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Original Article

Metabonomics analysis of sepsis and non-infected SIRS patients based on mass spectrometry

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Abstract: The aim of the current study was to explore the roles of metabolomics in early diagnosis of sepsis by comparing blood and urine metabolites between sepsis patients and non-infected systemic inflammatory response syndrome (SIRS) patients. Seventeen sepsis patients and 13 non-infected SIRS patients were enrolled in this study. Blood and urine samples were collected from all patients and used for the detection of metabolomics using tandem mass spectrometry (MS/MS). Data were analyzed using specific analysis software and interpretation database. There were no differences in age, sex, and APACHEII scores between the two groups (P>0.05). Data from patient serum and urinary metabolites were normalized, then used for establishment of a 3-Diamensional map of the PCA model using specific software. This map showed a discrepancy between SIRS and sepsis groups. A total of 93 substances were detected in serum. According to importance of variable projection (VIP) rankings, there were 11 substances with VIP>1. Of these, aspartate, free carnitine, ornithine, and glutamic acid were increased, while valine, serine, and leucine were decreased (P<0.05). A total of 175 substances were determined in the urine samples, of which 16 substances were with VIP>1.4, according to the VIP ranking. Metabolomics, based on mass spectrometry, showed significant differences in blood and urine metabolites between non-infected SIRS patients and sepsis patients.

Keywords: Sepsis, systemic inflammatory response syndrome, metabonomics, MS-MS

Introduction

Sepsis, a systemic inflammatory response caused by infection, is caused by various pathogenic microorganisms or toxins presenting in the body. It is an important cause of death in intensive care units (ICU). In the United States, 750,000 new cases of severe sepsis occur annually, costing nearly 17 billion U.S. dollars each year. However, more than 210,000 deaths occur each year, with a mortality rate of nearly 29%. It has become the third leading cause of death in the United States [1].

According to recommendations of the 2012 guidelines, critically ill patients should be routinely screened for severe sepsis, improving early diagnosis and treatment of sepsis [2]. Identification and effective control the formation and development of sepsis are keys to improving the success rate of emergency treatment. However, due to the complicated and diverse conditions of sepsis, sensitivity and specificity of existing indices are not high enough to make an accurate diagnosis early. Thus, they cannot be effective indices for clinical diagnosis [3, 4]. Currently, there is no combined prediction model able to provide accurate guidance for clinical work and scientific research, leading to the ineffectiveness of early intervention to reduce incidence and mortality of multiple organ dysfunction syndrome (MODS).

Sepsis has been less studied in the field of metabolomics research. In fact, in sepsis patients, due to abnormal levels of inflammatory cytokines, reactive oxygen species and hormones, changes of the body environment, increasing body temperatures, and hypoxia, levels and activities of metabolic enzymes are significantly affected, causing metabolism disorders on glucose, protein, fat, and nucleic acid. Consequently, concentrations or proportions of certain metabolites in body fluids change significantly

in the early stages of inflammation, reflecting the severity of the disease to some extent [5].

Metabolomics is the study of changes in all metabolites, produced by the organisms responding to the external stimuli, by analyzing a cluster of indicators via high-throughput determination and data processing. It reflects the changes of metabolic response in cells or tissues induced by external stimuli or genetic modifications [6]. Metabonomics has been widely used in the diagnosis of diseases, achieving great success [7]. In recent years, studies of metabolomics on sepsis have been carried out more and more [8, 9]. Pierrako et al. [10] found more than 170 biomarkers in sepsis by reviewing the literature. Izquierdo et al. [11] used the H-NMR method to analyze metabolites in lung tissues, bronchoalveolar lavage fluid, and serum from sepsis SD rats and non-septic SD rats. They found that, in sepsis rats, sarcosine, alanine, cyclohexanhexol, and ethanolamine levels were elevated in the lung tissues and acetoacetate was elevated in serum, while cyclohexanhexol and sarcosine levels were decreased in the alveolar fluid. A diagnostic model was then established with a specificity and sensitivity of 100% for sepsis. Liu et al. [12] used LC-MS technology to compare differences of metabolomics between thermal burn and sepsis rats. Schmerler et al. [13] analyzed the metabolites of sepsis patients and systemic inflammatory patients using LC-MS/MS combined with specific software. They found that the concentrations of two metabolites (glyceryl trityl phosphatidylcholine and acylcarnitine) were significantly higher in sepsis than in systemic inflammatory response syndrome (SIRS).

In the current study, differences in serum and urine metabolites between sepsis patients and non-infected SIRS patients were analyzed using GC-MS and MS-MS techniques. Certain biomarkers of interest were found via metabonomics analysis for early diagnosis of sepsis, laying the foundation for follow-up studies.

