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Original Article

Metabolic study in serum from patients with sepsis and severe sepsis

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Abstract: Sepsis is a whole-body inflammatory state that is caused by infection, which can threaten or reduce the quality of life. It is important to control sepsis progression within the shortest amount of time, to make an accurate diagnosis, and to choose the right treatment measures. In this study, we developed a serum metabolomic method based on gas chromatography-mass spectrometry (GC-MS) to evaluate the effect of sepsis in human body. The patients with sepsis were further divided into two subgroups, including sepsis and severe sepsis. Partial least squares-discriminate analysis (PLS-DA) revealed that sepsis induced metabolic perturbations. Compared to the healthy control group, the level of d-Galactose of sepsis group increased; the level of propanoic acid, d-Galactose of severe sepsis group increased, while the glycerol of severe sepsis group decreased. The results indicate that metabolomic methods based on GC-MS may be useful to elucidate sepsis through the exploration of biomarkers (propanoic acid, d-Galactose, glycerol).

Keywords: Metabolomics, GC/MS, sepsis, serum, human

Introduction

Sepsis is a life-threatening disease often encountered as hospital-acquired infection. It requires rapid detection and adequate treatment to prevent multi-organ dysfunction and fatal outcome. Despite various advances in antibiotic and supportive therapies, the mortality rate of sepsis-related diseases remains high in intensive care units (ICUs) [1, 2] due to delayed evaluation of illness severity and inappropriate treatment of the condition [3]. Accurate and timely evaluation of illness severity is therefore urgently needed to limit mortality, reduce costs, and improve outcome [4-6]. Present diagnostic biomarkers obtained from immunological or microbiological tests, RNA-profiling or protein-based assays [7-9] require long processing times hampering rapid therapeutic consequences. Although process automation has brought advancements for some biomarker [10], there is still a need for new biomarkers to improve diagnostics and therapy monitoring in sepsis.

Metabonomics is a well-developed platform for studying systems biology, leading to high-throughput screening processes in clinical diagnosis [11-13]. It alters the traditional concepts of single biomarker analysis by aiming at detecting and using holistic metabolic patterns for clinical diagnosis. Proton nuclear magnetic resonance (1H NMR) spectroscopy, liquid chromatography combined with mass spectrometry (LC/MS) and gas chromatography combined with mass spectrometry (GC/MS) are now routinely applied for detecting changes in metabolic profiles [14-23].

In this study, metabolic differences between serum samples from patients with sepsis and healthy controls were analyzed using gas chromatography mass spectrometry (GC-MS) metabolomics techniques. The patients with sepsis

Table 1. Demographic and clinical data of patients at the time of sepsis and sever sepsis diagnosis

Paramater	Total (n = 51)	Sepsis (n = 17)	Sever Sepsis (n = 34)	*p*-value
Age	45.2±20.7	42.9±14.9	46.3±17.5	0.499[a]
Gender (male)	28 (54.9%)	8 (47.1%)	18 (52.9%)	0.692[b]
Etiology of Sepsis Pneumonia	40 (78.4%)	13 (76.5%)	27 (79.4%)	0.811[b]
Urinary tract infection	11 (21.6%)	4 (23.5%)	7 (20.6%)	0.811[b]
CRP (mg/L)	130 (22-300)	70 (22-159)	160 (57-300)	<0.001[c]
WBC (× 10^9/L)	16.2 (5.1-28.2)	12.6 (7.5-1.9)	18.0 (5.1-28.2)	0.001[c]
PT (× 10^9/L)	120 (13-250)	180 (110-250)	90 (13-244)	<0.001[c]
LAC (mmol/L)	2.18 (0.53-11.23)	1.04 (0.53-2.43)	2.78 (0.59-11.23)	<0.001
Scr (μmol/L)	143 (34-320)	70 (34-121)	180 (88-320)	<0.001[c]

Abbreviations: CRP, C-reactionprotein; WBC, white blood cell; PT, platelet count; LAC, serum lactate; Scr, serum creatinine. [a]t test; [b]chi-square test; [c]Mann-Whitney U test.

