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Systemic Metabolic Changes of Traumatic Critically Ill Patients Revealed by an NMR-Based Metabonomic Approach

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Progression of critically ill patients from Systemic Inflammatory Response Syndrome (SIRS) to Multiple Organ Dysfunction Syndrome (MODS) accounts for more than 75% of deaths in adult surgical intensive care units. Currently, there is no practical clinical technique to predict the progression of SIRS or MODS. In this report, we describe an NMR-based metabonomic method to aid detection of these conditions based on abnormal metabolic signatures. We applied pattern recognition methods to analyze one-dimensional ^{1}H NMR spectra of SIRS and MODS patient sera. By using Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA), we could distinguish critically ill patients ($n = 52$) from healthy controls ($n = 26$). After noise reduction by Orthogonal Signal Correction (OSC), PLS-DA was also able to clearly discriminate SIRS and MODS patients. The corresponding coefficients indicated that spectra responsible for the discrimination were located in δ3.06−3.86 NMR integral regions from SIRS, mainly composed of sugars, amino acids and glutamine signals, and δ1.18−1.3 and δ4.02−4.1 integral regions of MODS serum samples, principally consisted of various proton signals of fatty acyl chains and glycerol backbone of lipids, along with creatinine and lactate. Our results are consistent with the clinical observations that carbohydrate and amino acid levels changes in the early course of critical illness (SIRS stage) and significant disturbances in fat metabolism and development of organ abnormalities become the characteristics in the late stage (MODS). These data suggest that NMR-based metabonomic approach can be developed to diagnose the disease progress of critically ill patients.

Keywords: NMR • metabonomics • pattern recognition • orthogonal signal correction • multiple organ dysfunction syndrome • systemic inflammatory response syndrome • sera

Introduction

Multiple organ dysfunction syndrome (MODS) is considered a leading cause of mortality in critically ill patients, accounting for more than 75% of deaths in adult surgical intensive care units. The early stage of MODS is systemic inflammatory response syndrome (SIRS), which is essentially a defensive reaction.[1] The development of MODS is accompanied by a series of metabolic changes involving many different pathways.[2] An abrupt rise of the secretion of stress hormones, inflammatory mediators and neurotransmitters, together with altered activity and alertness of patients, is observed.[3] Increases of energy consumption, as well as protein and lipid degradation, can lead to hyperglycemia, severe negative nitrogen balance, decreased immune responses and tissue healing capability, respiratory muscle atrophy, cell and systemic organ dysfunction.[2,3] Rapid assessment of patient condition is important for these SIRS and MODS critically ill patients, so a convenient and efficient method with minimal disturbance to patients is needed to assess metabolic disturbances and predict the development of MODS as early as possible.

Several indicators are available to determine the nutrition support in clinic. These indicators include body weight, concentrations of serum albumin, transferrin, and prealbumin, and measurements of nitrogen excretion, nonprotein respiratory quotient (NPRQ), nitrogen balance, energy expenditure, caloric intake, and cumulative caloric balance (CCB).[2] Although these indicators are useful for temporary nutrition support determination, they cannot predict whether a patient will develop MODS. In addition, most of the methods are usually used only in animal experiments because they are time-consuming and impractical in clinic. Furthermore, these indicators fail to assess the systemic metabolic state of patients, including metabolic changes of carbohydrates, proteins and

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lipids in patients. Although there are also some diagnostic methods such as angiography, biopsy, pulmonary and heart function tests that can be used to determine the clinical condition of a patient, these technologies are invasive and time-consuming, and critically ill patients are usually unable to withstand the tests. Some of biochemical parameters indicating the organ function are applied to detect the presence of MODS in clinic, but they cannot predict the progression of the disease in advance. Until now, there has been no convenient and efficient method to predict the development of MODS for a clinically ill patient.

NMR-based metabonomic approach is a newly developed technology that has been exploited to be a useful tool for disease diagnosis.[4−10] The approach uses NMR spectroscopy to quantitatively measure metabolic pattern changes in easily collected body fluids from a living organism in response to pathophysiological stimuli or genetic modifications.[4] The measurements of the metabolic changes are profiled using multivariate algorithms that are commonly used in chemometrics.[4] NMR allows rapid, nondestructive, nonselective and highly reproducible data collection.[4−7] NMR-based metabonomics has generated great interest as an important tool to diagnose and monitor the progress of clinical diseases.[4−7] Brindle et al.[8] reported for the first time the application of supervised partial least-squares-discriminant analysis (PLS-DA) to orthogonal signal-corrected data sets. This method could separate 90% of patients with stenosis of all three major coronary vessels from those with angiographically normal coronary arteries, with a specificity of more than 90%. In another study on hypertension, NMR-based metabonomics was not only able to group sera with different systolic blood pressure levels, but also reveal a relationship between serum metabolic profiles and blood pressure. They also demonstrated that the relationship was partly based on differences of lipoprotein particle composition between the samples.[9] Odunsi et al.[10] found that the principal component analysis (PCA) method could separate 38 serum specimens of epithelial ovarian cancer from 21 premenopausal normal samples and from 12 of benign ovarian cyst with an accuracy of 100%. In addition, it correctly categorized 37 of 38 (97.4%) cancer specimens from 31 of 32 (97%) postmenopausal control sera.

