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Metabolomic analysis suggests thiamine monophosphate as a potential marker for mesenchymal stem cell transplantation outcomes in patients with SLE

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ABSTRACT

Objective The objective of this research is to identify metabolic markers associated with successful treatment by evaluating the effect of mesenchymal stem cell transplantation (MSCT) on the metabolic profiles of patients with SLE.

Methods Plasma samples were collected from 20 patients with SLE before and after MSCT. Principal component analysis (PCA) was used to distinguish pretreatment and post-treatment groups and pathway analysis for identifying involved metabolic pathways. Clinical variables were monitored with a median follow-up time of 180 days. Pearson correlation and receiver operating characteristics (ROC) analysis were employed to associate metabolite changes with clinical outcomes and to predict treatment success.

Results We detected 18 121 metabolites, with 1152 showing significant changes post-treatment, which could be clearly distinguished between pretreatment and post-treatment groups through PCA. Pathway analysis indicated involvement in riboflavin and thiamine metabolism. Clinical improvements were observed at a median follow-up time of 180 days after MSCT, including decreased SLE Disease Activity Index scores, urine protein/creatinine ratios, and erythrocyte sedimentation rates, along with increased levels of complement C3 and C4, haemoglobin, and platelets. Pearson correlation indicated that specific metabolite changes were associated with clinical improvements, particularly increases in thiamine monophosphate (TMP) and asiaticoside levels. ROC analysis identified TMP level changes as the most predictive of treatment success, with a 35% increase indicating a good response to MSCT.

Conclusion This study concludes that TMP is a potential biomarker that can predict the efficacy of MSCT in treating SLE, providing valuable insights for clinical practice and further research.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Mesenchymal stem cell transplantation (MSCT) is a promising therapy for autoimmune diseases such as SLE due to its immunomodulatory effects.
⇒ Previous studies have identified metabolic changes in SLE, suggesting significant connections between metabolism and immune responses.
⇒ However, the influence of MSCT on metabolism and the specific metabolic predictors of MSCT outcomes in SLE remain largely unknown.

WHAT THIS STUDY ADDS

⇒ This study fills key gaps in understanding how MSCT affects metabolism in SLE. It reveals that changes in thiamine monophosphate (TMP) levels 24 hours after MSCT are a new biomarker candidate for predicting 6-month treatment responses, offering valuable insights for SLE treatment.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ It may help improve patient care and guide future research, leading to more personalised and effective treatments for SLE.

INTRODUCTION

SLE is an autoimmune condition that causes chronic inflammation and affects multiple organs and tissues. It is characterised by autoantibody production, immune complex deposition and inflammation of tissues.[1] Despite the availability of conventional drug therapy including glucocorticoids and various immunosuppressive drugs, patients with SLE continue to experience disease flares and reduced quality of life.[2] Since conventional drug therapies are non-curative, other treatments need to be explored. Recently, mesenchymal stem cells (MSCs) have been proposed as a new therapeutic option for patients with SLE, owing to their immunomodulatory and regenerative properties.[3 4] In preclinical studies and clinical trials, MSC transplantation (MSCT) has shown promising results in ameliorating the symptoms of SLE, such as

reducing disease activity, improving renal function and restoring the balance of T cell subsets.[5–8] However, the underlying mechanisms of MSCT in SLE are not yet fully understood.

Metabolomic studies of metabolites and their interactions in biological systems have become a powerful tool for unravelling the complex pathways and networks involved in disease pathogenesis and therapeutic interventions.[9 10] By comparing the metabolic profiles of patients with SLE before and after MSCT, it is possible to uncover the metabolic pathways that are disturbed in SLE and the metabolic changes induced by MSCT. In addition, it could also provide valuable insights into the mechanisms of MSCT in SLE and help to identify potential biomarkers for predicting treatment response. In this study, an untargeted metabolomics approach was used to search for variations in different plasma metabolites before and after MSCT.

