Serum Lactate:Creatinine Ratio as a Potential Therapeutic Markers of Liver Failure Patients Treated by Artificial Liver Support Systems

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Abstract

Artificial liver support systems (ALSSs) have been recommended as important approaches for treating liver fuilure (LF) patients. However, very few studies have focused on the screening of potential serum therapeutic markers of LF patients treated by ALSSs. Here, serum samples were obtained from 57 LF patients before and after ALSSs treatment and analyzed by metabonomics. The results 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 area under a receiver-operating characteristic curve (AUROC) value (0.650), which was higher than that of the prothrombin time activity (PTA, 0.562). A retrospective analysis showed that serum lactate:creatinine ratios among the LF patient groups 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 LF patients showed that the lactate-creatinine ratio in the death group remained unchanged, but fell markedly in the survival group (0.052 ± 0.005 vs. 0.025 ± 0.002) after ALSSs treatment. In comparison, the serum PTA levels were no statistical differences of in both the death group and survival group after ALSSs treatment. The AUROC of serum lactate-creatinine ratio and PTA after ALSSs treatment for diagnosis of survival group from death group was 0.682 and 0.591 respectively. These results indicate that the serum lactate-creatinine ratio may be more reliable than measures of PTA to evaluate the therapeutic effect of ALSSs treatment in LF patients.

1. Introduction

Liver failure (LF) 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 LF 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 therapeutic markers to identify LF 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 LF 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 (^{1}HNMR) 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 LF [18-23]. However, no study has yet focused on the screening of serum therapeutic markers of patients with liver failure who have been treated by ALSSs.

The present study adopted ^{1}HNMR-based metabonomics for the identification of differential metabolites in patients with LF 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 further performed to validate the markers for the evaluation of the prognosis of ALSSs treatment for LF patients.

2. 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); $NaH_2PO_4{\cdot}2H_2O$, and $K_2HPO_4{\cdot}12H_2O$ 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).

2.2 Patients for ALSSs treatment and collection of serum samples

Fifty-seven patients with LF 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 LF 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).

2.3 Sample preparation and ^{1}HNMR 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 ^{1}HNMR analysis in a Bruker AvanceII-600 MHz spectrometer (Germany) at a temperature of 298K with nuclear overhauser enhancement spectroscopy pulse sequence.

The ^{1}HNMR 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 ^{1}HNMR datanormalization

The ^{1}HNMR intensity of the signal for each sample was normalized by total normalization [26] 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 4.05ppm to 4.07ppm.

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

The clinical data were collected regarding patients with LF who were undergoing ALSSs 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 ALSSs treatment with their survival data was determined.

2.6 Predictive survival of LF after ALSSs treatment by lactate:creatinine ratio

Another retrospective cohort study was conducted on patients with *LF* who had received ALSSs treatment at the Fifth People's Hospital of Ganzhou between January 2018 and January 2020. The patients with LF (PTA: <40%) before ALSSs treatment, and their serum creatinine was in the normal range (44-110 μmol/L) were selected for analysis. The clinical data were collected before and after ALSSs treatment and the patients' survival time 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).

2.8 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 LF 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.

3. Results

3.1 General characteristics of the patients for ^{1}HNMR analysis

The information regarding patients with LF 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 group satisfied the diagnostic criteria for LF 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± 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 analysis on ^{1}H NMR data

In this paper, ^{1}HNMR data was processed by total normalization and creatinine normalization before multivariate analysis. The PCA and OPLS-DA scores plots are shown in Fig.1. 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 (Fig.1a). However, these groups could be partly separated by group in the OPLS-DA scores plot generated by creatinine normalization (Fig.1d). The established OPLS-DA model evaluated its accuracy and predictability with three parameters (R^2X, R^2Y and Q^2Y), when $R^2Y(cum)$ and $Q^2(cum)$ are >0.5, the model is accurate[27]. The values of R^2Xcum, R^2Ycum, 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[28].

