

ORIGINAL ARTICLE

Screening and Validation Potential Therapeutic Marker of Early to Middle Stage ACLF Patients Treated by ALSSs

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SUMMARY

Background: Artificial liver support systems (ALSSs) are important approaches for treating acute-on-chronic liver failure (ACLF) patients. Few studies have investigated potential serum therapeutic markers of ACLF patients treated by ALSSs.

Methods: Serum samples were obtained from 57 early to middle stage ACLF patients before and after ALSSs treatment and analyzed by metabolomics. The diagnostic values were evaluated by the area under receiver-operating characteristic curve (AUROC). A retrospective cohort analysis was further employed.

Results: Metabolomic study showed that serum ratios of lactate: creatinine in ACLF patients is significantly altered and then restored to normal levels after ALSSs treatment. A retrospective cohort analysis (n = 47) validated that the lactate: creatinine ratio of ACLF patients in the one-month death group remained unchanged after ALSSs treatment, but fell markedly in the survival group with AUROC of 0.682 for diagnosis of survival group from death group, which is a more sensitive measure than measures of prothrombin time activity (PTA) to evaluate the therapeutic effect of ALSSs treatment.

Conclusions: Our results demonstrated the greater the decline in the serum lactate: creatinine ratio with better effective treatments of ALSSs in the ACLF patients with early to middle stage, which presents a potential therapeutic biomarker of ALSSs treatment.

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KEYWORDS

Metabolomics, serum lactate:creatinine ratio, artificial liver support systems, acute-on-chronic liver failure

LIST OF ABBREVIATIONS

ALSSs - artificial liver support systems
ACLF - acute-on-chronic liver failure
AUROC - area under a receiver-operating characteristic curve
PTA - prothrombin time activity
PP - plasma perfusion
HP - hemoperfusion

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PE - plasma exchange
 PDF - plasma diafiltration
 ALF - acute liver failure
 HPLC-MS/MS - high-pressure liquid chromatography-mass spectrometry/mass spectrometry
 H NMR - proton nuclear magnetic resonance spectroscopy
 AST - aminotransferase
 ALT - alanine aminotransferase
 ALB - albumin
 INR - international normalized ratio
 MELD - The model for end-stage liver disease
 TBIL - total bilirubin
 PCA - principal component analysis
 OPLS-DA - orthogonal partial least squares discrimination analysis

INTRODUCTION

Acute-on-chronic liver failure (ACLF) 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]. ALSSs are mainly categorized into three types: non-bioartificial, bioartificial, and hybrid ALSSs. Non-bioartificial ALSSs have been widely used in clinical practice. They involve plasma perfusion (PP), hemoperfusion (HP), plasma exchange (PE), plasma diafiltration (PDF), etc. Studies have shown that the efficiency of ALSSs therapy falls as the severity of liver disease increases; hence, the efficiency of ALSSs therapy for early, middle, and late-stage ACLF has been shown to be 87.5%, 61.8% and 17.0%, respectively [5]. These findings indicate that 12.5% to 38.2% of patients with early and middle-stage ACLF are not cured by ALSSs and should be immediately recommended for liver transplantation. However, physicians need to be able to recognize patients for whom the use of ALSSs may be a life saver or ineffective. Therefore, the discovery of novel serum therapeutic 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 [6-9]. 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 lipo-

protein 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 [10]. Recent studies showed that alpha-fetoprotein, serum sodium, lactate, arterial blood ammonia, and phosphate were related to the prognosis of liver failure [11-14]. However, no study has yet focused on the screening of serum therapeutic markers of patients with ACLF who have been treated by ALSSs.

The present study adopted ^1H NMR-based metabolomics for the identification of differential metabolites in patients with ACLF 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. A retrospective cohort analysis was further employed to validate the markers for the evaluation of the prognosis of ALSSs treatment for early to middle stage ACLF patients. The flow of the participants for the study was presented as Figure 1.

MATERIALS AND METHODS

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 $\text{K}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ was 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-d₄ acid (TSP) was purchased from Sigma (USA).

Patients with ALSSs treatment and collection of serum samples

Fifty-seven patients with early to middle stage ACLF ($20\% < \text{PTA} < 40\%$) 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 ACLF patients. All samples were provided by the Second Affiliated Hospital of Nanchang University after approval from the ethics review committee on March 5th, 2016. Blood samples were taken between 6:00am and 8:30am after overnight fasting to eliminate any dietary interference [15]. The blood samples were centrifuged for 15 minutes at 4,000 rpm within four hours of collection, and the serum samples were separated and stored in a refrigerator at $-80\text{ }^\circ\text{C}$ until analysis [16]. The patients' demographic information and laboratory data were collected or calculated. These included the levels in serum of aspartate aminotransferase (AST), alanine aminotransfer-

ase (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).