In this report, we made an attempt to determine whether NMR-based metabonomic approach can be used to profile critically ill patients. We chose traumatic patients in order to increase the homogeneity of critical illness. Pattern recognition and signal correction technologies were used to analyze the one-dimensional 600 MHz ^{1}H NMR spectral data from serum samples. Our data indicate that this approach can distinguish critically ill patients from normal subjects, but more importantly distinguish SIRS patients from MODS.

Materials and Methods

Subjects. Total number of 52 serum samples (26 SIRS and 26 MODS) were collected from traumatic inpatients at the Affiliated Changhai Hospital of Second Military Medical University and the Affiliated Hospital of Nantong University. Traumatic insults resulted from road accidents, accidental traumas, fall injuries, and fire burns. SIRS patients were defined according to the 2003 American Thoracic Society (ATS) consensus conference criteria.[11] These patients had a mean age of 49.8 ± 20.3 years, 19 males and 7 females. MODS subjects (23 males and 3 females, 40.9 ± 11.8 years) were diagnosed according to the revised Fry-MODS diagnostic criteria of China published in 1997.[12,13] Among these patients, 7 had cardiovascular dysfunction, 15 had central nervous system and respiratory disorders, 21 and 14 had impairments of renal and liver function, respectively, and 13 had clotting abnormalities. All cases had different levels of gut and metabolic disturbances. Exclusion criteria for the cohort of patients under investigation were patients of cancer, endocrine disorders (e.g., diabetes, hyperthyroidism), severe obesity (body weight 20% greater than the ideal standard), or any other chronic organ impairments. Control sera were collected from healthy adult male volunteers for medical examination at the Affiliated Hospital of Nantong University, who had no inflammatory diseases in previous 2 weeks.

Serum Samples. Blood was drawn intravenously in the fasting state, without any other restrictions in diet, lifestyle, and physiological cycle. The blood was allowed to clot at room temperature, and then centrifuged to separate the upper serum. Aliquots of serum were stored at −80 °C until analysis.

One-Dimensional ^{1}H NMR Spectra. The stored sera were defrosted at room temperature. After centrifugation, 500 μL of upper serum and 50 μL of D_2O (as a field-frequency lock) were mixed in 5 mm NMR tubes for NMR analysis. All NMR spectra were recorded at 298 K on a Varian Unity INOVA 600 NMR spectrometer operating at 599.69 MHz ^{1}H frequency and equipped with a triple resonance probe and *z*-axis pulsed field gradient. Standard one-dimensional (1D) PRESAT approach using a single 90° pulse sequence was first employed to suppress the solvent peak, and 1D spin−echo spectra were recorded using the CPMG (Carr−Purcell−Meiboom−Gill) pulse sequence with a fixed total spin−spin relaxation delay of 120 ms to attenuate the broad NMR signals from slowly tumbling molecules such as proteins and retain those from low-molecular weight compounds and some lipid components. Typically, 64 transients and 16K data points were collected with a spectral width of 8,000 Hz, an acquisition time of 1.024 s, and a relaxation delay of 4 s. The free induction decay (FID) was zero-filled to 64K and an exponential line-broadening function of 0.3 Hz was applied to the FID prior to Fourier transformation. Both phase correction and baseline correction were carefully performed. The ^{1}H chemical shifts were referred to methyl doublet signal of lactate (δ1.33).

NMR Spectral Data Reduction. All NMR spectra were date-reduced to 245 integrated regions of equal width of 0.04 ppm (buckets) corresponding to the region of δ0.2−10.0 using the software package VNMR 6.1C (Varian, Inc.) for the purpose of decreasing the influences of pH and ionic strength on chemical shifts and facilitating the following pattern recognition analysis. The region δ6.0 to 4.5 was set to zero integral to eliminate the effects of variation both in the suppression of water resonance and in the urea resonance caused by partial cross-saturation.[8] The remaining spectral segments for each NMR spectrum were normalized to the total sum of the spectral area to partially compensate for differences in concentration of the many metabolities in the samples. Although serum samples are largely homeostatic, those from critically ill patients are not because these patients have disorders of water and electrolytes due to trauma (loss of blood), shock (reduction of effective blood volume), and kidney dysfunction (retention of sodium and water). Therefore, it is necessary to apply area normalization to NMR profiles of these serum samples in order to

