Serum Lactate: Creatinine Ratio as a Prognostic Marker for Liver Failure Patients Treated by artificial liver support systems

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Research

Keywords: liver failure, metabonomics, ALSSs treatment, prognostic markers, lactate:creatinine ratio

Posted Date: June 22nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-35762/v1>

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Abstract

Background: To screen potential serum prognostic markers for patients with liver failure treated by artificial liver support systems (ALSSs).

Methods: Serum samples were obtained from 57 liver-failure patients before and after ALSSs therapy and analyzed by proton nuclear magnetic resonance. The diagnostic values were evaluated by calculation of the area under a receiver-operating characteristic curve (AUROC). Two retrospective analyses were further employed.

Results: Metabonomics analysis showed that ratios of creatine:creatinine, taurine:creatinine, and lactate:creatinine were significantly altered and restored to normal levels after ALSSs treatment. The ratio of lactate:creatinine showed the highest AUROC value (0.650), which was higher than that of the prothrombin time activity (PTA, 0.562). A total of 105 cases of retrospective analysis showed that serum lactate:creatinine ratios among the liver-failure patients were 0.038 ± 0.002 (survival group, $n=48$), 0.048 ± 0.005 (three-month death group, $n=24$), and 0.052 ± 0.005 (one-month death group, $n=33$), which was significantly negatively correlated with survival ($r = -0.26$). Another retrospective cohort analysis ($n=81$) of the early to middle stage liver-failure patients treated by ALSSs showed that the AUROC of the lactate:creatinine ratio for diagnosis of those who survived for one subsequent month was 0.827, which was higher than that of PTA (0.628) and lactate (0.765).

Conclusion: The serum lactate:creatinine ratio may be a more sensitive measure than either the PTA or the lactate to evaluate the prognosis of ALSSs treatment in early to middle stage of liver-failure patients. This finding is worthy of further study in clinical applications.

Background

Liver failure is an acute breakdown of the functions of the organ due to chronic decompensated failure. It is characterized by rapidly deteriorating liver function and it manifests clinically as jaundice and coagulation dysfunction, extreme weakness, and ascites [1, 2]. It is also characterized by complex pathogenesis, rapid progression, and high mortality (short-term mortality of 50%–90%) [3]. Currently, artificial liver support systems (ALSSs) offer an important choice of treatment other than standard medical therapy and liver transplantation [4, 5]. ALSSs are mainly categorized into three types: non-bioartificial, bioartificial, and hybrid ALSSs [6, 7]. Non-bioartificial ALSSs have been widely used in clinical practice. They involve plasma perfusion (PP), hemoperfusion (HP), plasma exchange (PE), plasma diafiltration (PDF), etc. [8]. Studies have shown that the efficiency of ALSSs therapy falls as the severity of the liver disease increases; hence, the efficiency of ALSSs therapy for early, middle and late-stage liver disease has been shown to be 87.5%, 61.8% and 17.0% respectively [9]. These findings indicate that 12.5% to 38.2% of patients with early and middle-stage liver failure are not cured by ALSSs and should be immediately recommended for liver transplantation. However, physicians need to be able to recognize the patients for whom use of ALSSs may be a life saver or ineffective. Therefore, the discovery of novel

serum prognostic markers to identify liver-failure patients who would benefit from ALSSs treatment should help to improve the prognostic performance and would be of great theoretical significance and clinical value.

Metabonomics has developed rapidly over more than a decade, achieving great results in disease diagnosis, drug research and development, and other fields of human health care [10-14]. Yang L. et al. adopted for metabolomics analysis a rat model in which liver failure was induced by application of tetrachloromethane and alpha-naphthylisothiocyanate. The researchers observed significant differences between the control and experimental groups in bile acid [15]. Yang G. et al. compared serum differential metabolites of healthy individuals and patients with acute liver failure (ALF) by high-pressure liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS). They found that the concentrations of lysophosphatidylcholine and glycodeoxycholic acid in patients with ALF were significantly altered, and two compounds were defined as diagnostic biomarkers for ALF [16]. Amathieu et al. used the proton nuclear magnetic resonance spectroscopy (^1H NMR) to perform metabolomics analysis on the serum samples of patients with mild and severe chronic liver failure (CLF). They found that the levels of high-density lipoprotein and phosphorylcholine in patients with mild CLF were significantly higher than those in patients with severe CLF; and on the other hand, that the levels of lactate, pyruvate, glucose, amino acid, and creatinine were significantly higher in patients with severe CLF than in patients with mild liver failure [17]. Recent studies showed that alpha-fetoprotein, serum sodium, lactate, arterial blood ammonia, and phosphate were related to the prognosis of liver failure [18-23]. However, few studies have focused on the screening of serum prognostic markers of patients with liver failure who have been treated by ALSSs.

