Untargeted lipidomics reveals specific lipid abnormalities in systemic lupus erythematosus

Y. Wang[1,2], F. Guo[1], Y. Guo[1], Y. Lu[1], W. Ji[1], L. Lin[3], W. Chen[1], T. Xu[1,2], D. Kong[3], Q. Shen[3], Y. Zhu[3], P. Liu[1], J. Su[4], L. Wang[4], Y. Li[4], P. Gao[4], J. Shan[3], S. Liu[1]

[1]Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China; [2]School of Medicine & Holistic Integrative Medicine, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China; [3]College of Pharmacy, Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China; [4]Xuzhou Medical University, Xuzhou, Jiangsu, China.

Abstract

Objective

To identify potential lipid biomarkers by studying changes in the blood lipid profile of patients with systemic lupus erythematosus (SLE) using lipidomics.

Methods

Serum samples were collected from 115 SLE patients and 115 age- and sex-matched healthy controls (HCs). Lipid profiles were assessed using ultrahigh-performance liquid chromatography coupled with Q Exactive spectrometry, and possible lipid biomarkers were screened and evaluated by univariate and multivariate analyses.

Results

Metabolic phenotypes related to SLE disease activity index (SLEDAI) scores were detected in the serum of SLE patients, and these phenotypes indicated the activity of the disease. Alterations in energy metabolism, fatty acid metabolism and other pathways were observed in patients with SLE. Phosphatidylethanolamine (16:0/18:2), lysophosphatidylethanolamine (18:0), and acylcarnitine (11:0) can be used as biomarkers for the clinical diagnosis of SLE, and receiver operating characteristic (ROC) analysis indicated their effectiveness in diagnosing this disease.

Conclusion

Our study identified serum biomarkers related to disease activity in patients with SLE, providing a basis for its clinical diagnosis.

Key words

lipidomics, systemic lupus erythematosus, biomarkers, disease activity, UPLC-Q-exactive

*Yingzhuo Wang, MM**
*Feng Guo, PhD**
Yunke Guo, PhD
Yan Lu, PhD
Wei Ji, PhD
Lili Lin, PhD
Wenjun Chen, PhD
Tingting Xu, MM
Deshun Kong, PhD
Qiuxiang Shen, PhD
Youjuan Zhu, PhD
Ping Liu, PhD
Jinfeng Su, PhD
Lu Wang, PhD
Yuhua Li, PhD
Pan Gao, PhD
Jinjun Shan, PhD
Shijia Liu, PhD

**These authors contributed equally and share first authorship.*

Please address correspondence to:
Shijia Liu,
Affiliated Hospital of Nanjing University of Chinese Medicine, 155 Hanzhong Road, Qinhuai District, Nanjing, 210029, P.R. China.
E-mail: liushijia_2019@126.com

Received on March 29, 2021; accepted in revised form on May 24, 2021.

Data availability statement: the data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Funding: this work is financially supported by the National Natural Science Foundation of China (no. 82074241 and 81774096), the Open Projects of the Discipline of Chinese Medicine of Nanjing University of Chinese Medicine Supported by the Subject of Academic priority discipline of Jiangsu Higher Education Institutions (no. ZYX03KF031), the Jiangsu Provincial Medical Youth Talent (no. QNRC2016642).

Competing interests: none declared.

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease related to heredity, sex hormones, infection and other factors (1). It is estimated that the prevalence of SLE is 30–50 per 100,000 people. Lineage, race and nationality have a significant impact on the manifestations and severity of systemic lupus erythematosus (2, 3). Ninety percent of patients are women, generally of childbearing age (4). SLE has a variety of clinical manifestations, including rash, fever, and arthritis; organ-threatening complications may occur, such as lupus nephritis, autoimmune erythrocytopenia or nervous system diseases, in severe cases.

SLE is also one of the most heterogeneous diseases, which renders diagnosis as well as treatment and its efficacy challenging. Overall, excessive damage, morbidity and mortality still occur in SLE (5), indicating an existing large medical demand for therapies. Despite strong interest in studying the pathogenesis of SLE and identifying biomarkers, there are few biomarkers to date used for diagnosis, evaluating disease activity and predicting organ damage. In practice, the few markers widely used for SLE diagnosis or assessing disease activity are limited to antinuclear antibodies, complements and several autoantibodies (6). In recent years, several studies on new biomarkers have been published including circular RNAs, beta-2 microglobulin, IL-17A and protein deltex-1 (7). With the emergence of new technologies, the discovery of biomarkers has entered the "omics" era.

Lipids are components of cells and tissues and play an important role in life activities. Some studies have shown that glycolysis, the tricarboxylic (TCA) cycle, fatty acid oxidation and amino acid metabolism are seriously suppressed in patients with SLE compared with healthy people or those with other rheumatic diseases. For example, the levels of most amino acids and glycolysis and TCA cycle metabolites are reduced in patients with SLE, whereas fatty acids and markers of oxidative stress are increased (8, 9). Lipidomics is a new area of research in which whole lipids are systematically analysed. By comparing changes in lipid metabolic networks in different physiological states, key lipid biomarkers involved in metabolic regulation can be identified, ultimately revealing the mechanisms of lipids in various life processes (10). Lipidomics has wide application prospects for the identification of disease lipid markers and diagnosis. In this study, serum lipids (including acylcarnitine (ACar), phosphatidylcholine (PC), lysophosphatidylcholine, phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LPE), sphingomyelin (SM), triglyceride (TAG)) in patients with SLE were detected by ultrahigh-performance liquid chromatography coupled with Q Exactive (UPLC-QE) spectrometry. The changes in serum lipid metabolism suggest that these lipids may be used as potential biomarkers of SLE.