Table 1. Clinical and Biochemical Parameters of the Subjects Used in This Study[a]

items	SIRS group	MODS group	control group
n	26	26	26
males	19	23	26
females	7	3	0
age (years)	49.8 ± 20.3	40.9 ± 11.8	41.1 ± 9.7
Glc (mmol/L)	7.62 ± 2.26	7.56 ± 1.97	4.95 ± 0.40
BUN (mmol/L)	5.60 ± 3.64	19.13 ± 13.30[b]	4.96 ± 0.76
Cr (μmol/L)	70.54 ± 47.32	307.42 ± 230.00[b]	106.65 ± 7.83
UA (μmol/L)	150.83 ± 73.67	289.26 ± 154.143[b]	343.46 ± 39.01
GPT (U/L)	33.75 ± 29.97	97.74 ± 138.92	21.31 ± 8.02
TBi (μmol/L)	13.42 ± 6.86	23.08 ± 21.24	14.97 ± 3.36
GGT (U/L)	61.39 ± 45.97	109.67 ± 90.62	19.23 ± 8.08

[a] The subjects are SIRS patients, MODS patients, and healthy controls. The biochemical parameters are assayed according to the standard procedures on the HITACHI-7170 automated biochemical analyzer, including glucose (Glc), blood urea nitrogen (BUN), creatinine (Cr), and uric acid (UA), glutamate-pyruvate transaminase (GPT), total bilirubin (TBi), γ-glutamyltransferase (GGT). [b] $P < 0.01$, the significant difference between SIRS and MODS groups.

compensate for differences in concentration of many metabolites from the "dilution effect".

Pattern Recognition and Orthogonal Signal Correction. Chemometric analyses on the data sets were performed using the software package SIMCA-P (Version 10.0, Umetrics AB, Umea, Sweden). The 245 integral data for each spectrum were used as independent variables (*X*), and the class identity was taken as a response variable (*Y*). Because order of magnitude scores were not desired in our analysis, we focused on correlation and covariance. After tests of several available techniques in SIMCA-P software for scaling, the NMR data were preprocessed by the default unit variance scaling before orthogonal signal correction (OSC), and the mean-centering after OSC. As it is capable of reducing noise and enhancing the correlation between spectroscopic signals and target variables, OSC was used for pattern recognition.[14] OSC identified the first latent variable that was orthogonal or unrelated to *Y*, the class membership, and then removed the noncorrelated systematic variation and noise in *X*. Thereafter, principal component analysis (PCA) was performed. The unsupervised PCA method identifies several principal components (PCs) to describe the maximal variation in an independent variable matrix *X*. As another pattern recognition technique, partial least-squares (PLS) calculates several latent variables (LVs), similar to the principal components of PCA, which is able to attain the maximal regression extension of PCA to the response variable matrix *Y*. Partial least-squares-discriminant analysis (PLS-DA) is performed by the PLS algorithm against a "dummy matrix" (*Y*) composed of all orthogonal class vectors. As a supervised method, the validated PLS-DA model is able to predict the class membership for unknown samples.[15,16]

Results and Discussion

NMR-Based Metabonomics Distinguished SIRS from MODS Patients. To determine if NMR-based metabonomic technology can be used to profile such heterogeneous traumatic critically ill patients, we collected totally 78 serum samples from 26 SIRS patients, 26 MODS and 26 healthy males with no obvious inflammatory diseases in the previous 2 weeks. Statistically, there was no significant difference for the increased glucose levels between SIRS and MODS groups (Table 1). Although all parameters for renal (blood urea nitrogen (BUN), creatinine (Cr), and uric acid (UA)) and liver function (glutamate-pyruvate transaminase (GPT), total bilirubin (TBi), γ-glutamyltransferase (GGT)) were much higher in MODS than those in SIRS group, the differences of three indicators for liver function were statistically insignificant (Table 1). The standard deviation values of all biochemistry parameters in both SIRS and MODS critically ill patients were greater than those in healthy controls, consistent with the notion that critically ill patients are more heterogeneous in clinical symptoms (Table 1). The heterogeneity suggests that it would be difficult to assess the progression of SIRS and MODS critical illness using single or a few parameters, and that multivariate analysis may be a better tool for diagnosis of the disease.

We obtained 600 MHz ^{1}H NMR spectra using the sera and the data were subject to principle component analysis (PCA), an unsupervised pattern recognition technique. This algorithm automatically searches for components that represent the greatest variation in the data from different samples without knowing the identity of the samples, and uses the PCA score vectors to make clusters of these samples. This method is called an unsupervised analysis because the identity of samples is not assigned before the analysis (It is a blind test).[14,15] The PCA method clearly recognized the sera of healthy subjects from the traumatic critically ill patients, regardless of the age, sex, and injury (Figure 1A). These data suggest that ^{1}H NMR-based metabonomic approach can be applied to distinguish the SIRS and MODS critically ill patients from the healthy individuals.

To confirm our conclusion that ^{1}H NMR-based metabonomic approach can be applied to distinguish the critically ill patients from the healthy individuals, we also analyzed the NMR spectra using PLS-DA, a supervised algorithm commonly used for class discrimination. In contrast to PCA, this method is a nonblind test (supervised) because the class identity of samples is assigned before the PLS-DA calculation. The PLS-DA algorithm can obtain latent variables (LVs), which are similar to principle components in PCA, to explain the maximal separation between samples on the basis of their defined class membership. Therefore, PLS-DA can recognize the class membership of samples better than PCA.[14,15] The PLS-DA method not only separated the sera of healthy subjects from the traumatic critically ill patients, but also was able to distinguish the different classification trends of SIRS from MODS serum samples in its score plot (Figure 1B,C).

