL (umol/L)	15.277 ± 1.055	18.301 ± 1.769	0.141
DBIL (umol/L)	5.238 ± 0.310	7.974 ± 0.739	0.002
BA (umol/L)	7.542 ± 0.810	36.886 ± 5.470	0.000
TP (g/L)	68.442 ± 1.007	70.418 ± 1.911	0.354
ALB (g/L)	42.017 ± 0.655	37.082 ± 1.512	0.004
CHE (U/L)	7017.25 ± 380.968	4553.5 ± 289.843	0.000
PT (S)	12.15 ± 0.159	14.005 ± 0.299	0.000
PT%	93.483 ± 2.362	72.091 ± 2.169	0.000
APTT	30.5 ± 1.043	34.227 ± 0.768	0.007
TT (S)	18.704 ± 0.638	17.345 ± 0.336	0.073
FIB (g/L)	2.762 ± 0.133	2.347 ± 0.272	0.167
D-DI (mg/L)	0.182 ± 0.022	0.699 ± 0.160	0.004
INR	1.057 ± 0.015	1.247 ± 0.028	0.000
BUN (mmol/L)	7.675 ± 2.767	5.069 ± 0.273	0.374
Scr (umol/L)	70.068 ± 3.573	68.059 ± 2.999	0.672
Child-Pugh score	5.21 ± 0.085	6.59 ± 0.320	0.000
Hepatitis B DNA (+/-)	2/22	4/18	0.322
Antivirals (>1Y)	16/8	16/6	0.655

Continuous variables are expressed as the means ± standard errors. Letters indicate a significant difference ($p < 0.05$)

Discussion

HBC is a multifactorial pathological process in which the gut microbiota increasingly emerges as a pivotal contributor to disease progression. Characteristic dysbiosis in HBC patients is characterized by significantly altered α diversity, marked by enrichment of opportunistic pathogens and depletion of beneficial microbiota. This microbial imbalance drives intestinal barrier dysfunction, which manifests as increased permeability, small intestinal bacterial overgrowth (SIBO), and bacterial translocation [2]. Translocated pathobiont-derived metabolites—notably lipopolysaccharide (LPS)—migrate via portal circulation to hepatic tissue. LPS binding to Toll-like receptors (TLRs) activates proinflammatory cytokine cascades that accelerate hepatic fibrogenesis and cirrhosis progression [11]. Short-chain fatty acids (SCFAs) produced by beneficial microbiota exert hepatoprotective effects through multiple mechanisms: suppressing the proliferation of proinflammatory taxa, attenuating chronic inflammation, and modulating T-cell differentiation to ameliorate liver pathology. Depletion of these SCFA-producing consortia consequently accelerates HBC progression [12].

Compared with those in the control group, the HBC group presented significantly greater abundances of Bacilli, Lactobacillales, Prevotellaceae and Streptococcus; conversely, the abundances of Verrucomicrobia, Bacteroides and Akkermansiaceae were significantly lower. Trending microbiota has the potential to be used as a biomarker to monitor changes in HBC.

Opportunistic pathobionts including Bacilli, Prevotellaceae and Streptococcus may exacerbate hepatic pathology through metabolite-mediated mechanisms or immune interactions [13]. Chen et al. proposed that Streptococcus abundance progressively increases with HBC disease progression, highlighting its potential as a diagnostic biomarker for HBC [14]. Verrucomicrobia, Bacteroides and Akkermansiaceae are beneficial microbiota. Zheng et al. reported a notable decrease in

