# Original article

## A serum metabonomic study on the difference between alcohol- and HBV-induced liver cirrhosis by ultraperformance liquid chromatography coupled to mass spectrometry plus quadrupole time-of-flight mass spectrometry

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***Keywords:*** *metabonomics; liquid chromatography; liver cirrhosis; multivariate analysis*

**Background** Liver cirrhosis is the fatal consequence of chronic hepatitis, making early diagnosis of liver cirrhosis critical. Liver biopsy is still the standard diagnostic method for liver cirrhosis, although its use in a broad population with alcoholism or hepatitis B virus (HBV) infection remains difficult. In this study, we used a metabonomic approach to detect potential biomarkers for early diagnosis of liver cirrhosis.

**Methods** Serum specimens were collected prospectively from normal control subjects ($n$=22) and patients with alcoholic cirrhosis ($n$=18) or HBV-induced cirrhosis ($n$=19). The serum metabonome was analyzed using ultraperformance liquid chromatography (LC)/time-of-flight mass spectrometry (MS) integrated with chemometrics. The acquired LC-MS data were normalized and processed using principal component analysis (PCA) and partial least squares discrimination analysis (PLS-DA).

**Results** Significant differences in the metabonomics among the three groups were observed. Lysophosphatidyl cholines (LPCs) (LPC C16:0, LPC C18:0, LPC C18:2, LPC C18:3, LPC C20:3, LPC C20:5) were decreased in the serum of patients with hepatic cirrhosis, whereas bile acids (glycocholic acid, glycochenodeoxycholic acid), hypoxanthine, and stearamide were increased in the serum of patients with hepatic cirrhosis. These metabolites are considered "common" biomarkers for hepatic cirrhosis. Oleamide and myristamide were increased in the serum of patients with alcoholic cirrhosis but decreased in those with HBV-induced cirrhosis. These could be specific biomarkers for differential diagnosis between alcohol- and HBV-induced hepatic cirrhosis.

**Conclusions** There are significant metabonomic differences between alcohol- and HBV-induced liver cirrhosis. Metabonomics is a top-down systems biology tool for conducting research on clinical problems.

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Cirrhosis is the end stage of chronic damage to the liver. It is characterized by fibrosis resulting in the distortion and destruction of normal liver architecture. Functional liver tissue is destroyed and replaced by regenerating nodules that do not fully restore lost liver function. Cirrhosis may be due to various causes, with alcohol consumption and hepatitis B virus (HBV) being the main causes.[1,2] Alcoholism is the leading cause of cirrhosis in many industrialized countries, with 10%–15% of people with alcohol abuse developing cirrhosis.[3] Chronic infection with HBV accounts for 30% of hepatic cirrhosis globally.[4] In China, about 120 million people are carriers of HBV (almost a third of the people infected with HBV worldwide), with 30 million people in the country chronically infected. During a five-year period, 10%–20% of patients with chronic hepatitis develop cirrhosis.[5] Alcohol consumption in China is still low compared with that in industrialized countries, but with economic development, there has been evidence of a striking increase in alcohol consumption. The prevalence of alcoholic cirrhosis in the general population is likely to increase with the rise in alcohol consumption.[6] Cirrhosis precedes most cases of hepatocellular carcinoma (HCC), with 70%–90% of HCC developing from a background of chronic liver cirrhosis or inflammation.[7] These data clearly indicate the critical importance of early diagnosis of hepatic cirrhosis. Liver biopsy remains the standard diagnostic method for hepatic cirrhosis, but its use in a broad population with alcoholism or HBV infection remains difficult. There are also no efficient serum biomarkers for cirrhosis detection in HBV infection or