Sample preparation and ^1H NMR acquisition

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

The ^1H NMR data were pretreated with the 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 [17].

^1H NMR data normalization

The ^1H NMR intensity of the signal for each sample was normalized by total normalization [17] and creatinine normalization. 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 4.05 ppm to 4.07 ppm.

Survival of ACLF after ALSSs treatment

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

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 data were analyzed using SPSS 23.0 software (IBM Corp., Armonk, NY, USA).

Ethical approval

All experimental protocols were performed in accordance with the principles of the Declaration of Helsinki

ethical standards, and were approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University. The written informed consent was obtained from healthy people (control group) and patients with ACLF for metabonomic study. In the retrospective study, the data including serum creatinine and lactate were collected from electronic medical records. The written informed consent was waived by the Ethics Committee of the Second Affiliated Hospital of Nanchang University due to the retrospective analysis.

RESULTS

General characteristics of the patients for ^1H NMR analysis

The information regarding patients with early to middle stage ACLF is provided in Table 1. 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 treatment group satisfied the diagnostic criteria for ACLF 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.

Principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) analysis on ^1H NMR data

In this paper, ^1H NMR data was processed by total normalization and creatinine normalization before multivariate analysis. The principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) scores plots are shown in Figure 2. The results showed that the health group, pre-treatment and post-treatment of ALSSs groups were overlapped in the PCA scores plots by total normalization (Figure 2a). However, these groups could be partly separated by group in the OPLS-DA scores plot generated by creatinine normalization (Figure 2d). The established OPLS-DA model evaluated its accuracy and predictability with three parameters (R2X, R2Y and Q2), when R2Ycum and Q2cum are > 0.5 , the model is

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

Variables	Pre-treatment (n = 57)	Post-treatment (n = 57)	p-value	AUROC
Tbil ($\mu\text{mol/L}$)	346.95 \pm 14.51	281.44 \pm 23.42	< 0.05	0.640
Cr ($\mu\text{mol/L}$)	74.15 \pm 4.18	82.93 \pm 6.92	> 0.05	0.544
INR	2.29 \pm 0.14	2.05 \pm 0.11	> 0.05	0.621
MELD	26.35 \pm 0.49	23.61 \pm 0.86	< 0.01	0.634
PTA (%)	28.87 \pm 0.98	38.21 \pm 2.44	< 0.01	0.624
ALB (g/L)	31.67 \pm 0.48	32.18 \pm 0.44	> 0.05	0.536
ALT (IU/L)	779.94 \pm 110.76	71.13 \pm 12.12	< 0.01	0.938
AST (IU/L)	659.63 \pm 92.50	90.62 \pm 9.10	< 0.01	0.888
PLT ($10^9/\text{L}$)	110.71 \pm 4.97	87.81 \pm 5.91	< 0.01	0.689

Tbil - total bilirubin, Cr - creatinine, INR - international normalized ratio, MELD - model for end-stage liver diseases, PTA - prothrombin activity, ALB - albumin, AL - alanine aminotransferase, AST - aspartate aminotransferase, PLT - blood platelet.