Materials and methods

Study populations

This study was approved by the Institutional Review Board and the Ethics Committee of the First Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (2018NL-165-02). All volunteers who participated in the study were informed of the use of their blood and gave written informed consent.

Between November 2017 and May 2018, 115 patients with SLE were recruited from the Affiliated Hospital of Nanjing University of Chinese Medicine. At the same time, 115 healthy volunteers from the physical examination center of the Affiliated Hospital of Nanjing University of Chinese Medicine were selected as the control group. The SLE patients were included in accordance with the classification criteria of systemic lupus erythematosus of the American College of Rheumatology (ACR).

The inclusion criteria for the study were as follows: SLEDAI scores from 2 to 22 and age 27 to 55 years. All participants were Asian and treated at the Affiliated Hospital of Nanjing University of Chinese Medicine. There was no significant difference in age, sex or race between the patients with SLE and healthy subjects. Patients with a history of other diseases that affect

biological indicators and metabolic characteristics, such as cardiovascular disease, were excluded from the study. Serum samples from the SLE patients and healthy volunteers were collected in procoagulant vessels and stored at -80°C until analysis.

Materials

The materials and chemicals used are available in the online supplementary file.

Sample preparation

To 40 μL of serum sample thawed on ice, 225 μL ice-cold methanol containing a mixture of ISs was added and vortexed for 10 seconds. Then, the sample was mixed with 750 μL cold methyl tert butyl ether (MTBE) and vortexed for another 10 seconds. The mixture was shaken on an orbital mixer at 4°C for 10 min, after which 188 μL of room temperature LC/MS-grade water was added and vortexed for 20 s before centrifuging at 14,000 rcf at 4°C for 2 min. The upper phase was transferred to fresh tubes and dried in a vacuum centrifuge. The upper phase lipids were reconstituted with 110 μL of methanol:toluene (9:1) for UPLC-QE MS analysis. To ensure data quality for metabolic profiling, pooled quality control samples were prepared by mixing equal amounts of serum (10 μL) from the 115 patients with SLE and 115 HCs.

Table I. Clinical characteristics of the subjects.

Characteristic	Discovery set (n=153)		Validation set (n=77)	
	SLE	HC	SLE	HC
Number	76	77	39	38
Male	9	28	5	19
Female	67	49	34	19
Age (years), mean±SD	42.28 ± 12.89	33.3 ± 7.31	40.56 ± 16.76	33.73 ± 7.12
Body mass index (kg/m^2), mean ± SD	23.49 ± 3.13	—	21.41 ± 3.98	—
ESR (mm/h), mean (median)	21.87 (13)	—	42.93 (38.5)	—
CRP (mg/l), mean (median)	6.36 (2.09)	—	17.06 (12.9)	—
SLEDAI, mean±SD	6.62 ± 4.5	—	12.93 ± 5.3	—
Serum creatinine (μmol/l), mean ± SD	63.43 ± 20.71	—	77.18 ± 45.21	—
Creatinine (μmol/l), mean ± SD	57 ± 24.43	—	68.61 ± 40.63	—
Cholesterol (mmol/l), mean ± SD	4.44 ± 1.27	—	4.53 ± 0.77	—
Triglyceride (mmol/l), mean ± SD	1.55 ± 0.81	—	1.79 ± 0.52	—
LDL-C (mmol/l), mean ± SD	2.6 ± 0.81	—	2.65 ± 0.42	—
HDL-C (mmol/l), mean ± SD	1.37 ± 0.49	—	1.26 ± 0.45	—
Positive anti-nRNP/Sm	71.43%	—	91.67%	—
Positive anti-Sm	60.00%	—	75.00%	—
Positive anti-dsDNA	77.78%	—	62.50%	—
Positive anti-SSA	82.61%	—	80.00%	—
Positive anti-SSB	33.33%	—	15.38%	—
Positive anti-CentromerB	33.33%	—	25.00%	—
Positive anti-Histone	71.43%	—	66.67%	—
Positive anti-Nukleosome	71.43%	—	70.00%	—
Positive anti-Ribosomale	76.92%	—	75.00%	—
Positive anti-Ro-52	86.96%	—	80.00%	—
Positive anti-Scl-70	0.00%	—	0.00%	—
Positive anti-Jo-1	0.00%	—	0.00%	—
C3 (g/L), mean ± SD	0.67 ± 0.21	—	0.51 ±0.21	—
C4 (g/L), mean ± SD	0.14 ± 0.07	—	0.12 ±0.08	—
Ig A (g/L), mean ± SD	3.02 ± 1.03	—	2.04 ±0.52	—
Ig G (g/L), mean ± SD	16.02 ± 5.44	—	12.48 ±5.39	—
Ig M (g/L), mean ± SD	0.91 ± 0.56	—	0.99 ±0.72	—
Corticosteroid dose (mg equivalent/day)	11.93 ± 10.20	—	31.47 ±29.05	—
Hydroxychloroquine	62.26%	—	76.67%	—
Mycophenolate mofetil	9.43%	—	20%	—
Cyclophosphamide	3.77%	—	40%	—
Leflunomide	13.21%	—	6.67%	—
Methotrexate	1.89%	—	6.67%	—

SLEDAI: systemic lupus erythematosus disease activity index; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; WBC: white blood cell; PLT: platelet; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; C3: Complement 3; C4: Complement 4; Ig A: Immunoglobulin A; Ig G: Immunoglobulin G; Ig M: Immunoglobulin M.

Chromatography and MS

Untargeted lipidomic analysis was performed using the Dionex UltiMate 3000 Ultra-Performance Liquid Chromatography (UPLC) system (Santa Clara, CA, USA) coupled with a Q Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) via an electrospray ionisation (ESI) source.