However, SIRS and MODS were not completely separated in the LV score plot of PLS-DA. There were some overlaps between the two classes of samples (Figure 1B,C). The overlaps may be explained by at least two possibilities. One is that there are no significant metabolic differences between these SIRS and MODS patients, and the other is that the classification was obscured by the noise and uncorrelated systematic variation that are present in the NMR spectra.

Clinical observation indicates that early and late stages of critically ill patients have very different metabolic disturbances.[2,3] The reason that PLS-DA could not separate SIRS and MODS patients is probably because the multivariate algorithms consider each variable the same weight in calculation, ignoring different contribution of each variable. Variables from the NMR profiles can be or cannot be related to patient classification. Some of them can even be the noises. Consequently, less important variation and noise strongly influence the classification and the performance ability of the multivariate analysis was compromised. Therefore, we infer that the classification was obscured by spectral noise and systematic uncorrelated variation. To test this hypothesis, we applied the OSC filtration

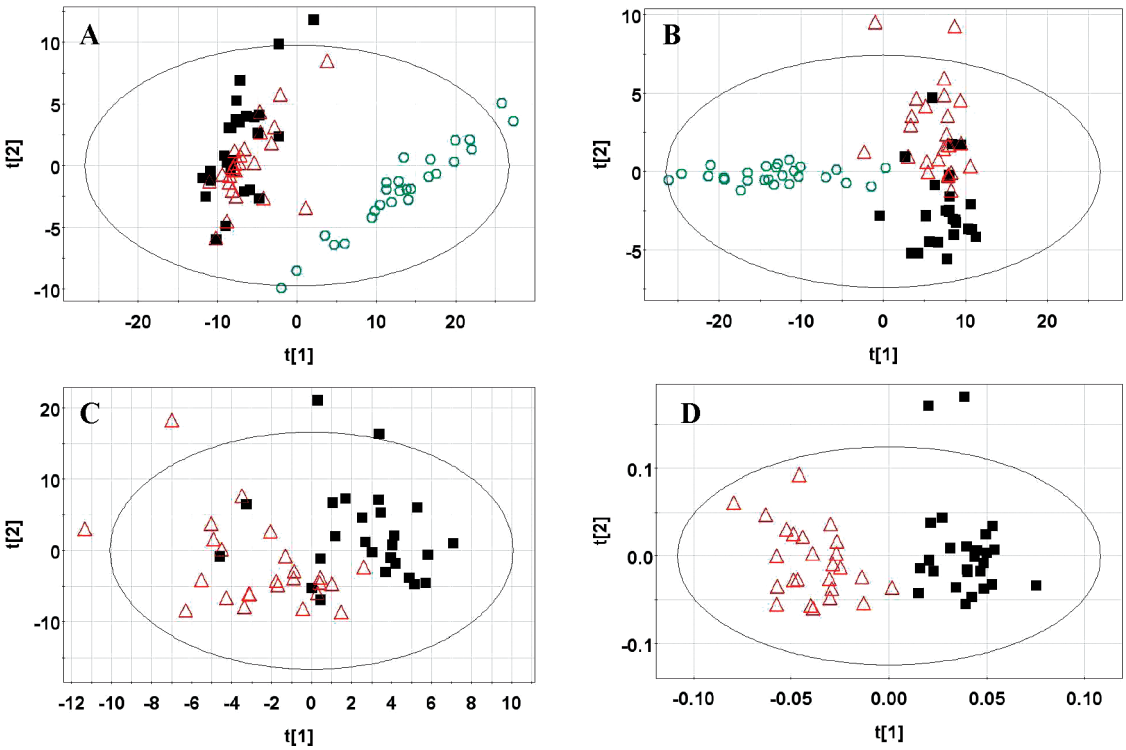

Figure 1. Pattern recognition analyses of serum ^{1}H NMR spectra before and after OSC. ^{1}H NMR spectra of sera from SIRS, MODS, and control groups were analyzed using PCA (A) and PLS-DA (B). ^{1}H NMR spectra of sera from SIRS and MODS were analyzed by PLS-DA before (C) and after (D) OSC, in which one OSC latent variable was removed from the analysis. (■ SIRS, red △ MODS, green ○ healthy control. $N = 26$ for each group of subjects. The ellipse in the score plot means 95% confidence region. The model parameters are shown in Table 2.)

