Metabolic dynamics in critically injured patients: a prospective cohort study integrated with 1H NMR metabolomics

doi: 10.6133/apjcn.201902/PP.0003
Published online: February 2019

Running title: Metabolic dynamics of critically injured patients

Yubo Zhou MD[1], Kai Wang MD[1], Jun Zeng MD[1,2], Wei Li MD[3], Jin Peng MD[2], Zhiyuan Zhou BSc[2,4], Pengchi Deng PhD[5], Mingwei Sun MD[1,2], Hao Yang PhD[2], Shijun Li MD[1], Charles Damien Lu[2], Hua Jiang MD[1,2]

[1]Department of Acute Care Surgery, Sichuan Provincial People's Hospital, Sichuan Academy of Medical Sciences, Chengdu, China
[2]Metabolomics and Multidisciplinary Laboratory for Trauma Research, Institute for Emergency and Disaster Medicine, Chengdu, China
[3]Department of Burns, Sichuan Provincial People's Hospital, Sichuan Academy of Medical Sciences, Chengdu, China
[4]Department of Biochemistry, Southwestern University for Medical Sciences, Luzhou, China
[5]Analysis and Test Center, Sichuan University, Chengdu, China

Authors' email addresses and contributions:
Yubo Zhou: 18981838177@163.com
Kai Wang: 371352604@qq.com
Jun Zeng: zengjun@medmail.com.cn
Wei Li: 7351933@qq.com
Jin Peng: pengjin7877@qq.com
Zhiyuan Zhou: 284359376@qq.com
Pengchi Deng: pcdeng@sina.com
Mingwei Sun: 653176424@qq.com
Hao Yang: 358688143@qq.com
Shijun Li: sclishijun@126.com
Charles Damien Lu: damienla@gmail.com.

Corresponding Author: Dr Hua Jiang, Department of Acute Care Surgery, Sichuan Provincial People's Hospital, Sichuan Academy of Medical Sciences, Chengdu 610072, China. Email: cdjianghua@gmail.com

ABSTRACT

Background and Objectives: By combining the techniques of metabolomics and computational biology, this research aims to explore the mechanism of metabolic dynamics in critically injured patients and develop a new early warning method for mortality. **Methods and Study Design:** A prospective cohort study was conducted, group plasma samples of critically injured patients were collected for 1H-NMR metabolomics analysis. The data was processed with partial least squares regression, to explore the role of enzyme-gene network regulatory mechanism in critically injured metabolic network regulation and to build a quantitative prediction model for early warning of fast death. **Results:** In total, 60 patients were enrolled. There were significant differences in plasma metabolome between the surviving patients and the deceased ones. Compared to the surviving patients, 112 enzymes and genes regulating the 6 key metabolic marker disturbances of neopterin, corticosterone, 3-methylhistidine, homocysteine, Serine, tyrosine, prostaglandin E2, tryptophan, testosterone and estriol, were observed in the plasmas of deceased ones. Among patients of different injury stages, there were significant differences in plasma metabolome. Progressing from T0 to T50 stages of injury, increased levels of neopterin, corticosterone, prostaglandin E2, tryptophan and testosterone, together with decreased levels of homocysteine, and estriol, were observed. Eventually, the quantitative prediction model of death warning was established. Cross-validation results showed that the predictive effect was good (RMSE=0.18408, R2=0.87 p=0.036). **Conclusions:** Metabolomics approaches can be used to quantify the metabolic dynamics of patients with critically injuries and to predict death of critically injured patients by plasma 1H-NMR metabolomics.

Key Words: critically ill injury, Kaplan-Meier curves, metabolism-dynamics, metabolomics, complex metabolic network, gene ontology

INTRODUCTION

Multidisciplinary comprehensive treatment of severely injured patients is an important issue for trauma and acute care surgery research. Improving survival rate of severely injured patients has become a major challenge and focus of research. The death of critically ill patients is mainly attributed to multiple organ dysfunction syndrome (MODS) caused by the primary disease, of which the incidence and mortality has not significantly reduced, even though clinical prevention is improving.[1]

Assessing patient conditions accurately, identifying MODS early, predicting the prognosis of patients, and administration of appropriate interventions are several important aspects of prevention and treatment of critically ill patients with MODS. There have been numerous studies conducted to find specific indicators to predict MODS, such as blood biochemistry indicators, inflammatory mediators, and cytology category indicators.[2-5] Although these indicators have shown value in clinical settings, the sensitivity and accuracy of the prediction system for MODS are not high enough to guide clinical therapy. Currently, there is a lack of high-sensitivity prediction method that can forecast MODS accurately; this limits early treatment measures to reduce the mortality of critically ill patients. Therefore, to establish a model that can be used to predict clinical outcome is key to early reduction of MODS and mortality, which also can help improve individualized treatment for patients.