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

Variable	Metabolite	$\delta^1\text{H}$	Integral interval	Relative levels in serum (mean \pm S.E.)				
				health	pre-treatment	post-treatment	VIP	AUROC
Var 1	Leucine	0.95 (t)	0.950 - 0.980	6.93 \pm 0.33 ^{a, b}	6.02 \pm 0.21	5.22 \pm 0.25 ^c	1.9797	0.640
Var 2	Isoleucine	1.02 (d)	1.005 - 1.025	9.20 \pm 0.23 ^{a, b}	6.30 \pm 0.22	5.37 \pm 0.17 ^c	1.5018	0.659
Var 3	Valine	1.03 (d)	1.030 - 1.055	7.53 \pm 0.17 ^{a, b}	4.56 \pm 0.16	4.09 \pm 0.19	1.2042	0.609
Var 4	3-Hydroxy-butyrate	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
Var 5	Alanine	1.48 (d)	1.470 - 1.510	12.78 \pm 0.47 ^{a, b}	8.93 \pm 0.40	7.64 \pm 0.39 ^c	1.2711	0.627
Var 6	Acetate	1.92 (s)	1.920 - 1.930	15.61 \pm 0.59 ^{a, b}	2.38 \pm 0.10	2.03 \pm 0.09 ^c	1.1692	0.614
Var 7	Pyruvic acid	2.40 (s)	2.376 - 2.380	0.02 \pm 0.00 ^{a, b}	0.03 \pm 0.00	0.03 \pm 0.00	0.5209	0.577
Var 8	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
Var 9	Glutamine	2.44 (m)	2.400 - 2.500	15.12 \pm 0.49 ^{a, b}	11.68 \pm 0.46	11.86 \pm 0.52	0.1191	0.527
Var 10	Citrate	2.54 (d)	2.540 - 2.580	5.08 \pm 0.18 ^{a, b}	2.74 \pm 0.09	3.06 \pm 0.14	1.3315	0.574
Var 11	Choline	3.20 (s)	3.210 - 3.230	7.26 \pm 0.28 ^{a, b}	3.90 \pm 0.15	3.28 \pm 0.09	0.7041	0.659
Var 12	Betaine	3.23 (s)	3.264 - 3.274	10.52 \pm 0.28 ^{a, b}	4.37 \pm 0.26	4.13 \pm 0.27	0.3550	0.551
Var 13	Carnitine	3.25 (s)	3.210 - 3.230	7.26 \pm 0.28 ^{a, b}	3.90 \pm 0.15	3.28 \pm 0.09	0.7041	0.659
Var 14	Glycine	3.56 (s)	3.560 - 3.568	2.18 \pm 0.14 ^{a, b}	3.03 \pm 0.14	2.62 \pm 0.13 ^c	0.8306	0.616
Var 15	Taurine	3.42 (t)	3.405 - 3.440	7.34 \pm 0.32 ^{a, b}	12.19 \pm 0.99	8.17 \pm 0.64 ^c	1.5859	0.644
Var 16	Creatine	3.98 (s)	3.971 - 3.981	1.17 \pm 0.05 ^b	0.51 \pm 0.02	0.51 \pm 0.02	0.2076	0.510
Var 17	β -glucose	5.23 (d)	5.230 - 5.250	3.32 \pm 0.13 ^{a, b}	2.76 \pm 0.15	3.17 \pm 0.24	1.2037	0.551
Var 18	Tyrosine	6.89 (d)	6.880 - 6.920	2.16 \pm 0.06 ^{a, b}	2.66 \pm 0.10	2.60 \pm 0.11	0.4837	0.535
Var 19	Phenylalanine	7.42 (m)	7.410 - 7.440	3.35 \pm 0.12	3.31 \pm 0.12	3.16 \pm 0.12	0.016	0.538
Var 20	Formate	8.45 (s)	8.455 - 8.465	0.55 \pm 0.03	0.48 \pm 0.02	0.52 \pm 0.02	1.2400	0.546
Var 21	Lactate	4.10 (m)	4.100 - 4.140	6.65 \pm 0.35 ^{a, b}	12.78 \pm 0.59	10.98 \pm 0.49 ^c	1.1346	0.650

^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.