The present study adopted ^1H NMR-based metabonomics for the identification of differential metabolites in patients with liver failure before and after ALSSs treatment. The area under a receiver-operating characteristic (AUROC) curve was used in the evaluation of the diagnostic values before and after ALSSs therapy. The correlations of serum lactate:creatinine ratio and prothrombin time activity (PTA) or survival of patients after ALSSs therapy were obtained by regression analysis. Two retrospective analyses were performed to validate the markers for the evaluation of the prognosis of ALSSs treatment for patients with liver failure.

Materials And Methods

2.1 Reagents and chemicals

Chromatographic-grade methanol and 3-trimethylsilyl-propionic acid were purchased from Merck Co., Ltd. (Darmstadt, Germany). Deuterated distilled water (D_2O) was provided by J&K Scientific Ltd. (Shanghai, China); $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, and $\text{K}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ used to prepare phosphate buffers were provided by Tianjin Weiyi Chemical Technology Co., Ltd. (Tianjin, China). The sodium salt of (trimethylsilyl)-propionic-2,2,3,3- d_4 acid (TSP) was purchased from Sigma (USA).

2.2 Patients for ALSSs treatment and collection of serum samples

Fifty-seven patients with liver failure received ALSSs treatment in the form of PDF or PP, and serum samples were taken from them before and after the treatment. Additionally, 50 serum samples were collected from people who attended routine physical examinations and whose gender and age profiles matched those of the liver-failure patients. All the samples were provided by the Second Affiliated Hospital of Nanchang University, and all cases were clinically diagnosed. Blood samples were taken between 6:00am and 8:30am after overnight fasting to eliminate any dietary interferences [24]. The blood samples were centrifuged for 15 min at 4000 rpm within four hours of collection, and the serum samples were separated and stored in a refrigerator at -80 °C until analysis [25]. The patients' demographic information and laboratory data were collected or calculated. These included the levels in serum of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB) and creatinine, and the calculated international normalized ratio (INR), PTA and score according to the model for end-stage liver disease (MELD). The present study was approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University.

2.3 Sample preparation and ¹HNMR acquisition

A titer of 200.0μl supernatant of the thawed serum was withdrawn and deproteinated by addition of 800.0μl methanol. Of this mixture, 700.0μl supernatant was evaporated to dry at 37°C with the SpeedVac system (Hersey Instrument Co., Ltd, China) and the residues were resuspended in 450.0μl of double distilled water. The solution was added to 50.0μl of phosphate-buffered saline (PBS) (pH7.4) and 50.0 μl of 5 mmol/L TSP solution and transferred into a 5mm NMR tube for ¹HNMR analysis in a Bruker Avancell-600 MHz spectrometer (Germany) at a temperature of 298K with nuclear overhauser enhancement spectroscopy pulse sequence.

The ¹HNMR data were pretreated with removal of the resonances of 3.36 ~ 3.37 ppm and 4.7 ~ 5.2 ppm to eliminate the influence of methanol and water peaks, according to the published method [26].

2.4 ¹HNMR data normalization and multivariate statistical analysis

The ¹HNMR intensity of the signal for each sample was normalized by total normalization [26] and creatinine normalization before multivariate analysis. The algorithms for creatinine normalization in each sample were as follows:

$$x_{j\text{new}} = x_{j\text{old}}/\text{creatinine}$$

where x_j represents one variable in one column and creatinine represents the integral value of creatinine from 4.05ppm to 4.07ppm. The multivariate statistical analysis methods chosen were principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), and these were performed by use of SIMCA-P software (version 12, Umetrics, Umea, Sweden).