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**Table 1.** Demographic information and liver function of the study population

| Variables | Control group (*n*=22) | Alcoholic cirrhosis (*n*=18) | HBV-induced cirrhosis (*n*=19) | **P* values | †*P* values |
|---|---|---|---|---|---|
| Sex (male/female) | 17/5 | 16/2 | 15/4 | 0.610 | 0.660 |
| Age (years) | 51.23±14.25 | 56.67±10.44 | 51.53±10.21 | 0.299 | 0.140 |
| HBsAg (negative/positive) | Negative | Negative | Positive | – | – |
| ALB (g/L) (35–55 g/L) | >35 | 31.95±4.21 | 30.75±5.22 | – | 0.296 |
| ALT (U/L) (3–50 U/L) | <50 | 112.50±120.39 | 188.63±245.84 | – | 0.063 |
| AST (U/L) (3–40 U/L) | <40 | 65.50±47.24 | 66.50±17.34 | – | 0.945 |
| TB (μmol/L) (1–22 μmol/L) | <22 | 126.11±126.96 | 95.42±146.11 | – | 0.173 |
| TBA (μmol/L) (1–12 μmol/L) | <12 | 77.28±55.25 | 70.35±67.56 | – | 0.542 |
| ALP (U/L) (30–115 U/L) | <115 | 117.83±58.57 | 125.34±80.91 | – | 0.444 |
| GGT (U/L) (0–54 U/L) | <54 | 201.56±244.39 | 260.25±264.25 | – | 0.538 |
| PT (seconds) (10.5–14.0 seconds) | <14 | 15.48±3.55 | 19.88±8.08 | – | 0.075 |
| Child-Pugh score | – | 7.98±1.95 | 8.06±1.92 | – | 0.863 |
| MELD score | – | 9.44±9.13 | 14.66±4.41 | – | 0.038 |

ALB: albumin; ALT: alanine aminotransferase; AST: aspartate transaminase; TB: total bilirubin; TBA: total biliary acid; ALP: alkaline phosphatase; GGT: gamma-glutamyl transpeptidase; PT: prothrombin time. MELD: model for end-stage liver disease. The value is represented as a form of mean±SD. **P* value of ANOVA. †*P* value of *t* test between disease groups.

alcoholism.

Metabonomics is concerned with the study of low molecular weight (MW) compounds (typically <1000 Da) in biofluids and tissue extracts to provide systemic views of biological processes.[8,9] It is being applied increasingly to specific biomarker discovery for clinical diagnosis and drug discovery.[10-12] Metabolites are the end products of cellular adjustment processes, and their levels can be regarded as the ultimate response of biological systems to genetic or environmental change.[13] Common tools used for metabonomic studies include nuclear magnetic resonance (NMR) spectroscopy and gas or high-performance liquid chromatography coupled to mass spectrometry (GC/MS and LC/MS, respectively).[14-16] Ultraperformance liquid chromatography coupled to mass spectrometry (UPLC/MS) is the tool with the highest resolution and this sensitive technique is considered a powerful tool in metabonomics because of its ability to obtain multiparametric metabolite profiles from biofluids rapidly and effectively.[17,18]

In this paper, gradient reversed-phase UPLC-positive electrospray ionization (ESI)-MS in a positive mode was used to examine the serum of patients with alcoholic cirrhosis or HBV-induced cirrhosis. By comparing metabolomic data analyzed through principal component analysis (PCA) and partial least squares discrimination analysis (PLS-DA) among patients with alcoholic cirrhosis and HBV-induced cirrhosis and healthy persons, we sought to identify positive serum biomarkers for alcoholic cirrhosis and HBV-induced cirrhosis.

## METHODS

### Study population and sample collection

Alcoholic cirrhosis (18 individuals) and HBV-induced liver cirrhosis (19 individuals) patients hospitalized at the First Affiliated Hospital of Zhejiang University School of Medicine (Hangzhou, China) during the period of 2008 to 2009 were enrolled in this study. Patients with HBV-induced cirrhosis were seropositive for hepatitis B surface antigen (HBsAg). Patients with alcoholic cirrhosis were HBsAg negative and had ethanol intake of 40 g/d for more than five years, and had ceased alcohol intake when obvious symptoms were observed. The diagnosis of liver cirrhosis was confirmed by liver biopsy. Patients with hepatitis delta virus, hepatitis C virus, or HIV coinfection were excluded. Patients with hepatocellular carcinoma or diabetes were also excluded. Blood samples from 22 healthy individuals who came to the First Affiliated Hospital of Zhejiang University School of Medicine for physical check-ups were taken as the controls. The healthy controls did not drink alcohol and were HBV negative. Their liver functions were normal and showed no evidence of disease. Table 1 summarizes the characteristics of the samples. All persons enrolled in this study gave their informed consent and were aware of the procedures undertaken, the use of serum samples, and any data obtained. The protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine. Blood samples were collected in tubes before breakfast upon admission and were stored at room temperature for 1–2 hours. After centrifugation at 3000 ×*g* for 10 minutes at 4°C, the serum was aliquoted into Eppendorf tubes (Eppendorf, Hamburg, Germany), 300 μl for each tube and stored at –80°C.