MATERIALS AND METHODS

Patient and public involvement

The study was conducted in accordance with the principles of the Helsinki Declaration. MSCT was generally used to treat refractory patients or help reduce the dosage of conventional drugs, especially glucocorticoids. Twenty study participants were randomly selected from a sample bank of 807 patients with SLE who had undergone MSCT at the Department of Rheumatology and Immunology, Affiliated Drum Tower Hospital. All patients fulfilled the 1997 revised American College of Rheumatology criteria for SLE and were evaluated for disease activity using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)-2000.[11 12] Those with a history of severe drug allergy, malignancies, New York Heart Association functional class III-IV, abnormal liver dysfunction not caused by the disease itself, severe infections, cognitive impairment or psychiatric abnormality, end-stage renal failure, or positive test results for HIV and other pathogenic microorganisms were excluded. No patients smoked or consumed alcohol. Patients were allowed to continue using corticosteroids and/or immunosuppressive therapy, but their dosage should remain stable or in a reduced state for at least 8 weeks prior to transplantation. Informed consent was obtained from all patients prior to MSCT.

After MSCT, patients were followedup for a median time of 180 days with an IQR of 164–241 days. Patients were reassessed for disease activity at follow-up. Based on SLEDAI Score improvements and changes in urine protein/creatinine ratios (uPCR), patients with SLE were divided into two categories: effective and ineffective groups. The effective group included patients with reduced SLEDAI scores post-MSC treatment and, if suffering from lupus nephritis (LN), a simultaneous decrease in uPCR. Those not meeting these criteria were assigned to the ineffective group. During follow-up, those who met the criteria for dose reduction, specifically the Definition of Remission In SLE criteria and the Lupus Low Disease Activity State, had their medication doses reduced accordingly.

Mesenchymal stem cell transplantation

The preparation of umbilical cord (UC) -MSCs was identical to that in the previous report.[13] All patients received an intravenous infusion of 1×10^6 UC-MSCs per kilogram of body weight. Fasting blood samples from 20 patients with SLE patients collected in the morning before infusion and 24 hours after MSCT. All blood samples were collected in EDTA-2K tubes and centrifuged to separate and collect plasma. Plasma aliquots of 150 µL each were then stored at –80°C.

Sample preparation

Frozen plasma samples were thawed at 4°C. A 100 µL aliquot was mixed with 400 µL of cold methanol/acetonitrile (1:1, v/v), and then centrifuged at 14 000 g for 20 min at 4°C for protein removal. The supernatant obtained was dried using a vacuum centrifuge, reconstituted in 100 µL acetonitrile/water (1:1, v/v) solvent and centrifuged again at 14 000 g for 15 min at 4°C. The supernatant was then applied for liquid chromatography-mass spectrometry analysis.

UHPLC-Q-Exactive Orbitrap MS

The analysis was conducted using a Vanquish Ultra High-Performance Liquid Chromatography (UHPLC) (Thermo) integrated with an Orbitrap (Q Exactive HF-X/Q Exactive HF, Thermo) at Shanghai Applied Protein Technology. Pooled quality control samples were injected at the beginning of the analytical sequence to assess the stability of the Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) system and ensure data quality. For Hydrophilic Interaction Liquid Chromatography (HILIC) separation, samples were processed through a 2.1 mm × 100 mm ACQUITY UPLC Bridged Ethyl Hybrid (BEH) Amide 1.7 µm column (Waters, Ireland). The mobile phase contained a mixture of A=25 mM ammonium acetate and 25 mM ammonium hydroxide in water, and B=acetonitrile in both Electrospray Ionization (ESI)-positive and ESI-negative modes. The gradient started with 98% B for 1.5 min, linearly decreased to 2% over 10.5 min, remained stable for 2 min, and then escalated to 98% in 0.1 min, with a 3 min re-equilibration period incorporated.

