



Original Article

Serum metabolomic profiling of patients with liver cirrhosis at different stages

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Article Info

Abstract



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An accurate and non-invasive diagnosis of the clinical stage is critical for effectively managing liver cirrhosis. This study aimed to identify serum metabolite biomarkers and clinical features that may reliably predict high-risk cirrhosis. This cross-sectional study recruited 94 cirrhotic patients (70 for identification cohort, 24 for validation cohort) from Minhang Hospital Affiliated with Fudan University between 2018 and 2021, who were analyzed by targeted quantitative metabolomics technique. Baseline clinical characteristics were collected, and different stage cirrhosis classification was performed according to the presence or absence of decompensated events. Potential metabolite biomarkers were screened, and a model for predicting the decompensation stage was created. Finally, the incidence of decompensated outcomes was analyzed. A total of 560 metabolites were detected in the identification cohort. Indole-3-propionic acid (IPA) was the most significantly decreased metabolic biomarker in the decompensated group ($P < 0.01$, $|\log_2FC| > 2$), having the strongest correlation with hyaluronic acid ($r = -0.50$, $P < 0.01$). It also performed well for differentiating decompensated cirrhosis with an area under the curve (AUC) of 0.79 (0.75 at internal validation). Another diagnostic model consisting of indole-3-propionic acid, hemoglobin, and albumin showed better predictive performance with an AUC of 0.97 (0.91 at internal validation). Also, 31 (44.29%) patients developed decompensated events at a median follow-up of 22.76 ± 15.24 months. The cumulative incidence of decompensated events based on IPA subgroups (IPA < 39.67 ng/ml and ≥ 39.67 ng/ml) showed a significant difference ($P < 0.01$). "Indole-3-propionic acid" and a diagnostic model of hemoglobin and albumin can non-invasively identify cirrhotic populations at risk for decompensation, aiding in future management of liver cirrhosis.

Keywords: Metabolomics; Liver cirrhosis; Decompensation; Hepatitis B virus; Indole-3-propionic acid

1. Introduction

Liver cirrhosis is a late-stage liver disease with a high global prevalence associated with high morbidity and mortality [1]. Each year, cirrhosis causes approximately 1 million deaths worldwide, while another 1 million deaths are caused by viral hepatitis and hepatocellular carcinoma [2]. Hepatitis B virus (HBV) is the major cause of liver cirrhosis in China. Liver cirrhosis can be classified into compensated cirrhosis (with a low risk of mortality) and decompensated cirrhosis (with a high risk of mortality). The transition from compensated to decompensated cirrhosis is characterized by complications such as gastroesophageal varices bleeding, ascites, and overt hepatic encephalopathy [3]. Thus, there is an urgent need to identify high-risk cirrhotic patients and find biomarkers that could predict cirrhosis progression, as this may help stratify cirrhosis patients and implement early prevention and intervention treatments.

The progression of cirrhosis and its complications have been associated with clinically significant portal hypertension (CSPH). Therefore, an important step in early prevention and the ability to predict the risk of decompensation. The ALBI-FIB4 Score [4], SHUNT test [5], and liver stiffness with platelet counts [6] can detect decompensation

cirrhosis by quantifying liver function. However, Baveno VII workshop recommends hepatic venous pressure gradient (HVPG) measurements as a gold standard to determine the presence of CSPH in virus-related cirrhotic patients [3].

Histopathological examination is the "gold standard" for diagnosing and evaluating cirrhosis [7]; nonetheless, this type of examination requires a collection of tissue (liver biopsy), which is an invasive procedure. On the other hand, other non-invasive methods such as imaging methods, elastography techniques, and serum biomarkers based on laboratory tests have shown moderate diagnostic accuracy in assessing portal hypertension of HBV-related cirrhosis [8-12]. Furthermore, studies have shown the potential diagnostic value of metabolic biomarkers in differentiating advanced liver fibrosis or hepatocellular carcinoma from chronic hepatitis B virus (CHB) or HBV-related cirrhosis [13-15]. In addition, we have recently identified and validated metabolomic signatures for distinguishing complications of esophagogastric variceal bleeding (EVV) from cirrhotic patients [16]. However, predicting the progression of liver cirrhosis remains challenging, while the application of metabolomics in this field is still unsatisfactory.

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In this study, we reported the results of a targeted quantitative metabolomics investigation from 94 cirrhotic patients at different stages in two independent cohorts. A total of 560 serum metabolites were detected by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). The present study aimed to find the link between serum metabolic biomarkers and liver functions as well as clinical decompensated events in patients with HBV-related cirrhosis. We further analyzed the metabolic profile of HBV-related cirrhotic patients, taking into account the possible impacts of etiology. In addition, the predictive value of these biomarkers in assessing cirrhosis was compared to other diagnostic and predictive tools, including the MELD score or Child-Turcotte-Pugh (CTP). We followed up with all patients and assessed the capacity of the potential biomarkers to predict the recurrence of decompensated events. Our study was initiated to find reliable biomarker signatures that could

differentiate cirrhotic patients at high risk or at an early stage of cirrhosis.