Lipid separation was carried out using a reversed-phase Waters Acquity UPLC CSH C18 column (100 mm×2.1 mm, 1.7 μm) maintained at 60°C by gradient elution, with a flow rate of 300 μL/min. The injection volume of each sample was 1 μL, as maintained at 4°C in an auto sampler. The mobile phase consisted of two solvents: 60% ACN in water, and isopropanol:ACN (9:1), both containing 5 mM ammonium formate and 0.1% formic acid. The lipids were separated with the elution gradient as follows: 0–4.0 min, 15% B; 4.0–5.0 min, 15–48% B; 5.0–22.0 min, 48–82% B; 22.0–23.0 min, 82-99% B; 23.0–24.0 min, 99% B; 24.0–24.2 min, 99-15% B; 24.2–30.0 min, 15% B.

The mass spectrometer was operated under positive and negative ion modes using a heated ESI source with a spray voltage of 3 kV (positive). For the ionisation mode, the sheath gas and auxiliary gas were kept at 45 and 10 (arbitrary units), and the capillary temperature and heater temperature were 300°C and 306°C, respectively. The S-Lens RF level was set at 50. The Orbitrap mass analyser was performed at a resolving power of 35,000 in full-scan mode (scan range: 150–2000 m/z; automatic gain control (AGC) target: 1e6) and of 175.00 in the top 10 data-dependent MS2 mode (stepped normalised collision energy: 20, 40 and 60; injection time: 50 ms; isolation window: 1.5 m/z; AGC target: 1e5), with a dynamic exclusion setting of 8.0 seconds.

Data analysis

The raw data from UPLC-QE MS were preprocessed by MS-DIAL, then the variables were identified by FiehnLab database. To reduce the concentration difference between samples, the peak

area of the data was normalised to R, features with missing values >80% were removed, and the remaining missing values were replaced by LoDs (1/5 of the minimum positive value of each variable) by MetaboAnalyst 4.0. The skewness distribution is more symmetrical through normalisation, and we carried out univariate and multivariate analyses of the determined blood lipids. The normalised data were imported into SIMCA software (version 14.1; Umetrics) for orthogonal partial least squares discriminant analysis (OPLS-DA) to identify lipid metabolites that contribute significantly to classification. Variable importance in the projection (VIP) was used to select variables according to the peak height obtained by the OPLS-DA model. We also assessed the significance of each lipid by correcting the *p*-values to obtain the false discovery rate (FDR) via the nonparametric test. Lipids with VIP >1.0, $p<0.05$, and FDR <0.05 were considered differential metabolites.

The candidate lipids were then further analysed to identify potential diagnostic biomarkers. Based on these potential biomarkers, a model was established by binary logistic regression and receiver operating characteristic curve (ROC) analysis using SPSS 25.0 software. In the binary logistic regression prediction model, forward stepwise regression and the Wald test were used to screen altered blood lipids and evaluate their significance. The diagnostic capacity of regression analysis was analysed and quantified by ROC curves, and the area under the ROC curve (AUC) was calculated to find the best combination of significantly changed lipid types. Correlation between these lipids and SLEDAI scores were determined by Spearman correlation analysis to identify biomarkers for SLE.

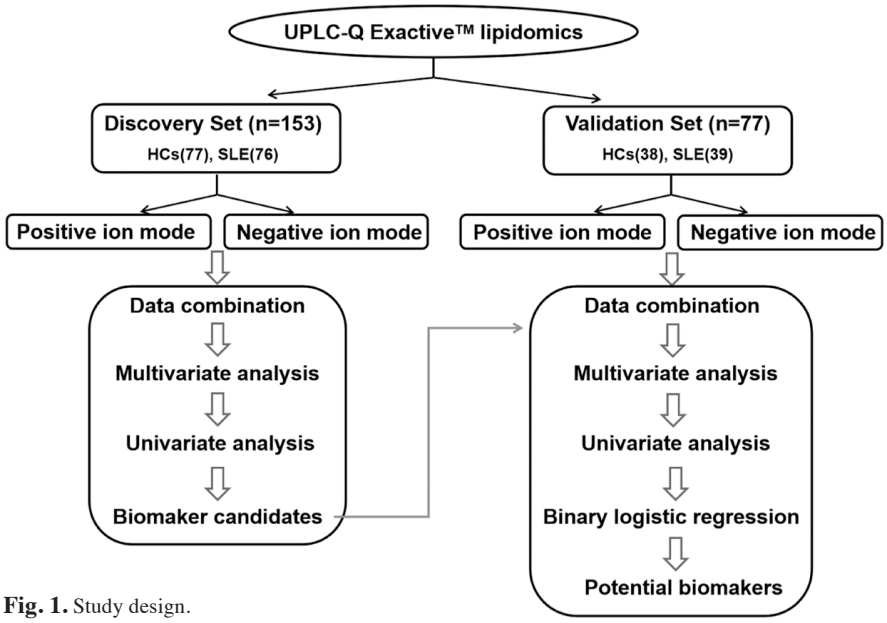

Fig. 1. Study design.

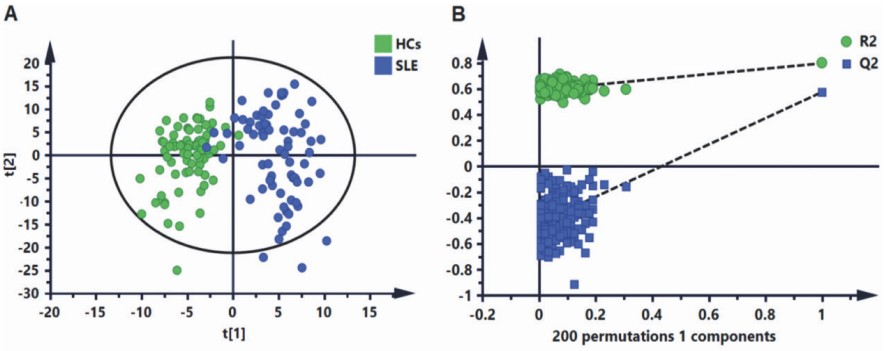

Fig. 2. Identification of potential lipid biomarkers for the diagnosis of SLE.
A: Orthogonal partial least squares discriminant analysis (OPLS-DA) score plot based on HCs and SLE groups in the Discovery Set. Green dot: healthy controls; blue dot: SLE patients.
B: 200 permutation tests of the OPLS-DA model.