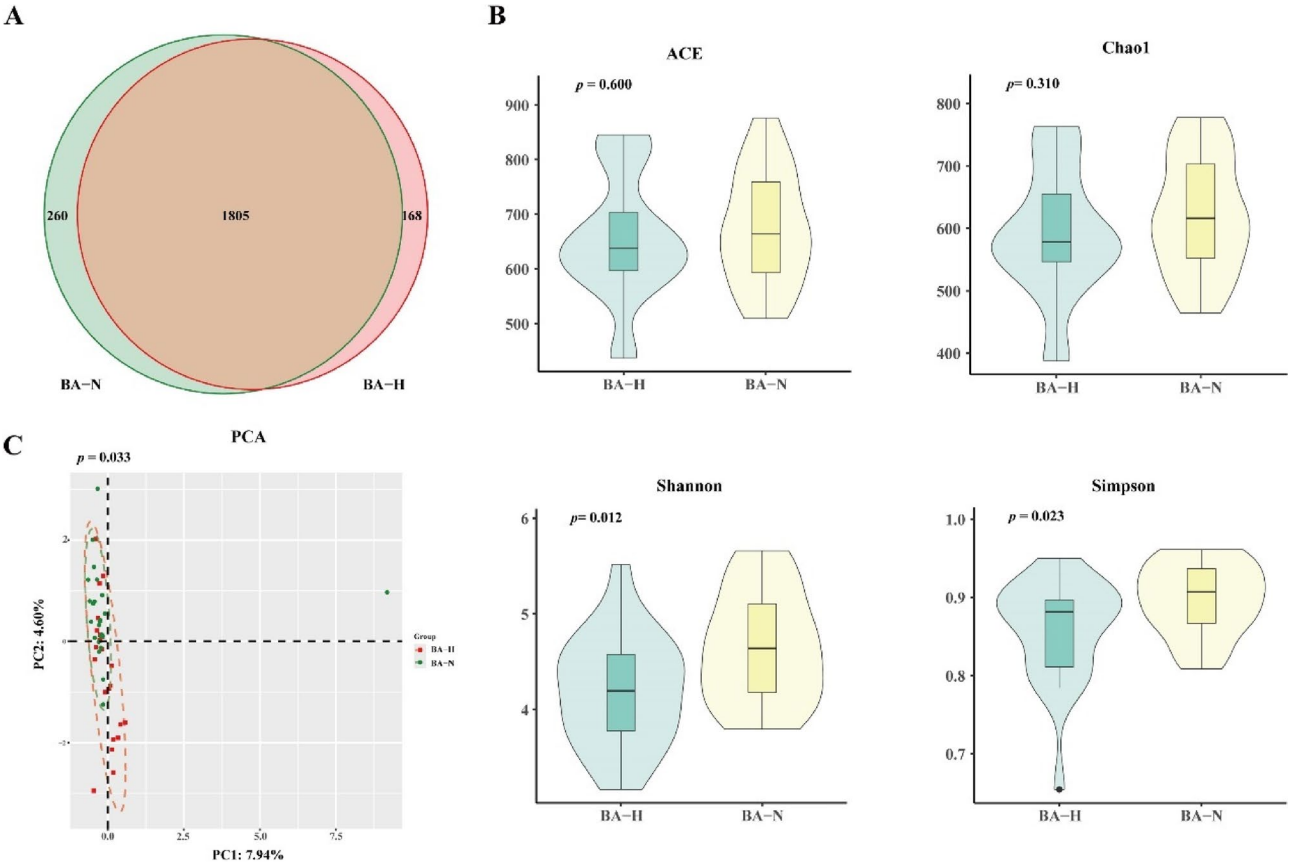

Fig. 4 Characteristics of the gut microbiota in the BA-N and BA-H groups. (**A**) There were 2,219 OTUs in both groups. A total of 260 and 168 unique OTUs were identified in the BA-N and BA-H groups. (**B**) α diversity index analysis of the ACE ($p > 0.05$), Chao1 ($p > 0.05$), Shannon ($p < 0.05$) and Simpson ($p < 0.05$). (**C**) β diversity analysis via PCA ($p < 0.05$).

Table 4 Differences in the relative abundance of the gut microbiota in the BA-N and BA-H groups

Gut microbiota	BA-*N*	BA-H	*p*-value
Bacilli	3.66 ± 1.498	14.813 ± 4.401	0.017
Lactobacillales	3.652 ± 1.494	14.809 ± 4.4	0.017
Clostridiales	32.453 ± 2.985	24.15 ± 2.723	0.047
Enterobacteriaceae	2.425 ± 0.96	8.448 ± 2.117	0.011
Veillonella	0.292 ± 0.116	1.763 ± 0.622	0.030
Streptococcus	2.972 ± 1.21	12.16 ± 4.19	0.034
Akkermansiaceae	2.767 ± 1.631	2.362 ± 1.215	0.719

Continuous variables are expressed as the means ± standard errors. Letters indicate a significant difference ($p < 0.05$).