### Sample preparation

The serum samples were thawed at 4°C. Acetonitrile (600 μl) was added to the serum (200 μl) and vortex mixed for 1 minute. The mixture was laid at room temperature for 10 minutes and centrifuged at 14 000 ×*g* for 10 minutes at 4°C. Aliquots (450 μl) of the resulting clear supernatants were placed into the glass inserts of the UPLC vials and prepared for UPLC/TOFMS analysis. Serum samples from the control and patient groups were analyzed blindly in a random order.

### Liquid chromatograph

A 2-μl aliquot of the supernatant from the last step was injected and chromatographed on a (2.1 mm×100.0 mm, ACQUITYTM 1.7 μm BEHC) C18 column (Waters, Milford, MA, USA) maintained at 40°C using an ACQUITY™ UPLC system (Waters, Milford, MA). The

mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Flow rate was 450 μl/min. A linear gradient was optimized as follows: 0–2.5 minutes, 3%–50% B; 2.5–6.5 minutes, 50%–100% B; the composition was held at 100% B for 4.5 minutes, then for 11–14 minutes at equilibration with 3% B. The column eluent was directed to the mass spectrometer for analysis.

**Mass spectrometry**

Mass spectrometry was performed using a Waters Micromass quadrupole time-of-flight (Q-TOF) premier (Waters Micromass Technologies, Manchester, UK) operating in ESI modes with V optics. The capillary and cone voltages were set to 3000 V and 35 V, respectively. The source temperature was maintained at 100°C with a cone gas flow of 50 L/h, a desolvation temperature of 350°C, and a gas flow of 600 L/h. The Q-TOF premier MS acquisition rate was set to 0.3 second with a 0.1 second interscan delay. The scan range was from 50 to 1000 m/z. Data were collected in centroid mode. All analyses were acquired using lock spray to ensure mass accuracy and reproducibility. Leucine-enkephalin was used as lock mass (m/z 556.2771) at a concentration of 200 ng/ml and a flow rate of 10 μl/min. Argon was used as the collision gas. Structures of the serum biomarkers were elucidated by MS/MS fragmentation with collision energies ranging from 20 to 40 eV.

**Statistical analysis**

The data files were processed using the MarkerLynx Applications Manager version 4.1 (Waters, UK). This application manager integrated peaks in the UPLC-MS data using ApexTrack peak detection. The track peak parameters were set as follows: peak width at 5% height of 10 second, peak-to-peak baseline noise calculated automatically, minimum intensity of 100, mass window of 0.01 Da, retention time window of 0.2 minute, noise elimination level of 3, and mass tolerance of 0.01 Da with exclusion of deisotopic data. Data were only used for a period of 0–6.5 minutes. The data were exported and analyzed by PCA and partial least squares discrimination analysis (PLS-DA) using SIMCA-P 12.0 (Umetrics AB, Umea, Sweden). Statistical analysis was performed using the SPSS statistical software (SPSS Inc., USA), Windows version 16.0. $P$ <0.05 was considered statistically significant.