Results

Basic characteristics of the participants

In this study, 153 participants (76 SLE and 77 HCs) were allocated to the discovery set to evaluate candidate biomarkers; 77 participants (39 SLE and 38 HCs) were allocated to the validation set to test the biomarkers identified. The sex, age and other clinical information of the patients and HCs are summarised in Table I.

Serum lipid profiling of UPLC-QE/MS

The workflow of this study is shown in Figure 1. In untargeted lipidomics analysis, we examined serum samples by positive and negative electrospray ionisation (ESI) modes with two injections, and a total of 510 lipids were determined. We further applied OPLS-DA (Fig. 2A) as a multivariate pattern analysis model to identify serum lipid profile differences between the groups in the discovery data set. Without overfitting of the model (Fig. 2B), there was an obvious separation between the SLE and HC groups, indicating a significant change in lipid metabolism patterns between them. According to the statistical significance criteria of VIP >1, $p<0.05$ and FDR <0.05, 156 lipids with significant changes were selected from the discovery data set (Supplementary Table S1) and compared with the altered lipids in the validation data set for further screening and verification.

Definition and verification of potential biomarkers for SLE

A validation set was used to evaluate the reliability of the differential metabolites and define meaningful biomarkers. The above differential lipids were further verified in the validation set, and 77 potential biomarkers with significant differences between the two comparisons (SLE and HCs) were selected (Suppl. Table S2). Moreover, the change trend of these biomarkers in the validation set was consistent with that in the discovery set. Significance anal-

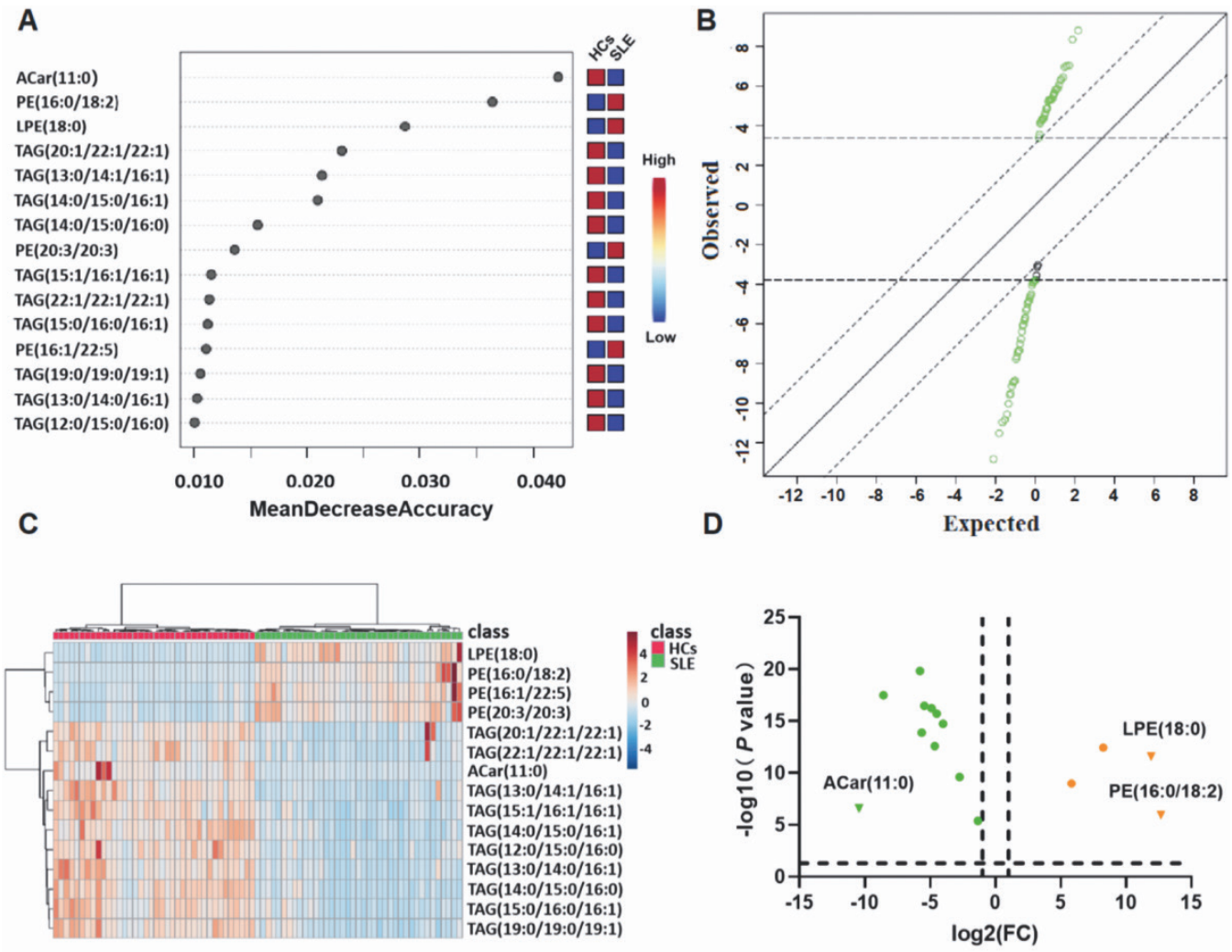

Fig. 3. Lipidomic profiling of serum samples from 15 lipid species that distinguish HCs and SLE.
A: Significant features identified by random forest. **B**: The result of the SAM scatter plot of observed scores plotted *versus* expected scores with a delta value of 3.1. Significant lipid species are represented in green. **C**: A Heatmap of the differential lipid species between HCs and SLE. Brown: increased levels; blue: decreased levels. Rows: serum samples; Columns: lipid species. **D**: Comparison of the abundance of 15 different lipid species. Coloured spots are lipids that change significantly. Green: increased levels; orange: decreased levels.