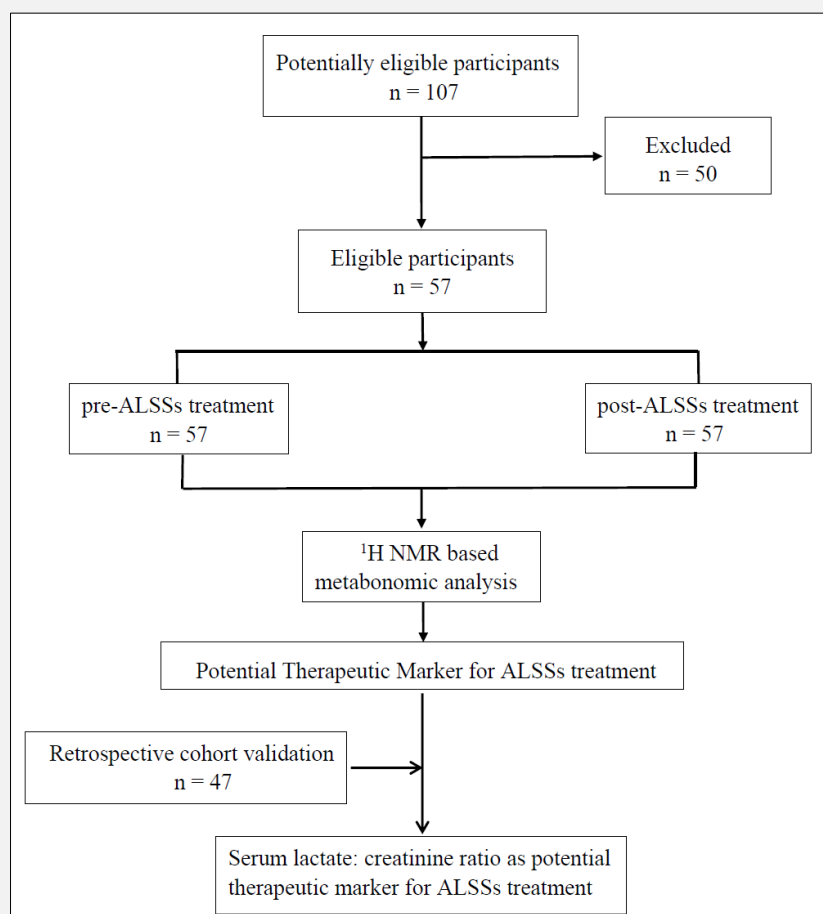


Figure 1. Flow of participant selection for the study.

accurate [18]. The values of R^2_{Xcum} , R^2_{Ycum} , and Q^2 in the OPLS-DA model based on total normalization are 0.302, 0.408, and 0.378, respectively, whereas those in the model based on creatinine calibration are 0.512, 0.428, and 0.414. Thus, the OPLS-DA model based on creatinine normalization has good predictive power. The results of the sevenfold cross-validation and permutation test (200 times) show an R^2 of 0.488 and Q^2 of -0.13, indicating that the model is not over-fitted and the model is effective.

Changes of metabolites in ACLF 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 [17]. 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 3 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 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,