## RESULTS

Typical LC/MS base peak intensity (BPI) chromatograms of serum samples from the alcoholic cirrhosis, HBV-induced cirrhosis, and healthy control groups are shown in Figure 1. Chromatography peaks were detected using Micromass MarkerLynx with a 0 to 6.5 minutes retention time, and then normalized data were fed to SIMCA-P. Pareto variance (Par) was used for all data scaling. To understand the metabolic changes in alcoholic cirrhosis and HBV-induced cirrhosis globally, unsupervised PCA was selected to analyze the data in this article. Figure 2 shows the PCA score plot. All healthy persons were clustered together while the alcoholic cirrhosis patients were a little closer to HBV-induced cirrhosis patients. This suggests that compared with the healthy controls, the metabolism of patients with liver cirrhosis had changed considerably, but that the metabolism of alcohol- and HBV-induced cirrhosis patients had some degree of similarity.

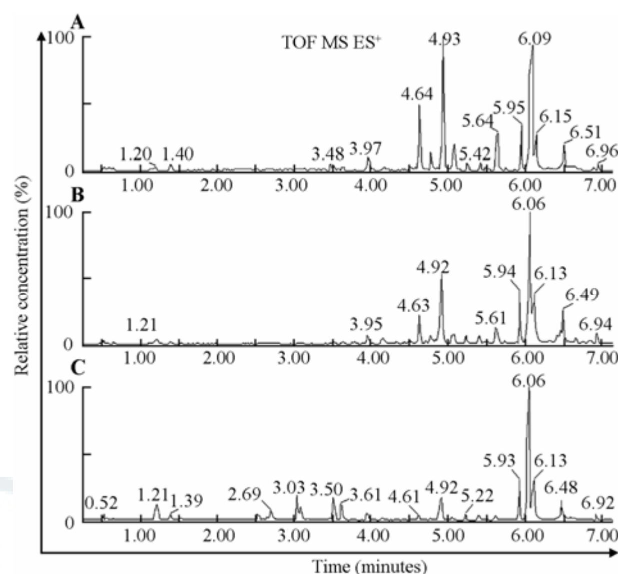


**Figure 1.** Typical UPLC Q-TOF MS base peak intensity (BPI) chromatograms of a serum sample. **A:** Healthy control subjects; **B:** HBV-induced cirrhosis group; **C:** Alcoholic cirrhosis group.

Subsequently, in order to better characterize the serum metabolite profile of cirrhosis patients, supervised PLS-DA models were built based on the three groups and the groups with individual hepatic cirrhosis. Clear separation was achieved among the three groups (Figure 3A) and between alcohol- and HBV-induced cirrhosis (Figure 3B). Components that played important roles in the separation were chosen according to the variable importance in the projection (VIP) parameter. The VIP of PLS-DA is a major parameter for the detection of potential biomarkers whereas VIP values reflect the correlation of the metabolites to diseases. The following steps were employed to detect potential biomarkers among thousands of variables. First, variables were selected as candidates according to their VIP values. The top 10 VIP among the three groups and between the groups with individual hepatic cirrhosis were selected as biomarkers (Tables 2 and 3). The information on relative concentration was included and affected the separation because Par was used for all data scaling, resulting in the variables of high concentration accounting for more influence than those of low concentration. To study the change in metabolites with lower relative concentrations, the independent $t$ test was used between the cirrhosis groups, and analysis of variance (ANOVA) among the three groups for the remaining variables. The identified metabolites, as shown in Table 4, were also selected.