The ESI source conditions were configured as follows: Ion Source Gas1 (Gas1) was set to 60, Ion Source Gas2 (Gas2) was set to 60, curtain gas was set to 30, and the source temperature was maintained at 600°C. The IonSpray Voltage Floating was regulated at ±5500 V. For MS-only acquisition, the instrument was adjusted to scan over the m/z range of 80–1200 Da, with the resolution and accumulation time set at 60 000 ms and 100 ms, respectively. In the case of auto MS/MS acquisition,

the instrument was set to scan over the m/z range of 70–1200 Da, with the resolution and accumulation time set at 30 000 ms and 50 ms, respectively, excluding a time span of 4 s.

MS-based untargeted metabolomics

Multivariate statistical analyses, including principal component analysis (PCA) and hierarchical clustering, were performed to reveal relationships and differences among the samples. Volcano plots and pie charts were used to display the features and categories of different metabolites (fold change (FC) >1.5 or < 1/1.5 and value of p≤0.05). Enriched metabolic pathways were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Heatmaps were generated to illustrate the selected differentially abundant metabolites.

Analysis of metabolomics data

Due to the fact that the raw data for most metabolite levels do not conform to a normal distribution, a logarithmic transformation (data <–$\log_2$(data)) was applied to make the overall data more closely approximate a normal distribution. Regarding the difference in metabolite levels before and after treatment, data analysis included the use of paired t-tests and non-parametrical tests, and was conducted using R software (V.4.2.3). For the log-transformed data, the Shapiro-Wilk test was used to assess the normality of the distribution of differences in metabolite levels before and after treatment. If the distribution was found to satisfy normality (p>0.05), paired t-tests were performed; otherwise, the Wilcoxon signed-rank test was applied for non-normally distributed data.

FC refers to the ratio of two quantities, showing how much a quantity has increased or decreased between two conditions. The plasma metabolite levels after MSCT were divided by those before MSCT to calculate the FC. $\log_2$FC is the logarithm to base 2 of the FC. A $\log_2$FC greater than 0 indicates an increase in levels after treatment compared with before treatment, while a $\log_2$FC less than 0 indicates a decrease.

We used the programming language R for PCA, hierarchical clustering analysis, volcano plots, pie charts and correlation heatmap analysis. KEGG enrichment analysis was conducted using MetaboAnalyst V.5.0 (https://www.metaboanalyst.ca/).

Correlation analysis

Clinical data of patients were collected before MSCT and during follow-up. To assess the relationship between differential metabolites and clinical efficacy indicators (SLEDAI Scores and uPCR), correlation analysis was performed. Values of p were calculated to determine the statistical significance of the correlations, with a threshold of p<0.05 considered statistically significant. The analysis was conducted using GraphPad Prism (V.9.0) (GraphPad Software, California, USA).

Receiver operating characteristics analysis

Receiver operating characteristics (ROC) analysis was used to evaluate the prognostic value of changes in differential metabolites following MSCT. An area under the ROC curve (AUC) of 1.0 represents perfect discrimination, whereas an AUC of 0.5 indicates no discriminative power. The optimal cut-off value for each metabolite was determined based on the Youden Index, which maximises the sum of sensitivity and specificity. Statistical significance was assigned to AUC values with p<0.05. ROC analysis was performed using GraphPad Prism (V.9.0) (GraphPad Software, California, USA).

RESULTS

Clinical characteristics of the enrolled patients

Clinical characteristics of the enrolled patients were described in table 1. Because over 80% of metabolomics data were missing for cases 17 and 19, they were not included in the analysis (online supplemental figure S1). Patient 4 was also excluded from the subsequent analysis because follow-up contact could not be established. Among the remaining 17 patients, there were 15 women and 2 men, with ages ranging from 17 years to 66 years and a median age of 33 (IQR: 29–42) years. The median disease duration was 120 (IQR: 84–192) months, the median SLEDAI Score was 11 (IQR: 5–13), and over two-thirds (13 cases) had renal nephritis. The vast majority of patients were given both glucocorticoids and hydroxychloroquine, and about half also received other immunosuppressants. No patients received intravenous steroids at the time of MSCT, and corticosteroid exposures listed in table 1 represent oral prednisone equivalent doses. For more detail, the full SLEDAI item prevalence of each patient with SLE is provided in online supplemental table S1.