ysis via random forest and microarray was applied for further selection of significantly altered lipid metabolites (Fig. 3A-B), and 15 lipids were retained, as shown in Table II. The heatmap of Fig. 3C and the volcano map of Fig. 3D show the relative intensity distribution and the significance of the differences of these metabolites, respectively.

Subsequently, the above 15 differential lipid metabolites were analysed by binary logic regression using SPSS 25.0. The optimal model was constructed by the forwarding stepwise optimisation algorithm (Wald), and PE (16:0/18:2), LPE (18:0) and ACar (11:0) were determined to be reliable lipids in the regression model. PE (16:0/18:2) and LPE (18:0) levels were significantly increased but ACar (11:0) level decreased in patients with SLE (Fig. 4). The ROC values of the three lipids and their combination are provided in Figure 5. For SLE and HCs, PE (16:0/18:2), LPE (18:0), ACar (11:0) and their combination showed AUCs of 0.976, 0.962, 0.989 and 1.000, sensitivities of 92.31%, 89.74%, 97.44% and 100%, and specificities of 100%, 100%, 97.37% and 100%, respectively.

Next, correlations between PE (16:0/18:2), LPE (18:0), ACar (11:0) and SLEDAI were determined (Fig.6A-B-C). According to the latest SLE staging standard, scores of 0~6, 7~12 and >12 are considered mild (or remission), moderate and severe disease activity, respectively. The results showed that there were significant differences in the levels of the three biomarkers in different disease status of SLE. As shown in Figure 6D and E, the combination of PE (16:0/18:2), LPE (18:0) and ACar (11:0) distinguishes remission SLE from active SLE and HCs, with a coincidence rate of 67.92% and 92.06%, respectively. In addition, 0.304 as the cut-off value can differentiate between active SLE and HCs (Fig. 6F). Therefore, the combination of PE (16:0/18:2), LPE (18:0) and ACar (11:0) is ideal biomarker to differentiate between patients with SLE and healthy subjects.

It has been reported that antibodies to PE (aPE) is related to antiphospholipid antibodies (aPL). Therefore, we performed spearman correlation analysis between

Table II. Identified differential lipid metabolites between the SLE and HCs.

Metabolite	[a]VIP	[b]*p*-value	[c]FDR	[d]FC
ACar (11:0)	2.082	<0.001	<0.001	0.132
LPE (18:0)	2.171	<0.001	<0.001	2.967
PE (16:0/18:2)	2.161	<0.001	<0.001	5.858
PE (16:1/22:5)	2.026	<0.001	<0.001	3.277
PE (20:3/20:3)	2.003	<0.001	<0.001	3.558
TAG (12:0/15:0/16:0)	1.905	<0.001	<0.001	0.449
TAG (13:0/14:0/16:1)	2.004	<0.001	<0.001	0.435
TAG (13:0/14:1/16:1)	1.962	<0.001	<0.001	0.279
TAG (14:0/15:0/16:0)	2.155	<0.001	<0.001	0.324
TAG (14:0/15:0/16:1)	2.053	<0.001	<0.001	0.454
TAG (15:0/16:0/16:1)	2.035	<0.001	<0.001	0.438
TAG (15:1/16:1/16:1)	1.915	<0.001	<0.001	0.421
TAG (19:0/19:0/19:1)	2.001	<0.001	<0.001	0.541
TAG (20:1/22:1/22:1)	1.623	<0.001	<0.001	0.419
TAG (22:1/22:1/22:1)	1.691	<0.001	<0.001	0.285

[a]VIP was obtained from the OPLS-DA model with a threshold of 1.0. [b]*p*-values were obtained from one-way ANOVA. The value of [c]FDR was obtained from the adjusted *p*-value in the metaboanalyst 4.0. The value of [d]FC was obtained by comparing those metabolites in patients with SLE with the HCs. VIP: variable importance in the projection; FC: fold change; FDR: false discovery rate.

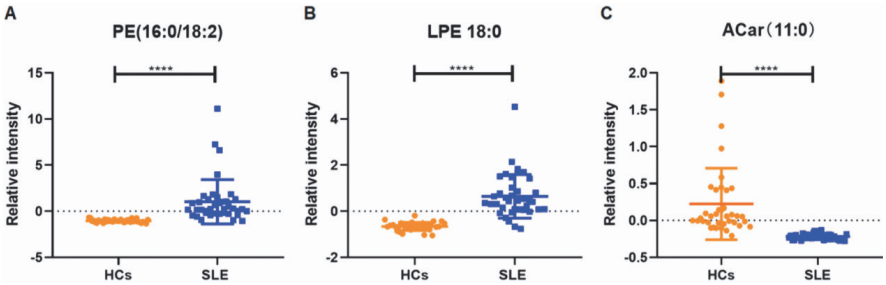

Fig. 4. Serum relative intensities of PE (16:0/18:2), LPE (18:0) and ACar (11:0) in the HCs and SLE. ****$p<0.0001$.