From systems biology perspective, critically ill patients experience acute metabolic disorders, which involve multiple organs and systems. The entire process is regulated by hormones, enzymes, and small-molecule chemical groups. Adding in clinical intervention, these factors together make up dynamic and complicated network.[6-7] Therefore, if the metabolomes of critically ill patients can be identified and their dynamics understood, the outcome of patients can be predicted. Metabolomics can visualize the MODS process by detecting all small-molecule metabolites in plasma.[8-14] In recent years, some metabolomics studies have been conducted on the critical and complex mechanisms of MODS. For example, Jiang and Peng established the animal model of spinal cord injury based on 1H-nuclear magnetic resonance (1H-NMR), and elucidated the active lipid metabolic pathway after injury; there were significant changes in the levels of aspartate, citrulline, and arginine.[14,15] However, to date, there is no report on the metabolic dynamic of critically ill patients by 1H-NMR.

In this study, we established a systems biology approach by combining the techniques of metabolomics and computational biology. We adopted this plasma 1H-NMR metabolomics-based approach to quantify the metabolic dynamics of patients with critically injuries and predict their mortality by analyzing metabolites and the enzyme–gene regulatory network.

MATERIALS AND METHODS

Subjects

A prospective cohort study was conducted. Patients with injure admitted to emergency center of Sichuan Provincial People's Hospital in Chengdu, Sichuan, from June 2014 to December 2015 were recruited, provided written informed consent, and were enrolled in this prospective cohort study. Our primary exposures were trauma type and time since injury. The clinic data

were collected in continues phase every week until they died or discharged. Both groups were followed for entire period of standard therapies. Selected patients' plasma samples (5mL) were collected for 1H-NMR metabolomics (DRX 600MHz NMR, Bruker Biospin Rheinstetten, Germany) analysis. Plasma metabolites of dead patients were compared with those of survivors. The inclusion criteria was as follows: (1) patients with severe traumatic brain injury (Glasgow coma score <8), (2) patients with thoracic-abdominal trauma (The acute physiology and chronic health evaluation II (APACHEII) >10 and injury severity score (ISS) >17), (3) patients with severe burn (burn area covering more than 50% TBSA). All patients are from Emergency Medical Center, and Burns Surgery of Sichuan Provincial People's Hospital. The exclusion criteria were as follows: patients with carcinoma; patients with diabetes; patients with hyperthyroidism; patients with chronic heart diseases. Written informed consent was obtained from the patients or their guardian (if the patient was unconscious).

The Sichuan Provincial Academy of Medical Sciences & Sichuan Provincial People's Hospital Medical Ethics Committee approved the research, and the clinical registration number is NCT02164786.

Collection and preparation of blood samples and NMR measurements

Venous blood samples were collected from the forearm vein at 8.00 am on the morning of enrollment day (T0), and on the second (T1), third (T3), fifth (T5), seventh (T7), 14th (T14), 20th (T2), 28th (T28), 35th (T35), 42nd (T42) and 49th (T49) days. All plasma samples were centrifuged at 16000 rpm for 10 min, and 50μL of deuterium oxide (D2O) was added to 450 μL of the plasma in 5-mm Wilmad NMR tubes to lock the field frequency for the 600-MHz 1H-NMR spectrometer with an inverse broad band probe. 1D-NMR spectra were collected at room temperature (300k); the spectral width was 20 ppm, and 32 transients ware collected into 256 data points. The zgp pulse sequence was used to suppress the water peak and visualize the latent biomarkers of smaller molecules.

Data analysis and pattern recognition

Clinical data were described as median (interquartile range) or mean ± standard deviation (SD). Differences between means were assessed by Student's t-test or Wilcox test. Differences in counts or percentages were evaluated by Fisher's exact probability test. Differences were considered significant if a two-tail p value was <0.05.

All plasma 1H-NMR spectra were baseline corrected and phased with mestRenova (version 6.1.1, MestreLab Research, Spain), and the chemical shifts were referenced to a creatinine peak at Δ3.05. All data were introduced into a Matlab (R2015b, The Math Works, Inc. Natick, MA, USA) data structure, where each row comprised the integral descriptors for an NMR spectrum. We use deuterium water to suppress water resonance.

Matlab R2015b was used for computing and mathematical modeling (The Math-Works Inc., Natick, MA). All multivariate statistics and pattern recognition were performed using the Eigenvector Toolbox (ver 6.2.1) with PLS on the Matlab. Orthogonal signal correction partial least squares-discriminant analysis (OPLS-DA) was performed on the 1H-NMR data to reduce data dimensionality.