2.5 A retrospective cohort study for correlation of lactate:creatinine ratio with survival of patients with liver failure

The clinical data were collected regarding patients with liver failure who were undergoing ALSSs or other patients receiving basic medical treatment at the Fifth People's Hospital of Ganzhou between February 2017 and January 2019. The patients were followed up for at least six months after treatment through review of their medical records and call visits. The correlation of the patients' lactate:creatinine ratios measured before treatment with their survival data was determined.

2.6 Predictive prognosis of patients after ALSSs treatment by lactate:creatinine ratio

Another retrospective cohort study was conducted on patients with *liver failure* who had received ALSSs treatment at the Fifth People's Hospital of Ganzhou between January 2018 and January 2020. The inclusion criteria for this study were patients with liver failure in the early to middle stage (PTA: 20-40%) before ALSSs treatment, and their serum creatinine was in the normal range (44-110 $\mu\text{mol/L}$). The clinical data were collected before ALSSs treatment and the patients' survival times were monitored after treatment.

2.7 Statistical analysis

The clinical data regarding lactate, creatinine and PTA to be investigated in the two retrospective cohort studies were obtained from the clinical laboratory of the Fifth People's Hospital of Ganzhou. The quantitative data were tested using an independent sample *t*-test. A *p* value of less than 0.05 was considered to indicate statistical significance. All the data were analyzed using SPSS 23.0 software (IBM Corp., Armonk, NY, USA).

Results

3.1 General characteristics of the patients for ^1H NMR analysis

The information regarding patients with liver failure is provided in Table 1. The data regarding the healthy control group are provided in Table S1. The group treated by ALSSs comprised 54 males and three females, and the control group comprised 46 males and four females. All the subjects in the ALSSs group satisfied the diagnostic criteria for liver failure as described in 2012 in the Clinical Practice Guideline for Liver Failure promulgated by the Liver Failure and Artificial Liver Group, Chinese Society of Infectious Diseases, Chinese Medical Association. The exclusion criteria comprised: presence of mental or neurological disorders; allergic constitution; inability to tolerate blood products during ALSSs; or an incomplete record of liver failure due to various factors. The ALSSs-treated and control groups were comparable as they exhibited no statistically significant differences in age or gender ($P > 0.05$).

The PTA of the serum samples taken after ALSSs treatment were observed to have significantly increased (28.87 ± 0.98 before treatment vs. $38.21 \pm 2.44\%$ after treatment, $P < 0.001$). In contrast, the

MELD score and levels of total bilirubin (TBIL), ALT and AST in serum after ALSSs treatment were markedly decreased. No significant differences were observed in serum creatinine levels or INRs between samples taken before and after ALSSs treatment.

3.2 PCA and PLS-DA analyses of ^1H NMR data

In the research described in this paper, ^1H NMR data were processed by total normalization and creatinine normalization before multivariate analysis. The scores plots of PCA and OPLS-DA are shown in Figure 1. The results show that the total normalization data regarding the healthy group and that of the ALSSs-treated group both pre- and post-treatment overlaps in the PCA scores plot (Figure 1a). However, the scores plot of OPLS-DA generated from the creatinine-normalized data show partial separation according to group (Figure 1d). The accuracy and predictability of the established OPLS-DA model were evaluated by use of three parameters ($R^2\text{X}$, $R^2\text{Y}$ and $Q^2\text{Y}$). The values of $R^2\text{X}_{\text{cum}}$, $R^2\text{Y}_{\text{cum}}$, and Q^2 in the OPLS-DA model based on total normalization were 0.302, 0.408, and 0.378, respectively, whereas those in the model based on creatinine calibration were 0.512, 0.428, and 0.414. Thus, the OPLS-DA model based on creatinine normalization was found to show superior predictive power compared with that based on total normalization. The results of the sevenfold cross-validation and permutation test (200 times) show a value of R^2 of 0.488 and a value of Q^2 of -0.13, indicating that the model is not over-fitted and is effective (see Figure S1) [27].

3.3 Changes of metabolites in liver-failure patients after ALSSs treatment

A total of 21 serum metabolites in ^1H NMR spectra were identified and confirmed (Table 2) by comparing their chemical shifts and coupling patterns with the corresponding values according to the method described in a previous paper [26]. Our results show significant differences between samples taken before and after ALSSs treatment in the ratios of serum leucine to creatinine, isoleucine to creatinine, acetate to creatinine, alanine to creatinine, creatine to creatinine, taurine to creatinine, and lactate to creatinine (Independent-sample t-test, $P < 0.05$). Figure 2a shows that variables 15 (taurine:creatinine), 16 (creatine:creatinine), and 21 (lactate:creatinine) were closely correlated with ALSSs treatment. In addition, it can be clearly seen that these three ratios returned to normal levels after ALSSs treatment (Figure 3).