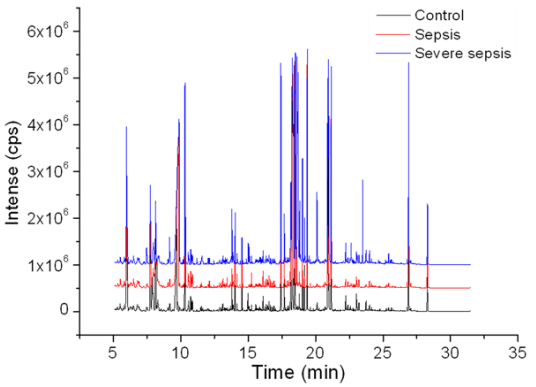

Figure 1. Typical GC-MS total ion chromatogram of serum from sepsis, severe sepsis group with healthy control group.

were further divided into two subgroups, including sepsis and severe sepsis, to screen for potential metabolic biomarkers of sepsis during these different stages of disease.

Material and methods

Chemicals

HPLC-grade n-heptane and acetonitrile were purchased from Tedia Reagent Company (Shanghai, China). Pyridine and methylhydroxylamine hydrochloride were purchased from Aladdin Industrial, Inc. (Shanghai, China). Trimethylchlorosilane (TMCS) and N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were purchased from Sigma-Aldrich (Shanghai, China).

Instrumentation and conditions

Agilent 6890N-5975B GC/MS, HP-5MS (0.25 mm × 30 m × 0.25 mm), were from Agilent Company (Santa Clara, California, USA). The GC oven was initially set at 80°C and was kept at this temperature for 5 minutes. The temperature was then gradually increased to 260°C at a rate of 10°C/min, and then kept at 260°C for 10 minutes. Mass detection was conducted first in EI mode with electron energy of 70 eV, then in full-scan mode with m/z 50-550, and finally, by splitless mode injection [17, 20].

Patients and blood samples

Fifty-one patients with sepsis were enrolled from the emergency department or intensive care unit (ICU) of the second affiliated hospital of Wenzhou Medical University, between January 2014 and January 2015. The inclusion criteria according to Surviving Sepsis Campaign Guidelines for Management of Severe Sepsis and Septic Shock: 2012 [24, 25]. Patients <18 years old, patients with venous nutrition or a metabolic disease were excluded. The samples for measurement were fasting blood and obtained within 24 hours after sepsis diagnosis. The healthy control was randomly selected in medical examination center of The Second Affiliated Hospital of Wenzhou Medical University over the same period. The study was approved by the ethics committee of The Second Affiliated Hospital of Wenzhou Medical University. All patients gave written informed consent.

Blood samples were collected from the healthy controls, sepsis and severe sepsis group, respectively. The blood samples were collected and then centrifuged at 4000 *g* for 10 min at 4°C. The serum was stored at -80°C until measurement.

Sample preparation

The 250 μL of acetonitrile was added to 100 μL of serum, kept in an ice-bath for 15 min, and