Establishing metabolome and upstream genes

The variable importance for the projection (VIP) score was used to screen metabolites. The VIP score greater than 1 as a threshold, was considered to be significant. Then we use The Human Metabolome Database (HMDB) to identify key metabolites and enzymes that catalyze metabolites.[15] To investigate the underlying mechanisms of interactions of those metabolites, which were up or down regulated, we use differential equations to establish a series of simulation global metabolic networks based on mass action and Mimon's law. After identified key metabolites through HMDB, the enzymes ID were converted to Kyoto Encyclopedia of Genes and Genomes (KEGG) enzyme IDs. Then we use the Matlab API to map these enzymes into metabolic pathways. Finally, up or down regulated metabolites and disturbed pathways related to death were visualized. Following this, a linear discriminant formula was established as a quantitative prediction model of mortality. The root means square error (RMSE) was calculated to verify the validity of the equations.

Computing platform and tools

Computations were performed using a high-performance computing platform (CPU Xeon E7-8848x4, 512GB DDR3 1333Mhz) at the Metabolomics and Multidisciplinary Laboratory of Sichuan Academy of Medical Sciences. All computing software (Matlab R2013b; MestReNova 6.1.1 MestreLab Research, Spain； Human Metabolome Database (HMDB) http://www.hmdb.ca/; G: profiler website; and R 3.11, cran.r-project.org) were run on Windows 7 operating system.

RESULTS

Patients and clinical assessments

In total, 60 patients were enrolled between June 2014 and December 2015 and were divided into three groups: 19 patients with severe traumatic brain injury, 26 patients with thoracic or abdominal trauma and 15 patients with severe burns. 342 plasma samples from 60 injured patients were obtained. The patients' average age was 53±18.4 years and the mean body mass index (BMI kg/m^2) was 22.8±2.06, and none withdrew consent. Table 1 demonstrates that the three groups of patients were comparable for basic demographic data. There was no significant variance among the patient's groups in terms of age, BMI, and severity of illness. Demographic and clinical characteristics for the cohort of deaths and survivors were showed in Table 2. The dead cohort was severely injured, as evidenced by long length of ICU stays and high morbidity of MODS.

The survival curves revealed that the surviving percentage of patients with severe burns was significantly lower than those in the other two groups (Z=-3.32, p=0.00090; Z=-3.45 p=0.00057; Figure 1s). Mortality increased significantly on T10, T40, and T50; therefore, the plasma specimens from these days were analyzed as the metabolism-network points.

Plasma metabolome of all patients

There were significant differences in plasma metabolome between the surviving and the deceased patients (Figure 1 and 2s). Compared to the surviving patients, altered levels of neopterin, corticosterone, 3-methylhistidine, homocysteine, serine, tyrosine, prostaglandin E2, tryptophan, testosterone, and estriol were observed in the plasma samples of the deceased patients (Figure 2). Of these, six metabolic markers-neopterin, 3-methylhistidine, prostaglandin E2, homocysteine, testosterone, and estriol- were significantly altered. Gene ontology (GO) analysis showed that these six key metabolic markers were regulated by 112 enzymes and genes (Table 4). Among patients at different stages of injury, there were significant differences in plasma metabolome (Figure 3s). All metabolomes of critically ill patients are observed dynamically in the three-dimensional space. Increased levels of neopterin, corticosterone, prostaglandin E2, tryptophan and testosterone, together with decreased levels of homocysteine, and estriol were observed from T0 to T50 stages of injury (Table 3). Furthermore we used the heat map to reveal significant metabolites in patients at different stages of injury (Figure 3). Eventually, the following quantitative predictive model for mortality was established: PC1 = corticosterone * 0.1017- melatonin * 0.1075 + homocysteine * 0.0771- tryptophan * 0.0813 + prostaglandin E2 * 0.095 + estradiol * 0.1042-

testosterone * 0.1083. Cross-validation results showed that the predicted effect was good (RMSE=0.18784, R2=0.83, p=0.036).

DISCUSSION

Critical illness is a global medical problem, which is gaining increasing attention by researchers. Most of this kind of research is dedicated to understand the mechanism by which the mortality of critically ill patients with MODS can be reduced. Despite the large number of such studies, the dynamical mechanism of MODS is still unclear. To explore the mechanism of metabolic dynamics in critically injured patients, we established a systems biology approach, based on metabolomics and computational biology, for early prediction of mortality in such patients.

In this study, changes in the plasma metabolic pool in critically injured patients were measured dynamically by 1H-NMR metabolomics methods. The first important finding was the increased levels of corticosterone, prostaglandin E2, and testosterone, together with decreased levels of melatonin, homocysteine, and estriol, in the plasma samples of deceased patients, compared to that of the survivors. The increase in corticosterone levels in the plasma indicates that the critically injured patients were predisposed to critical illness-related corticosteroid insufficiency (CIRCI). Researchers have found that critically ill patients with CIRCI are closely associated with poor prognosis.16 Annane et al reported that the incidence of CIRCI in critically ill patients was 60%, which is closely related to the incidence of MODS and increase in mortality.[17,18] Walker showed that the mortality of trauma patients with CIRCI reached up to 34%.[19] This is possibly because the dysfunction of the adrenal gland reduces the secretion of and resistance to corticosteroid.[20] Further, deregulation of genes whose products downregulate proinflammatory mediators in critically ill patients leads to increase in the levels of systemic inflammatory mediators and immune response dysfunction.