Bacteroidaceae and Verrucomicrobia in patients with HBC, which is consistent with our findings [15]. Furthermore, Verrucomicrobia is positively correlated with diamine oxidase, a biomarker for intestinal injury, and negatively correlated with cirrhosis severity [16].

Lactobacillales are generally considered beneficial, and Kassa et al. suggested that Lactobacillales exerts beneficial effects on intestinal health by modulating host immunity and preserving intestinal barrier integrity through the prevention of endotoxin translocation [17]. Interestingly, our study revealed a significant increase in this microbiota among HBC patients, with an abundance positively correlated with elevated BA levels. Yang et al. reported a positive correlation between Lactobacillus abundance and the progression of noncancer disease stages in hepatitis B virus-infected patients. Predictive functional profiling adjusted for confounders revealed negative associations with environmental adaptation and immune system pathways, suggesting that excessive Lactobacillus accumulation may promote proinflammatory cytokine production and exert detrimental effects [18]. As many factors and the environment influence the composition of the gut microbiota, the same bacteria may play distinctive roles in different intestinal states. Therefore, these findings highlight the need for further functional studies to clarify the role of Lactobacillales in HBC progression.

The Akkermansiaceae gram-negative anaerobic mucin-degrading bacterium from the phylum Verrucomicrobia has potential as a newly discovered probiotic, showing promise for regulating lipid metabolism and suppressing liver inflammation. In our study, HBC patients presented a significantly reduced abundance of the mucin-degrading bacterium Akkermansiaceae compared with controls.