3.3 Changes of metabolites in LF patients after ALSSs treatment

A total of 21 serum metabolites in ^{1}HNMR 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 2 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 LF patients

Of 105 inpatients with LF, 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 LF patients

A total of 81 inpatients with LF 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 ALSSs treatment. Our results showed that the lactate-creatinine ratio in the death group remained unchanged after ALSSs treatment (without statistical differences). However, the lactate-creatinine ratio in the survival group fell

markedly (0.052 ± 0.005 vs. 0.025 ± 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 (Fig.5).

The AUROC (95% confidence intervals) of the lactate-creatinine ratio after ALSSs treatment for diagnosis of survival group from death group was 0.682 (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% (Fig.6). These results indicate that the lactate-creatinine ratio may be more reliable than measures of PTA to evaluate the therapeutic effect of ALSSs treatment in LF patients.

4. Discussion

LF develops rapidly and leads to high short-term mortality. Studies [6, 29-31] 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 LF patients, whereas levels of conjugated bile acids increased significantly. These metabolites are considered to be common biomarkers of LF [32]. 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 therapeutic markers of LF patients treated by ALSSs.

In the present study, serum metabolites related to ALSSs treatment were identified by ^{1}HNMR-based metabonomics. To eliminate or reduce errors in sampling, ^{1}HNMR data were processed by total normalization and creatinine calibration [33-36]. Our results show that processing of ^{1}HNMR data by creatinine calibration offered improved discrimination between patients after ALSSs treatment compared with total normalization. This was because the peak area of ^{1}HNMR was correlated with the concentrations of metabolites and the number of hydrogen molecules they contained [37, 38], 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 [39]. 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 [40]. Patients with LF 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 LF [41-43]. 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 [44]. 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 ^{1}HNMR 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 LF patients. 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 potential therapeutic biomarker of LF.

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 LF. Studies have shown that the prognosis is poor for LF patients with low PTA [45, 46]. Therefore, in clinical practice, PTA data are often used to predict and evaluate the therapeutic effect of ALSSs [47-50] and to offer prognoses for LF patients. Our findings show that the lactate:creatinine ratio is negatively correlated with PTA ($r = - 0.186$, $p = 0.025$, Figure 4b). Furthermore, a retrospective cohort study suggests that the lactate-creatinine ratio in the survival LF 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 offer greater diagnostic efficacy to draw up accurate prognoses of likely survival periods for LF patients compared with serum PTA.

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

5. Conclusions

Few studies have focused on the screening of potential serum therapeutic markers of LF patients after ALSSs treatment. Through use of ^{1}HNMR metabonomics in this study, the lactate:creatinine ratio in serum has been found to be a good indicator to distinguish between LF patients 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 LF patients who have undertaken ALSSs treatment, and that this marker may offer superior results to those offered by measurement of PTA. Application of the novel marker in predicting the outcomes of ALSSs treatment in LF patients 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; ^{1}HNMR: 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.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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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 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.

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Tables

Table 1.

The characteristics of patients with liver failure before and after ALSSs treatment (mean ± S.E.)

Varaiables	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; AL: alanine aminotransferase; AST: aspartate aminotransfera; PLT:blood platelet.

Table 2.