2. Materials and Methods

2.1. Study Population and Data Collection

Two datasets were enrolled in this study. The study workflow is presented in Figure 1. Cohort 1 consisted of 70 inpatients with cirrhosis, 35 with decompensated and 35 with compensated cirrhosis, who were recruited from Minhang Hospital Affiliated with Fudan University between June 2018 and December 2021. Data obtained from the identification cohort were used to screen serum metabolite biomarkers and establish predictive models (Table 1). Subgroup analysis was conducted based on the etiology of cirrhosis, which meets the diagnostic criteria for chronic hepatitis B in the EASL 2017 Clinical Practice Guidelines for Hepatitis B Virus Infection Management. HBV-related cirrhosis was diagnosed according to the “guidelines

Table 1. Baseline clinical characteristics of patients in the identification study.

Group	compensated cirrhosis		decompensated cirrhosis	
	Total	HBV-related	Total	HBV-related
Number	35	22	35	21
Age (years)	54.4±14.3	50.9±2.6	57.1±12.3	54.4±2.8
Male sex, n(%)	25(71.4)	17(81.0)	26(74.3)	17(77.3)
BMI (kg/m ²)	25.0±3.9	24.8±0.9	23.5±3.1	23.8±0.7
cirrhosis history(years)	3.5±6.2	4.6±1.6	7.3±10.3*	5.3±1.4
Etiology				
Virus,n (%)	22(62.9)	22(100)	21(60)	21(100)
Alcohol,n (%)	2(5.7)	/	5(14.3)	/
Schistosomiasis,n (%)	4(11.4)	/	4(11.4)	/
Other, n (%)	7(20.0)	/	5(14.3)	/
Diabetes history, n (%)	9(25.7)	5(22.7)	11(31.4)	6(28.6)
Hypertension history, n (%)	10(28.6)	3(13.6)	12(34.3)	4(19.0)
Hyperlipidemia history, n (%)	4(11.4)	3(13.6)	3(8.6)	0(0)
GEV, n (%)	16(45.7)	14(63.6)	30(85.7)**	19(90.5)
PVT, n (%)	3(8.6)	3(13.6)	11(31.4)*	5(23.8)
Child-Pugh Class A, n (%)	32(91.4)	20(90.9)	10(28.6)**	7(33.3)**
MELD score	7.9±4.6	9.1±1.0	10.7±4.7**	12.1±1.0*
Hemoglobin (g/L)	131.6±22.7	129.2±27.0	97.7±22.1**	96.6±18.8**
ALT (U/L)	86.7±141.6	79.5±32.7	30.2±19.7	30.6±3.9
AST (U/L)	60.9±76.3	57.7±18.0	47.3±39.3	51.2±10.0
TBIL (μmol/L)	23.3±24.5	24.3±6.1	35.5±26.4**	34.4±5.5
DBIL (μmol/L)	12.7±19.0	13.2±4.8	18.7±15.8**	19.1±3.4*
Albumin (g/L)	40.8±4.7	40.8±5.1	30.9±5.8**	31.3±6.0**
PT (s)	13.4±2.3	13.9±0.5	15.4±2.3**	15.7±0.6*
INR	1.1±0.1	1.2±0.0	1.3±0.2**	1.4±0.1**
Hyaluronic acid (ng/ml)	232.2±253.3	248.9±79.8	501.6±324.6**	458.5±84.8*
Laminin (ng/ml)	123.9±62.0	117.1±11.8	192.6±111.7*	185.6±30.2*
Fasting plasma glucose (mmol/L)	5.6±1.8	5.8±0.5	6.1±3.7	6.3±1.0
Total cholesterol (mmol/L)	3.4±1.1	3.1±0.3	3.4±1.3	3.1±0.2
Triglycerides (mmol/L)	1.2±0.7	1.2±0.2	1.0±0.3	1.0±0.1
Uric acid (μmol/L)	302.6±82.9	284.9±13.4	300.8±107.9	301.6±23.4
HBV-DNA (×10 ³)	8574.6±4188.4	11690.8±6273.7	1014.6±431.9	901.5±652.0

Data is presented as mean±SD or in raw numbers (%).

Abbreviation: ALT: Alanine transaminase; GEV: gastroesophageal varices; PVT: portal vein thrombosis;

*P<0.05, **P<0.01

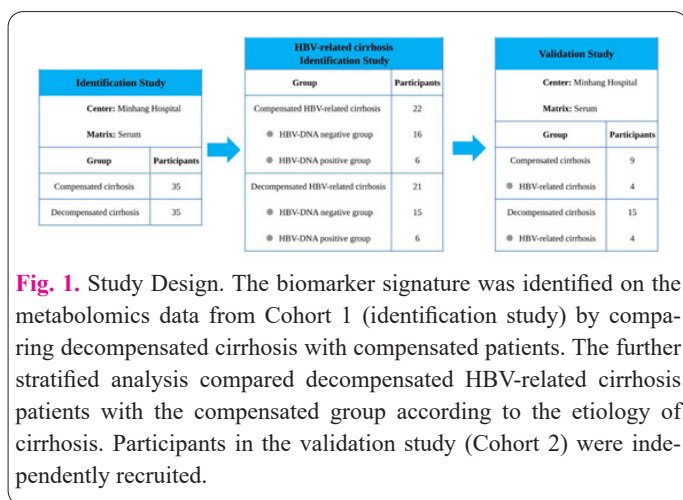


Fig. 1. Study Design. The biomarker signature was identified on the metabolomics data from Cohort 1 (identification study) by comparing decompensated cirrhosis with compensated patients. The further stratified analysis compared decompensated HBV-related cirrhosis patients with the compensated group according to the etiology of cirrhosis. Participants in the validation study (Cohort 2) were independently recruited.

of prevention and treatment for chronic hepatitis B (2019 version)” (17). To analyze the etiological impact, we further performed a stratified study according to HBV-DNA levels in compensated HBV-related cirrhosis. All patients were followed up, and their liver decompensated events, complications, and cirrhosis progression after every 3-6 months were recorded. The primary outcome was the cirrhosis stage and time of the first or recurring decompensated events; the secondary outcomes were the mortality on follow-up and complications of cirrhosis.