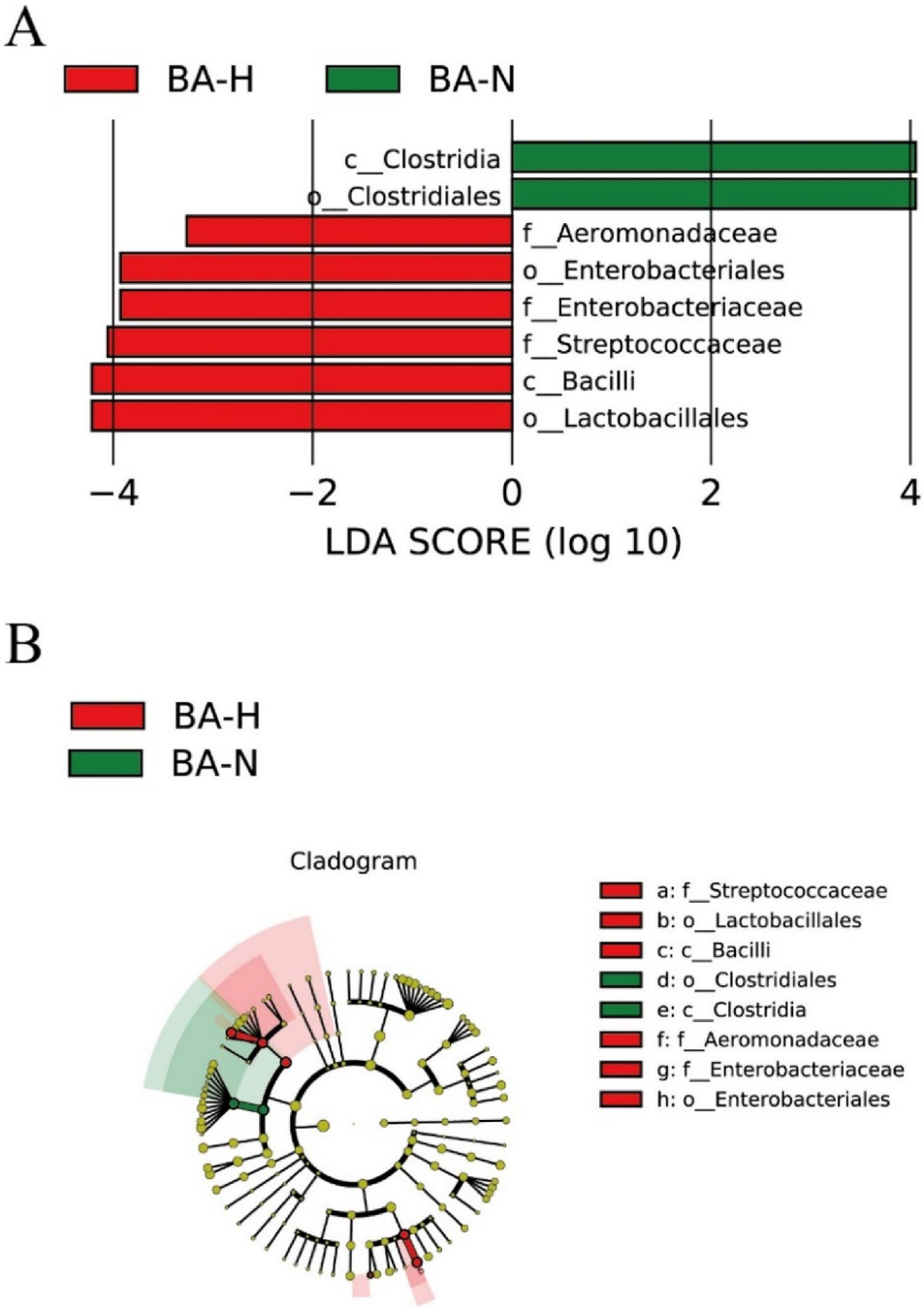

Fig. 5 Comparison of differences in the microflora via the LEfSe online tool. **A** Histogram of the linear discriminant analysis (LDA) scores for differentially abundant genera between the BA-N and BN-H groups. **B** Taxonomic cladogram for significant differences between the BA-N and BN-H groups

Raftar et al. reported that Akkermansia and its extracellular vesicles can ameliorate hepatic stellate cell activation, resulting in significant anti-inflammatory effects in liver and adipose tissues [19]. Rao et al. reported that treatment with Akkermansia for six weeks significantly alleviated nonalcoholic fatty liver disease (NAFLD) in mice, maintaining effective antiNAFLD activity even four weeks after treatment [20]. Oguri et al. reported significant depletion of Akkermansia muciniphila in CCl_4-induced cirrhotic mice. Compared with vehicle control mice, mice subjected to 4 weeks of oral supplementation with Akkermansia muciniphila, presented substantially reduced hepatic fibrosis areas and blood ammonia levels. Notably, fibrotic improvement persisted after treatment cessation, demonstrating sustained therapeutic effects [21]. In a human trial, Depommier et al. supplemented overweight/obese subjects for 3 months with placebo or pasteurized A.muciniphila (10^{10}

bacteria per day). Compared with placebo, pasteurized A.muciniphila improved insulin sensitivity, and reduced insulinemia and plasma total cholesterol. This study shows that A.muciniphila intervention was safe, effective and well-tolerated [22]. However, systematic studies concerning HBC in Akkermansiaceae are lacking. The participants may subsequently be orally administered Akkermansia for longitudinal analysis.

Experimental evidence confirms that BA exerts antimicrobial effects and suppresses microbial overgrowth, while concurrently enhancing intestinal barrier integrity. A reduction in the bowel lumen BA concentration contributes to gut dysbiosis in cirrhotic patients. Conversely, the gut microbiota modulates hepatic BA synthesis via the ileal FXR-FGF19 signaling axis. Dysbiosis leads to hepatic cholestasis and subsequent hepatocyte injury [23].

This study revealed that elevated BA levels in HBC patients were significantly correlated with increased Child−Pugh scores ($p < 0.05$), indicating an association between BA and HBC developmente. The cross-sectional nature of this study limits our ability to establish causal relationships between these variables. We conducted a stratified analysis comparing the gut microbiota profiles of HBC patients with normal versus elevated BA levels. This investigation aims to delineate microbial signatures associated with BA dysregulation and elucidate their interplay in cirrhosis progression.

Compared with the BA-N group, the BA-H group presented significant differences in both alpha diversity and beta diversity. The BA-H group presented increased Bacilli, Lactobacillales, Streptococcus, Veillonella and Enterobacteriaceae abundances and decreased Clostridiales abundances. The mean relative abundance of Akkermansiaceae decreased but did not reach statistical significance. Notably, Aeromonadaceae, Enterobacteriales, Enterobacterialceae, Bacilli, Streptococcaceae and Lactobacillales were significantly enriched among these taxa.