Relative quantitative data of metabolite in each group by creatinine calibration

Varible	Metabolite	δ^1H	Integral interval	Relative levels in serum (Mean ± S.E.)				
				Health	pre-treatment	post-treatment	VIP	AUROC
Var 1	Leucine	0.95(t)	0.950-0.980	6.93 ± 0.33^{ab}	6.02 ± 0.21	5.22 ± 0.25^{c}	1.9797	0.640
Var 2	Isoleucine	1.02(d)	1.005-1.025	9.20 ± 0.23^{ab}	6.30 ± 0.22	5.37 ± 0.17^{c}	1.5018	0.659
Var 3	Valine	1.03(d)	1.030-1.055	7.53 ± 0.17^{ab}	4.56 ± 0.16	4.09 ± 0.19	1.2042	0.609
Var 4	3-Hydroxybutyrate	1.20(t)	1.170-1.210	4.75 ± 0.21	5.15 ± 0.33	5.31 ± 0.29	0.9054	0.561
Var 5	Alanine	1.48(d)	1.470-1.510	12.78 ± 0.47^{ab}	8.93 ± 0.40	7.64 ± 0.39^{c}	1.2711	0.627
Var 6	Acetate	1.92(s)	1.920-1.930	15.61 ± 0.59^{ab}	2.38 ± 0.10	2.03 ± 0.09^{c}	1.1692	0.614
Var 7	Pyruvic acid	2.40(s)	2.376-2.380	0.02 ± 0.00^{ab}	0.03 ± 0.00	0.03 ± 0.00	0.5209	0.577
Var 8	Glutamate	2.34(m)	2.320-2.360	12.05 ± 0.46	12.03 ± 0.44	11.92 ± 0.49	0.0585	0.528
Var 9	Glutamine	2.44(m)	2.400-2.500	15.12 ± 0.49^{ab}	11.68 ± 0.46	11.86 ± 0.52	0.1191	0.527
Var 10	Citrate	2.54(d)	2.540-2.580	5.08 ± 0.18^{ab}	2.74 ± 0.09	3.06 ± 0.14	1.3315	0.574
Var 11	Choline	3.20(s)	3.210-3.230	7.26 ± 0.28^{ab}	3.90 ± 0.15	3.28 ± 0.09	0.7041	0.659
Var 12	Betaine	3.23(s)	3.264-3.274	10.52 ± 0.28^{ab}	4.37± 0.26	4.13 ± 0.27	0.3550	0.551
Var 13	Carnitine	3.25(s)	3.210-3.230	7.26 ± 0.28^{ab}	3.90 ± 0.15	3.28 ± 0.09	0.7041	0.659
Var 14	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
Var 15	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

Var 16	Creatine	3.98(s)	3.971-3.981	1.17 ± 0.05[b]	0.51 ± 0.02	0.51 ± 0.02	0.2076	0.510
Var 17	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
Var 18	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
Var 19	Phenylalanine	7.42(m)	7.410-7.440	3.35 ± 0.12	3.31 ± 0.12	3.16 ± 0.12	0.016	0.538
Var 20	Formate	8.45(s)	8.455-8.465	0.55 ± 0.03	0.48 ± 0.02	0.52 ± 0.02	1.2400	0.546
Var 21	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