Table 2. Chemometric Parameters for the Cross-Validation of the PLS-DA Models[a]

PLS-DA	*A*	R2X (cum)	R2Y (cum)	Q2 (cum)
three groups	4	0.712	0.826	0.667
SIRS and Control groups	3	0.72	0.989	0.962
MODS and Control groups	2	0.676	0.971	0.955
SIRS and MODS groups	3	0.465	0.758	0.435
SIRS and MODS groups after OSC	1	0.248	0.862	0.831

[a] On the basis of NMR spectra of sera from traumatic SIRS and MODS patients, and healthy controls, the PLS-DA models were constructed before and after OSC filtered the first latent variable. The component number (*A*), R2X (cum), R2Y (cum) and Q2 (cum) were parameters of the cross-validation, which carried out a leave-one-out procedure to determine the quality and predictability of a PLS-DA model. After application of OSC, the PLS-DA model for SIRS and MODS was improved.

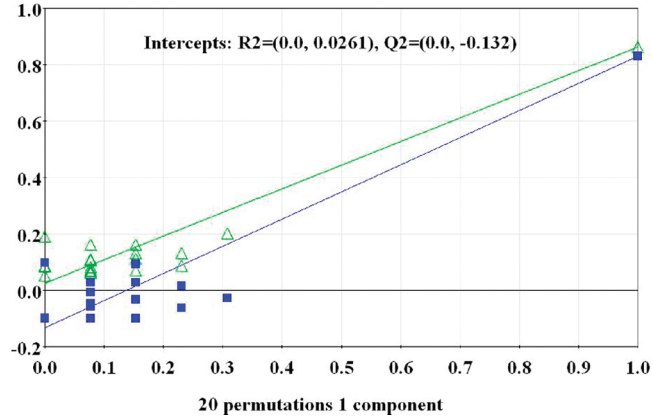

Figure 2. Validation of the PLS-DA model for SIRS and MODS using a permutation test. The original PLS-DA model was calculated using the OSC-filtered NMR data from 26 SIRS and 26 MODS patients. The data shown on the left bottom are new R2Y (cum) and Q2 (cum) values after the response variables of samples are randomly permuted for 20 times with the first statistically significant component and those on the top right are from the original calculation. Green △ is R2Y (cum) and blue ■ is Q2 (cum).

to the NMR spectral data from critically ill patients. The OSC can eliminate small systematic variation and noise that are unrelated to classification from our NMR data in advance, so that our multivariate analysis can focus on the variables that are more important to the classification. After removal of the systematic uncorrelated variation and noise from the NMR profiles by the application of OSC filtration, the SIRS and MODS samples were correctly separated in the score plot of PLS-DA (Figure 1D). These data indicate that NMR-based metabonomic approach can be applied to distinguish SIRS and MODS patients, and also suggest that the metabolic differences between SIRS and MODS stages are generally less significant than those between critically ill patients and healthy individuals. This is consistent with the clinical characteristics of critical illness. In clinic, when the stress is triggered by insults, the body metabolism changes inevitably and differs considerably from that of healthy population. However, SIRS and MODS represent a continuation of a disease and different degrees of the severity of stress and the occurrence of complications and organ dysfunction.[1]

Validation of the PLS-DA Model for SIRS and MODS. To validate the constructed PLS-DA model for SIRS and MODS, we performed cross validation by the default leave-one-out procedure. The component number (*A*), R2X (cum), R2Y (cum) and Q2 (cum) were calculated to describe the quality of the

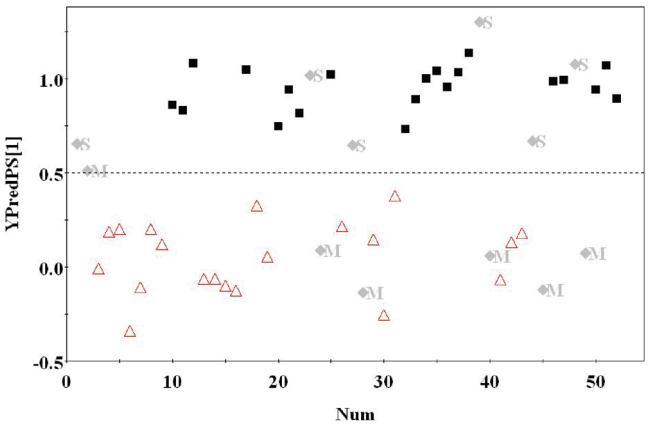

Figure 3. Evaluation of the external predictability of the PLS-DA model for SIRS and MODS. A new PLS-DA model was constructed using OSC-filtered NMR data from 20 SIRS and 20 MODS samples and used to predict the class membership of 6 SIRS and 6 MODS samples that were randomly excluded as the external test set prior to the model building. SIRS should have a value of more than 0.5 in *y*-axis and MODS less than 0.5. Note only one MODS test sample higher than a priori cutoff of 0.5 in *y*-axis. (■ SIRS, red Δ MODS, gray ◆ test sets. S and M represent SIRS and MODS, respectively.)