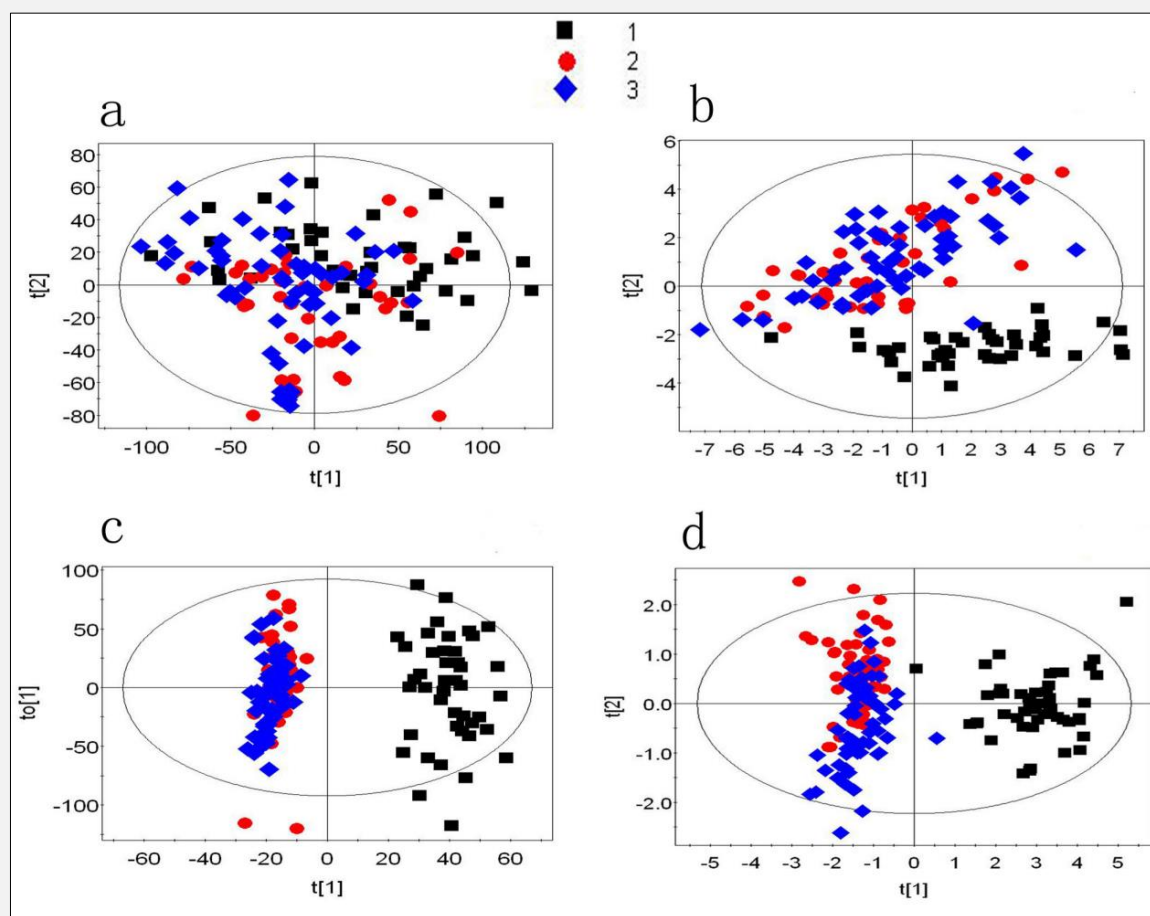


Figure 2. Scores plots of PCA and OPLS-DA generated by data from ^1H NMR spectroscopy.

The colored dots are designated as 1, 2, and 3 and 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.

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.

Use of lactate:creatinine ratio and PTA to predict one-month survival of ACLF patients

A total of 47 inpatients with ACLF who received ALSSs treatment, including 42 males and five females, from ages 26 to 66 years old, were employed for retrospective cohort analysis in this study. Of these, 17 survived for more than six months, while 30 died within one month after ALSSs treatment. Our results showed that the lactate-creatinine ratio in the death group remained unchanged after ALSSs treatment (without sta-

tistical differences). However, the lactate-creatinine ratio in the survival group fell markedly (0.050 ± 0.005 vs. 0.030 ± 0.002) after ALSSs treatment ($p < 0.05$). In comparison, the serum PTA levels in both the death group and survival group obviously increased, but no statistical differences of those were observed after ALSSs treatment (Figure 5).

The AUROC (95% confidence interval) of the lactate-creatinine ratio after ALSSs treatment for diagnosis of the survival group from the death group was 0.689 (0.502 - 0.862), the sensitivity was 44.8%, and the specificity was 82.1%. Whereas, The AUROC of PTA after ALSSs treatment for diagnosis of those was 0.591 (0.40 - 0.783), the sensitivity was 40.7%, and the specificity was 76.8% (Figure 6). These results indicate that the lactate-creatinine ratio may be more reliable than mea-

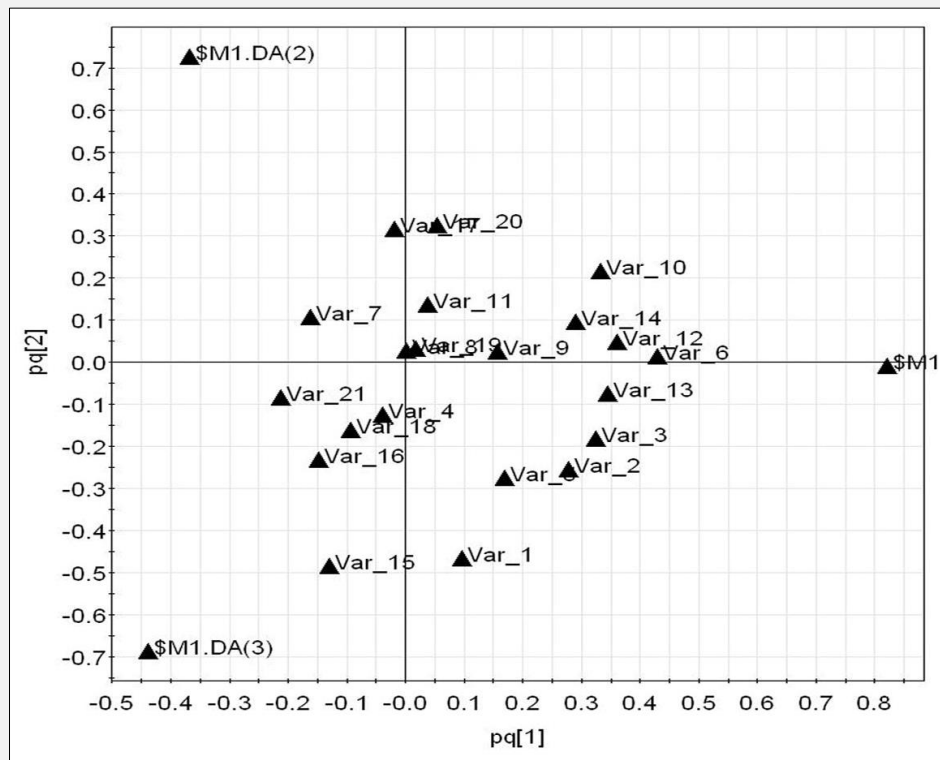


Figure 3. Loadings plot of OPLS-DA generated by ¹H NMR data, indicating which of 21 metabolites are consistent with the metabolites of Table 2.