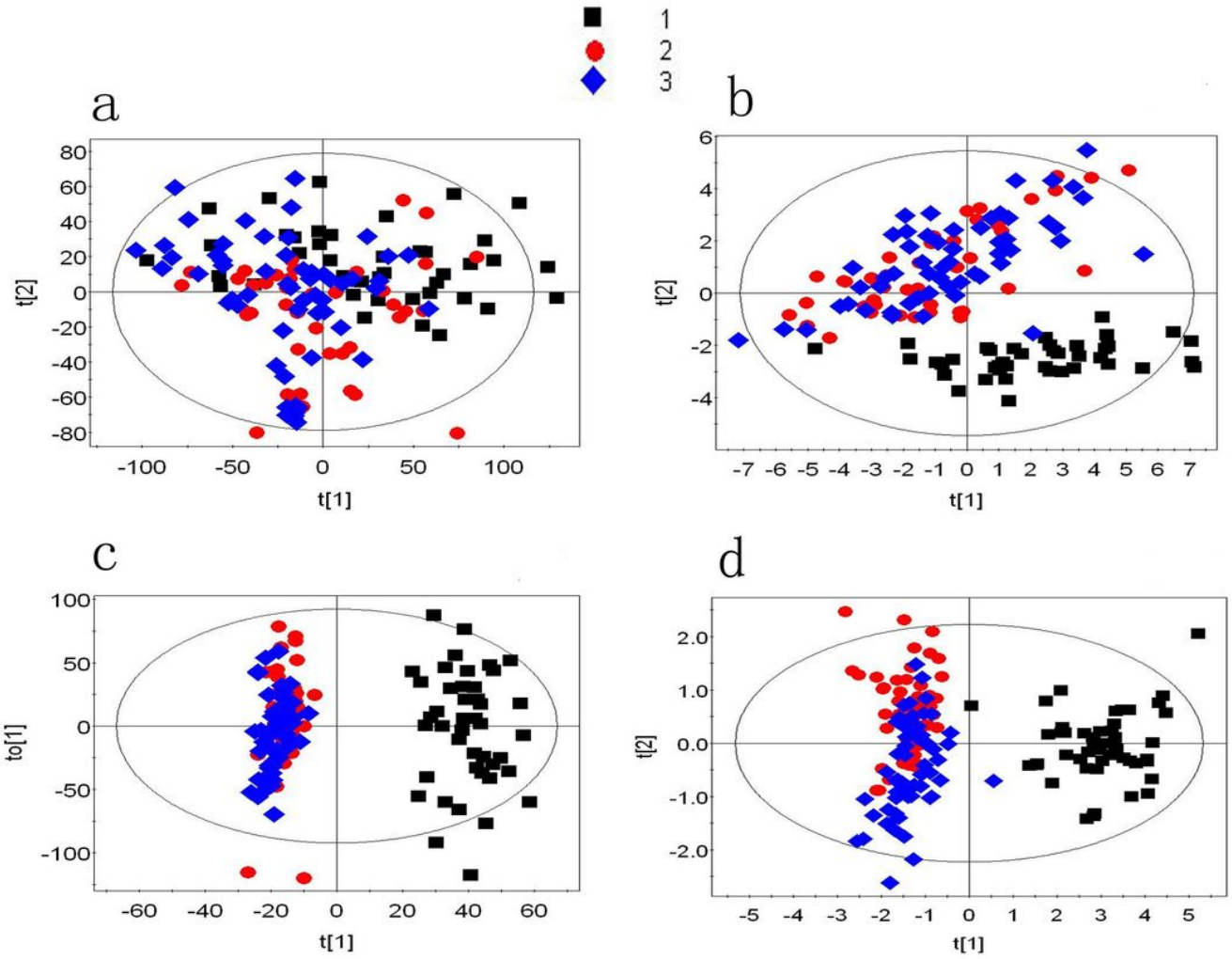

Figure 1

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.