According to our subgroup analysis, the abundance of Akkermansia mucoptin tended to decrease with increasing BA, suggesting that the bacterium may exert a compensatory protective effect through the gut–liver axis. Lactobacillus increases with increasing BA, suggesting that Lactobacillus may be involved in disease progression through metabolite interaction networks in the HBC microenvironment. These two bacteria may be related to the effect of BA on the HBC process.

Bile salt hydrolase (BSH) is an important uncoupling enzyme for the conversion of primary BA to secondary BA, and compared with conjugated BA, unconjugated BA have stronger antimicrobial activity, Clostridiales expresses BSH activity, which weakened the FXR-FGF19 signaling pathway when Clostridiales decreases, resulting in increased liver BA synthesis Cholestasis and hepatocyte damage occur, and the antibacterial ability of BA in the intestine decreases, resulting in a vicious cycle. The increase in Veillonella abundance in our study was the same as that reported by Wei. Wei et al. reported that Veillonella can hydrolyze conjugated bile salts, impairing micelle formation and contributing to cirrhosis [24]. This results in the onset of cholestasis and exacerbates cirrhosis progression. In alcoholic cirrhosis, the increase in primary BA is associated with Enterobacteriaceae, and its bacterial abundance is positively correlated with the mean percent abundance of primary BA [25]. Currently, engineered probiotics have been shown to modulate the composition of intestinal bile acids by increasing the production of secondary bile acids, and fecal microbiota transplantation in combination with FXR agonists has a synergistic effect on reducing portal endotoxin levels [26]. In summary, monitoring the trending microbiota and further study of its influence on the disease change mechanism can provide new ideas forinterventions in the treatment of HBC.

Conclusions

Compared with that in the control group, the intestinal microbiota of the HBC group was characterized mainly by a decrease in beneficial microbiota and an increase in opportunistic pathogens. Clostridia, which participate in BA metabolism, are negatively correlated with the development of HBC, whereas Bacilli, Enterobacteriales, Streptococcaceae, Veillonella and Lactobacillales are positively correlated with HBC advancement. Notably, Akkermansiaceae abundance was reduced in the HBC group, and its abundance tended to decrease as BA levels increased, Lactobacillales was markedly enriched in HBC patients, with its abundance increasing proportionally with increasing BA. These findings provide critical insights for investigating the gut microbiota–BA crosstalk in HBC, facilitating the discovery of novel biomarkers for disease monitoring and the development of microbiota-targeted therapeutic strategies modulating BA metabolism to intervene in HBC progression.

This study has several limitations. First, the cross-sectional design of this study limited the ability to establish causal relationships between bile acid levels and gut microbiota composition, and the lack of longitudinal follow-up prevented the assessment of how these relationships evolved with disease progression or therapeutic interventions; We must also admit that we have neglected the discussion of potential confounding factors other than diet, age, medications, geography, seasons, etc. Second, the presence of esophagogastric varices in most of the HBC group was another limitation of this study; Third, this study preliminarily discussed the relationship between BA and the intestinal microbiota in the context

of HBC, but owing to the relatively small sample size, no bile acid composition analysis and metabolomics analysis was performed, so the exact mechanism of change and accurate correlation analysis could not be carried out.

Therefore, we will expand the sample size in the future, through multicenter cooperation, in-depth analysis of BA and signature intestinal microbiota-related genes, and functional metabolic pathways, and further verification of the conclusions through animal experiments and clinical trials.To explore the mechanisms by which HBC induces intestinal microflora imbalance more deeply and discover novel microbial biomarkers, the relationships between the intestinal microflora and BAs in the context of HBC should be further investigated, new biomarkers for evaluating and monitoring the progression of liver cirrhosis should be identified, and new insights for the treatment of HBC through BA metabolism modulation by the intestinal microflora should be provided.