Materials and methods

Subjects

Inpatients in the Department of Intensive Medicine of the Eastern Branch of the First Affiliated Hospital and the Sixth Affiliated Hospital of Sun Yat-sen University, from September 2013 to December 2015, were enrolled in this study. Inclusion criteria for the sepsis group included: 1) Aged 16-80 years, with no gender limitations; 2) With causes of sepsis, including infections and non-infection factors (severe trauma, severe pancreatitis, major surgery, cardiopulmonary resuscitation, etc.); 3) Struck by the above factors for 24 hours and in line with SIRS standards jointly proposed by ACCP/SCCM in 1991 [14]; and 4) Informed consent was obtained from the patients or their guardians. Exclusion criteria: 1) Patients that received anti-tumor drugs, radiation therapy, and immunosuppressive agents. Patients that received transplantation, patients with human immunodeficiency virus infection (AIDS), patients with autoimmune diseases, such as systemic lupus erythematosus (SLE), patients with agranulocytosis, and long-term dialysis patients; 2) Patients with brain death; 3) Patients complicated with all types of mental illness; 4) Pregnancy or suspected pregnant women; 5) Patients using intravenous or enteral nutrition; and 6) Patients that could not finish the plans on schedule though informed consent was provided.

Inclusion criteria for non-infected SIRS patients were as follows: 1) Aged 16-80 years, without gender limitations; 2) Developed from severe trauma, severe burns, major surgery, shock, or other non-infection factors and in line with SIRS standards jointly proposed by ACCP/SCCM in 1991; and 3) Informed consent was obtained from the patients or their guardians. Exclusion criteria were in accord with those for the sepsis group. Infected patients were also excluded.

This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University. Written informed consent was obtained from all participants.

Specimen collection and observation indices

Three drops of blood were taken from the fingers or heels of each subject, then dropped on a blood filter paper. Each blood spot should have a diameter of no less than 8 mm. After the blood spots were naturally dried, the filter paper was stored in a sealed plastic bag and kept at -20°C until testing. Additionally, 2 mL urine was collected in a special collection bag from each subject and stored at -20°C until testing.

Immediately after the patients were enrolled, blood and urine samples were collected and sent to the Lab of Genetics and Metabolism of the Sixth Hospital Affiliated to Sun Yat-sen University. They were analyzed using a tandem mass spectrometer (API 3200 QTRAP, Applied Biosystems, US) and gas chromatography-mass spectrometry (JEOL JMS-Q1000GC). Ninety-three indices were detected in blood samples and 175 substances were detected in urine samples. Routine blood tests, biochemistry, liver function, liver metabolism, C-reactive protein, serum procalcitonin, and blood gas analysis were determined at the Clinical Laboratory of the Hospital. Patient vital signs, body temperatures, and 24-hour urine output were recorded and APACHE II scoring was performed [15].

Determination of blood indices

Dried blood filter paper was made into a circular filter paper piece, with a diameter of 3 mm by a punch. The circular filter paper pieces (equal to 3.2 μL whole blood) were placed into 96-well plates. Next, 100 μL methanol, containing amino acids and acylcarnitine isotopic internal standards, was added to each well of the plate and incubated at room temperature for 20 minutes. Acylcarnitine and amino acids in the blood filter were extracted. The extract was centrifuged and placed in another 96-well polypropylene plate. After heating dry at 50°C, 60 μL N-butanol hydrochloride (3 mol/L) was added. The plate was then covered with a Teflon membrane and incubated at 65°C for 15 minutes. It was then dried at 50°C, followed by deliquescence with 100 μL 80% acetonitrile in each well. Eventually, the plate was covered with aluminum film for detection.

Software Chemo View Version 1.2 (Applied Biosystems) was used for quantitative analysis. Based on ion peak intensities of isotopic internal standards and various butyl esterified amino acids and acylcarnitines, concentrations of amino acids and acylcarnitines in each sample were automatically calculated following known concentrations of internal controls. Ninety-three metabolites were detected in each blood sample.

Determination of urine samples

Urine specimens were analyzed by gas chromatography-mass spectrometry. Chromatographic conditions were set as follows: splitless injections, injection volume 1 μL, inlet temperature 260°C, ion source temperature 200°C, and interface temperature 220°C. The programmed temperature started at 60°C and was held for 2 minutes. It was then raised to 220°C at a speed of 17°C/min, followed a speed of 15°C/min to 325°C. It was then held for 10 minutes. The carrier gas was helium, with a flow rate of 1 mL/min. Mass spectrometry conditions were as follows: ionization EI, electron energy 70 eV, MS scan range: 50-650 m/z, scan mode was full scan.

Data obtained from the tests were analyzed using a proprietary analytical software and interpretation database (Japan MILS) to characterize and quantify the peaks detected. Detection values of creatinine were taken as the internal standard and the ratio of the peak area of the detected substances and creatinine was calculated. Obtained data was compared with the upper limit of the normal range of the ratio to obtain the magnification between detected substances and the upper limit of normal range. A total of 175 urine metabolites were detected in each urine specimen.

Statistical analysis

SPSS22.0 software was used for statistical analysis. Measurement data are shown as mean ± standard deviation (SD) and compared using t-test between groups. Multivariate statistical analysis software SIMCA-P11.0 (Umeteics, Umea, Sweden) was used for principal component analysis (PCA) and partial least squares discrimination analysis (PLS-DA). After PLS-DA analysis, all values with a VIP value>1.0 were statistically analyzed (SigmaStat 3.5, Germany) and *t*-test was used to compare the two groups. $P<0.05$ indicates statistical significance.