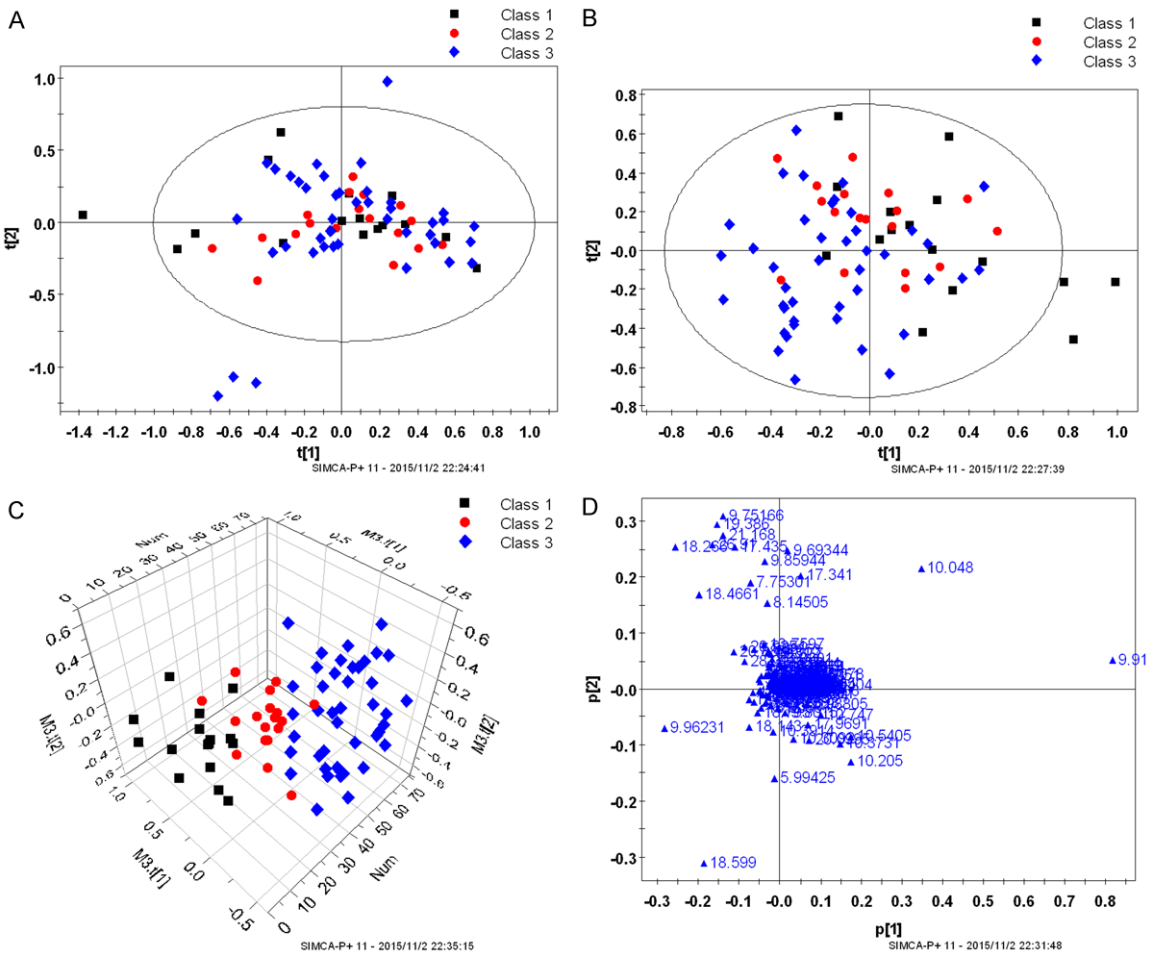

Figure 2. PCA (A) and PLS-DA (B) and PLS-DA 3D (C) score results of serum samples, healthy control group (Class 1), sepsis group (Class 2), severe sepsis group (Class 3); the corresponding load diagram (D).

then were centrifuged at 10000 *g* for 10 minutes at 4°C. The 150 μL of the supernatant was transferred to a GC vial and evaporated to dryness under a stream of nitrogen gas. Methoximation was carried out at 70°C for 24 h after 50 μL of methylhydroxylamine hydrochloride (15 mg/mL in pyridine) was added. The 50 μL MSTFA (with 1% TMCS as the catalyst) was added and kept at 70°C for another hour, and then vortexed after adding 150 μL n-heptane.

Data analysis

The GC/MS data was exported into Microsoft Excel, with the peaks normalized to the total sum of spectrum prior to multivariate analyses. The resulting data was processed through principal component analysis (PCA) and partial least squares discriminate analysis (PLS-DA) using SIMCA-P 11.5 software (Umetrics, Umea, Sweden).

Statistical analysis was carried out using SPSS software (Version 18.0, SPSS). Independent samples T-test was applied in order to detect significant differences in all metabolites between two groups. A *P* value of <0.05 was considered statistically significant.

Results and discussion

Patient characteristics

A total of 51 sepsis patients with mean age (± SD) 45.2 (±20.7) years were enrolled into this study, and 54.9% of which were male. The etiologies of Sepsis were Pneumonia (78.4%) and Urinary tract infection (21.6%).

Table 1 also displayed the demographic and clinical features of sepsis (n = 17) and severe sepsis (n = 34) which were grouped according to organ dysfunction, tissue perfusion inade-

Table 2. Summary of the changes in relative levels of metabolites in sepsis human serum

NO.	Renten time/min	Metabolite	VIP	Sepsis	Severe sepsis
1	10.048	glycerol	4.8963	-	↓*
2	10.5405	l-Threonine	2.61269	-	-
3	5.99425	Propanoic acid	2.27649	-	↑*
4	18.599	glucitol	2.23328	-	-
5	26.91	Benzoic acid	1.84821	-	-
6	18.143	d-Galactose	1.84648	↑*	↑*
7	18.266	d-Glucose	1.66799	-	-
8	19.386	Tetradecanoic acid	1.6577	-	-
9	21.168	Hexadecanoic acid	1.53902	-	-
10	10.747	Butanedioic acid	1.46604	-	-
11	8.35901	L-Isoleucine	1.43588	-	-
12	18.4661	d-Mannose	1.35218	-	-
13	13.812	L-Proline	1.32481	-	-
14	10.5751	l-Threonine	1.31808	-	-
15	10.3092	glycerol	1.31184	-	-
16	8.14505	Butanoic acid	1.0523	-	-
17	20.0995	Myo-Inositol	1.05128	-	-
18	7.75301	2-Propenoic acid	1.03784	-	-
19	13.7597	Xylitol	1.02737	-	-