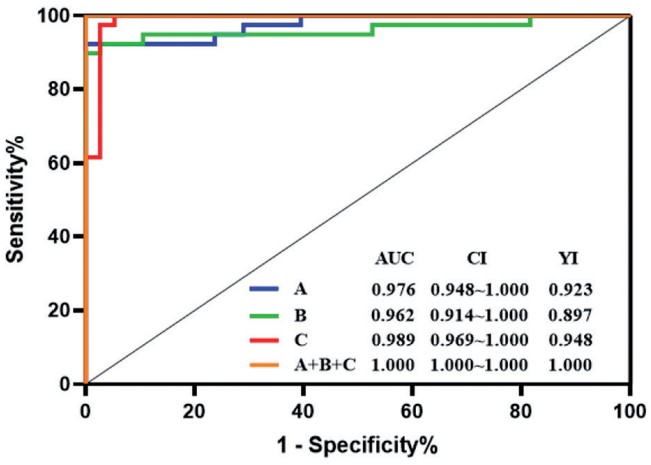

Fig. 5. Receiver operating characteristic curve analysis (ROC) of PE (16:0/18:2), LPE (18:0) and ACar (11:0) and their combination.
A: PE (16:0/18:2)
B: LPE (18:0)
C: ACar (11:0)
AUC: area under the curve; CI: confidence interval; YI: Youden index.

PE (16:0/18:2) and anticardiolipin antibody (ANA) IgG (Suppl. Fig. S1). The result shows that PE (16:0/18:2) is associated with ANA IgG.

Discussion

SLE is a complex autoimmune disease with a variety of clinical manifestations and serological markers that has attracted much attention. Although the exact pathogenesis of SLE remains unclear, there is much research on this disease. At present, markers used for SLE diagnosis are limited to antinuclear antibodies, anti-ds-DNA antibodies, several autoantibodies and complements. Therefore, we urgently need biomarkers for SLE diagnosis. Some previous studies were limited by the analysis platform (11) or sample size (12, 13), and these types of studies cannot well identify a sufficient number of biomarkers. Liposomes have shown wide application prospects in the identification of disease lipid markers, diagnosis, drug targets and the discovery of lead compounds. We used the UPLC-QE method to conduct a comprehensive study of serum lipids in patients with SLE using a sample size larger than that in previous studies-making the results more effective. We screened more than 510 lipids, identified the types of lipids associated with SLE, and verified the biomarkers related to disease activity in a verification set. It was found that levels of serum PE and LPE were significantly increased but that ACar was decreased in patients with SLE.

Elevated levels of oxidative stress are involved in the pathogenesis of SLE. Increased oxidative stress leads to abnormal lipid metabolism in SLE patients (14). It increases the levels of oxidised low-density lipoprotein (ox-LDL) and proinflammatory cytokines (15), as well as damages the function of high-density lipoprotein (HDL) and induces autoimmune response through oxidative modification of autoantibodies (16, 17). Oxidative stress contributes to cardiovascular disease, which is the main cause of morbidity and mortality of SLE (18). PE is among the most abundant phospholipids, second only to phosphatidylcholines, in the mammalian cell membrane and play an important role in biological processes such as apoptosis and cell signal transduction (19). The content of PE in mitochondria is significantly higher than that in other organelles, and some studies have shown that mitochondrial dysfunction plays an important role in the pathogenesis of SLE (20). Mitochondrial dysfunction in abnormal immune cells of patients with SLE can lead to increased oxidative stress, which in turn contributes to abnormal lipid metabolism (21, 22). Oxidised lipids play an important signal transduction role in inflammation and the immune response. Oxidised phospholipids are recognised by the immune system and bind to C-reactive protein (CRP) through IgG and