Results

General data

General information of the 17 cases included in the sepsis group, including gender, age, and diagnosis, is shown in **Table 1**. Information of the 13 cases of non-infected SIRS patients is shown in **Table 2**. There were no significant differences in age, gender, and APACHE II scores between the two groups ($P>0.05$, **Table 3**).

Table 1. General information of the sepsis group

No.	Gender	Age	Clinical diagnosis	WBC count ($\times 10^9$/L)	Hypersensitive CRP (mg/L)	PCT (ng/mL)	APACHE II scores
1	M	66	Acute diffuse peritonitis, gastric perforation	12.63	17.55	>100	17
2	F	63	Gangrenous appendicitis and perforation	2.78	11.82	2.73	11
3	F	50	Primary diffuse peritonitis, sigmoid colon perforation, pneumonia	30.42	20.61	>100	18
4	M	75	Acute suppurative obstructive cholangitis, pulmonary infection	11.98	15.92	16.97	27
5	M	67	Pulmonary infection	8.66	12.13	1.86	22
6	M	68	Acute pyelonephritis, pneumonia	23.07	14.63	>100	21
7	F	75	Severe pneumonia	18.68	15.8	36.25	27
8	F	72	Aspiration pneumonia, coronary heart disease	3.81	13.17	2.51	31
9	F	57	Urinary tract infection	5.92	13.5	>100	19
10	F	72	Pulmonary infection	16.04	19.12	0.85	21
11	M	68	Acute incomplete intestinal obstruction, duodenal perforation	15.76	11.24	1.23	10
12	M	64	Severe pneumonia	12.09	65.46	>100	18
13	F	45	Sigmoid colon perforation, abdominal infection, septic shock	22.26	21.52	143.85	19
14	M	58	Secondary status epilepticus, aspiration pneumonia, sepsis	15.67	47.8	4.49	24
15	M	44	Large area cerebral infarction, severe pneumonia	14.48	13.22	25.16	19
16	M	43	Brainstem hemorrhage, blood infection, pulmonary infection	20.15	30.21	198.23	20
17	M	76	Cervical spinal cord injury and paralysis, pulmonary infection	12.85	12.42	0.47	20

Note: M, male; F, female; WBC, white blood cells; CRP, C reactive protein; PCT, procalcitonin; APACHE II, Acute Physiology and Chronic Health Evaluation II.

Table 2. General information of the non-infected SIRS group

No.	Gender	Age	Clinical diagnosis	APACHE II scores
1	F	55	Myasthenia gravis	12
2	F	57	Lumbar spinal stenosis (L3-5)	7
3	M	65	Right femoral neck fracture	18
4	M	65	Emphysema (bilateral multiple bullae)	17
5	F	57	Left femur intertrochanteric fracture	9
6	M	66	Lumbar spinal stenosis	12
7	M	53	Lumbar spinal stenosis	14
8	M	11	Four-ventricle large cell medulloblastoma	8
9	F	53	Meningioma, pontine hemangiomas complicated with venous malformations	25
10	M	62	Gallstone	11
11	M	50	Cervical 3/4 dislocation, hypertension	14
12	M	24	Multiple bilateral rib fractures, bilateral lung contusion, heart contusion	4
13	M	44	Brainstem hemorrhage	23

Note: M, male; F, female; APACHE II, Acute Physiology and Chronic Health Evaluation II.

Metabolomics in blood samples

Data from blood determination were normalized using software SIMCA-P11.0. A 3D graph of the PCA model of blood metabolites was established between sepsis and non-infected SIRS groups (**Figure 1**), indicating that the two groups distributed without cross, suggesting that there were significant differences in blood metabolites between sepsis and non-infected SIRS. In addition, data of the sepsis group was relatively scattered, while data of the non-infected SIRS group was relatively concentrated. Results suggest that the metabolisms of sepsis patients were significantly varied.

The 93 substances detected in blood samples were ranked from high to low, according to VIP ranking. The top ranked substances were aspartic acid (ASP), free carnitine (C0), ornithine (Orn), valine (Val), proline (Pro), glutamic acid (Glu), serine (Ser), Acetyl-L-Carnitine (C2),

Table 3. Comparison of general information between the two groups

Groups	Gender (M/F)	Age	APACHE II scores
Non-infected SIRS	9/4	50.92±16.36	13.38±6.12
Sepsis	10/7	62.53±11.20	20.24±5.25
P	>0.05	>0.05	>0.05

Note: M, male; F, female.