3.4 ROC analysis of metabolites in patients before and after ALSSs treatment

Analysis of receiver-operating characteristics (ROC) was conducted before and after ALSSs treatment, and the identified metabolites were used as variables. The results are provided in Table 2. The AUROC values of the creatine:creatinine ratio, taurine: creatinine ratio, and lactate:creatinine ratio were 0.633, 0.644, and 0.650, which were higher than the MELD scores (AUROC=0.593) and prothrombin activity (PTA, AUROC=0.562, Table 1). Therefore the lactate:creatinine ratio was found to show the highest diagnostic efficiency regarding ALSSs treatment.

3.5 Correlation of lactate:creatinine ratio with survival of liver-failure patients

Of 105 inpatients with liver failure, 48 survived for more than six months after ALSSs treatment (the survival group), 24 died within three months of treatment, and 33 died within one month of treatment. These data were employed for retrospective analysis. The clinical data are presented in Table 3. The results (Figure 4a) show that the survival group exhibited a lactate:creatinine ratio before treatment of 0.038 ± 0.002 (n=48), the group who died within three months had a lactate:creatinine ratio of 0.048 ± 0.005 (n=24) and the group who died within one month had a corresponding ratio of 0.052 ± 0.005 (n=33). These data indicate that the groups of patients who died showed higher lactate:creatinine ratios than the group of patients who survived for more than six months. The correlation analysis shows that the lactate:creatinine ratio is negatively correlated with survival period of liver-failure patients ($r = -0.26$, $p = 0.001$).

In addition, the result shows that the correlation coefficient between the serum lactate:creatinine ratio and PTA was -0.186 ($p = 0.025$, Figure 4b).

3.6 Use of lactate:creatinine ratio and PTA to predict one-month survival of liver-failure patients after ALSSs treatment

A total of 81 inpatients with liver failure (the early to middle stage before ALSSs treatment) who received ALSSs treatment, were included for retrospective cohort analysis in this study. Of these, 49 survived for more than six months, while 32 died within one month after treatment. The results show that the lactate:creatinine ratio before treatment for those who died within one month (0.057 ± 0.004 , n=49) was markedly higher than that of those who survived for more than six months (0.035 ± 0.002 , n=32). The AUROC (95% confidence intervals) of the lactate:creatinine ratios for potential diagnosis of those who could be expected to survive more than six months against those who would be expected to die within one month was 0.827 (0.735 - 0.918); the sensitivity was 81.3% and the specificity was 73.9% with the cut-off value of 0.041.

In comparison, the AUROCs (Figure 5) of the PTA and lactate data for potential diagnosis of the survival group were 0.628 (0.503 - 0.754) and 0.765 (0.661 - 0.869), respectively. The sensitivities of the PTA and lactate were 52.1% and 87.1%, and the specificities of the PTA and lactate were 75.0% and 61.7% with the cut-off values of 30.84% and 2.45 respectively. These results indicate that use of the lactate:creatinine ratio may be more reliable than measures of PTA and lactate to evaluate the prognosis of ALSSs treatment in liver-failure patients.

Discussion

Liver failure develops rapidly and leads to high short-term mortality. Studies [6, 28-30] have shown that ALSSs therapy can remove harmful substances from the body, maintain homeostasis, temporarily assist or replace liver function, facilitate hepatocyte regeneration and recovery of liver function, and improve the survival rate of patients with liver failure. Hu P. [11] found that levels of ALB, creatinine, TBIL, INR, PTA, and ALT in serum changed significantly before and after ALSSs treatment. Hu S. et al. [13] explored the curative effect of hybrid-bioartificial liver on patients with liver failure related to infection by hepatitis B

virus, and found that ALT, ALB, and TBIL were significantly altered after ALSSs treatment. Lian et al. found that the amount of serum metabolites, including phosphatidylcholines and lysophosphatidylcholines, decreased significantly in liver-failure patients, whereas levels of conjugated bile acids increased significantly. These metabolites are considered to be common biomarkers of liver failure [31]. In addition, alpha-fetoprotein, serum sodium, lactate, arterial blood ammonia, and phosphate have been related to the prognosis of liver failure [18-23]. To date, very few studies have focused on the screening of potential serum prognostic markers to predict the outcome for liver-failure patients treated by ALSSs.