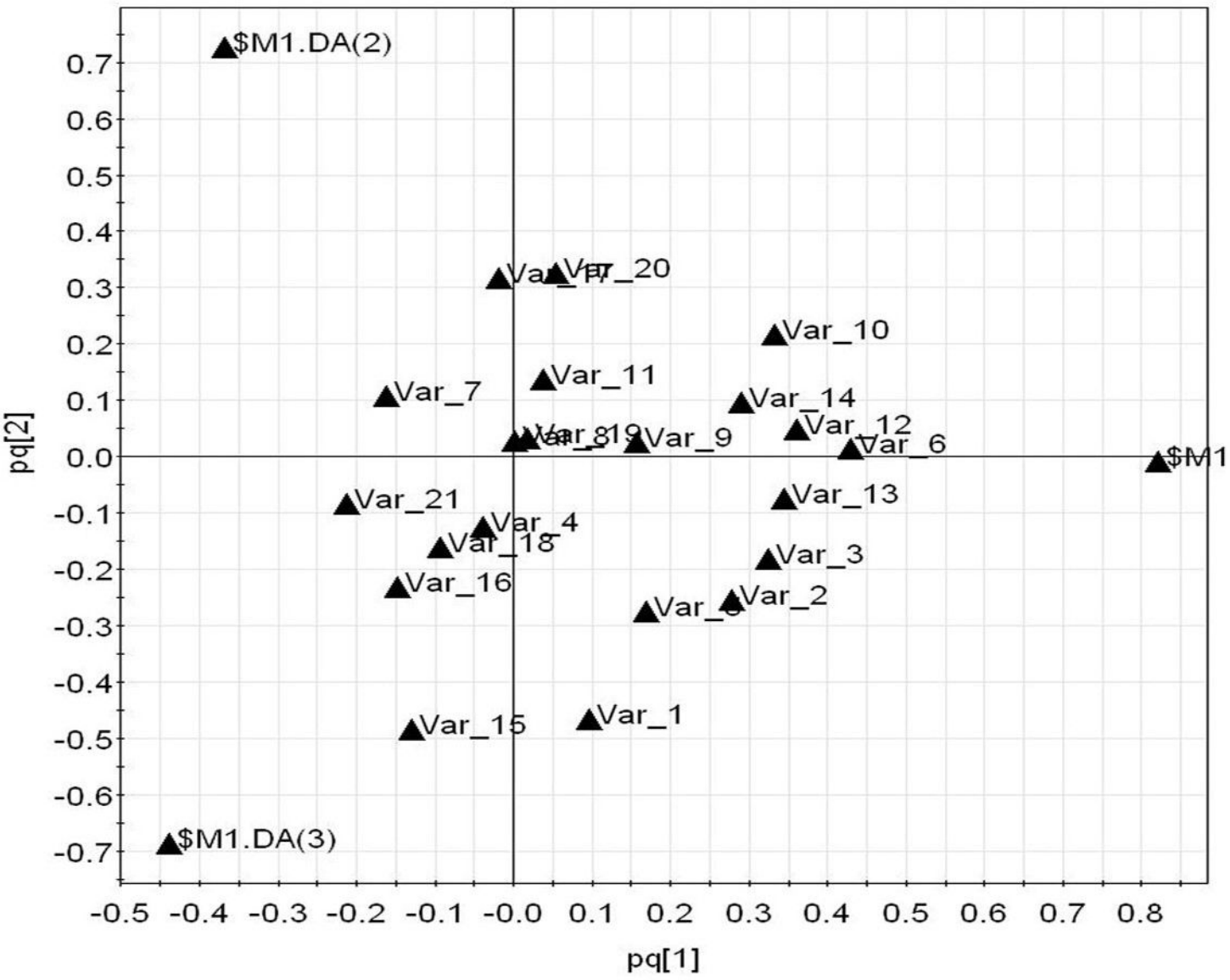

Figure 2

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