Cohort 2, included 24 inpatients with cirrhosis recruited at the same hospital between March 2018 and February 2019, for whom the same targeted quantitative metabolomics analysis was completed in 2020. Data obtained from the validation cohort were used to further validate the performance of metabolic biomarkers, and the established predictive model was screened from the identification cohort.

Inclusion criteria were the following: (1) patients diagnosed with liver cirrhosis according to the international guidelines by liver biopsy or clinical data and imaging findings (2); (2) aged between 18-80 years; (3) consented to give serum samples within 24 hours of admission and provide adequate clinical information. The definition of decompensated events is the occurrence of gastroesophageal variceal bleeding, ascites, and obvious hepatic encephalopathy (3).

Exclusion criteria were: tumor history, infection on admission, pregnancy, probiotics or antibiotics used within 2 weeks, endoscopy or surgery performed within 2 months, combined with severe respiratory and circulatory failure, poorly controlled diabetes, insufficient clinical data, or decline to participate in this study.

Baseline clinical features, laboratory tests, and treatment history were collected. There were no significant differences in age, sex, and body mass index (BMI) distributions between decompensated and compensated cirrhotic patients. Figure 1 and Table 1 show an overview of the cohorts. Routine liver tests, abdominal ultrasonography, and recording of decompensation events were performed semiannually until the patients lost or died or until the end of the study period (June 2022). Time to decompensation was calculated as the number of months from the study date until the first or recurring decompensation event. All samples were stored in a freezer at -80°C until analysis. The diagnosis and the sample collection were performed using the same protocols.

The study was approved by the Ethics Committee of

Minhang Hospital Affiliated to Fudan University (2018-009-001X). All participants signed informed consent.

2.2. Metabolite profiling

A UPLC-MS/MS system (Acquity UPLC-Xevo TQ-S, Waters Corp., USA) was used to quantify all targeted metabolites. This project was carried out by Metabo Profile (Shanghai, China). In addition, all the criteria of targeted metabolites were obtained from Sigma-Aldrich (St. Louis, USA), Steraloids Inc. (Newport, USA) and TRC Chemicals (Toronto, Canada).

All samples were analyzed within 48 hours after extraction and derivatization. We quantified the concentrations of 560 metabolites from 29 different classes using a targeted metabolomic protocol with fully quantitative Metabolic Chip Q600 technology. Furthermore, serum samples were assessed for quality using a sample control procedure (ISO9001, QAIC/CN/170149) [18].

2.3. Statistical Analysis

Raw data files generated by UPLC-MS/MS were processed for each metabolite using TMBQ software (v1.0, Metabo-Profile, China). For the metabolite variables, three types of statistical analysis were extensively performed: (1) multivariate statistical analysis: orthogonal partial least square discriminant analysis (OPLS-DA) was applied to distinguish differences in serum metabolic profiles and calculate variable importance for the projection (VIP) scores for assessing the importance of each variable; (2) univariate statistical analysis: including ANOVA test, Wilcoxon-Mann-Whitney test, Student's T-Test, correlation analysis, etc.; (3) defining potential metabolic biomarker: Random Forest (RF) was performed to identify candidate biomarkers from differential metabolites, and further verify their importance.

Clinical data statistical analyses were performed using SPSS 21 and expressed as continuous variables with normal distribution presented as mean \pm standard deviation (SD) or median value (IQR). Category variables were presented as a percent. Univariate analysis was performed to identify significantly different variables. Standard stepwise logistic regression was performed with significantly differential metabolic and clinical variables to build a predictive model. We combined 2-3 screened metabolic and clinical characteristic variables based on the sample size to avoid overfitting. The performance of the metabolic biomarker and the predictive model was assessed by area under the curve receiver operating characteristic (AUROC) curves, Youden Index, sensitivity, and specificity. The selected metabolic biomarker and model underwent internal validation. A P-value was considered to be statistically significant. We did not use imputation for missing variables.

3. Results

3.1. Baseline Characteristics of Study Cohort

In cohort 1, 70 serum samples were collected from cirrhotic inpatients, including 35 decompensated and 35 compensated cases of cirrhosis, and targeted metabolomics analysis was used to screen for differential biomarkers. We analyzed 43 HBV-related cirrhotic patients, consisting of 22 decompensated and 21 compensated cases, according to the etiology of liver cirrhosis. A further stratified analysis was then performed according to HBV-DNA expres-

sion levels in compensated HBV-related cirrhosis to assess the impact of etiology on the serum metabolites in cirrhosis progression. There were no significant differences in age, gender, and BMI among the groups (all $P>0.05$).

3.2. Metabolomic analysis in samples from cirrhotic patients

The metabolomics data were acquired through UPLC-MS/MS with strict quality control, after which 560 known metabolites from serum samples were used for statistical analysis. The distribution over ontology class is shown in Figure 2A. The relative abundance of each metabolite in a different group is shown in Figure 2B.