Abbreviations

HBC	Hepatitis B-related cirrhosis
BA	Bile acid
HBV	Hepatitis B virus
BMI	Body mass index
WBC	White blood cell count
RBC	Red blood cell count
PLT	Platelet count
HGB	Hemoglobin levels
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
GGT	Gamma–glutamyl transferase
TBIL	Total bilirubin
DBIL	Direct bilirubin
ALP	Alkaline phosphatase
TP	Total protein
ALB	Albumin
CHE	Cholinesterase
Scr	Serum creatinine
BUN	Blood urea nitrogen
PT	Prothrombin time
PTA	Prothrombin activity
PT%	Prothrombin activity
APTT	Activated partial thromboplastin time
INR	International standardized ratio
D-DI	D-dimer
FIB	Fibrinogen quantification
TT	Thrombin time
OUT	Operational taxonomic units
PCA	Principal component analysis
LEfSe	Linear discriminant analysis effect size
SCFA	Short-chain fatty acid
FXR-FGF19	Farnesoid X receptor-fibroblast growth factor 19
LPS	Lipopolysaccharide
NAFLD	Nonalcoholic fatty liver disease

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Author contributions
Changzheng Li contributed to conceptualization, writing– review & editing and supervision; Ningbo Hao contributed to methodology, writing– review & editing and supervision; Yannan Li contributed to methodology, formal analysis and writing– original draft; Jing Xie, Tian Tian, Dan Zhang, Xiaolin Wang and Wenjun Deng contributed to the investigation.

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Data availability
Sequence data that support the findings of this study have been deposited in the Genome Sequence Archive (GSA) with the primary accession code CRA026122（https://ngdc.cncb.ac.cn/gsa/）.

Declarations

Ethics approval and consent to participate
This study has been performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center (KY2024009). All participants were required to provide informed consent prior to participation.

Consent for publication
All patients signed consent forms for data collection and publication.

Competing interests
The authors declare no competing interests.

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References

1. Laivacuma S, Oblate O, Derovs A. Gut microbiota and the gut-liver axis in liver disease: from chronic viral hepatitis to cirrhosis, hepatocellular carcinoma, and microbiome-based therapies. Microorganisms. 2025;13(5):1053.
2. Li YG, Yu ZJ, Li A, Ren ZG. Gut microbiota alteration and modulation in hepatitis B virus-related fibrosis and complications: molecular mechanisms and therapeutic inventions. World J Gastroenterol. 2022;28(28):3555–72.
3. Abenavoli L, Scarpellini E, Paravati MR, Scarlata GGM, Boccuto L, Tilocca B, Roncada P, Luzza F. Gut microbiota and critically ill patients: immunity and its modulation via probiotics and immunonutrition. Nutrients 2023, 15(16):3569.
4. Dusheiko G, Agarwal K, Maini MK. New approaches to chronic hepatitis B. N Engl J Med. 2023;388(1):55–69.
5. Shu W, Shanjian C, Jinpiao L, Qishui O. Gut microbiota dysbiosis in patients with hepatitis B virus-related cirrhosis. Ann Hepatol. 2022;27(2):100676.
6. Wang X, Chen L, Wang H, Cai W, Xie Q. Modulation of bile acid profile by gut microbiota in chronic hepatitis B. J Cell Mol Med. 2020;24(4):2573–81.
7. Wang J, Xu H, Liu Z, Cao Y, Chen S, Hou R, Zhou Y, Wang Y. Bile acid-microbiota crosstalk in hepatitis B virus infection. J Gastroenterol Hepatol. 2024;39(8):1509–16.
8. Collins SL, Stine JG, Bisanz JE, Okafor CD, Patterson AD. Bile acids and the gut microbiota: metabolic interactions and impacts on disease. Nat Rev Microbiol. 2023;21(4):236–47.
9. Song Y, Lau HC, Zhang X, Yu J. Bile acids, gut microbiota, and therapeutic insights in hepatocellular carcinoma. Cancer Biol Med. 2023;21(2):144–62.
10. Ni J, Huang R, Zhou H, Xu X, Li Y, Cao P, Zhong K, Ge M, Chen X, Hou B, et al. Analysis of the relationship between the degree of dysbiosis in gut microbiota and prognosis at different stages of primary hepatocellular carcinoma. Front Microbiol. 2019;10:1458.
11. Zeng Y, Chen S, Fu Y, Wu W, Chen T, Chen J, Yang B, Ou Q. Gut microbiota dysbiosis in patients with hepatitis B virus-induced chronic liver disease covering chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. J Viral Hepat. 2020;27(2):143–55.
12. Ouyang J, Zaongo SD, Zhang X, Qi M, Hu A, Wu H, Chen Y. Microbiota-Meditated immunity abnormalities facilitate hepatitis B virus Co-Infection in people living with HIV: A review. Front Immunol. 2021;12:755890.
13. Padilha MDM, Melo FTV, Laurentino RV, da Silva A, Feitosa RNM. Dysregulation in the microbiota by HBV and HCV infection induces an altered cytokine profile in the pathobiome of infection. Braz J Infect Dis. 2025;29(1):104468.
14. Chen Z, Xie Y, Zhou F, Zhang B, Wu J, Yang L, Xu S, Stedtfeld R, Chen Q, Liu J, et al. Featured gut microbiomes associated with the progression of chronic hepatitis B disease. Front Microbiol. 2020;11:383.