The altered levels of estradiol and testosterone in the plasma samples of the critically ill patients in this study indicate that the circulating levels of sex hormone are disrupted after injury. Researchers have found that high levels of estrogen in plasma plays a protective role to brain tissue.[21,22] Owing to anti-cell oxidation, and by reducing neurotoxin levels and vasodilatation, estradiol ensures sufficient blood supply to the brain.[23] However, testosterone has a side effect on brain tissues in patients with severe traumatic brain injury.[24, 25] To protect the brain tissue effectively in critically ill patients, plasma testosterone may be converted to estradiol by aromatase.

Based on our clinical experience and the model established previously.[14,15] we selected six specific, significantly altered metabolites as biomarkers in this study, which can distinguish surviving patients from the deceased ones. Analysis showed that these six key metabolic markers (Table 4) were regulated by 112 enzymes and genes. Eventually, the quantitative prediction model of mortality was established as follows: PC1 = corticosterone * 0.1017- melatonin * 0.1075 + homocysteine * 0.0771- tryptophan * 0.0813 + prostaglandin E2 * 0.095 + estradiol * 0.1042- testosterone * 0.1083. The model identified morbid and surviving cases effectively at early stages, and the risk of death was clinically and objectively assessed in the critically ill patients to provide an effective intervention as early as possible.

The second important finding of this study was the significant differences between the plasma metabolic fingerprint of critically ill patients at different clinical stages (T0, T10, T40, T50), which provides a new metabolomics perspective for dynamic observation of the overall developmental process of critical illness. By examining the clinical evolution of various stages of critically ill patients in three dimensions, we revealed the mechanism of critical illness in metabolic network disturbance, and characterized the dynamics of metabolic network changes in critical illness. Throughout the evolution of disease, the levels of the following plasma metabolites were significantly altered: increased levels of valproic acid, neopterin, corticosterone, endogenous hydrogen sulfide, prostaglandin E2, homocysteine, ornithine, γ- aminobutyric acid, testosterone and tryptophan, together with decreased levels of cholesterol, 1-pentene-3-one, butyric acid, progesterone, estradiol, linoleic acid, glycyl-proline, and L-sphingosine.

Metabolomics represents an advance over other approaches, yet some limitations are relevant to the interpretation of this study and are related to the current capacities of 1H-NMR and pathway analysis. Major limitations of this study are the small sample size and the imbalance of patient subtypes, which contributed to the significantly higher mortality from burns patients. However, these disadvantages may be alleviated because injured patients share similar mechanism of biochemical response originating from stress. Injury is a biochemical trigger that alters the metabolism of proteins, carbohydrates, nucleic acids, amino acids and lipids.[26] The alteration of these biochemical process presents unique metabolic signatures that provide information on the response to the pathophysiological insults. Thus, these biochemical responses can be depicted by metabolomics dynamic model, which is the purpose of our study.[27,28] To this end, our study is an important attempt to identify the shared metabolites and derangements in metabolic pathways overtime after injury.

Conclusion

In summary, metabolomics has become one of the important tools and theoretical frameworks to solve complex clinical problems. In this study, we established a model of metabolic dynamics of critical illness based on 1H-NMR metabolomic methods; and developed a new way to monitor critically ill patients dynamically through changes in their metabolic profiles. Morbid and surviving cases can be identified effectively with this model at the early stages. However, sample size and plasma specimen for metabolomics analysis of this study are small, and further analysis of spectra of small metabolites can go deeper along the route of metabolites-enzyme-gene. Our study contributes towards the development of a new generation of clinical-decision support systems.

ACKNOWLEDGEMENTS

We thank Mr. Zhan Liu of Metabolomics and Multidisciplinary Laboratory of Sichuan Academy of Medical Sciences for his helping on manuscript preparation.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest. This work was supported by grants from the Sichuan Department of Science and Technology (No.2014FZ0125 and 2015SZ0110). The funders did not contribute to the study design, data collection and analysis, decision to publish, or manuscript preparation

REFERENCES

1. Zeng J, Yang H, Jiang H. Challenges and opportunities for traumatic shock resuscitation research: integration of data analysis computational science，systems biology and trauma science. Journal of Traumatic Surgery. 2013;15:186-9.
2. Husum H, Strada G. Injury Severity Score versus New Injury Severity Score for penetrating injuries. Prehosp Disaster Med. 2002;17:27-32.
3. Tian LH, Gao W, Tang CH, Liu KJ. Predictive assessment of scoring systems for MODS in severe traumatic patients. Journal of Traumatic Surgery. 2006;8:202-4.
4. Liang HP, Wang ZG. Progress in predicting the outcome of complications and death after trauma. Chinese Journal of Trauma. 2006;22:186-9.
5. Koretz RL. Nutritional supplementation in the ICU. How critical is nutrition for the critically ill? Am J Respir Crit Care Med. 1995;151:570-3.
6. Van Ommen B. Nutrigenomics: exploiting systems biology in the nutrition and health arenas. Nutrition. 2004;20:4-8.