In the present study, serum metabolites related to ALSSs treatment were identified by ^1H NMR-based metabolomics. To eliminate or reduce errors in sampling, ^1H NMR data were processed by total normalization and creatinine calibration [32-35]. Our results show that processing of ^1H NMR data by creatinine calibration offered improved discrimination between patients after ALSSs treatment compared with total normalization. This was because the peak area of ^1H NMR was correlated with the concentrations of metabolites and the number of hydrogen molecules they contained [36, 37], so creatinine calibration not only eliminated errors in sampling but also enabled accurate calculation of the relative levels of metabolites. Table 2 shows that the ratios of leucine:creatinine, isoleucine:creatinine, acetate:creatinine, alanine:creatinine, creatine:creatinine, taurine:creatinine and lactate:creatinine were significantly altered after ALSSs treatment. Alanine and glycine are amino acids that are not essential in the body, but they are linked with amino acid and energy metabolism and are of great significance. Taurine is an important metabolite of bile-acid metabolism and has great biological significance in the cholesterol binding of bile acid, antioxidation, osmoregulation, and calcium signaling [38]. In addition, our results show clearly that the ratios of creatine:creatinine, taurine:creatinine and lactate:creatinine returned to normal levels after liver failure patients with ALSSs treatment and that the AUROCs of those were higher than the AUROCs of the MELD and PTA. The ratio of lactate:creatinine displayed the highest AUROC for use in discriminating between pre- and post-ALSSs treatment.

Lactate is metabolized by the liver, after which it is secreted and excreted by the kidneys. It is the end product of glycolysis in the body. Lactate levels in blood can be elevated by strenuous exercise, severe anemia, cell poisoning, respiratory and circulatory failures and severe infection especially involving septic shock, which can lead to tissue hypoxia and increased anaerobic metabolism [39]. Patients with liver failure due to abnormal liver function rather than causes such as acetaminophen overdose or viral attack usually experience metabolic disorders, acid-base imbalances, decreased liver function over time, and decreased metabolism, which result in recycling and accumulation of lactate. The level of lactate in serum has become an important objective indicator to evaluate the prognosis of patients with cirrhosis and liver failure [40-42]. In addition, it has been shown that the rate of lactate clearance is a good and independent predictor of death in critically ill patients with cirrhosis and acute-on-chronic liver failure (ACLF) [43]. Creatinine is a metabolite that is a byproduct of muscle metabolism. It is derived from creatine, arginine, and glycine. In general, creatinine in serum is found in a fairly constant ratio and its level is proportional to muscle mass. In the kidney, creatinine excretion is mainly through glomerular

filtration and tubular secretion, since almost no creatinine is reabsorbed through the renal tubules. Therefore, clinically, measured creatinine levels in blood and urine are often used to calibrate the metabolite content of these body fluids to reduce errors caused by sampling, instrumentation, and so on. Our results show that creatinine calibration of our ^1H NMR data was better than total normalization to discriminate between patients before and after ALSSs treatment. In addition, our retrospective analyses indicate that the lactate:creatinine ratio in serum is negatively correlated with length of survival time of patients with liver failure. Our results indicate that higher lactate:creatinine ratios are associated with earlier deaths, and this finding suggests that the lactate:creatinine ratio can be used as a biomarker for the prognosis of liver failure.

PTA is commonly used in the clinic as an indicator to assess coagulation function. PTA significantly decreases when hepatocytes are severely damaged or necrotic. The rate of decline in PTA increases with the severity of hepatocyte damage, and the decline in PTA is positively correlated with the severity of the liver failure. Studies have shown that the prognosis is poor for liver failure patients with low PTA [44, 45]. Therefore, in clinical practice, PTA data are often used to predict and evaluate the therapeutic effect of ALSSs [46-49] and to offer prognoses for patients with liver failure. Our findings show that the lactate:creatinine ratio is negatively correlated with PTA ($r = -0.186$, $p = 0.025$, Figure 4b). Furthermore, the retrospective cohort study suggests that data regarding the lactate:creatinine ratio offer greater diagnostic efficacy to draw up accurate prognoses of likely survival periods for liver-failure patients compared with serum PTA or lactate. The results indicate that the lactate:creatinine ratio might be useful as a biomarker to assess the likely effect of ALSSs treatment in liver failure patients with the early to middle stage.