model (Table 2). R2X (cum) and R2Y (cum) represent percentages of respective *X* and *Y* variables used to calculate the PLS-DA model; Q2 (cum) reflects the predictive capacity of the PLS-DA model. Generally, the higher R2X (cum) and R2Y (cum), the more modeled variation used in the calculation. The higher the Q2 (cum) is, the better the predictability of the PLS-DA model is.[14] In this study, both PLS-DA models for classification of patients and controls obtained almost the same highest validation statistics. Both R2Y (cum) and Q2 (cum) are over 95%, whereas the PLS-DA model for SIRS and MODS patients before OSC filtration had the lowest values of R2Y (cum), and Q2 (cum) (Table 2). After application of the OSC that deleted 33.66% of variance and noise in NMR spectral data, the component number in the PLS-DA model reduced from three to one. It has been known that a perfect PLS-DA model from single response variable should obtain one component, whereas two or more components generally indicate some variance and noise exist in data. After application of OSC that deleted one LV, R2X (cum) decreased from 46.5% to the minimum of 24.8%, suggestive of the least systematic variance and noise remained in the PLS-DA model. The predictability parameter Q2 (cum) increased to 83.1%, suggesting that the PLS-DA model after OSC was able to correctly predict 83.1% of unknown samples (Table 2). The improvement of validation statistics, R2X (cum), R2Y (cum) and Q2 (cum), illustrated that the application of a single round of OSC was capable of optimizing the PLS-DA model for classification of SIRS and MODS samples.

To further validate the PLS-DA model for classification of SIRS and MODS samples, the class variables of samples were randomly permutated for 20 times. All Q2 (cum) values calculated from the permuted data were not more than 20%, far lower than 83.1% of the original value in the validation plot, suggesting that our original PLS-DA model for SIRS and MODS was not random. Also, Q2 (cum) intercepted the *y*-axis at −0.132. Such a small Q2 intercept indicated that the randomly permutated data could not yield a model with any predictive ability, and the original PLS-DA model for SIRS and MODS was not over fitting and trustworthy (Figure 2).

To evaluate the external predictability of our PLS-DA model for SIRS and MODS, six individuals from each group were randomly excluded as an external test set, and a new one-component PLS-DA model was generated from the remaining samples. This new PLS-DA model had essentially the same statistical parameters as the original one, with one statistically significant component, R2Y (cum) = 0.893 and Q2 (cum) =

Table 3. Metabolite Assignment of Integral Fragments Statistically Important for the Separation of SIRS from MODS[a]

key	chemical shift	CoeffCS[1]	VIP[1]	metabolites
1	δ3.70−3.74	7.33	4.68	glucose, fructose, leucine, glutamine
2	δ3.66−3.70	5.52	3.52	galactose, furanose, isoleucine, N-acetyl galactosamine
3	δ3.78−3.82	5.28	3.37	fructose, furanose, fucose, galactose, uridine, glycerol, ornithine
4	δ3.58−3.62	3.94	2.51	furanose, valine, sarcosine, $-CH_2-N^+(CH_3)_3$ in phosphatidylcholine or sphingomyelin headgroup, ethanol
5	δ3.74−3.78	3.79	2.42	glucose, fucose, alanine, arginine, glutamate, lysine
6	δ3.62−3.66	3.54	2.26	fucose, isoleucine, glycerol, choline, myo-inositol
7	δ3.38−3.42	3.08	1.97	glucose, proline
8	δ3.14−3.18	2.16	1.38	phenylalanine
9	δ3.30−3.34	2.10	1.34	tryptophan, proline, tyrosine
10	δ3.34−3.38	2.01	1.28	unknown 3
11	δ3.82−3.86	1.95	1.24	glucose, furanose, methionine, unknown 1
12	δ3.06−3.10	1.74	1.11	spermine, unknown 2
13	δ1.26−1.30	−13.69	8.73	(CH_2)n and $CH_2CH_2CH_2CO$ in fatty acyl chain of lipids (mainly VLDL), unknown 4, isoleucine
14	δ1.22−1.26	−8.27	5.28	(CH_2)n and $CH_3CH_2(CH_2)$n in fatty acyl chain of lipids (mainly LDL, HDL), unknown 4
15	δ4.02−4.06	−4.18	2.67	CH_2OCOR in glycerol backbone of lipids, choline, creatinine, fructose
16	δ4.06−4.10	−2.26	1.44	choline, lactate, galactose, myo-inositol
17	δ1.18−1.22	−1.70	1.09	$CH_3CH_2(CH_2)$n in fatty acyl chain of lipids, fucose, β-hydroxybutyric acid
18	δ3.10−3.14	−1.60	1.02	phenylalanine, spermine, histidine

[a] These integral fragments derived from the PLS-DA calculation on the OCS filtered NMR data from SIRS and MODS patients. On the basis of the first statistically significant component of PLS-DA algorithm, each integral region (bin) had the variable importance in projection (VIP) >1 and centered and scaled coefficients (CoeffCS) >1.5, the magnitude of which represented the relative importance of this bin for the classification of SIRS and MODS patients.