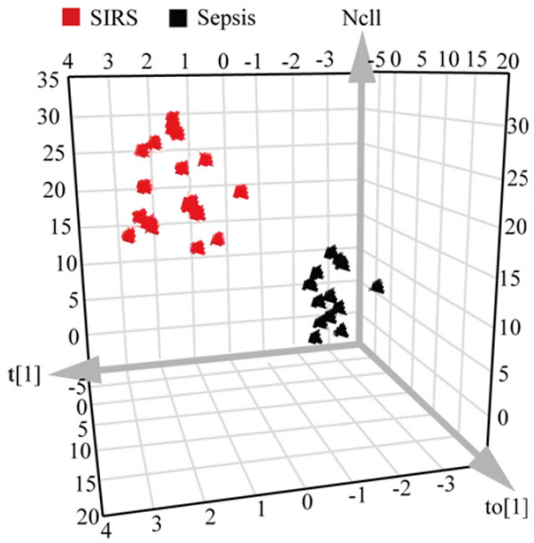

Figure 1. 3D graph of the PCA model established by the blood metabolites of sepsis and non-infected SIRS patients.

alanine (Ala), phenylalanine (Phe), leucine (Leu), citrulline (Cit), glutaminate (Gln), glycine (Gly), lysine (Lys), asparagine (Asn), piperamide (Pip), and histidine (His) (**Figure 2**). Moreover, 11 substances were found to be VIP>1, including ASP, C0, Orn, Val, Pro, Glu, Ser, C2, Ala, Phe, and Leu. A PLS-DA model was established using the VIP substances contributing to differences between the two groups after normalization (**Figure 3**). It was found that Asp, C0, Orn, and Glu were remarkably increased (P<0.05), while Val, Ser, and Leu were markedly reduced in the sepsis group (P<0.05).

Metabolomics in urine samples

Data from GC-MS were normalized using software SIMCA-P11.0. A 3D graph of the PCA model of urinary metabolites in sepsis and non-infected SIRS groups was established (**Figure 4**). This model showed no cross between distributions of the two groups, suggesting that levels of metabolites in urine samples were notably different between non-infected SIRS and sepsis groups.

Ranked from high to low, the top rank lists of VIP>1.4 among the 175 substances detected in urine samples were uric acid, 2-hydroxybutyric acid, phenyl glycolic acid, methylmalonic acid, 2-hydroxyisocaproic acid, 4-hydroxybutyric acid, acetoacetic acid, phenylacetic acid, 2-methylmalonic acid, propionyl, 3-methylmalonicacid,4-hydroxyproline,4,5-dihydroxypyruvate, 2-hydroxyphenylacetic acid, and N-(3-methyl-l-oxo-2-butanoyl) glycine (**Figure 5**).

Discussion

Relationship between the metabolism of sepsis and possible biomarkers detected in blood samples

In this study, aspartate, free carnitine, ornithine, and glutamic acid were found to be increased, while valine, serine, and leucine were decreased in the blood of patients with sepsis, according to metabonomics analysis. The top ranked urine metabolites with VIP>1.4 were uric acid, 2-hydroxybutyric acid, phenylglycolic acid, methylmalonic acid, 2-hydroxyisocaproic acid, 4-hydroxybutyric acid, acetoacetic acid, phenylacetic acid, 2-methylmalonic acid, propionyl, 3-methylmalonic acid, isovaleric acid, 4-hydroxyproline, 4,5-dihydroxypyruvate, 2-hydroxyphenylacetic acid, and N-(3-methyl-1-oxo-2-buthyl) glycine. A series of pathophysiological changes occur in the body when sepsis happens, especially in the neuroendocrine system. Levels of catecholamines, adrenal cortex hormones, glucagon, insulin, growth hormone, TNF-α, IL-6, and others are significantly increased [16]. These hormones or inflammatory factors make low utilization of exogenous nutrients. Thus, the body energizes mainly by decomposing its own proteins and mobilizing adipose tissue. This reduces the proportion of energy supply by glucose oxidation [17]. Therefore, the proportions of protein, fat, sugar, and metabolites are significantly changed. The metabolic disruption of these biomarkers, detected in this study, involved oxidative stress, energy metabolism, inflammatory response, and so forth. Therefore, these biomarkers may be potential markers for early diagnosis of sepsis. They may also be auxiliary indicators for diagnosis of sepsis.

	1	2	3
1	Var ID (Primary)	Var ID (Var. Sec. ID:1)	M3.VIP [1]
2	Var_4	Asp	3.96039
3	Var_24	C0	3.80511
4	Var_15	Orn	3.66825
5	Var_23	Val	3.05676
6	Var_18	Pro	3.01654
7	Var_92	C0/(C16+C18)	2.85592
8	Var_8	Glu	2.22094
9	Var_19	Ser	1.84481
10	Var_25	C2	1.46561
11	Var_1	Ala	1.26319
12	Var_90	(0+2+3+16+18:1)/Cit	1.19111
13	Var_16	Phe	1.14628
14	Var_12	Leu	1.07236
15	Var_55	Orn/Cit	0.916466
16	Var_5	Cit	0.893066
17	Var_7	Gln	0.781057
18	Var_9	Gly	0.770797
19	Var_69	C5-OH/C8	0.764402
20	Var_13	Lys	0.74577
21	Var_3	Asn	0.696478
22	Var_58	Val/Phe	0.531492
23	Var_17	Pip	0.491743
24	Var_11	His	0.47918
25	Var_57	Tyr/Cit	0.408414

Figure 2. VIP ranking of blood metabolites obtained via regression coefficient chart (Top 24).