Although the serum lactate:creatinine ratio were screened and validated for the possible prediction of the survival outcomes of liver failure patients treated by ALSSs, certain limitations existed in our study. First, we did not classify the liver failure patients caused by the hepatitis B, hepatitis C virus, and drug-induced hepatitis. Second, whether different artificial liver treatment modes will affect the lactate:creatinine ratios and survival outcomes of liver failure patients has not been studied. Finally, more large-scale prospective multicenter studies are needed to verify the applicability of the lactate:creatinine ratio as a serum prognostic markers to predict the outcome for liver-failure patients treated by ALSSs.

Conclusions

Few studies have focused on the screening of potential prognostic markers in serum to predict the survival outcomes of patients with liver failure after ALSSs treatment. Through use of ^1H NMR metabonomics in this study, the lactate:creatinine ratio in serum has been found to be a good indicator to distinguish between patients with liver failure before and after ALSSs treatment. Further two retrospective analysis showed that the lactate:creatinine ratio can be used as a novel marker to evaluate the likely prognosis for liver-failure patients who have undertaken ALSSs treatment, and that this marker may offer superior results to those offered by measurement of serum lactate or PTA in the early to middle stage liver-failure patients. Application of the novel marker in predicting the outcomes of ALSSs treatment in

liver-failure patients with early to middle stage may help avoid unnecessary ALSSs and perform liver transplantation.

Abbreviations

ALSSs: artificial liver support systems; AUROC: the area under a receiver-operating characteristic curve; PTA: prothrombin time activity; PP: plasma perfusion; HP: hemoperfusion; PE: plasma exchange; PDF: plasma diafiltration; ALF: acute liver failure; HPLC-MS/MS: high-pressure liquid chromatography-mass spectrometry/mass spectrometry; ¹HNMR: proton nuclear magnetic resonance spectroscopy; CLF: chronic liver failure; TSP: sodium salt of (trimethylsilyl)-propionic-2,2,3,3-d₄ acid; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; INR: international normalized ratio; MELD: the model for end-stage liver disease. PBS: phosphate-buffered saline; PCA: principal component analysis; PLS-DA: partial least squares discriminant analysis. ROC: receiver-operating characteristics.

Declarations

Ethics approval and consent to participate

All experimental protocols were approved by the committee of the Second Affiliated Hospital of Nanchang University.

Consent for publication

Not applicable.

Availability of data and materials

The data are included in the article as figures and Tables.

Competing interests

The authors declare that they have no competing interests.

Funding

The work was supported by a grant from the Key research and development program of Jiangxi Province (No. 20181BBG70029 and 20171BBG70086).

Authors' contributions

Baogang Xie and Shuilin Sun accomplished the conception and design of the research;

Ling Chen, Guanlin Zhou and Lili Guo performed the experiments; Ruijin geng, Yunquan Cai and Wenlong Yang analyzed and interpreted the data; Baogang Xie, Ling Chen and Shuilin Sun drafted the manuscript;

All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Tables

Table 1. The characteristics of patients with liver failure before and after ALSSs treatment (mean $\pm$ S.E.)

Variables	pre-treatment (n=57)	post-treatment (n=57)	P-value	AUROC
Tbil (μmol/L)	346.95 ± 14.51	281.44 ± 23.42	0.019	0.640
Cr (μmol/L)	74.15 ± 4.18	82.93 ± 6.92	0.272	0.544
INR	2.29 ± 0.14	2.05 ± 0.11	0.190	0.621
MELD	26.35 ± 0.49	23.61 ± 0.86	0.007	0.634
PTA(%)	28.87 ± 0.98	38.21 ± 2.44	0.001	0.624
ALB(g/L)	31.67 ± 0.48	32.18 ± 0.44	0.438	0.536
ALT(IU/L)	779.94 ± 110.76	71.13 ± 12.12	0.000	0.938
AST(IU/L)	659.63 ± 92.50	90.62 ± 9.10	0.000	0.888
PLT(10 ⁹ /L)	110.71 ± 4.97	87.81 ± 5.91	0.004	0.689

Note: Tbil: total bilirubin; Cr: creatinine; INR: international normalised ratio; MELD: model for end-stage liver diseases; PTA: prothrombin activity; ALB: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PLT: blood platelet.