3.3. Discrimination of decompensated and compensated cirrhosis by multivariate statistics

We further investigated whether the metabolic profiles of decompensated and compensated cirrhosis could be distinguished by multivariate statistical methods. An unsupervised principal component analysis (PCA) showed the most overlapping and partial separating in the principal component (PC) 1 and 2, which explained 35.6% and 9.4% variation of the dataset, respectively. Thus, a supervised modeling method of OPLS-DA was used to further visualize the differences between decompensated and compensated cirrhosis (Figure 3A).

To analyze within-subject metabolic changes introduced by etiology of cirrhosis, OPLS-DA analyses were performed among cirrhotic patients with hepatitis B as the sole cause (decompensated HBV-related cirrhosis vs. compensated group, Figure 3B), as well as a stratified study according to HBV-DNA level in compensated HBV-related cirrhosis (positive HBV-DNA vs. negative control,

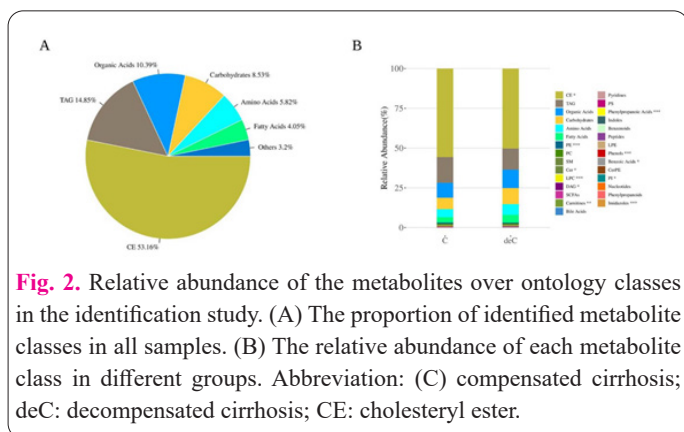


Fig. 2. Relative abundance of the metabolites over ontology classes in the identification study. (A) The proportion of identified metabolite classes in all samples. (B) The relative abundance of each metabolite class in different groups. Abbreviation: (C) compensated cirrhosis; deC: decompensated cirrhosis; CE: cholesteryl ester.

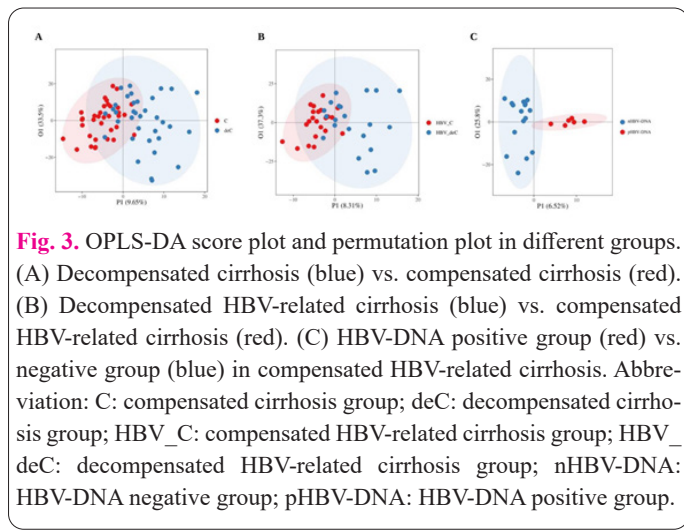


Fig. 3. OPLS-DA score plot and permutation plot in different groups. (A) Decompensated cirrhosis (blue) vs. compensated cirrhosis (red). (B) Decompensated HBV-related cirrhosis (blue) vs. compensated HBV-related cirrhosis (red). (C) HBV-DNA positive group (red) vs. negative group (blue) in compensated HBV-related cirrhosis. Abbreviation: C: compensated cirrhosis group; deC: decompensated cirrhosis group; HBV_C: compensated HBV-related cirrhosis group; HBV_deC: decompensated HBV-related cirrhosis group; nHBV-DNA: HBV-DNA negative group; pHBV-DNA: HBV-DNA positive group.