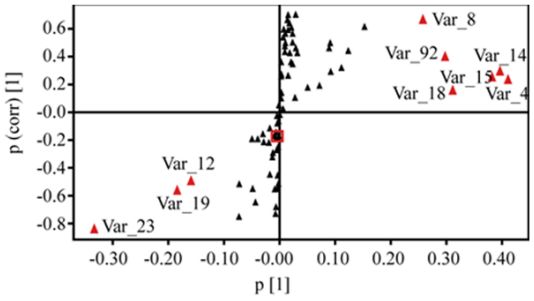

Figure 3. OPLS-DS model of the differently expressed substances between the two groups.

In sepsis, protein is in a high catabolism state *in vivo*, especially skeletal muscle protein degradation [18]. At the same time, the liver is predominantly responsible for the uptake of amino acids, oxidative utilization, and gluconeogenesis, resulting in the concentrations of most amino acids decreasing significantly [19]. Most amino acids (except branched-chain amino acids) are transamined or deaminated to convert into other amino acids. They then synthesize albumin or generate urea in the liver. Aspartic acid, a structural unit of protein, is not only a synthetic precursor of lysine, threonine, isoleucine, methionine, other amino acids, and purine and pyrimidine bases in organisms, but is also involved in the ornithine cycle, promoting the generation of urea from oxygen and carbon dioxide. One part of the urea cycle, ornithine is associated with urea production. It combines with carbamyl phosphate to form phosphoric acid and citrulline, which converts into arginine and splits into ornithine and urea. Moreover, ornithine can interconverse with arginine, glutamic acid, and proline *in vivo*, playing an important role in metabolism. Glutamic acid is deaminated into glutamine by glutamate dehydrogenase, which can promote the oxidation of glutamine when the body is in a state of insufficient energy and hypoxia. Thus, it plays a vital regulatory role in the body's energy metabolism [20]. Under stress, glutamine metabolism is mainly carried out by incomplete oxidation. Partial oxidation metabolites mainly include glutamic acid, aspartic acid, and ammonia. Therefore, in blood collected from sepsis patients, the contents of aspartic acid, ornithine, and glutamic acid are high.

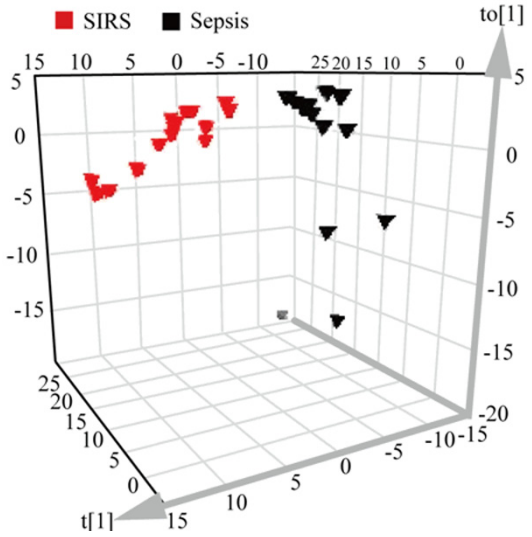

Figure 4. 3D graph of the PCA model established by the urine metabolites of sepsis and non-infected SIRS patients.

Valine and leucine, branched-chain amino acids, work with isoleucine to promote body growth, repair muscles and tissues, regulate blood glucose, and provide the body with nec-

	1	2	3	4
1	Var ID (Primary)	Var ID(Var. Sec. ID:1)	M3.VIP [1]	1.89456 * M3.VIP [1] cvSE
2	Var_111	Uric acid	1.45169	0.99559
3	Var_7	2-hydroxy-butyric acid	1.44592	0.293164
4	Var_14	Malonic acid 1	1.44592	0.293164
5	Var_17	Methylmalonic acid	1.44592	0.293164
6	Var_22	2-hydroxy-isocaproic acid	1.44592	0.293164
7	Var_23	4-hydroxy-butyric acid	1.44592	0.293164
8	Var_25	Acetoactic acid 1	1.44592	0.293164
9	Var_28	Acetoactic acid 2	1.44592	0.293164
10	Var_29	Phenylacetic acid	1.44592	0.293164
11	Var_34	2-methylacetoacetic acid	1.45169	0.293164
12	Var_39	Propionyglycine 1	1.44592	0.293164
13	Var_45	Malonic acid 2	1.44592	0.293164
14	Var_47	Propionyglycine 2	1.44592	0.293164
15	Var_48	3-methylglutaric acid	1.44592	0.293164
16	Var_52	isovalerylglycine-1	1.44592	0.293164
17	Var_57	4-hydroxy-proline	1.44592	0.293164
18	Var_59	4,5-dihydroxyhexonic acid	1.44592	0.293164
19	Var_61	2-hydroxyphenylacetic acid	1.44592	0.293164
20	Var_62	3-methylcrotonylglycine	1.44592	0.293164