15. Zheng R, Wang G, Pang Z, Ran N, Gu Y, Guan X, Yuan Y, Zuo X, Pan H, Zheng J, et al. Liver cirrhosis contributes to the disorder of gut microbiota in patients with hepatocellular carcinoma. Cancer Med. 2020;9(12):4232–50.
16. Efremova I, Maslennikov R, Medvedev O, Kudryavtseva A, Avdeeva A, Krasnov G, Romanikhin F, Diatroptov M, Fedorova M, Poluektova E et al. Gut microbiota and biomarkers of intestinal barrier damage in cirrhosis. Microorganisms 2024, 12(3):463.
17. Kassa Y, Million Y, Gedefie A, Moges F. Alteration of gut microbiota and its impact on immune response in patients with chronic HBV infection: a review. Infect Drug Resist. 2021;14:2571–8.
18. Yang XA, Lv F, Wang R, Chang Y, Zhao Y, Cui X, Li H, Yang S, Li S, Zhao X, et al. Potential role of intestinal microflora in disease progression among patients with different stages of hepatitis B. Gut Pathog. 2020;12:50.
19. Raftar SKA, Ashrafian F, Abdollahiyan S, Yadegar A, Moradi HR, Masoumi M, Vaziri F, Moshiri A, Siadat SD, Zali MR. The anti-inflammatory effects of Akkermansia muciniphila and its derivates in HFD/CCL4-induced murine model of liver injury. Sci Rep. 2022;12(1):2453.
20. Negi CK, Babica P, Bajard L, Bienertova-Vasku J, Tarantino G. Insights into the molecular targets and emerging pharmacotherapeutic interventions for nonalcoholic fatty liver disease. Metabolism. 2022;126:154925.
21. Oguri N, Miyoshi J, Nishinarita Y, Wada H, Nemoto N, Hibi N, Kawamura N, Miyoshi S, Lee STM, Matsuura M, et al. Akkermansia muciniphila in the small intestine improves liver fibrosis in a murine liver cirrhosis model. NPJ Biofilms Microbiomes. 2024;10(1):81.
22. Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, Falony G, Raes J, Maiter D, Delzenne NM, et al. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med. 2019;25(7):1096–103.
23. Zhu F, Zheng S, Zhao M, Shi F, Zheng L, Wang H. The regulatory role of bile acid microbiota in the progression of liver cirrhosis. Front Pharmacol. 2023;14:1214685.
24. Wei X, Yan X, Zou D, Yang Z, Wang X, Liu W, Wang S, Li X, Han J, Huang L, et al. Abnormal fecal microbiota community and functions in patients with hepatitis B liver cirrhosis as revealed by a metagenomic approach. BMC Gastroenterol. 2013;13:175.
25. Kakiyama G, Hylemon PB, Zhou H, Pandak WM, Heuman DM, Kang DJ, Takei H, Nittono H, Ridlon JM, Fuchs M, et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. Am J Physiol Gastrointest Liver Physiol. 2014;306(11):G929–937.
26. Yan W, Zhang K, Guo J, Xu L. Bile acid-mediated gut-liver axis crosstalk: the role of nuclear receptor signaling in dynamic regulation of inflammatory networks. Front Immunol. 2025;16:1595486.

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