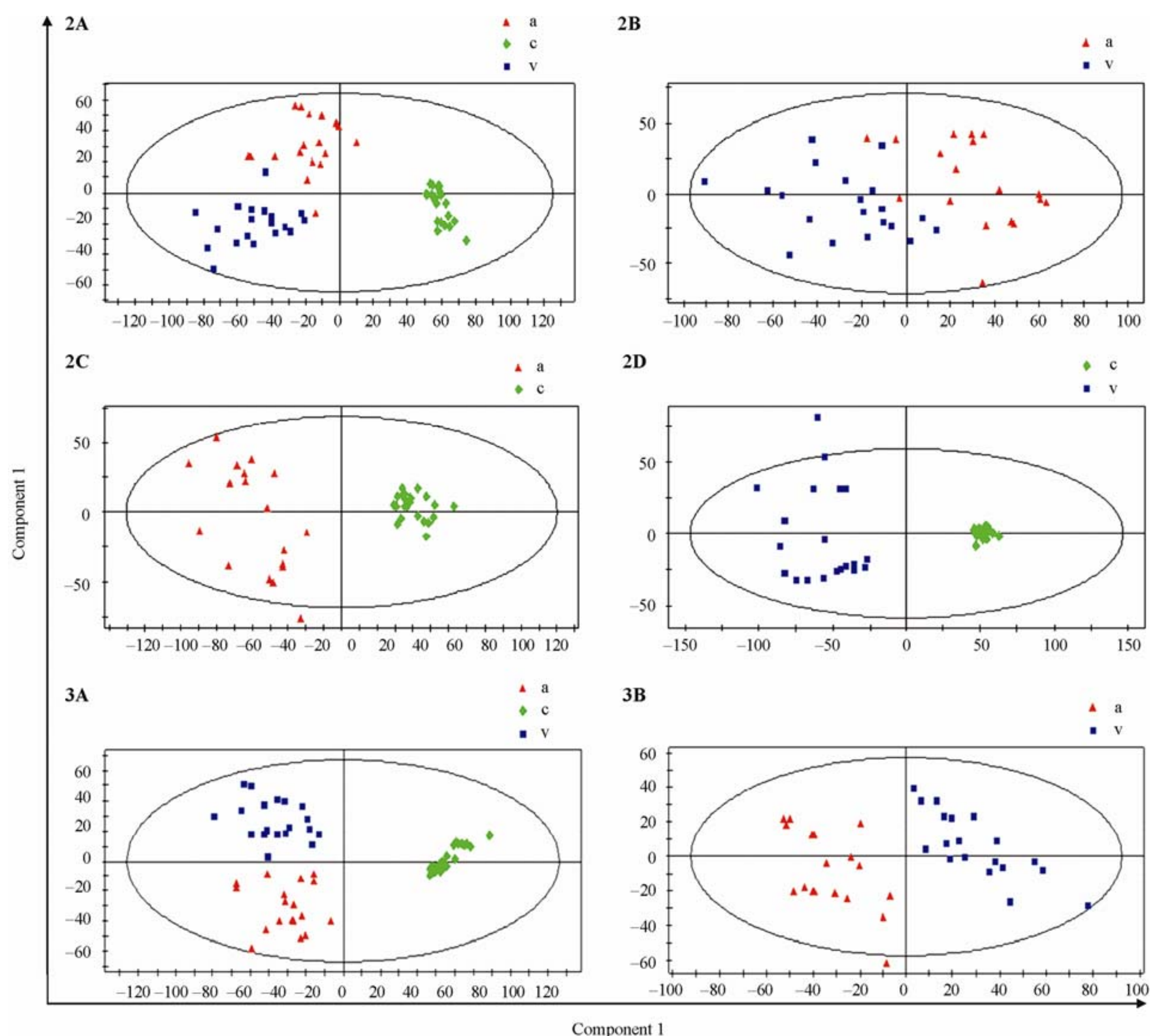


**Figure 2.** PCA analysis of alcohol- and HBV-induced liver cirrhosis and healthy control subjects. a: Alcoholic hepatic cirrhosis patients, c: healthy control group, and v: HBV-induced hepatic cirrhosis patients. **2A:** PCA score plot for the first two components showing separation among the three groups. **2B:** PCA score plot for the first two components showing separation between the groups with hepatic cirrhosis. **2C:** PCA score plot for the first two components showing separation between the healthy control group and alcoholic hepatic cirrhosis patients. **2D:** PCA score plot for the first two components showing separation between the healthy control group and HBV-induced hepatic cirrhosis patients. X and Y axis represent component 1.

**Figure 3.** PLS-DA analysis of alcohol- and HBV-induced liver cirrhosis and healthy control subjects. a: Alcoholic hepatic cirrhosis patients, c: healthy control group, and v: HBV-induced hepatic cirrhosis patients. **3A:** PLS-DA score plot for the first two components showing the separation among the three groups. **3B:** PLS-DA score plot for the first two components showing the separation between the groups with hepatic cirrhosis. X and Y axis represent component 1.