7. Wang Y, Holmes E, Tang H, Lindon JC, Sprenger N, Turini ME, Bergonzelli G, Fay LB, Kochhar S, Nicholson JK. Experimental metabonomic model of dietary variation and stress interactions. J Proteome Res. 2006;5:1535-42.
8. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica. 1999;29:1181-9.
9. Weljie AM, Newton J, Mercier P, Carlson E, Slupsky CM. Targeted profiling: quantitative analysis of 1H NMR metabolomics data. Anal Chem. 2006;78:4430-42.
10. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. Mass Spectrom Rev. 2007;26:51-78.
11. Lindon JC, Nicholson JK. Spectroscopic and statistical techniques for information recovery in metabonomics and metabolomics. Annu Rev Anal Chem (Palo Alto Calif). 2008;1:45-69.
12. Aberg KM, Alm E, Torgrip RJ. The correspondence problem for metabonomics datasets. Anal Bioanal Chem. 2009;394:151-62.
13. Lindon JC, Holmes E, Bollard ME, Stanley EG, Nicholson JK. Metabonomics technologies and their applications in physiological monitoring, drug safety assessment and disease diagnosis. Biomarkers. 2004;9:1-31.
14. Jiang H, Peng J, Zhou ZY, Duan Y, Chen W, Cai B, Yang H and Zhang W. Establishing 1H nuclear magnetic resonance based metabonomics fingerprinting profile for spinal cord injury: a pilot study. J Chinese Med. 2010;123:186-9.
15. Peng J, Zeng J, Cai B, Yang H, Cohen MJ, Chen W, Sun MW, Lu CD, Jiang H. Establishment of quantitative severity evaluation model for spinal cord injury by metabolomic fingerprinting. PLoS One. 2014;9:e93736.
16. Cohan P, Wang C, McArthur DL, Cook SW, Dusick JR, Armin B et al. Acute secondary adrenal insufficiency after traumatic brain injury: a prospective study. Crit Care Med. 2005;33:2358-66.
17. Annane D, Maxime V, Ibrahim F, Alvarez JC, Abe E, Boudou P. Diagnosis of adrenal insufficiency in severe sepsis and septic shock. Am J Respir Crit Care Med. 2006;174:1319-26.
18. Annane D, Meduri GU, Marik P. Critical illness-related corticosteroid insufficiency and community-acquired pneumonia: back to the future! Eur Respir J. 2008;31:1150-2.
19. Walker ML, Owen PS, Sampson C, Marshall J, Pounds T, Henderson VJ. Incidence and outcomes of critical illness-related corticosteroid insufficiency in trauma patients. Am Surg. 2011;77:579-85.
20. Meduri GU, Golden E, Freire AX, Taylor E, Zaman M, Carson SJ, Gibson M, Umberger R. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest. 2007;131:954-63.
21. Groswasser Z. Gender and traumatic brain injury. J Neurosurg. 2001;94:862-4.
22. Li Z, Zou WH, Gu JH. The clinical value of dynamic monitoring of hormones and blood glucose in patients with brain injury. Zhejiang Practical Medicine. 2007;12:189-91.

23. Zhi DS, Zhang Q. New Progresses in drug treatment of traumatic brain injury. Chinese Journal of Trauma. 2005;21:50-2.
24. Barreto G, Veiga S, Azcoitia I, Garcia-Segura LM, Garcia-Ovejero D. Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone. Eur J Neurosci. 2007;25:3039-46.
25. Offner PJ, Moore EE, Biffl WL. Male gender is a risk factor for major infections after surgery. Arch Surg. 1999;134:935-8.
26. D'Alessandro A, Nemkov T, Moore HB, Wither M, Nydam T, Slaughter A, Silliman CC, Banerjee A, Hansen KC. Metabolomics of trauma-associated death: shared andfluid-specific features of human plasma vs lymph. Blood Transfusion. 2016;14:185-94.
27. Parent BA, Seaton M, Sood RF, Gu H, Djukovic D, Raftery D, O'Keefe GE. Use of Metabolomics to Trend Recovery and Therapy After Injury in Critically Ill Trauma Patients. JAMA Surg. 2016, 151:e160853. doi: 10.1001/jamasurg.
28. Jayaraman SP, Anand RJ, DeAntonio JH, Mangino M, Aboutanos MB, Kasirajan V et al. Metabolomics and precision medicine in trauma: the state of the field. Shock. 2018;50:5-13.