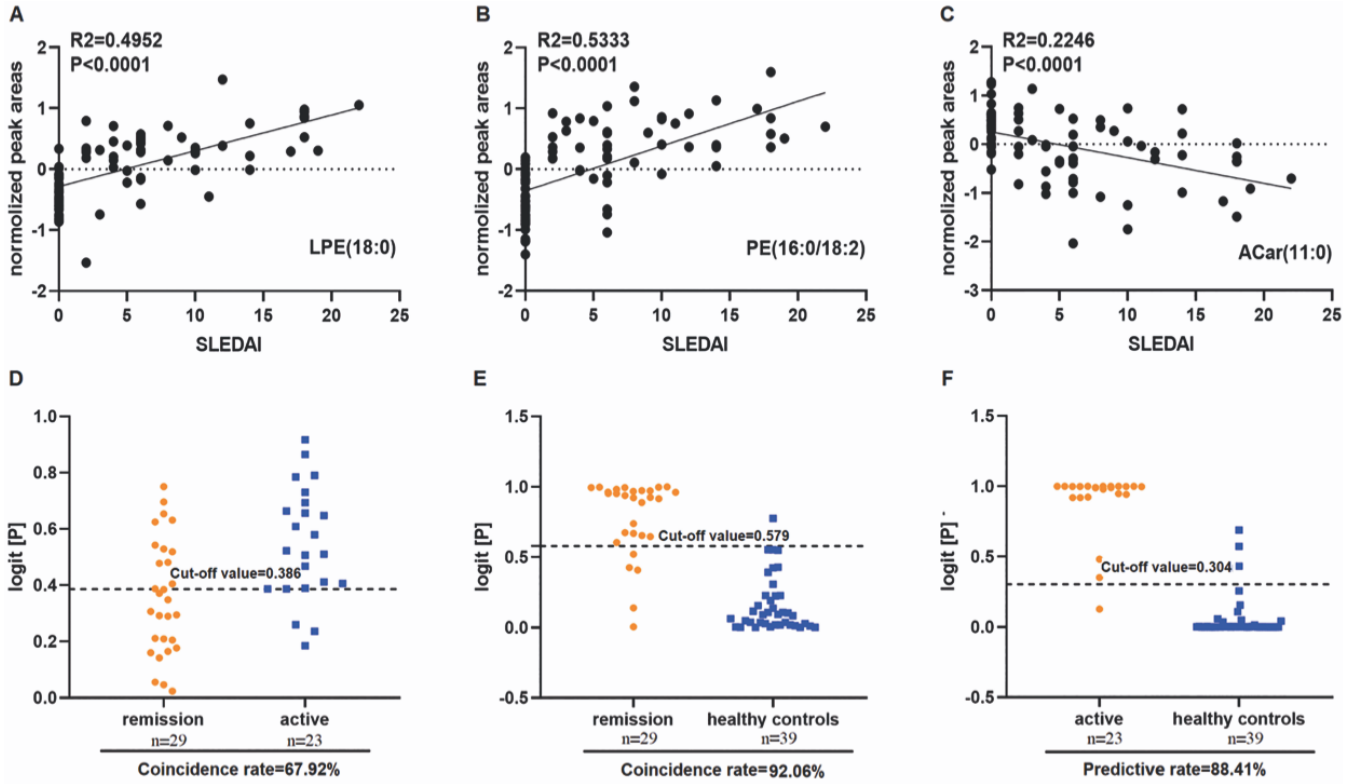

Fig. 6. Diagnosis of disease activity in systemic lupus erythematosus using PE (16:0/18:2), LPE (18:0) and Acar (11:0).
A: Spearman correlation analysis between PE (16:0/18:2) and SLEDAI scores. **B**: Spearman correlation analysis between LPE (18:0) and SLEDAI scores. **C**: Spearman correlation analysis between Acar (11:0) and SLEDAI scores. **D**: Diagnostic coincidence rate for the comparison between remission *vs*. active SLE. **E**: Diagnostic coincidence rate for the comparison between remission SLE *vs*. HCs. **F**: Diagnostic prediction rate for thecomparison between active SLE *vs*. HC.

IgM antibodies, thus activating various intracellular signalling mechanisms. In addition, the existence of PE has always been associated with thrombosis. It has been reported that aPE was common in patients with SLE (23, 24) and its presence is also related to thrombosis (25). APL are heterogeneous autoantibodies that specifically target phospholipid-binding proteins or their complexes with phospholipids. In some cases, aPE has been reported to be associated with aPL (26, 27). This is consistent with the correlation between PE (16:0/18:2) and ANA IgG in our results, indicating the disorder of lipid metabolism and the occurrence of cardiovascular disease in patients with SLE. LPE is a haemolytic metabolite of PE. As intercellular signalling molecules [28), LPE plays a role in the occurrence and development of inflammation (29). Our result has shown that the level of LPE in patients with SLE is increased, which is consistent with that in Changfeng Hu's study. ACar, produced by mitochondria and peroxidase enzymes, is an intermediate of oxidative catabolism of fatty acids and amino acids, mainly transporting long-chain fatty acids through the mitochondrial membrane for β-oxidation (30). ACar has a role in regulating energy metabolism, cardiac function, inflammation, cellular stress, ion balance and membrane permeability (31). Some studies have shown that the level of ACar in patients with SLE is decreased (32, 33), suggesting alteration of the fatty acid metabolism pathway. Based on the above results, abnormal lipid metabolism caused by SLE may induce cardiovascular disease.

Our study provides a reference for the diagnosis of SLE. Although the sample size was expanded, there were still some shortcomings. All the participants were Asian and were from the same research center, which limits the scope of application of the experimental results. In future studies, multiethnic, cross-centre verification should be carried out to confirm the results.

In conclusion, we used UPLC-QE to study serum lipid metabolism in patients with SLE. The results showed that PE and LPE are upregulated and ACar downregulated. These lipids are risk factors for oxidative stress, fatty acid oxidation and energy metabolism disorders in patients with SLE. Furthermore, the diagnostic value of PE (16:0/18:2), LPE (18:0) and ACar (11:0) in SLE was further verified. In addition, these three lipids were correlated with SLEDAI scores, indicating that they can not only be used for the diagnosis of SLE, but also further determine the disease activity of SLE. These three lipids may serve as biomarkers for the clinical diagnosis of SLE and have great potential in distinguishing SLE patients from healthy people.

Acknowledgments
We thank all the participants who contributed their efforts to this study, as well as the staff of the Affiliated Hospital of Nanjing University of Traditional Chinese Medicine who helped us collect samples.