Each potential biomarker was identified according to three criteria: accurate mass, retention time, and tandem MS (MS/MS). First, the MW of the potential biomarker was determined and used to search against the METLIN (*metlin.scripps.edu*), HMDB (*www.hmdb.ca*), and KEGG (*www.genome.jp/kegg/ligand.html*) databases. Mass accuracy from the TOF was usually less than 5 ppm. The possible compounds matching the potential biomarkers were obtained. Second, the identity of the compounds was confirmed by collecting MS/MS based on a Q-TOF. This method isolated the ion base on the quadrupole and then fragmented it using low-energy impact with a collision gas, producing a characteristic fragmentary pattern. The MS/MS spectrum was used to identify by comparison the fragmentation pattern of the possible compound. The LC run was repeated using the same parameters for profiling, and fragmentation data on the target unknown metabolites were obtained. Finally, standard compounds were used to confirm the identified metabolites.

Concentrations of five categories of substances in the serum of patients with cirrhosis changed significantly. The concentrations of seven saturated or unsaturated lysophosphatidylcholines (LPCs) decreased by 1.33- to 8.09-fold. The concentrations of myristamide and oleamide increased in the serum of patients with alcoholic cirrhosis but decreased in the serum of patients with HBV-induced cirrhosis. The levels of stearamide and four bile acids (BAs) increased in the serum of patients with

**Table 2.** Top 10 potential biomarkers in discriminating among healthy control, alcoholic cirrhosis, and HBV-induced cirrhosis groups according to VIP values

| Name | Ionization state | Molecular formula | m/z observed | m/z calculated | Relative error (ppm) | Retention time (minutes) | VIP* | *P* values† | Fold change compared with controls | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Alcoholic | HBV |
| LPC C16:0 | $[M+H]^+$ | C24H50NO7P | 496.3310 | 496.3303 | 1.41 | 4.92 | 14.98 | <0.001 | 2.44§ | 2.94§ |
| Oleamide | $[M+H]^+$ | C18H35NO | 282.2854 | 282.2857 | –1.06 | 6.06 | 12.51 | <0.001 | 1.19‡ | 1.35§ |
| LPC C18:2 | $[M+H]^+$ | C26H50NO7P | 520.3435 | 520.3403 | 6.14 | 4.61 | 9.14 | <0.001 | 2.70§ | 3.13§ |
| LPC C18:0 | $[M+H]^+$ | C26H54NO7P | 524.3655 | 524.3640 | 2.86 | 5.62 | 8.31 | <0.001 | 2.78§ | 3.57§ |
| UN | $[M+H]^+$ | – | 991.6730 | – | 2.02 | 4.91 | 6.82 | <0.001 | 7.14§ | 9.09§ |
| UN | $[M+H]^+$ | – | 518.3195 | – | 2.32 | 4.91 | 6.31 | <0.001 | 1.96§ | 2.33§ |
| UN | $[M+H]^+$ | – | 497.3438 | – | 3.02 | 4.91 | 6.24 | <0.001 | 5.69§ | 5.99‡ |
| LPC C20:3 | $[M+H]^+$ | C28H52NO7P | 546.3559 | 546.3560 | –0.18 | 5.61 | 5.40 | <0.001 | 2.86§ | 3.33§ |
| LPC C20:5 | $[M+H]^+$ | C28H48NO7P | 542.322 | 542.3246 | –4.79 | 4.61 | 5.30 | <0.001 | 2.86§ | 3.03§ |
| UN | $[M+H]^+$ | – | 414.3014 | – | – | 3.03 | 4.99 | <0.001 | 66.73‡ | 5.30‡ |

LPC: lysophosphatidylcholine; UN: unidentified; m/z: mass charge ratio; M: mass; H: hydrion. *Variable importance in the projection; †*P* values of ANOVA.
‡: up-regulated. §: down-regulated.