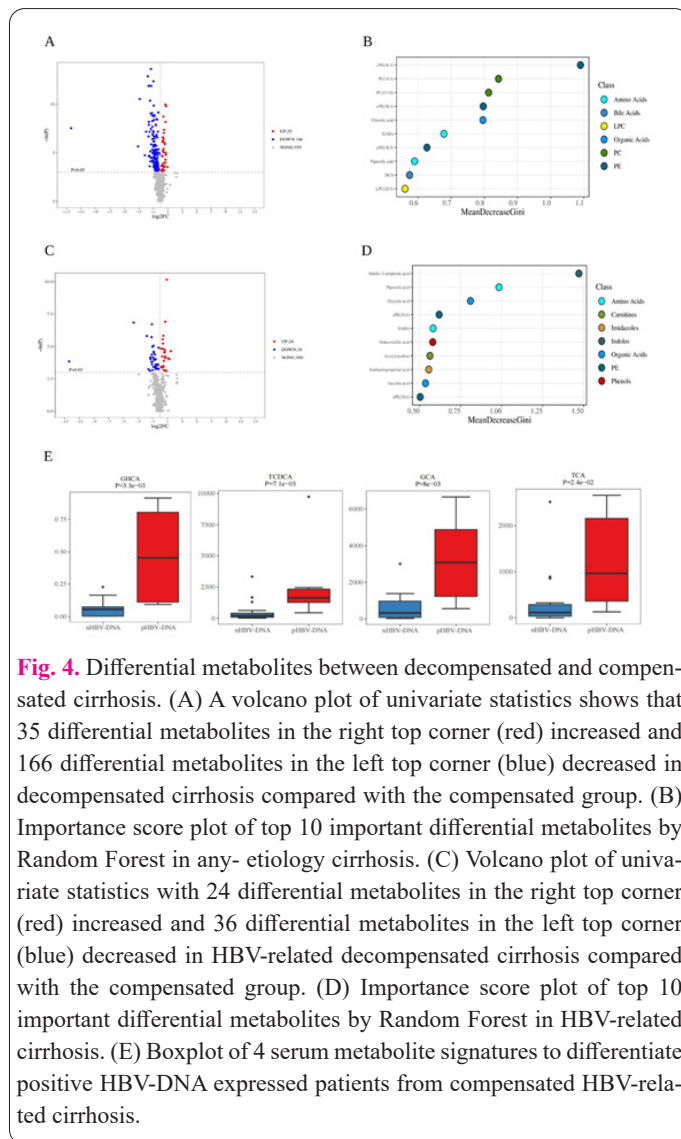


Fig. 4. Differential metabolites between decompensated and compensated cirrhosis. (A) A volcano plot of univariate statistics shows that 35 differential metabolites in the right top corner (red) increased and 166 differential metabolites in the left top corner (blue) decreased in decompensated cirrhosis compared with the compensated group. (B) Importance score plot of top 10 important differential metabolites by Random Forest in any- etiology cirrhosis. (C) Volcano plot of univariate statistics with 24 differential metabolites in the right top corner (red) increased and 36 differential metabolites in the left top corner (blue) decreased in HBV-related decompensated cirrhosis compared with the compensated group. (D) Importance score plot of top 10 important differential metabolites by Random Forest in HBV-related cirrhosis. (E) Boxplot of 4 serum metabolite signatures to differentiate positive HBV-DNA expressed patients from compensated HBV-related cirrhosis.

Figure 3C). The score plots for each statistical analysis showing a significant alteration in the metabolic profiles are presented in Figure 3. These results indicated that the progression from compensated cirrhosis to the decompensated stage was a major contributor to the alteration of the sera metabolome.

3.4. Defining potential metabolic biomarkers for decompensated cirrhosis

From the 560 analyzed serum metabolites, OPLS-DA indicated separability between decompensated and compensated cirrhosis ($R^2Y=0.53$, $Q^2Y=0.37$), and 221 differential metabolites with $VIP>1.0$ were identified as significant variables for liver cirrhosis staging (Figure 4A). Subsequently, 133 of these metabolites were statistically significant under the threshold value of $P<0.05$ and $|\log_2FC|>0$ univariate analysis. We carried out RF and selected the top 5 candidate biomarkers according to the importance scores, including ePE (36:4), PC (34:4), PC(42:10), ePE (38:4), and glycolic acid (Figure 4B).

To further improve the classification performances, we analyzed cirrhotic patients with the etiology of chronic HBV infection under the same biomarker selective criteria (Figure 4C). The top 5 candidate biomarkers sorted by RF importance scores in HBV-related cirrhosis were indole-3-propionic acid, pipecolic acid, glycolic acid, ePE (36:4), and GABA (Figure 4D).

In the current study, indole-3-propionic acid was the most significantly decreased metabolite ($P < 0.01$, $|\log_2FC| > 2$) in cirrhosis, especially in HBV-related cirrhosis. On the other hand, the differential performance of glycolic acid and ePE (36:4) did not seem to be affected by the etiology of cirrhosis. Then we stratified patients according to HBV-DNA level in compensated HBV-related cirrhosis and found that patients with positive HBV-DNA expressed had significantly higher GHCA, TCDCA, GCA, and TCA levels compared to negative controls ($P < 0.05$, $|\log_2FC| > 2$, Figure 4E). A list of the differential metabolic biomarkers is shown in Table 2.

3.5. Correlation analysis of metabolic biomarkers and clinical characteristics

Spearman's correlation analysis was performed to understand better the association of metabolic biomarkers with cirrhosis progression (Figure 5A). As expected, indole-3-propionic acid had the strongest correlation with hyaluronic acid, a recognized marker of liver fibrosis ($r = -0.50$, $P < 0.01$). Several bile acids (GHCA, TCDCA, and TCA) were positively associated with the levels of laminin, aspartate transaminase (AST), total bilirubin (TBIL), and direct bilirubin (DBIL) ($0.50 < r < 0.70$, $P < 0.01$). Glycolic acid, PC (42:10), and PC (34:4) were significantly associated with hemoglobin (Hb), albumin (ALB), prothrombin time (PT), and international normalized ratio (INR) ($0.50 < r < 0.70$, $P < 0.01$). However, ePE (36:4), ePE (38:4), pipecolic acid, and GCA showed weak associations ($0.20 < r < 0.50$, $P < 0.05$) but not GABA ($P > 0.05$). Levels of

these serum metabolites were not significantly associated with BMI and age ($P > 0.05$).