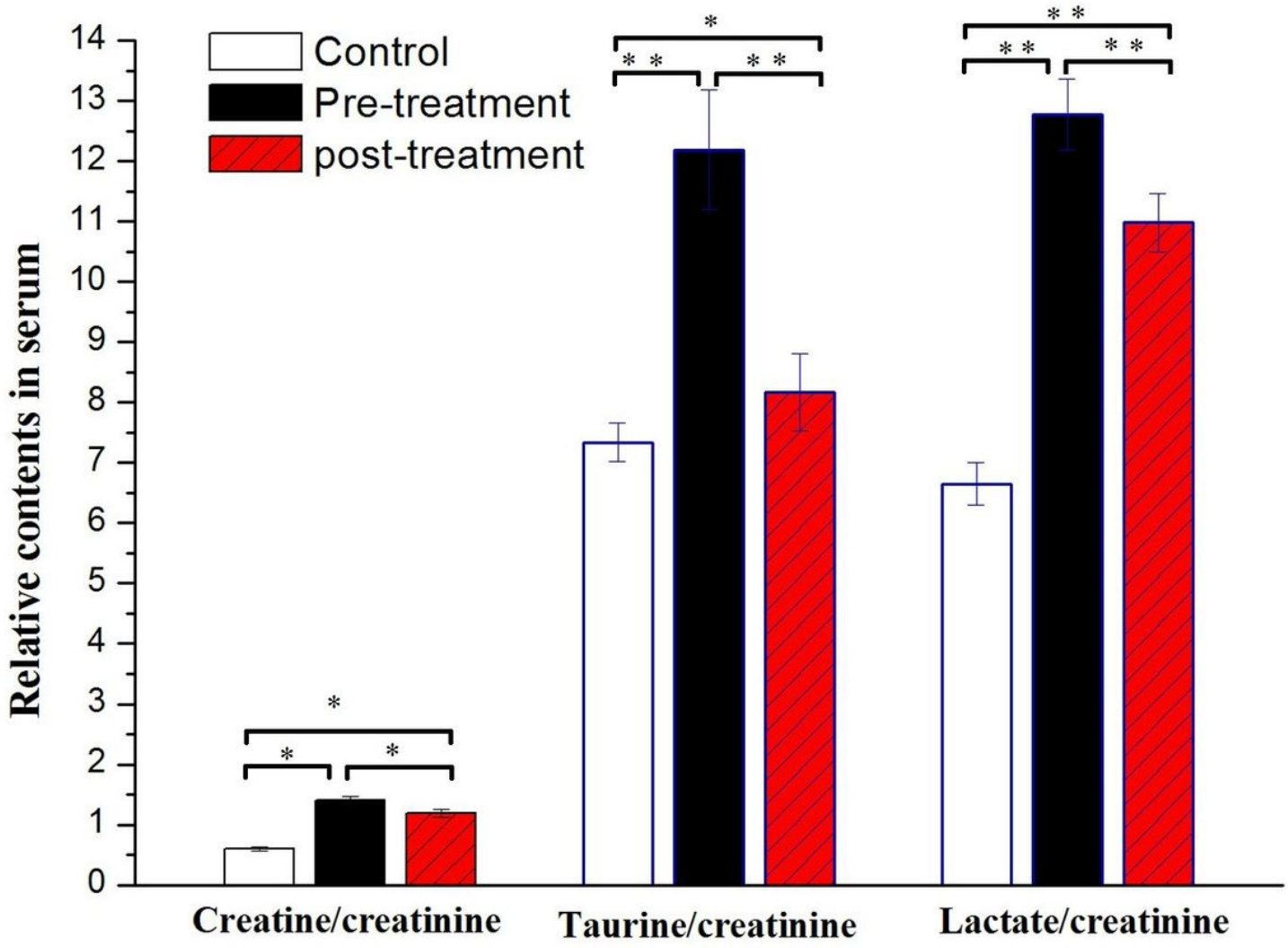

Figure 3

The alteration of potential serum markers in LF patients who were treated by ALSSs.