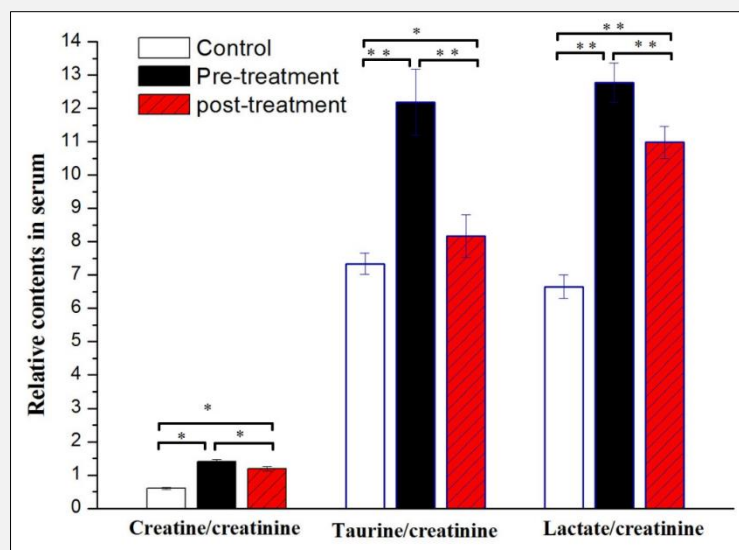


Figure 4. The alteration of potential serum markers in ACLF patients who were treated by ALSSs.

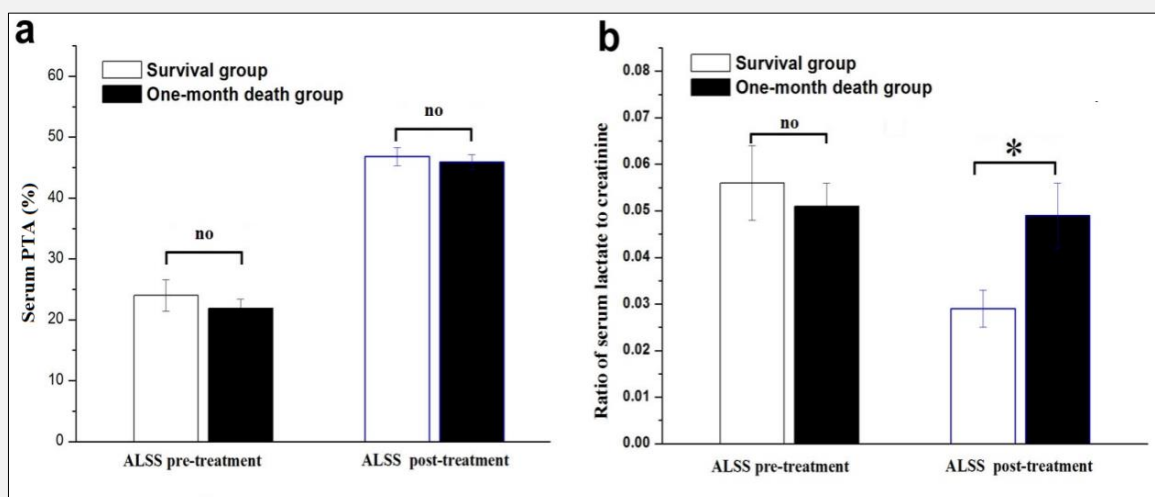


Figure 5. The alterations of serum lactate:creatinine ratio and PTA in ACLF patients before and after ALSSs treatment and in the survival group (n = 17) and the death group (n = 30).