**Table 3.** Top 10 potential biomarkers in discriminating between alcoholic cirrhosis and HBV-induced cirrhosis according to VIP values

| Name | Ionization state | Molecular formula | m/z observed | m/z calculated | Relative error (ppm) | Retention time (minutes) | VIP* | *P* value† |
|---|---|---|---|---|---|---|---|---|
| Oleamide | $[M+H]^+$ | C18H35NO | 282.2854 | 282.2857 | –1.06 | 6.06 | 20.93 | <0.001 |
| UN | $[M+H]^+$ | – | 414.3014 | – | – | 3.03 | 5.99 | 0.006 |
| UN | $[M+H]^+$ | – | 265.2709 | – | – | 6.05 | 4.81 | 0.001 |
| UN | $[M+H]^+$ | – | 497.3438 | – | – | 4.91 | 3.93 | 0.006 |
| UN | $[M+H]^+$ | – | 247.2447 | – | – | 6.05 | 3.56 | 0.008 |
| LPC C18:0 | $[M+H]^+$ | C26H54NO7P | 524.3655 | 524.3640 | 2.86 | 5.62 | 3.33 | 0.048 |
| UN | $[M+H]^+$ | – | 437.2345 | – | – | 1.68 | 3.26 | 0.014 |
| UN | $[M+H]^+$ | – | 393.2075 | – | – | 1.61 | 3.03 | 0.024 |
| UN | $[M+H]^+$ | – | 481.2604 | – | – | 1.75 | 2.98 | 0.013 |
| Myristamide | $[M+H]^+$ | C14H29NO | 228.2351 | 228.2347 | 1.75 | 5.23 | 2.92 | <0.001 |

UN: unidentified; m/z: mass charge ratio. *Variable importance in the projection. †*P* values of independent *t* test.

**Table 4.** Potential biomarkers selected and identified according to the *P* values of ANOVA

| Name | Ionization state | Molecular formula | m/z observed | m/z calculated | Relative error (ppm) | Retention Time (min) | *P* values* | Fold change compared with controls | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Alcoholic | HBV |
| GCDCA | $[M+Na]^+$ | C26H43NO5 | 472.3033 | 472.3039 | –1.27 | 3.50 | <0.001 | 22.76† | 23.73† |
| GCA | $[M+H]^+$ | C26H43NO6 | 466.3160 | 466.3169 | –1.93 | 3.03 | <0.001 | 45.81† | 55.17† |
| Hypoxanthine | $[M+H]^+$ | C5H4N4O | 137.0474 | 137.0470 | 2.92 | 0.67 | <0.001 | 13.38† | 13.01† |
| L-acetylcarnitine | $[M+H]^+$ | C9H17NO4 | 204.1388 | 204.1386 | 0.97 | 0.67 | 0.001 | 1.81† | 2.46† |
| Stearamide | $[M+H]^+$ | C18H37NO | 284.2957 | 284.2953 | 1.4 | 6.49 | 0.006 | 1.28† | 1.21† |

GCDCA: glycochenodeoxycholic acid; GCA: glycocholic acid; m/z: mass charge ratio. **P* values of ANOVA. †: up-regulated.

cirrhosis (shown in Table 4). The concentration of hypoxanthine in the serum of patients with alcoholic cirrhosis and HBV-induced cirrhosis increased by 12.38- and 12.01-fold, respectively, whereas L-acetylcarnitine increased by 0.81-fold in the serum of patients with alcoholic cirrhosis and 1.46-fold in the serum of patients with HBV-induced cirrhosis. Oleamide and myristamide are therefore considered as potential biomarkers for the differential diagnosis of alcohol- and HBV-induced liver cirrhosis. Lysophosphatidylcholine, glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), L-acetylcarnitine, and hypoxanthine are considered as "common" potential biomarkers for liver cirrhosis.

## DISCUSSION

LPC is of great importance *in vivo* and takes part in various physiological functions. Yin et al[11] also found that LPC decreased in patients with liver cirrhosis or hepatocellular carcinoma. Lysophospholipase D (lyso-PLD) in the human body can convert LPC to lysophosphatidic acid (LPA). In liver cirrhosis patients, lyso-PLD activity was increased, resulting in a decrease in LPC concentration in the serum.[19,20] LPA stimulates fibroblast proliferation, such as hepatic stellate cells (HSCs) which play a central role in the development of hepatic fibrosis. LPA inhibiting HSC apoptosis has been observed in rats.[21] Therefore, the increase in LPC broken down into LPA may be one of the mechanisms leading to liver cirrhosis.