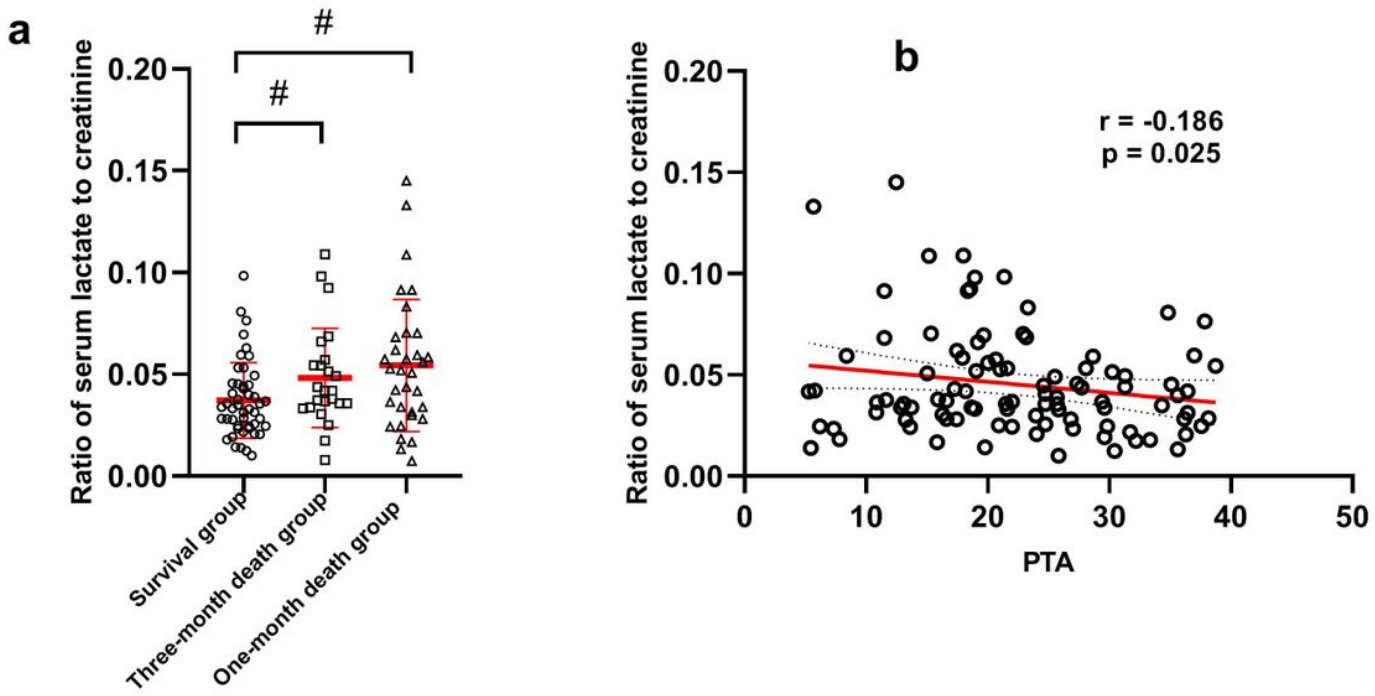

Figure 4

(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 LF patients (n = 105).

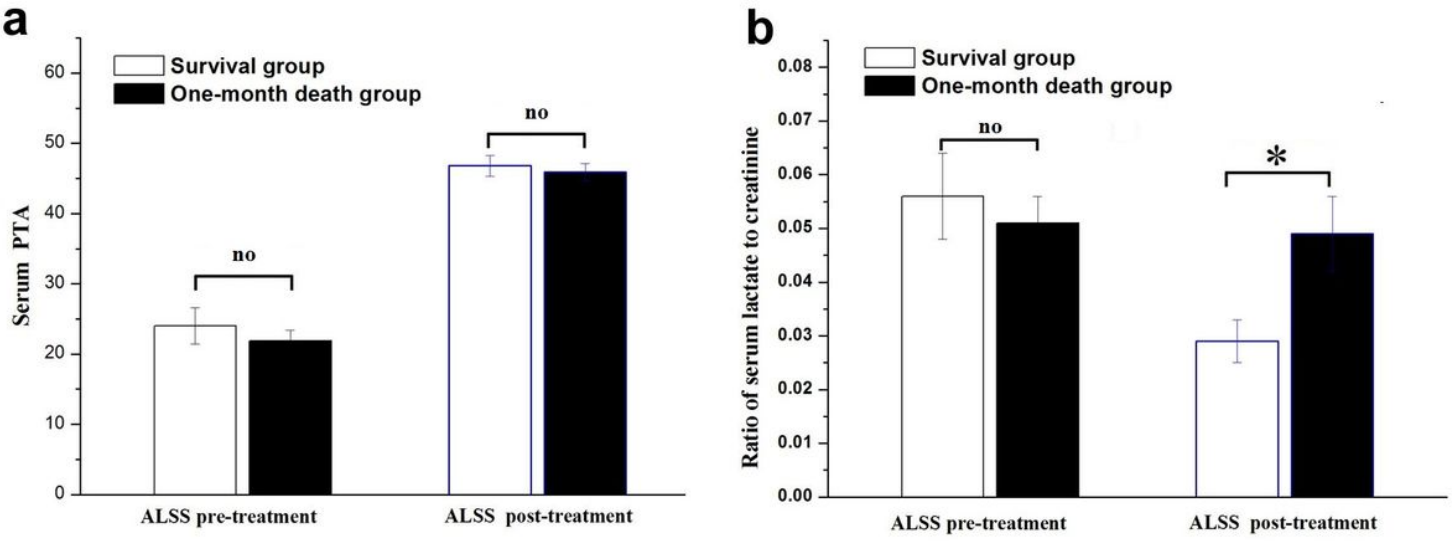

Figure 5

The alterations of serum lactate:creatinine ratio and PTA before and after ALSSs treatment and in the survival group (n=49) and the death group (n=32).