Table 1. Basic demographic data of all patients

Variances	STBI (N=19)	Trauma (N=26)	Severe burns (N=15)	*p*
Demographic indicators				
Age, median (SD), y	46.1±15.7	63.5±11.6	37.6±9.1	0.17
gender				0.24
Male, n (%)	10 (52.6)	13 (50.0)	8 (53.3)	
Female, n (%)	9 (47.4)	13 (50.0)	7 (46.7)	
Basic information on admission				
Systolic blood pressure, median (SD), mmHg	135±35.1	127±12.86	124±12.18	0.048
Diastolic blood pressure, median (SD), mmHg	82±14.78	76±11.19	79±11.39	0.166
Axillary temperature, median (SD), ℃	36.36±0.311	36.59±0.24	36.88±0.56	0.195
Heart rate, median (SD), bpm	86 ±25.62	79±11.19	112±24.65	0.012
ISS, median (IQR)	22 (18-28)	21 (17-26)	24 (19-29)	0.25
APACHE II, median (IQR)	13 (11-17)	12 (11-14)	17 (11-19)	0.11
BMI, median (SD), (kg/m^2)	25±1.71	21.1±2.15	22.5±1.36	0.125
Outcomes				
Death, n (%)	2 (10.5)	1(3.80)	12 (80.0)	<0.05
MODS				<0.05
Yes, n (%)	5 (26.3)	2 (7.8)	9 (75.0)	
No, n (%)	14 (73.7)	24 (92.2)	3 (25.0)	
Length of ICU, median (IQR), day	7 (3-9)	3 (2-5)	23 (12-46)	<0.05
Physiological and biochemical indices				
K+, median (SD), mmol/L	3.71±0.92	4.19±0.29	3.54±0.33	0.95
Na+, median (SD), mmol/L	138.42±2.73	138.62±3.56	144.1±4.33	0.17
Cl-, median (SD), mmol/L	102.0±2.95	102.35±3.79	106.93±5.17	0.74
Ca++, median (SD), mmol/L	2.13±0.14	2.11±0.15	2.23±1.66	0.36
Glu, median (SD), mmol/L	7.91±2.44	5.82±2.22	9.45±2.44	0.41
TP, median (SD), g/L	64.25±6.37	60.17±8.15	43.02±8.13	0.39
WBC, median (SD), 10^9/L	13.23±5.84	6.78±2.82	23.5±10.22	0.018
HGB, median (SD), g/L	122.84±18.78	122.04±17.55	157.9±22.26	0.04
ALB, median (SD), g/L	39.08±5.59	37.68±4.50	25.64±5.44	0.02
BUN, median (SD), mmol/L	4.81±1.57	5.21±1.61	8.93±2.96	0.66
Cr, median (SD), umol/L	60.27±21.64	75.5±18.62	111.8±59.3	0.00
ALT, median (SD), U/L	57.63±70.6	26.07±38.5	66.11±135.0	0.359
AST, median (SD), U/L	73.16±128.2	28.26±21.0	186.5±431.09	0.166

STBI: severe traumatic brain injury; APACHEII: The acute physiology and chronic health evaluation II; ISS: injury severity score; MODS: multiple organ dysfunction syndrome; WBC: white blood cell; HGB: hemoglobin; ALB: albumin; BUN: blood urea nitrogen; Cr: creatinine; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Table 2. Demographic and clinical characteristics for the cohort of deceased and survivors

Variances	Survivors (N=45)	Deceased (N=15)	*p*
Demographic indicators			
Age, median (SD), y	54.7±15.4	41.9±15.8	0.548
Gender			0.120
Male, n (%)	35 (77.8)	13 (86.7)	
Female, n (%)	10 (22.2)	2 (13.3)	
Basic information on admission			
Systolic blood pressure, median (SD), mmHg	128±20.31	129±28.70	0.410
Diastolic blood pressure, median (SD), mmHg	79±12.84	77±11.97	0.674
Axillary temperature, median (SD), ℃	36.56±0.39	36.67±0.44	0.474
Heart rate, median (SD), bpm	82±19.36	111±23.69	0.151
ISS, median (SD), bpm	20 (17-25)	25 (18-29)	0.20
APACHE II, median (SD), bpm	12 (11-14)	17 (11-19)	0.11
BMI, median (SD) ,(kg/m^2)	22.6±2.53	22.4±1.40	0.057
Outcomes			
MODS			<0.05
Yes, n (%)	5 (11.1)	11 (73.3)	
No, n (%)	40 (8.9)	4 (26.7)	
Length of ICU (day), median (IQR), day	5 (2-10)	22 (10-46)	<0.05

APACHEII: The acute physiology and chronic health evaluation II; ISS: injury severity score; MODS: multiple organ dysfunction syndrome ; ICU: intensive care unit.