Figure 5. VIP ranking of urine metabolites obtained via regression coefficient chart (Top 19).

essary energy. Isoleucine supplies the necessary energy for the body's normal growth, tissue repair, and regulation of blood sugar. In the high metabolic state of sepsis, in addition to supplying energy for muscles, branched-chain amino acids provide a nitrogen source for the synthesis of glutamine and other amino acids. If the body remains in a persistent high metabolic state, irreversible damage can occur when the protein and branched-chain amino acids reserves in muscle are exhausted. Furthermore, during the compensatory periods of hyperkinetic sepsis, hepatic uptake of amino acids is accelerated. The synthesis of intrahepatic acute proteins rises rapidly. Since the amino acid delivery system is inhibited, the muscle intake of amino acids is hindered. With the increased lysis of myoprotein, amino acids flow into the liver from the periphery. The liver uptakes amino acids and synthesizes glycogen and protein. This may also be one reason for the decreased content of plasma branched-chain amino acids [21]. The present study also found that valine and leucine contents were low in patients with sepsis.

Serine plays an important role in the metabolism of fatty acids and fats and the growth of muscle. Under high energy consumption conditions of sepsis, mobilization of fat is strengthened, especially in the circumstance of decompensation that free fatty acids become the preferred oxidative energy supply largely used by oxidation. Thus, serine reserves are exhausted. Carnitine is closely related to energy metabolism. It transports fatty acids from the cytoplasm to the mitochondria during lipid fragmentation of the energy metabolism in cells. It has been reported that the content of carnitine in the body is significantly increased in the early stage of sepsis, while it is remarkably decreased in persistent sepsis [22]. Inoue et al. pointed out that carnitine inhibits intracellular calcium transport in endothelial cells, thereby inhibiting cell relaxation. They also speculated that carnitine may play important roles in endothelial cell dysfunction [23].

Relationship between metabolic changes in sepsis and potential biomarkers detected in urine

This experiment screened many biomarkers, including uric acid, 2-hydroxybutyric acid, phenylglycolic acid, methylmalonic acid, 2-hydroxyisocaproic acid, 4-hydroxybutyric acid, acetoacetic acid, phenylacetic acid, methylmalonic acid, isopentanoic acid, 4-hydroxyproline, 4,5-dihydroxypyruvate, 2-hydroxyphenylacetic acid, and N-(3-methyl-1-oxo-2-butanoyl) glycine. It is believed that all the above substances are related to occurrence and development of sepsis in the process of urinary metabolism.

The association between uric acid and inflammation has drawn much attention. Chuang et al. [24] showed that elevated uric acid levels in sepsis patients was related to the decrease of uric acid excretion and increase of uric acid production. When severe sepsis and septic shock occur, ischemia or tissue hypoxia happens in multiple organs. Consequently, the activated xanthine oxidase in microvascular endothelial cells converts xanthine and hypoxanthine into uric acid. It can also increase the production of uric acid by activating leukocytes.

Hydroxybutyric acid and acetoacetic acid are two ketone bodies. Their increase may be related to elevated fatty acid oxidation. Under high energy consumption conditions of sepsis, mobilization of fat is strengthened, especially in decompensation that free fatty acids become

the preferred source for energy supply by oxidation and are largely consumed.

Pyruvic acid is the pivot of the interconversion among sugar, proteins, and fat. Under the relatively anaerobic conditions of sepsis, pyruvate dehydrogenase activity is decreased. This leads to the conversion into the tricarboxylic acid cycle being blocked, resulting in an increase of plasma pyruvic acid levels. Adenosine triphosphate is the allosteric activator of pyruvate carboxylase. If the energy supply is insufficient, pyruvic acid metabolism through the carboxylate branch is decreased. This can also lead to an increased content of pyruvate [20]. In addition, many proteins are decomposed in sepsis, causing plasma free amino acids to increase. Alanine, serine, glycine, cysteine, and tryptophan can also be transaminated into pyruvate [16].

A study by Cerra et al. [25] reported that proline levels are increased in sepsis patients. This can be used as a reference index for increased mortality. In this study, blood and urine samples of sepsis and non-infected SIRS patients were analyzed at the same time. This method is superior to only blood or urine used. Present results may reflect the metabolic characteristics of sepsis more completely and truly. Due to its high sensitivity and less sample amounts required, GC-MS was used as the detection platform. It is suitable for the detection of trace substances. With a standard mass spectrometry database, a variety of metabolites can be detected at a time by GC-MS. This is in line with the rapid and simple demands of clinical testing methods. Moreover, statistical analysis of data by combining unsupervised mode (PCA), supervised mode (PLS-DA), and t-tests can ensure the reliability of results. It avoids the drawbacks of time-consumption and high costs from the previous single substance or index detected or combined with multiple indicators, but also avoids the risk of lagging in relevant indicators or examinations. These factors improve early diagnosis of sepsis and promotes early treatment of sepsis.