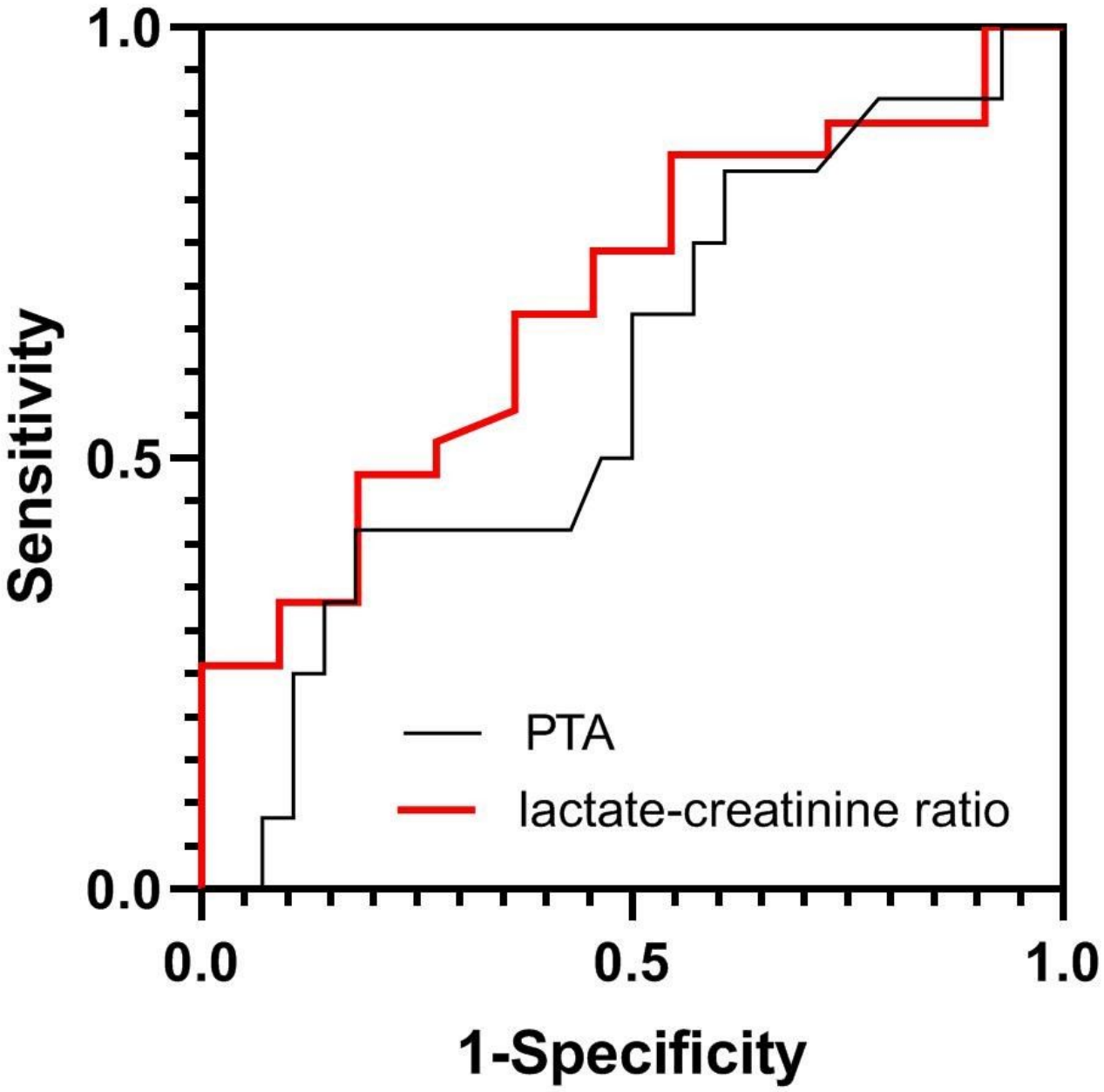

Figure 6

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