Note: Variable importance in the projection (VIP) was acquired from the PLS-DA model with a threshold of 1.0. Marks indicate the direction of the change, i.e. ↓ for decrease, ↑ for increase, - for no change. Compared sepsis, severe sepsis group with healthy control group, *P<0.05, as indicated by the statistical analysis T-test.

quacy or hypotension. There were no significant differences in age, gender and etiology of sepsis between the two groups (P>0.05). Further analysis showed that the combined factors including CRP, white blood cell count and serum creatinine were all significantly higher in severe sepsis group when compared with the sepsis group (P<0.05). The comparison also showed that severe sepsis group had lower platelet count than sepsis group (P<0.05).

Metabolomics study

Metabolomics is a newly emerging omics approach to the investigation of metabolic phenotype changes induced by environmental or endogenous factors. It has shown promising results in healthcare fields, especially in disease diagnosis and drug-toxicity assessment, as reviewed recently. Although genomics, transcriptomics and proteomics have been used in many studies of sepsis, these results do not directly reflect the changes in metabolism that take place during sepsis. Only the metabolome can reveal the final downstream products that result from the preceding gene and protein expression.

Figure 1 provides the typical metabolic profiles of serum acquired through GC-MS technique. Metabolic profile data pretreatment resulted in a final dataset consisting of seventy metabolic features from GC-MS analyses. The endogenous metabolites in the serum were identified using the NIST 2005 mass spectrometry database.

In order to explore the metabolic profile changes of patient after sepsis and severe sepsis, we compared the GC-MS spectrum of PCA and PLS-DA of the sepsis group with the control group (**Figure 2**). **Figure 2** PCA (a) and PLS-DA (b) score chart showed that the first principal components of the sepsis group were not distinguished clearly from the control group. PLS-DA 3D (**Figure 2C**) score chart showed that sepsis group were distinguished from the control group clearer than 2D **Figure 2A** and **2B**, the corresponding load diagram was shown in **Figure 2D**.

Changes in metabolite

Metabolomics comprises the measurement of endogenous metabolites, including amino acids, nucleic acid precursors, lipids, and degradation products of chemical intermediates in catabolism and biosynthesis. The advantage of metabolomics is that it provides the most functional measure of cellular status and can help to describe an organism's phenotype [20].

In this study, the changes of metabolites between sepsis groups and their control group were shown in **Table 2**. Compared to the healthy control group, the level of d-Galactose of sepsis group increased, the level of propanoic acid, d-Galactose of severe sepsis group increased, while the glycerol of severe sepsis group decreased. These finding may be useful for new evidences in sepsis study. Additional prospective studies will be required to better understand these observations.

It is widely accepted that the serum lactate levels were increased significantly in many patients with sepsis because of severe hypoperfusion and tissue hypoxia [26]. Many studies

show that serum lactate levels were associated with mortality in septic patients [27, 28]. Nevertheless, it is sometimes difficult to distinguish severe sepsis from sepsis by measuring serum lactate levels. In this study, we found the propanoic acid was increased significantly in patients with severe sepsis, but not in general sepsis. Propanoic acid is regarded as an important precursor for the synthesis of lactate, and they also can be transformed into each other [29]. We also found the d-Galactose was increased simultaneously both in sepsis and severe sepsis. D-Galactose can stimulate neutrophil accumulation, and increased the mortality in septic mice by pathogen-associated molecular patterns [30]. So, we can draw a conclusion that the propanoic acidis and d-galactose were associated with sepsis. Especially propionic acid may be a potential marker for predicting severe sepsis.

Conclusion

These biomarkers (propanoic acid, d-Galactose, glycerol) were the additional evidence for sepsis patient. We demonstrated that metabolomic methods based on GC-MS could provide a useful tool for exploring biomarkers to elucidate sepsis. Propionic acid may be a potential marker for predicting severe sepsis.

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Disclosure of conflict of interest

None.

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