Table 3. Demographic and Clinical Characteristics for the cohort of deceased and survivors

Chemical shift (ppm)		HMDB number				Metabolites				Tendecy†
0.16	1.08	00067	00151	00153	01423	Cholesterol	Estradiol	Estriol	Coenzyme A	↓*
0.52	1.12	01877	03276	06483	03265	Valproic acid	Hydrogen sulfide	D-Aspartic acid	Hesperidin	↑
0.68	0.72	31607	00039	01429	00479	1-Penten-3-one	Butyric acid	phosphate	3-Methylhistidine	↓*
1.2	1.04	01220	01547	00895	00133	Prostaglandin E2	Corticosterone	Acetylcholine	Guanosine	↑*
0.76	1.36	01830	00721	00251	00904	Progesterone	Glycyl proline	Taurine	Citrulline	↓*
0.88	1.48	00845	00214	00742	01389	Neopterin	Ornithine	Homocysteine	Melatonin	↑*
1	1.84	00234	00112	30396	00767	Testosterone	Gamma-Aminobutyric acid	Tryptophan	Pseudouridine	↑
1.32	1.96	00673	00252	03966	02391	Linoleic acid	Sphingosine	Selenomethionine	Uvaol	↓
3.24	1.36	01220	11628	00101	01569	Prostaglandin E2	Glycyrrhetinic acid	Deoxyadenosine	Epi-coprostanol	↑

†↓: indicates decrease, ↑: indicates increase.
*p<0.05.

Table 4. Enzymes and genes that are regulated by the six key metabolic markers

Metabolites	Enzymes		Genes	
Cortcosterone	Cytochrome P450 11B1, mitochondrial	3-oxo-5-beta-steroid 4-dehydrogenase	CYP11B1	AKR1D1
	Corticosteroid 11-beta-dehydrogenase isozyme 2	Corticosteroid 11-beta-dehydrogenase isozyme 1	HSD11B2	HSD11B1
Prostaglandin E2	Carbonyl reductase [NADPH] 1	Putative adenosylhomocysteinase 3	PTGER1	CBR3
	15-hydroxyprostaglandin dehydrogenase	Prostaglandin E synthase	HPGD	PTGES
Homocysteine	Methionine synthase	Betaine--homocysteine S-methyltransferase 1	MTR	BHMT
	Cystathionine beta-synthase	Adenosylhomocysteinase	AHCYL2	CBS
Estradiol	Estradiol 17-beta-dehydrogenase 2	3-keto-steroid reductase	HSD17B2	HSD17B7
	Estrogen receptor beta	Cytochrome P450 3A4	ESR2	CYP3A4
Testosterone	Amine oxidase A	Estrogen sulfotransferase	MAOA	SULT1E1
	UDP-glucuronosyltransferase 2B28	3-oxo-5-beta-steroid 4-dehydrogenase	UGT2B28	AKR1D1
Melatonin	Acetylserotonin O-methyltransferase	Ribosyldihydronicotinamide dehydrogenase	ASMT	NQO2
	Myeloperoxidase	Eosinophil peroxidase	MPO	EPX
	Indoleamine 2,3-dioxygenase 1	Cytochrome P450 2C9	IDO1	CYP2C9
	Estrogen receptor	Indoleamine 2,3-dioxygenase 2	ESR1	IDO2
	Calreticulin	Melatonin receptor type 1A	CALR	MTNR1A

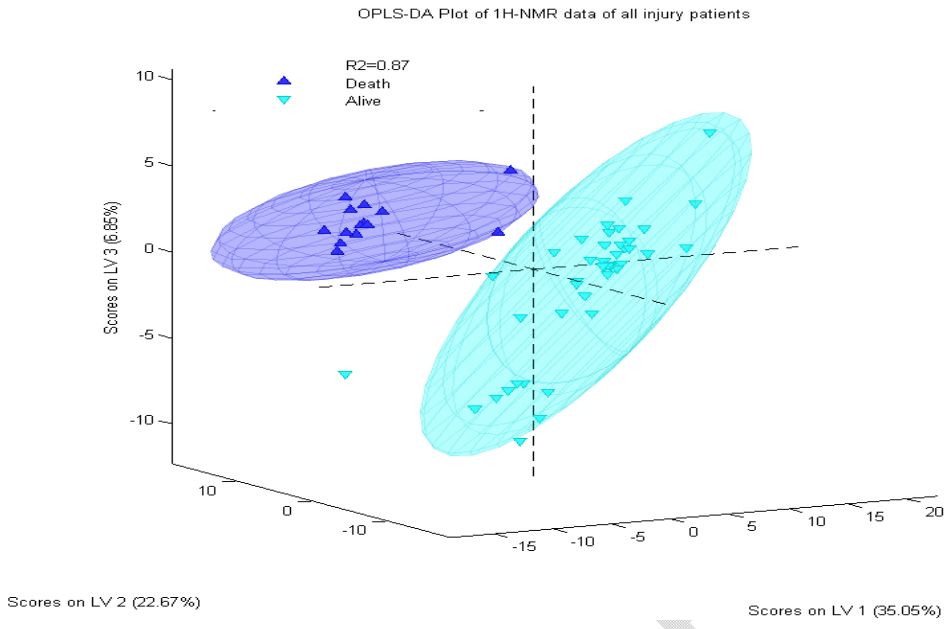

Figure 1. OPLS-DA of all patients. Dark blue triangles indicate deaths; light blue triangles represent the survivors. OPLS-DA: Orthogonal Signal Correction Partial Least Squares-Discriminant Analysis