References

1. TRAGER J, WARD MM: Mortality and causes of death in systemic lupus erythematosus. *Curr Opin Rheumatol* 2001; 13: 345-51.
2. LEWIS MJ, JAWAD AS: The effect of ethnicity and genetic ancestry on the epidemiology, clinical features and outcome of systemic lupus erythematosus. *Rheumatology* (Oxford) 2017; 56 (Suppl. 1): i67-i77.
3. PONS-ESTEL BA, BONFA E, SORIANO ER *et al*.: First Latin American clinical practice guidelines for the treatment of systemic lupus erythematosus: Latin American Group for the Study of Lupus (GLADEL, Grupo Latino Americano de Estudio del Lupus) Pan-American League of Associations of Rheumatology (PANLAR). *Ann Rheum Dis* 2018; 77: 1549-57.
4. DÖRNER T, FURIE R: Novel paradigms in systemic lupus erythematosus. *Lancet* 2019; 393: 2344-58.
5. GORDON C, AMISSAH-ARTHUR MB, GAYED M *et al*.: The British Society for Rheumatology guideline for the management of systemic lupus erythematosus in adults. *Rheumatology* (Oxford) 2018; 57: e1-45.
6. KIM HA, LEE HS, SHIN TH *et al*.: Polyamine patterns in plasma of patients with systemic lupus erythematosus and fever. *Lupus* 2018; 27: 930-8. doi:
7. SIGNORINI V, ELEFANTE E, ZUCCHI D, TRENTIN F, BORTOLUZZI A, TANI C: One year in review 2020: systemic lupus erythematosus. *Clin Exp Rheumatol* 2020; 38: 592-601.
8. DING H, MOHAN C: Connective tissue diseases: promises and challenges of metabolomics in SLE. *Nat Rev Rheumatol* 2016; 12: 627-8.
9. WU T, XIE C, HAN J *et al*.: Metabolic disturbances associated with systemic lupus erythematosus. *PLoS One* 2012; 7: e37210.
10. LI M, DU C, GUO N *et al*.: Composition design and medical application of liposomes. *Eur J Med Chem* 2019; 164: 640-53.
11. OUYANG X, DAI Y, WEN JL, WANG LX: ^{1}H NMR-based metabolomic study of metabolic profiling for systemic lupus erythematosus. *Lupus* 2011; 20: 1411-20.
12. YU Y, CAO Y, CHEN Y, WEN C, XU Z: Plasma metabonomics study of systemic lupus erythematosus based on liquid chromatography–mass spectrometry. *Se Pu* 2010; 28: 644-8 (In Chinese).
13. ZHANG Q, YIN X, WANG H *et al*.: Fecal metabolomics and potential biomarkers for systemic lupus erythematosus. *Front Immunol* 2019; 10: 976.
14. LU L, HU C, ZHAO Y *et al*.: Shotgun lipidomics revealed altered profiles of serum lipids in systemic lupus erythematosus closely associated with disease activity. *Biomolecules* 2018; 8: 105.
15. FROSTEGÅRD J, SVENUNGSSON E, WU R *et al*.: Lipid peroxidation is enhanced in patients with systemic lupus erythematosus and is associated with arterial and renal disease manifestations. *Arthritis Rheum* 2005; 52: 192-200.
16. MCMAHON M, GROSSMAN J, FITZGERALD J *et al*.: Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 2541-9.
17. KURIEN BT, HENSLEY K, BACHMANN M, SCOFIELD RH: Oxidatively modified autoantigens in autoimmune diseases. *Free Radic Biol Med* 2006; 41: 549-56.
18. HU C, ZHOU J, YANG S *et al*.: Oxidative stress leads to reduction of plasmalogen serving as a novel biomarker for systemic lupus erythematosus. *Free Radic Biol Med* 2016; 101: 475-81.
19. VANCE JE: Phosphatidylserine and phosphatidylethanolamine in mammalian cells: two metabolically related aminophospholipids. *J Lipid Res* 2008; 49: 1377-87.
20. YANG SK, ZHANG HR, SHI SP *et al*.: The role of mitochondria in systemic lupus erythematosus: a glimpse of various pathogenetic mechanisms. *Curr Med Chem* 2020; 27: 3346-61.
21. PERL A: Oxidative stress in the pathology and treatment of systemic lupus erythematosus. *Nat Rev Rheumatol* 2013; 9: 674-86.
22. MOREL L: Immunometabolism in systemic lupus erythematosus. *Nat Rev Rheumatol* 2017; 13: 280-90.
23. BERTOLACCINI ML, MURRU V, SCIASCIA S, SANNA G, KHAMASHTA MA: The clinical value of testing for antibodies to phosphatidylethanolamine (aPE) in patients with systemic lupus erythematosus (SLE). *Thromb Res* 2012; 130: 914-8.
24. BRANCH DW, ROTE NS, DOSTAL DA, SCOTT JR: Association of lupus anticoagulant with antibody against phosphatidylserine. *Clin Immunol Immunopathol* 1987; 42: 63-75.
25. SANMARCO M, GAYET S, ALESSI MC *et al*.: Antiphosphatidylethanolamine antibodies are associated with an increased odds ratio for thrombosis. A multicenter study with the participation of the European Forum on antiphospholipid antibodies. *Thromb Haemost* 2007; 97: 949-54.
26. KARMOCHKINE M, BERARD M, PIETTE JC *et al*.: Anti-phosphatidylethanolamine antibodies in systemic lupus erythematosus. *Lupus* 1993; 2: 157-60.
27. STAUB HL, HARRIS EN, KHAMASHTA MA, SAVIDGE G, CHAHADE WH, HUGHES GRV: Antibody to phosphatidylethanolamine in a patient with lupus anticoagulant and thrombosis. *Ann Rheum Dis* 1989; 48: 166-9.
28. QAMAR T, GHARAVI AE, LEVY RA, LOCKSHIN MD: Lysophosphatidylethanolamine is the antigen to which apparent antibody to phosphatidylethanolamine binds. *J Clin Immunol* 1990; 10: 200-3.
29. LIU-WU Y, HURT-CAMEJO E, WIKLUND O: Lysophosphatidylcholine induces the production of IL-1beta by human monocytes. *Atherosclerosis* 1998; 137: 351-7.
30. TEAV T, GALLART-AYALA H, VAN DER VELPEN V, MEHL F, HENRY H, IVANISEVIC J: Merged targeted quantification and untargeted profiling for comprehensive assessment of acylcarnitine and amino acid metabolism. *Anal Chem* 2019; 91: 11757-69.
31. MAKRECKA M, KUKA J, VOLSKA K *et al*.: Long-chain acylcarnitine content determines the pattern of energy metabolism in cardiac mitochondria. *Mol Cell Biochem* 2014; 395: 1-10.
32. LI Y, LIANG L, DENG X, ZHONG L: Lipidomic and metabolomic profiling reveals novel candidate biomarkers in active systemic lupus erythematosus. *Int J Clin Exp Pathol* 2019;12: 857-66.
33. BELLOCCHI C, FERNÁNDEZ-OCHOA Á, MONTANELLI G *et al*.: Identification of a shared microbiomic and metabolomic profile in systemic autoimmune diseases. *J Clin Med* 2019; 8: 1291.