The AUROC was 0.79 for IPA in the detection of decompensated cirrhosis with 39.67 ng/ml as the optimum cut-off (Youden index 0.63), which was superior to glycolic acid (AUC=0.77, $P < 0.01$, Youden index 0.49) and

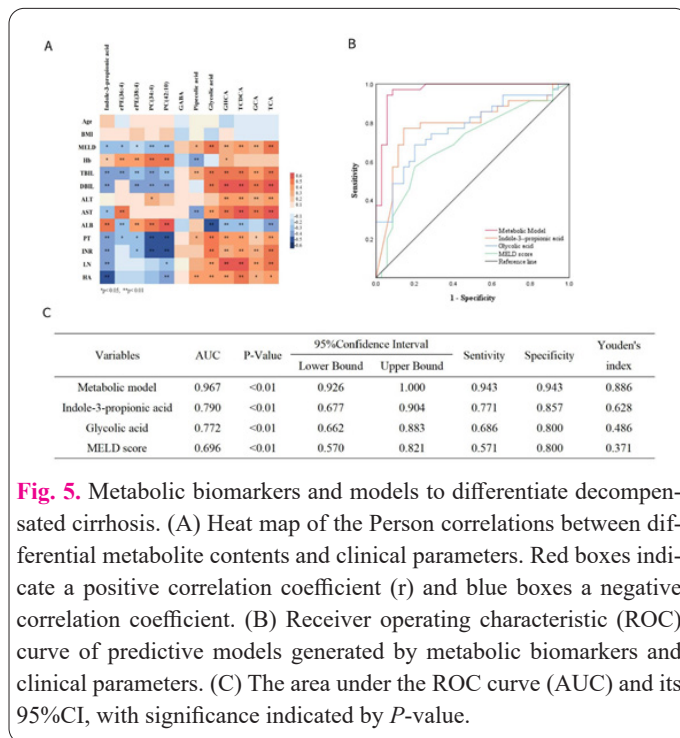


Fig. 5. Metabolic biomarkers and models to differentiate decompensated cirrhosis. (A) Heat map of the Person correlations between differential metabolite contents and clinical parameters. Red boxes indicate a positive correlation coefficient (r) and blue boxes a negative correlation coefficient. (B) Receiver operating characteristic (ROC) curve of predictive models generated by metabolic biomarkers and clinical parameters. (C) The area under the ROC curve (AUC) and its 95%CI, with significance indicated by P-value.

Table 2. List of metabolites selected for the biomarker signature.

Metabolite name	Ontology class	HMDB	KEGG	decompensated cirrhosis vs. compensated cirrhosis				pHBV-DNA vs. nHBV-DNA compensated HBV-related cirrhosis					
				log2FC	p-value	FDR	VIP	log2FC	P value	FDR	VIP		
Indole-3-propionic acid	Indoles	HMDB0002302	NA	-2.88	<0.01	<0.01	0.84	-3.60	<0.01	0.17	0.91		
ePE (36:4)	PE	NA	C04475	-1.56	<0.01	<0.01	1.33	-1.10	<0.01	0.17	1.30		
ePE (38:4)	PE	NA	C04475	-1.45	<0.01	<0.01	1.25	-1.80	<0.01	0.26	1.34		
PC (34:4)	PC	HMDB0008007	C00157	-1.34	<0.01	<0.01	1.81	-0.89	<0.01	0.30	1.26		
PC(42:10)	PC	HMDB0008675	C00157	-1.29	<0.01	<0.01	1.96	-0.86	<0.05	0.30	1.42		
GABA	Amino Acids	HMDB0000112	C00334	0.48	<0.01	<0.01	1.47	0.59	<0.05	0.30	1.29		
Pipecolic acid	Amino Acids	HMDB0000716	C00408	0.67	<0.01	<0.05	1.83	0.91	<0.01	0.02	2.21		
Glycolic acid	Organic Acids	HMDB0000115	C00160	0.76	<0.01	<0.01	2.26	0.71	<0.01	0.17	2.38		
GHCA	Bile Acids	HMDB0240607	NA								3.03	<0.01	2.77
TCDCA	Bile Acids	HMDB0000951	C05465								2.94	<0.01	1.88
GCA	Bile Acids	HMDB0000138	C01921								3.24	<0.01	2.68
TCA	Bile Acids	HMDB0000036	C05122								3.07	<0.05	2.02

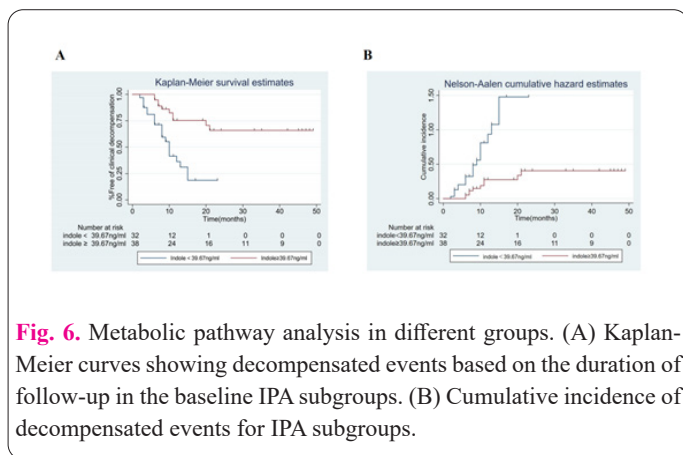


Fig. 6. Metabolic pathway analysis in different groups. (A) Kaplan-Meier curves showing decompensated events based on the duration of follow-up in the baseline IPA subgroups. (B) Cumulative incidence of decompensated events for IPA subgroups.

MELD score (AUC=0.70, $P<0.01$, Youden index 0.37) (Figure 5B). Binary logistic regression analysis revealed that hemoglobin, albumin, and IPA < 39.67 ng/ml were independent risk factors for decompensated cirrhosis detection ($P<0.01$). The cumulative incidence of decompensated outcomes was significantly higher in patients with IPA < 39.67 ng/ml ($P<0.01$).