Table 2. Relative quantitative data of metabolite in each group by creatinine calibration

Metabolites	$\delta^1\text{H}$	Integral interval	Relative levels in serum (Mean $\pm$ S.E.)				
			Health	pre-treatment	post-treatment	VIP	AUROC
Leucine	0.95(t)	0.950-0.980	6.93 $\pm$ 0.33 ^{ab}	6.02 $\pm$ 0.21	5.22 $\pm$ 0.25 ^c	1.9797	0.640
Isoleucine	1.02(d)	1.005-1.025	9.20 $\pm$ 0.23 ^{ab}	6.30 $\pm$ 0.22	5.37 $\pm$ 0.17 ^c	1.5018	0.659
Valine	1.03(d)	1.030-1.055	7.53 $\pm$ 0.17 ^{ab}	4.56 $\pm$ 0.16	4.09 $\pm$ 0.19	1.2042	0.609
3-Hydroxybutyrate	1.20(t)	1.170-1.210	4.75 $\pm$ 0.21	5.15 $\pm$ 0.33	5.31 $\pm$ 0.29	0.9054	0.561
Alanine	1.48(d)	1.470-1.510	12.78 $\pm$ 0.47 ^{ab}	8.93 $\pm$ 0.40	7.64 $\pm$ 0.39 ^c	1.2711	0.627
Acetate	1.92(s)	1.920-1.930	15.61 $\pm$ 0.59 ^{ab}	2.38 $\pm$ 0.10	2.03 $\pm$ 0.09 ^c	1.1692	0.614
Pyruvic acid	2.40(s)	2.376-2.380	0.02 $\pm$ 0.00 ^{ab}	0.03 $\pm$ 0.00	0.03 $\pm$ 0.00	0.5209	0.577
Glutamate	2.34(m)	2.320-2.360	12.05 $\pm$ 0.46	12.03 $\pm$ 0.44	11.92 $\pm$ 0.49	0.0585	0.528
Glutamine	2.44(m)	2.400-2.500	15.12 $\pm$ 0.49 ^{ab}	11.68 $\pm$ 0.46	11.86 $\pm$ 0.52	0.1191	0.527
Citrate	2.54(d)	2.540-2.580	5.08 $\pm$ 0.18 ^{ab}	2.74 $\pm$ 0.09	3.06 $\pm$ 0.14	1.3315	0.574
creatine	3.19(s)	3.200-3.215	0.58 $\pm$ 0.02	0.55 $\pm$ 0.02	0.58 $\pm$ 0.03	0.4012	0.535
creatinine	3.00(s)	3.025-3.035	0.6 $\pm$ 0.04 ^{ab}	1.41 $\pm$ 0.06	1.19 $\pm$ 0.06 ^c	1.0395	0.633
Choline	4.04(s)	4.040-4.060	1.17 $\pm$ 0.05 ^b	0.51 $\pm$ 0.02	0.51 $\pm$ 0.02	0.2076	0.510
Phosphorylcholine	3.20(s)	3.200-3.210	2.07 $\pm$ 0.09 ^{ab}	1.53 $\pm$ 0.07	1.29 $\pm$ 0.06 ^c	0.8916	0.625
Carnitine	3.20(s)	3.210-3.230	7.26 $\pm$ 0.28 ^{ab}	3.90 $\pm$ 0.15	3.28 $\pm$ 0.09	0.7041	0.659
Betaine	3.26(s)	3.264-3.274	10.52 $\pm$ 0.28 ^{ab}	4.37 $\pm$ 0.26	4.13 $\pm$ 0.27	0.3550	0.551