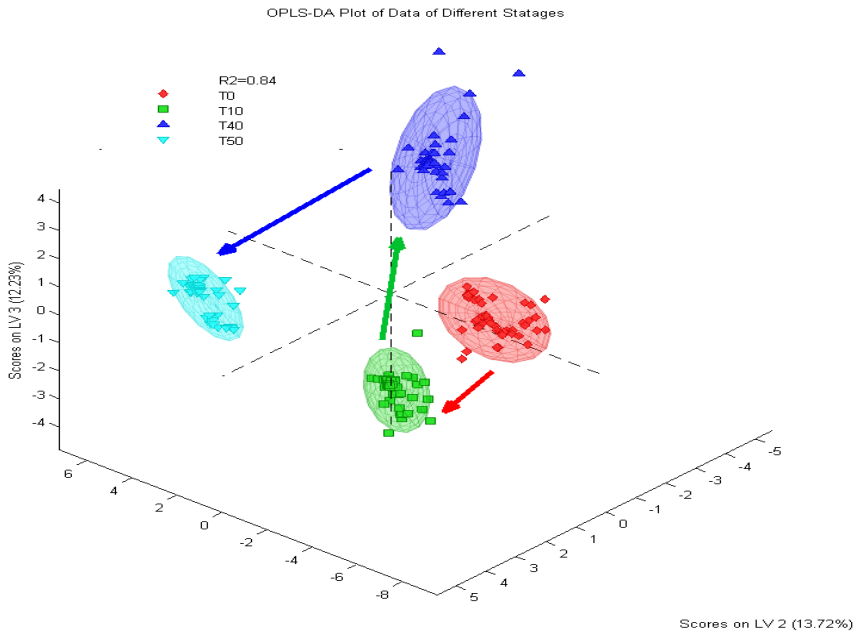

Figure 2. OPLS-DA can distinguish plasma metabolic fingerprints at different stages of critical illness. OPLS-DA: Orthogonal Signal Correction Partial Least Squares-Discriminant Analysis.

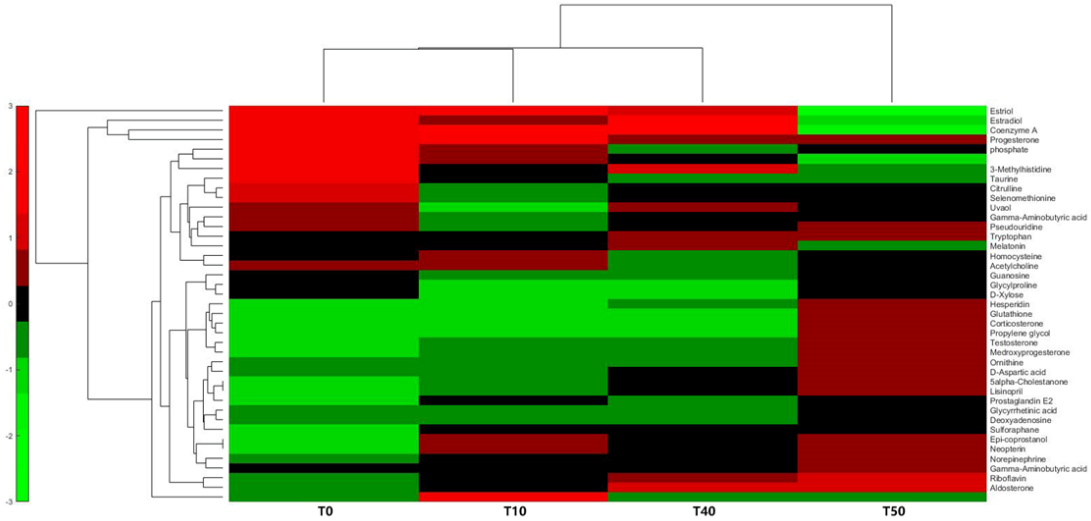

Figure 3. The heat map of significant metabolites in patients at different stages of injury. (The red color represents high concentration, and the green color represents low concentration. The heat map display relatively decreased metabolites (estriol, estradiol, coenzyme A, 3-Methylhistidine and taurine), and some increased metabolites (hesperidin, guanosine and testosterone). The heat map is generated using NMR spectrometry-based metabolomics.