The clinical and metabolic model for identifying the cirrhotic population with decompensated events was built as follows: $\text{logit}(P=\text{decompensated cirrhosis})=0.273*\text{ALB}-3.006*\text{IPA score}+0.052*\text{Hb}-14.314$. IPA score equal to 0 or 1 based on serum IPA ≥ 39.67 ng/ml or < 39.67 ng/ml, where ($P=\text{decompensated cirrhosis}$) is the predicted probability of decompensated cirrhosis, which showed the best performance with AUC 0.97 (95%CI: 0.926-1.000, $P<0.01$), high sensitivity of and specificity of 94.3% (Figure 5C). Taken together, metabolic changes occurred with the progression of cirrhosis, which allowed us to differentiate cirrhosis from decompensated events and further explore the metabolic changes associated with cirrhosis at different stages.

3.6. Validation of the predictive model in an independent cirrhotic cohort

We used another independent cirrhosis cohort (Cohort 2, $n=24$), which completed a quantitative UPLC-MS/MS test in 2020 in order to validate the diagnostic potential of metabolic biomarkers. Similar to the results of the identification study, the level of indole-3-propionic acid significantly decreased in decompensated cirrhosis compared to compensated group ($P<0.05$). The clinical and metabolic model also showed a higher diagnostic performance than indole-3-propionic acid alone (AUC 0.91 vs. 0.75, $P<0.01$). Also, both indole-3-propionic acid and the metabolic model showed specificity for distinguishing decompensated cirrhosis.

3.7. Predictors of decompensated events

Overall, 31 (44.29%) patients developed decompensated events at a median follow-up of 22.76 ± 15.24 months. At a follow-up of 6 months, 17.14% of patients had decompensated events. More events were seen in patient with low IPA expression (IPA < 39.67 ng/ml) than in those with IPA expression (IPA ≥ 39.67 ng/ml) (12.9% vs. 4.3%, $P<0.01$), Figure 6A. The cumulative incidence of decompensated events based on indole-3-propionic acid subgroups (IPA < 39.67 ng/ml and ≥ 39.67 ng/ml) also showed a significant difference ($P<0.01$, Figure 6B).

4. Discussion

Cirrhosis is the 11th most common cause of death, accounting for 3.5% of all deaths worldwide [19]. Stage transition from compensated to the decompensated stage is a critical factor affecting mortality in cirrhotic patients. Therefore, early detection of decompensated cirrhosis is critical for evaluating prognosis and designing treatment.

Recently, a new Early Prediction of Decompensation (EPOD) score, calculated from bilirubin, platelet count, and albumin, was suggested for predicting the three-year risk of decompensation in compensated cirrhotic patients [20]. Other studies focused on serum biomarkers, such as HVP, albumin, vitamin D, anemia, and interleukin-6 [21-23]. Unfortunately, there is still an urgent need for a simple, routinely performed clinical biomarker that could predict liver cirrhosis progression. Also, a more expansive view reflecting multiple systematic changes in the development of cirrhosis is needed.

The metabolomics approach has been reported to provide a reliable diagnostic possibility for the fibrosis stages in chronic hepatitis B viral-infected patients compared to the healthy group [14]. In order to further explain the pathophysiology of the cirrhosis progression, we performed a cross-sectional analysis using clinical data and metabolic analyses from two independent cohorts of hospitalized cirrhotic patients. Our study demonstrated that specific metabolites in serum obtained at admission could be used to predict the development of cirrhosis. The serum metabolite indole-3-propionic acid, identified and validated by univariate statistical analysis, showed the most significant decrease among the etiology cirrhosis groups and HBV-related cirrhosis subgroups.

Indole-3-propionic acid (IPA) is a tryptophan metabolite. According to previous studies, *Clostridium sporogenes* metabolizes tryptophan into indole and subsequently IPA [24]. IPA can improve insulin sensitivity and blood; it can correct intestinal microbial disorders, maintain the intestinal barrier, suppress the intestinal immune response, and inhibit liver lipid synthesis [25, 26]. Our study showed IPA had the strongest correlation with hyaluronic acid ($r=-0.50$, $P<0.01$), which was considered a non-invasive biomarker for assessing liver fibrosis and monitoring disease progression [27]. In addition, hyaluronic acid (HA) was reported to correlate with cirrhosis severity [28]. In the identification study, the AUC of IPA for predicting decompensated cirrhosis was 79%, with a sensitivity of 77.1% and satisfied specificity of 85.7%, regardless of etiology. Furthermore, IPA has been reported to improve liver fibrosis by directly inhibiting LPS-induced activation of hepatic NF- κ B signaling [24]. In the gut, IPA acts as an anti-inflammatory agent [29]. Thus, targeting the intestinal tryptophan metabolism by regulating endogenous gut microbiota may be a useful strategy to prevent and delay the progression of liver cirrhosis. However, whether IPA participates in cirrhosis progression remains unclear, similar to underlying molecular mechanisms. Further studies should address these findings and the detailed mechanisms.

Another specific metabolite detected in this study was glycolic acid, which is consistent with Yoo *et al.* [30]. It is an independent factor associated with liver cirrhosis development in the identification cohort. Another cross-sectional study also identified glycolic acid with high accuracy for discriminating decompensated HCV-related cirrhosis (CTP ≥ 7) [31]. We found that glycolic acid is associated

with liver functions, such as TBIL, DBIL, ALB, PT, and INR ($P < 0.05$, $0.50 < r < 0.70$). Also, increased levels of glycolic acid may reflect cirrhosis status.