Taurine	3.42(t)	3.405-3.440	7.34 ± 0.32 ^{ab}	12.19 ± 0.99	8.17 ± 0.64 ^c	1.5859	0.644
Glycine	3.56(s)	3.560-3.568	2.18 ± 0.14 ^{ab}	3.03 ± 0.14	2.62 ± 0.13 ^c	0.8306	0.616
β-glucose	5.23(d)	5.230-5.250	3.32 ± 0.13 ^{ab}	2.76 ± 0.15	3.17 ± 0.24	1.2037	0.551
Tyrosine	6.89(d)	6.880-6.920	2.16 ± 0.06 ^{ab}	2.66 ± 0.10	2.60 ± 0.11	0.4837	0.535
Phenylalanine	7.42(m)	7.410-7.440	3.35 ± 0.12	3.31 ± 0.12	3.16 ± 0.12	0.016	0.538
Formate	8.45(s)	8.455-8.465	0.55 ± 0.03	0.48 ± 0.02	0.52 ± 0.02	1.2400	0.546
Lactate	4.10(m)	4.100-4.140	6.65 ± 0.35 ^{ab}	12.78 ± 0.59	10.98 ± 0.49 ^c	1.1346	0.650

Note: a denotes the difference of health and ALSSs pre-treatment; b denotes the difference of health and ALSSs post-treatment; c denotes the difference of ALSSs pre-treatment and post-treatment. ^{ab} Significant difference (p < 0.05) of the health group comparing with pre-treatment group; ^c Significant difference (p < 0.05) of the pre-treatment group comparing with post-treatment group.

Table 3. Retrospective analysis data - basic clinical information pre-treatment artificial liver in patients with liver failure

Varaiables	Survival group (n=48, mean ± S.E.)	Three-month death group (n=24, mean ± S.E.)	One-month death group (n=33, mean ± S.E.)
Lactate (mmol/L)	2.61 ± 0.19 ^a	3.39 ± 0.31 ^b	4.33 ± 0.35
Tbil (μmol/L)	335.23 ± 15.05 ^a	392.77 ± 53.04	395.24 ± 29.07
Dbil (μmol/L)	256.78 ± 16.29	274.90 ± 34.13	239.57 ± 22.55
PTA(%)	26.11 ± 1.22	24.23 ± 1.98 ^b	17.50 ± 1.66
Creatinine (μmol/L)	75.66 ± 6.63	81.06 ± 12.59 ^b	90.77 ± 10.73
CRP (mg/L)	7.76 ± 0.81	8.33 ± 0.74	8.91 ± 1.02
Lactate/creatinine (*1000)	35.41 ± 3.12 ^a	39.52 ± 1.53 ^b	51.31 ± 5.64

Note: Tbil, total bilirubin; Dbil,direct bilirubin; prothrombin time activity; CRP,C-reaction protein. ^a Significant difference ($p < 0.05$) of the survival group comparing with three-month death group; ^b Significant difference ($p < 0.05$) of the three-month death group comparing with one-month death group.

Figures

Figure 1

Scores plots of PCA and OPLS-DA generated by data from 1HNMR spectroscopy. 1, 2, and 3 represent the healthy control, pre-ALSSs treatment and post-ALSSs treatment groups. (a) Scores plot of PCA by total

normalization; (b) Scores plot of PCA by creatinine normalization; (c) Scores plot of OPLS-DA by total normalization; (d) Scores plot of OPLS-DA by creatinine normalization.

Figure 2

Loadings Plot of OPLS-DA and metabolic pathways deduced from 1HNMR data, indicating which of 21 metabolites are consistent with the metabolites of Table 2. (a) Loadings plot of OPLS-DA generated by 1HNMR data; (b) Significantly altered metabolic pathways post-treatment with ALSSs. All matched pathways are shown according to p-values from the pathway enrichment analysis (y-axis) and pathway impact values from the pathway topology analysis (x-axis) with the most impacted pathways colored in red.

Figure 3

The alteration of potential serum markers in patients with liver failure who were treated by ALSSs.

Figure 4

(a) Serum lactate:creatinine ratios in groups of patients with liver failure who survived more than six months after treatment, died within three months or died within one month of treatment. # denotes $p < 0.05$ between groups by independent sample t-test. (b) Correlation plot of PTA – serum lactate:creatinine ratio in liver-failure patients ($n = 105$).

Figure 5

ROC analysis of lactate:creatinine ratio, serum lactate and PTA as potential diagnostic markers for likely survival periods of patients with liver failure after ALSSs treatment (n=81).

Supplementary Files

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