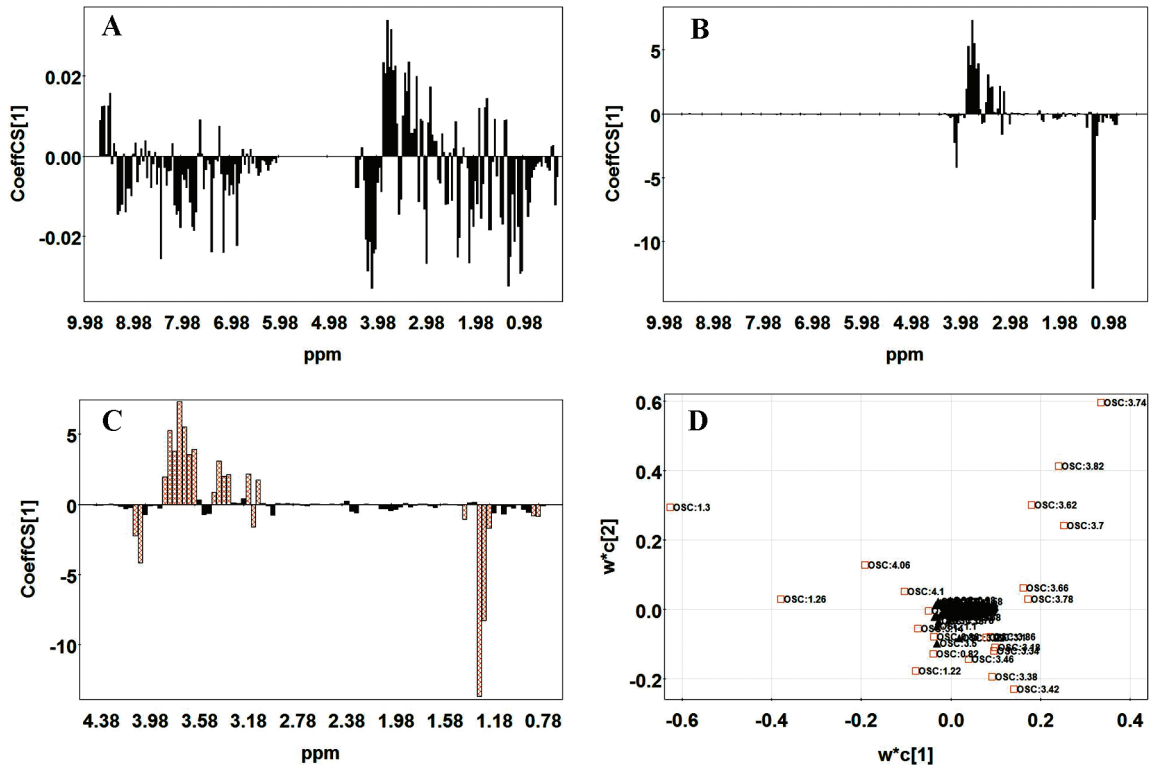

Figure 4. Coefficient and loading plots of PLS-DA model from SIRS and MODS samples before and after removing one OSC latent variable. (A) Coefficient plot of PLS-DA before OSC; (B) coefficient plot of PLS-DA after OSC; (C) zoomed coefficient plot of PLS-DA after OSC; (D) loading plot of PLS-DA after OSC. (The statistically significant coefficient fragments and loading values are marked as red.)

0.871. When this new model was applied to predict the excluded 12 external test-set samples, 11 samples were correctly classified using a priori cutoff of 0.5, with a 95% confidence interval (Figure 3).

Systemic Metabolism of Critical Illness Revealed by NMR-Based Metabonomic Method. To determine what variables were responsible for classification of SIRS and MODS patients, we used regression coefficient plots deriving from PLS-DA models to locate key regions in the NMR spectra. We chose the principal regions of interest based on chemometric standards such as variable importance in projection (VIP) and centered and scaled coefficients (CoeffCS). The magnitude of VIP and CoeffCS of a variable represents its relative importance for the classification. VIP > 0.7 is widely used for the choice of regions of interest in multivariate data.[14] In our study, a more stringent criterion was used to narrow down the region of interest. We used VIP > 1 and corresponding CoeffCS > 1.5 to locate the NMR integral regions of interest from the PLS-DA model for SIRS and MODS after OSC filtration (Table 3). The statistically significant integral fragments were red-marked in the coefficient and loading plots (Figure 4C,D). The application of OSC reduced the principle NMR integral fragments responsible for classification and facilitated the assignment of metabolites (Figure 4A,B).