Defensive mechanisms against oxidative stress in cirrhosis reduce with a significant decrease in ascorbic acid and an increase in malondialdehyde (MDA) levels, which is an index of lipid peroxidation [32]. In addition, glycolic acid is one of the oxidated metabolites of dehydroascorbic acid, oxidized by ascorbic acid and free radicals [33]. Finally, glycolic acid is metabolized to oxalic acid, which is excreted in urine via the kidney [30]. Given that glycolic acid may reflect the changes in the oxidative stress status at a different stage of cirrhosis, IPA is also reported to induce anti-oxidative effects *in vitro* and *in vivo* [26]. Yet, more studies are needed to evaluate the mechanism between IPA, glycolic acid, and the progression of cirrhosis.

We constructed a diagnostic model, which included IPA, hemoglobin, and albumin to better discriminate decompensated cirrhosis from cirrhotic patients with AUC 0.97 (95%CI 0.926-1.000, $P < 0.01$), high sensitivity, and specificity of 94.3%. These results highlight the potential value of IPA and the diagnostic model in differentiating decompensated cirrhosis. Therefore, screening high-risk cirrhotic patients with decompensated events using these predictive metabolic biomarkers is recommended and can reduce the costs associated with unnecessary inspections, including imaging, endoscopy, and HVPG determination.

Bile acids are metabolized by gut bacteria-derived enzymes and have a profound effect on each other [34]. The conjugated bile acids are believed to indicate liver dysfunction in cirrhosis or chronic hepatitis [35]. In this study, the GHCA, TCDCA, GCA, and TCA levels in this positive subgroup were significantly higher than those in the negative controls ($P < 0.05$, $|\log_2FC| > 2$). BA compositions were reported to significantly differ among liver disease patients with different etiologies [36]. However, we found that several bile acids of GHCA, TCDCA, and TCA were positively associated with the levels of laminin, AST, TBIL, and DBIL ($0.5 < r < 0.7$, $P < 0.01$), which is consistent with other studies that reported a significant increase [37]. Our results highlighted their potential as biomarkers for clinical stratification of HBV-related cirrhosis, assessing the risk and monitoring the progression. Considering the small sample size, the results on high HBV-DNA-expressed cirrhotic patients need to be further validated and examined.

The major strengths of this study are the collection of serum samples within 24 hours of admission by UPLC-MS/MS metabolomic technology that quantified the concentrations of 560 metabolites from 29 different classes, thus narrowing down the significant metabolites and clinical variables. This metabolomic study offers a practical strategy for screening decompensated cirrhosis. The predictive nature of these metabolic biomarkers could potentially help doctors screen high-risk cirrhotic patients, especially HBV-related cirrhosis. In addition, our results could be used as an indicator for further examinations of cirrhotic patients, ultimately reducing the cost of unnecessary inspections, promoting early preventive treatment, and closely monitoring high-risk patients. Furthermore, participants in cohort 2 (validation set) were independent of cohort 1 (identification set), and this new set of cirrhotic patients confirmed the robustness of our metabolic biomarker and model combined with clinical features.

This study has some limitations: (i) the etiology of cirrhosis was a confounding factor in our model, but key findings were not altered in the HBV-related cirrhosis subgroup. A small sample size of patients with cirrhosis with other etiologies did not allow us to perform further analysis; neither did the cirrhosis subgroup with high HBV-DNA expression. (ii) All the participants were Chinese from a single center. Future large-scale multicenter validation studies should include cirrhosis with other etiologies and other race/ethnicity participants. (iii) Improving cirrhosis outcomes is the most important task in a clinic. We are still collecting prognostic information to further validate the reproducibility of current findings and the predictive value of the model. We plan to report the outcome results in the future. (iv) The cost of targeted quantitative metabolome analysis is high. However, if future validation studies further validate the robustness of the metabolic biomarkers, specific tests may be conducted only for IPA to decrease the cost.

5. Conclusions

In summary, we found that compensated cirrhotic patients, especially with HBV-related cirrhosis, who progress to decompensated cirrhosis, indeed have dysmetabolism. Indole-3-propionic acid, a metabolic biomarker, as well as the clinical and metabolic diagnostic model consisting of hemoglobin, and albumin, were defined and validated as an effective tool for the discrimination of decompensated cirrhosis by a single-center cross-sectional study. Further validation and potential translation of these metabolite changes could help to guide the management of cirrhotic patients by assessing the risk and monitoring the progression. For high-risk cirrhotic patients, therapeutic portal vein decompression strategies should be initiated as early as possible to improve long-term prognosis and reduce medical costs. Our study pointed to a correlation between metabolomics and the advancement of liver cirrhosis, yet we have not been able to establish a direct functional link. Further study is needed to investigate the underlying mechanisms in the future.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interests

The authors declare that they have no competing interests.

Ethics approval

The study was approved by the Ethics Committee of Minhang Hospital Affiliated to Fudan University (2022-028-01K). All participants signed informed consent.

Consent for publication

Not applicable.

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None.

Author contributions

Study concept and design: Ying Chen, Qingqing Fang; acquisition of data: Xiaojuan Li, Yu Li, Wei Chen; analysis and interpretation of data: Ying Chen, Qingqing Fang, Xinxin Xu; drafting of the manuscript: Xiaojuan Li, Xin-

xin Xu; critical revision of the manuscript for important intellectual content: Ying Chen, Qingqing Fang.

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