There were some inconsistencies in present results, compared with other metabolomics studies. Detected potential biomarkers were not consistent completely [26]. The reasons may include: 1) Different sources of blood samples, venous blood, or peripheral blood used; 2) Different sample processing methods, such as serum or plasma used; and 3) Different determination methods and different test objects [27].

Due to experimental conditions and time constraints, the number of cases in this study was small. It should be expanded in further studies. The lack of detection of polyunsaturated fatty acid (PUFAs), nucleic acids, and other small molecular substances in this experiment may have resulted in the deletion of some important diagnostic information. Confounding factors in clinical studies are difficult to rule out, such as body weight, other diseases that may affect the metabolism, and iatrogenic factors. This may contribute to results bias. In addition, different organs, tissues, and cells respond unevenly to metabolism. Thus, overall metabolites analysis may ignore some internal differences, resulting in loss of information. Therefore, some researchers have proposed cell and subcellular metabonomics [28, 29]. This has brought a new direction and challenges to metabolomics.

In conclusion, metabolomics research methods, based on mass spectrometry, can be used to detect blood and urine metabolites of patients with sepsis and non-infected SIRS patients, providing accurate qualitative and quantitative analysis results. Compared with non-infected SIRS patients, sepsis patients have significant differences in blood and urine metabolites. This study found some of the differently expressed biomarkers. These include aspartic acid, free carnitine, ornithine, glutamic acid, valine, serine and leucine blood metabolites, uric acid, hydroxybutyric acid, acetoacetate, pyruvic acid, and proline in urinary metabolites. These can be used to identify sepsis patients from non-infected SIRS patients.

Conclusion

Metabolomics, based on mass spectrometry, showed significant differences in the blood and urine metabolites between non-infected SIRS patients and sepsis patients. This study found some of the differently expressed biomarkers. These include aspartic acid, free carnitine, ornithine, glutamic acid, valine, serine and leucine blood metabolites, uric acid, hydroxybutyric acid, acetoacetate, pyruvic acid, and proline in urinary metabolites. These can be used to iden-

tify sepsis patients from non-infected SIRS patients.

Disclosure of conflict of interest

None.

Abbreviations

GC-MS, Gas chromatography-tandem mass spectrometry; SIRS, Systemic inflammatory response syndrome; PCA, Principal component analysis; PLS-DA, Partial least square-discriminant analysis; VIP, Variable importance in projection; MODS, Multiple organ dysfunction syndrome; 1H/13C-NMR, 1H/13C-nuclear magnetic resonance; HPLC-MS, High performance liquid chromatography-mass spectrometry; FT-IR, Fourier Transform Infrared Spectrometer; Ala, alanine; Arg, arginine; Asn, asparagines; Asp, aspartic; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

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References

[1] Buehler SS, Madison B, Snyder SR, Derzon JH, Cornish NE, Saubolle MA, Weissfeld AS, Weinstein MP, Liebow EB and Wolk DM. Effectiveness of practices to increase timeliness of providing targeted therapy for inpatients with bloodstream infections: a laboratory medicine best practices systematic review and meta-analysis. Clin Microbiol Rev 2016; 29: 59-103.

[2] Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R; Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving sepsis campaign guidelines committee including the pediatric subgroup. surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013; 39: 165-228.

[3] Sponholz C, Sakr Y, Reinhart K and Brunkhorst F. Diagnostic value and prognostic implications of serum procalcitonin after cardiac surgery: a systematic review of the literature. Crit Care 2006; 10: R145.

[4] Balcl C, Sungurtekin H, Gürses E, Sungurtekin U and Kaptanoglu B. Usefulness of procalcitonin for diagnosis of sepsis in the intensive care unit. Crit Care 2003; 7: 85-90.

[5] Christaki E and Giamarellos-Bourboulis EJ. The beginning of personalized medicine in sepsis: small steps to a bright future. Clin Genet 2014; 86: 56-61.

[6] Skibsted S, Bhasin MK, Aird WC and Shapiro NI. Bench-to-bedside review: future novel diagnostics for sepsis-a systems biology approach. Crit Care 2013; 17: 231.

[7] Rogers AJ and Matthay MA. Applying metabolomics to uncover novel biology in ARDS. Am J Physiol Lung Cell Mol Physiol 2014; 306: L957-961.

[8] Mickiewicz B, Tam P, Jenne CN, Leger C, Wong J, Winston BW, Doig C, Kubes P and Vogel HJ; Alberta Sepsis Network. Integration of metabolic and inflammatory mediator profiles as a potential prognostic approach for septic shock in the intensive care unit. Crit Care 2015; 19: 11.

[9] Mickiewicz B, Duggan GE, Winston BW, Doig C, Kubes P and Vogel HJ; Alberta Sepsis Network. Metabolic profiling of serum samples by 1H nuclear magnetic resonance spectroscopy as a potential diagnostic approach for septic shock. Crit Care Med 2014; 42: 1140-1149.