Of the 17 patients, 6 were on long-term Angiotensin II Receptor Blocker (ARB) therapy for hypertension management. Specifically, five patients were taking valsartan (80 mg once per day), and one patient was taking irbesartan (150 mg once per day). Their ARB dosage remained unchanged before, during and after MSCT, as well as throughout the follow-up period.

Among the 17 patients, 12 tolerated MSCT well. The remaining five patients experienced minor side effects within 24 hours post-MSCT treatment: one had a fever, one had a headache, one had chills, one had chest tightness and one had dizziness. These symptoms were all transient and resolved spontaneously.

Differential metabolites associated with MSCT in patients with SLE

In order to explore the impact of MSCT on patients with SLE, plasma metabolites of patients before and 24 hours after MSCT were compared. A total of 18 121 metabolites were identified in 34 plasma samples from 17 patients with SLE. Among them, 10 491 metabolites were identified in the positive ion (POS) mode and 7630 in the negative ion (NEG) mode. Samples before and after treatment could be distinguished by unsupervised PCA in both POS mode (figure 1a) and NEG mode

Table 1 Characteristics of the enrolled patients with SLE

Patient	Age (years)	Disease duration (months)	SLEDAI Score	Presence of LN	Glucocorticoid dose*	Concomitant medication†	Time to follow-up (days)	Glucocorticoid dose‡	Concomitant medication§
1	30	120	4	Y	5	HCQ, TAC, MMF	193	0	HCQ, MMF
2	20	48	17	Y	5	TAC, MMF	164	2.5	TAC, MMF
3	40	120	20	Y	50	HCQ, TAC	299	45	HCQ, TAC
5	40	204	11	Y	15	HCQ	169	12.5	HCQ
6	70	120	4	N	10	HCQ, LEF	180	0	HCQ, LEF
7	30	132	11	Y	20	HCQ	553	20	HCQ
8	30	48	13	Y	30	HCQ	86	15	/
9	20	96	18	Y	5	HCQ, MMF, LEF	171	5	/
10	50	132	4	N	15	TAC	180	10	TAC
11	40	192	17	Y	27.5	TAC	180	15	TAC
12	40	264	8	N	5	HCQ	50	5	HCQ
13	30	72	1	N	5	HCQ	169	2.5	HCQ
14	60	288	12	Y	5	HCQ, TAC, MMF	105	5	HCQ, TAC, MMF
15	40	96	5	N	0	HCQ, MMF	657	0	HCQ
16	20	2	11	Y	30	TAC, MMF	155	20	TAC, MMF
18	30	84	9	Y	5	HCQ, MMF	241	5	HCQ, MMF
20	30	216	12	Y	5	HCQ, MMF	351	2.5	HCQ, MMF

Due to the confidentiality agreement, we only provide the approximate age of patients without sex.
The follow-up time listed in table 1 corresponds to the timing of lab testing as shown in table 2, as well as the timing of the post-MSCT calculation of the SLEDAI Score.
*Glucocorticoid dose: daily dosage of prednisone administered prior to MSCT.
†Concomitant medication: other concurrent medications administered prior to MSCT.
‡Glucocorticoid dose: daily dosage of prednisone administered during the follow-up period after MSCT.
§Concomitant medication: other concurrent medications administered during the follow-up period after MSCT.
HCQ, hydroxychloroquine; LEF, leflunomide; LN, lupus nephritis; MMF, mycophenolate mofetil; MSCT, mesenchymal stem cell transplantation; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; TAC, tacrolimus.