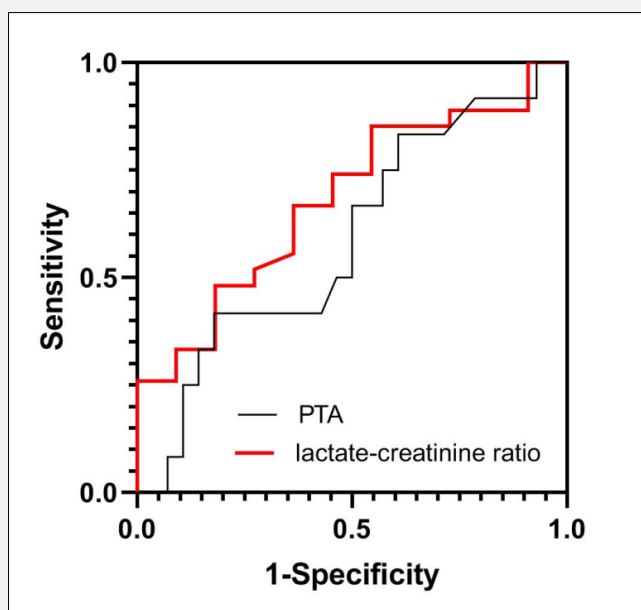


Figure 6. ROC analysis of lactate:creatinine ratio, serum lactate, and PTA as potential diagnostic markers for likely survival periods of patients with ACLF after ALSSs treatment (n = 47).

sures of PTA to evaluate the therapeutic effect of ALSSs treatment in ACLF patients.

DISCUSSION

ACLF develops rapidly and leads to high short-term mortality. Studies [19-21] have shown that ALSSs therapy can remove harmful substances through mechanical, physical and chemical or biological devices *in vitro* 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. Previous study [5] showed that 12.5 - 38.2% of early and middle-stage ACLF patients should be immediately recommended for liver transplantation. Hu P [7] found that levels of ALB, creatinine, TBIL, INR, PTA, and ALT in serum changed significantly before and after ALSSs treatment. Hu S. et al. [9] 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 ACLF patients, whereas levels of conjugated bile acids increased significantly. These metabolites are considered to be common biomarkers of ACLF [22]. In addition, alpha-fetoprotein, serum sodium, lactate, arterial blood ammonia, and phosphate have been related to the prognosis of liver failure [11-14]. To date, very few studies have focused on the screening of potential serum therapeutic markers of ACLF patients treated by ALSSs.

In the present study, serum metabolites related to ALSSs treatment in early and middle-stage ACLF patients 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 [23,24]. 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, 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 signalling [25]. In addition, our re-

sults 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 [26]. Patients with ACLF 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 [27]. 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 [28]. 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. Our results suggest that the lactate:creatinine ratio can be used as a potential therapeutic biomarker of early to middle stage ACLF. 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 ACLF. Studies have shown that the prognosis is poor for ACLF patients with low PTA [29,30]. Therefore, in clinical practice, PTA data are often used to predict and evaluate the therapeutic effect of ALSSs [30-32] and to offer prognosis for ACLF patients. The retrospective cohort study suggests that the lactate-creatinine ratio in the survival ACLF group fell markedly, but no statistical differences of serum PTA levels were observed in the death group from the survival group after ALSSs treatment, suggesting that the lactate:creatinine ratio offers greater diagnostic efficacy to draw up accurate prog-

noses of likely survival periods for early to middle stage ACLF patients compared with serum PTA.

Although the serum lactate:creatinine ratio were screened and validated for the possible prediction of the survival outcomes of ACLF patients treated by ALSSs, certain limitations existed in our study. First, we did not classify the ACLF 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 ratio and survival outcomes of ACLF 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 therapeutic marker of ACLF patients treated by ALSSs.

CONCLUSION

Few studies have focused on the screening of potential serum therapeutic markers of ACLF patients after ALSSs treatment. Through the use of ¹H NMR metabolomics in this study, the lactate:creatinine ratio in serum has been found to be a good indicator to distinguish between ACLF patients before and after ALSSs treatment. Further a retrospective analysis showed that the lactate:creatinine ratio can be used as a novel marker to evaluate the likely prognosis for early to middle stage ACLF patients who have undertaken ALSSs treatment. This marker may offer superior results to those offered by measurement of PTA. Application of the marker in predicting the outcomes of ALSSs treatment in early to middle stage ACLF patients may help avoid unnecessary ALSSs and perform liver transplantation.

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Data Availability:

The study data presented may be made available from the corresponding author upon reasonable request.

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Declaration of Interest:

The authors declare that they have no competing interests.

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