[10] Pierrakos C and Vincent JL. Sepsis biomarkers: a review. Crit Care 2010; 14: R15.

[11] Izquierdo-García JL, Nin N, Ruíz-Cabello J, Rojas Y, de Paula M, López-Cuenca S, Morales L, Martínez-Caro L, Fernández-Segoviano P, Esteban A and Lorente JA. A metabolomic approach for diagnosis of experimental sepsis. Intensive Care Med 2011; 37: 2023-2032.

[12] Liu XR, Zheng XF, Ji SZ, Lv YH, Zheng DY, Xia ZF and Zhang WD. Metabolomic analysis of thermally injured and/or septic rats. Burns 2010; 36: 992-998.

[13] Schmerler D, Neugebauer S, Ludewig K, Bremer-Streck S, Brunkhorst FM and Kiehntopf M. Targeted metabolomics for discrimination of systemic inflammatory disorders in critically ill patients. J Lipid Res 2012; 53: 1369-1375.

[14] American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992; 20: 864-74.

[15] Rizzi G, Startseva X, Wolfrum M, Lüscher TF, Ruschitzka F, Fröhlich GM and Enseleit F. Unfa-

vorable donor pretransplant APACHE II, SAPS II, and SOFA scores are not associated with outcome: implications for heart transplant donor selection. Transplant Proc 2016; 48: 2582-2587.
[16] Peng J and Du B. Sepsis-related stress response: known knowns, known unknowns, and unknown unknowns. Crit Care 2010; 14: 179.
[17] Verhoeven JJ, den Brinker M, Hokken-Koelega AC, Hazelzet JA and Joosten KF. Pathophysiological aspects of hyperglycemia in children with meningococcal sepsis and septic shock: a prospective, observational cohort study. Crit Care 2011; 15: R44.
[18] Su L, Li H, Xie A, Liu D, Rao W, Lan L, Li X, Li F, Xiao K, Wang H, Yan P, Li X and Xie L. Dynamic changes in amino acid concentration profiles in patients with sepsis. PLoS One 2015; 10: e0121933.
[19] Wijnands KA, Brink PR, Weijers PH, Dejong CH and Poeze M. Impaired fracture healing associated with amino acid disturbances. Am J Clin Nutr 2012; 95: 1270-1277.
[20] Zupke C, Sinskey AJ and Stephanopoulos G. Intracellular flux analysis applied to the effect of dissolved oxygen on hybridomas. Appl Microbiol Biotechnol 1995; 44: 27-36.
[21] Wernerman J, Hammarqvist F, Gamrin L and Essén P. Protein metabolism in critical illness. Baillieres Clin Endocrinol Metab 1996; 10: 603-615.
[22] Lanza-Jacoby S, Holahan M and Reibel DK. Changes in tissue levels of carnitine during E. coli sepsis in the rat. Circ Shock 1988; 24: 29-34.
[23] Inoue N, Hirata K, Akita H and Yokoyama M. Palmitoyl-L-carnitine modifies the function of vascular endothelium. Cardiovasc Res 1994; 28: 129-134.
[24] Chuang CC, Shiesh SC, Chi CH, Tu YF, Hor LI, Shieh CC and Chen MF. Serum total antioxidant capacity reflects severity of illness in patients with severe sepsis. Crit Care 2006; 10: R36.
[25] Cerra FB, Caprioli J, Siegel JH, McMenamy RR and Border JR. Proline metabolism in sepsis, cirrhosis and general surgery. The peripheral energy deficit. Ann Surg 1979; 190: 577-586.
[26] Langley RJ, Tsalik EL, van Velkinburgh JC, Glickman SW, Rice BJ, Wang C, Chen B, Carin L, Suarez A, Mohney RP, Freeman DH, Wang M, You J, Wulff J, Thompson JW, Moseley MA, Reisinger S, Edmonds BT, Grinnell B, Nelson DR, Dinwiddie DL, Miller NA, Saunders CJ, Soden SS, Rogers AJ, Gazourian L, Fredenburgh LE, Massaro AF, Baron RM, Choi AM, Corey GR, Ginsburg GS, Cairns CB, Otero RM, Fowler VG Jr, Rivers EP, Woods CW and Kingsmore SF. An integrated clinico-metabolomic model improves prediction of death in sepsis. Sci Transl Med 2013; 5: 195ra95.
[27] Su L, Huang Y, Zhu Y, Xia L, Wang R, Xiao K, Wang H, Yan P, Wen B, Cao L, Meng N, Luan H, Liu C, Li X and Xie L. Discrimination of sepsis stage metabolic profiles with an LC/MS-MS-based metabolomics approach. BMJ Open Respir Res 2014; 1: e000056.
[28] Liu X, Ren H and Peng D. Sepsis biomarkers: an omics perspective. Front Med 2014; 8: 58-67.
[29] Delanghe JR and Speeckaert MM. Translational research and biomarkers in neonatal sepsis. Clin Chim Acta 2015; 451: 46-64.