BAs are the major endogenous metabolic products of cholesterol and are involved in lipid absorption and generation of bile flow. BAs are synthesized from cholesterol in the liver through the action of hepatic enzymes and then excreted into the small intestine via the bile duct in the forms of glycine and taurine conjugates. Four cytochrome P450 enzymes play important roles in bile acid biosynthesis.[22,23] In healthy humans, only small quantities of bile acids are found in the peripheral circulation and urine. However, many diseases, such as cirrhosis and hepatobiliary diseases, affect the concentration of bile acids. When hepatic trauma occurs, hepatic clearance of bile acids decreases, leading to a rise

in serum bile acids. Therefore, bile acid has been considered as a marker of liver injury for years.[24] GCDCA and GCA are conjugated bile acids. High levels of GCDCA and GCA in patients with cirrhosis could indicate the occurrence of hepatic injury. BAs induce up-regulation of hepatocyte-derived monocyte chemotaxis protein-1 (MCP-1), resulting in HSC recruitment in diverse causes of cholestatic liver injury; this is a key early event in liver fibrogenesis in these conditions.[25] Liver cirrhosis leads to an increase in bile acid, and the bile acid, in turn, can cause liver fibrosis through MCP-1-mediated HSC, thus forming a vicious circle.

In the present study, the concentration of L-acetylcarnitine increased in the serum of patients with cirrhosis compared with that of the healthy controls. This result is in agreement with a previous study in which Krähenbähl et al[26] also found that patients with cirrhosis had increased acetylcarnitine. Carnitine is a vitamin-like compound synthesized in the liver, kidneys, and brain. It is an essential cofactor in the transfer of long-chain fatty acids across the inner mitochondrial membrane for oxidation.[27] Changes in carnitine metabolism could impair mitochondrial fatty acid oxidation.

An intriguing result of the present study is that oleamide and myristamide, which are fatty acid amides, increased in the serum of patients with alcoholic cirrhosis but decreased in patients with HBV-induced cirrhosis. Oleamide is the prototype long-chain primary fatty acid amide lipid messenger.[28] A previous study found that oleamide accumulated in the cerebrospinal fluid under conditions of sleep deprivation and induced physiological sleep in animals.[29] Thus, it is possible that oleamide is a fatty acid amide signaling molecule in the cardiovascular system.[30] Oleamide is generated from oleoylglycine by the neuropeptide processing enzyme peptidylglycine alpha-amidating monooxygenase (PAM), or the direct amidation of oleic acid via oleoyl coenzyme A by cytochrome c using ammonia as the nitrogen source.[28] Fatty acid amide change in patients with cirrhosis may be related to disordered fatty acid metabolism, which in turn may be correlated with disordered metabolism of carnitine. Ethanol can cause impairment of synthesized lipid transportation and inhibition of fatty acid oxidation. Alcoholic cirrhosis is already activated in the early stages of steatosis and steatohepatitis.[31,32] An *in vitro* study showed that hepatic lipid accumulation will induce the release of factors that accelerate the activation and proliferation of HSC and result in fibrogenesis and cirrhosis.[33] Oleamide and myristamide increased in the serum of patients with alcoholic cirrhosis, which may be the result of disordered lipid metabolism that may be one of the mechanisms leading to alcoholic cirrhosis. Stearamide has been found to be increased in the serum of patients with alcohol- or HBV-induced cirrhosis. HBV could induce lipid metabolism mediated by HBV protein X (HBx). HBx expression induces lipid accumulation in hepatic cells mediated by the induction of sterol-regulatory-element-binding protein 1, a key regulator of lipogenic genes in liver disorder.[34,35] HBx may have a direct role in the development of liver cirrhosis by paracrine activation of HSC.[36] The exact mechanism of fatty acid amide difference between alcohol- and HBV-induced cirrhosis is not fully understood and warrants further study.

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