An integral region with positive coefficient indicated that the concentration of the metabolites within this region was relatively higher in MODS samples, and those with negative coefficient indicate there were more corresponding metabolites present in SIRS sera. The NMR spectral signals of almost all the metabolites in human serum sample have been assigned successfully in previous published literature.[17] On the basis of these known information, the major metabolites in the regions of interest of proton NMR profiles were assigned and summarized in Table 4. The spectra responsible for the discrimination of SIRS were located in δ3.06−3.86 NMR integral regions, and principally comprised of the signals of sugars (glucose, fructose, galactose, furanose, fucose, etc.), amino acids (leucine, valine, arginine, etc.), and glutamine (Table 4). The integral regions influential for MODS were δ1.18−1.3 and δ4.02−4.1, composed of the proton signals of (CH_2)n, $CH_2CH_2CH_2CO$, $CH_3CH_2(CH_2)$n in fatty acyl chain, CH_2OCOR in glycerol backbone of lipids (mainly, VLDL, LDL, and HDL), creatinine, and lactate (Table 4).

Table 4. Summary of the Major Metabolites within the Integral Regions of Interest That Were Important for the Separation of SIRS from MODS Groups

group	integral regions of interest	major metabolites
SIRS	δ3.06−δ3.86	glucose, fructose, galactose, furanose, fucose, etc.; leucine, valine, arginine, etc.; glutamine.
MODS	δ1.18−δ1.3	fatty acyl chain and glycerol backbone of lipids;
	δ4.02−δ4.1	creatinine; lactate.

The assigned metabolites influential for SIRS were mainly carbohydrates and amino acids, which were consistent with metabolic responses in early stage of critical illness (Figure 5). During this stage, stress reaction to insults can trigger the rapid breakdown of body reserve of glycogen and decomposition of proteins from skeletal muscles, resulting

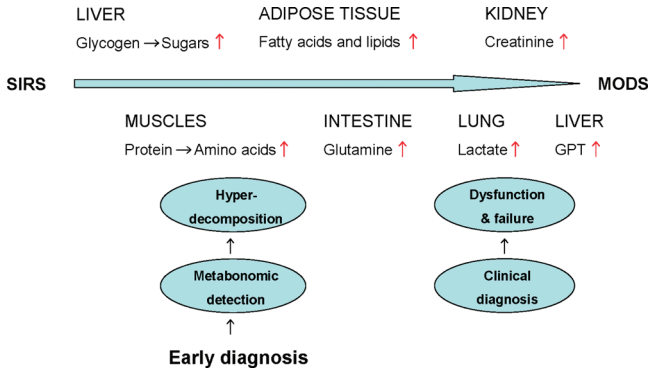

Figure 5. NMR-based metabonomic approaches reveal potential biomarkers that may be used to detect the presence of MODS in traumatic critically ill patients earlier than those currently used in clinical settings.

in the rise of sugars and amino acids (principally branched chain amino acids) in blood.[2,3] With the progression of the stress, the liver can transform redundant glucose into fatty acids and lipids, and may lead to intrahepatic cholestasis and even liver dysfunction. At the same time, the stored fat is also mobilized and oxidized as the main caloric source, substituting for the role of the exhausting glycogens and proteins. As the end products of steatolysis, free fatty acid and glycerol increase in blood.[2,3] Obviously, these metabolic disturbances in lipids are consistent with the metabolite information from the NMR integral regions of interest of MODS (Figure 5).

Moreover, severe stress can reduce the body synthesis and intake of glutamine.[2,18] Therefore, it is easy to explain that SIRS patients have higher levels of glutamine in sera than MODS subjects (Figure 5). Because the rise of serum creatinine is the indicator of kidney dysfunction, the NMR spectra of creatinine became one of principal metabolites for the separation of MODS (Figure 5). A number of factors may contribute to hyperlactataemia in acute severe illness, such as global tissue hypoxia and catecholamine therapy in shock, low ventilation and gas exchange in acute lung injury. In general, blood lactate level can indicate the degree of cellular derangements, and the duration of lactic acidosis often carries a bad prognosis in clinic.[19] Consequently, lactate was responsible for the classification of MODS in this study (Figure 5). Wang et al.[20] investigated the early life maternal separation stress in rats using the same NMR-based metabonomic approach. The biochemical response to this stress was characterized by decreased levels of total lipoproteins and increased levels of amino acids, glucose, lactate, creatine, and citrate in blood plasma. Their findings in rats are essentially similar to ours in critically ill patients.

The assignment of the major metabolites not only explains our constructed PLS-DA model for the separation of SIRS from MODS groups, but also reveals potentially biomarkers for early diagnosis of MODS. Our study indicates that the NMR-based metabonomic approach may be used to detect the presence of MODS in traumatic critically ill patients earlier than those currently used in clinical settings (Figure 5).

Abbreviations: SIRS, Systemic Inflammatory Response Syndrome; MODS, Multiple Organ Dysfunction Syndrome; CPMG, Carr−Purcell−Meiboom−Gill; NMR, nuclear magnetic resonance; FID, free induction decay; OSC, orthogonal signal correction; PCA, principal components analysis; PLS-DA, partial least-squares-discriminant analysis; LV, latent variable; VIP, variable importance in projection; CoeffCS, centered and scaled coefficients; PR, pattern recognition; Glc, glucose; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid; GPT, glutamate-pyruvate transaminase; TBi, total bilirubin; GGT, γ-glutamyl-transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

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