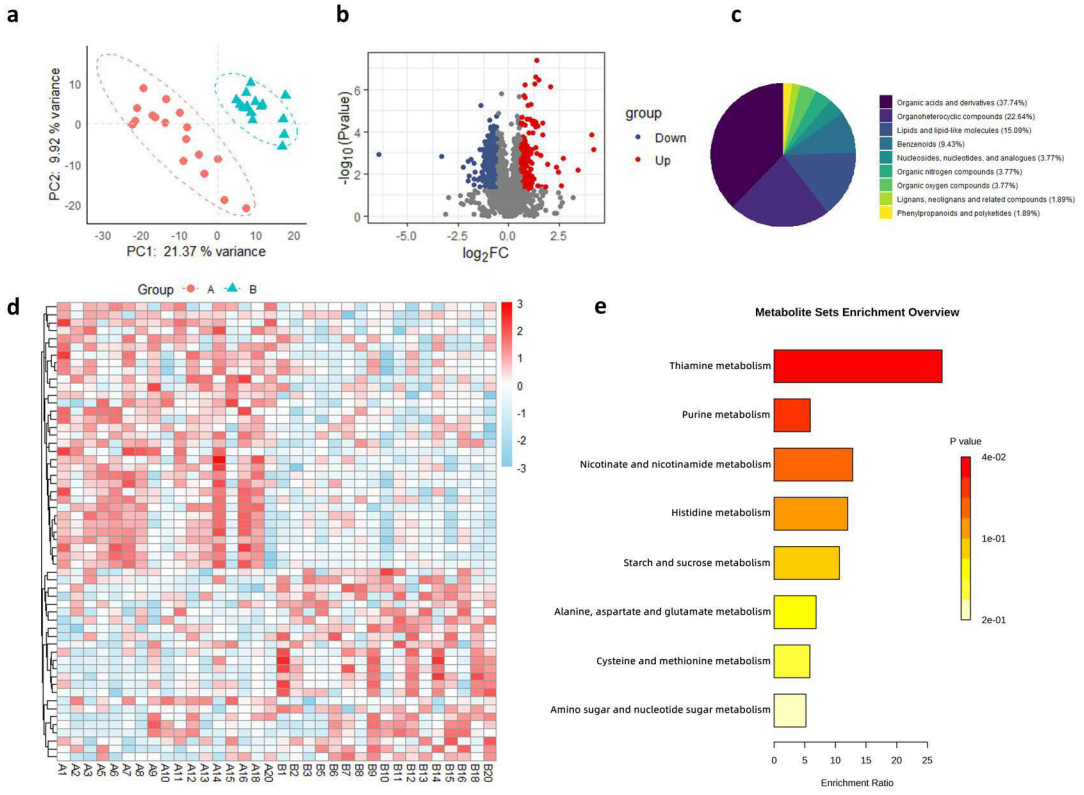

Figure 1 Differential metabolites associated with MSCT in cationic mode. (a) Principal component analysis of the metabolomics before and after MSCT. Each symbol represented an individual sample. Group A represents pre-MSCT, Group B represents post-MSCT. (b) Differential metabolites identified by volcano plots (highlighted with colours). (c) Categories of recognisable differential metabolites depicted in pie charts. (d) Heatmap of recognisable differential metabolites. The colour bar (top right) indicates the scale of standardised metabolite levels. (e) KEGG pathway analysis of differential metabolites. The colour of the band represents the p value of the enrichment analysis, and the length of the band indicates the enrichment ratio. KEGG, Kyoto Encyclopedia of Genes and Genome; MSCT, mesenchymal stem cell transplantation.

(figure 2a). A total of 1152 metabolites were changed after MSCT, of which 207 metabolites were upregulated and 411 downregulated in POS mode (figure 1b) and 94 metabolites were upregulated and 440 metabolites were downregulated in NEG mode (figure 2b).

However, only 93 of these differential metabolites could be identified, with lipids and lipid-like molecules accounting for the largest proportion (35.46%) in the POS mode (figure 1c), while in the NEG mode (figure 2c), lipids and lipid-like molecules accounted for only the third place (15.09%), with organic acids and their derivatives taking the first place (37.74%). As shown in the heatmap, 34 metabolites were downregulated and 23 metabolites were upregulated in the POS mode (figure 1d), and 31 metabolites were downregulated and five metabolites were upregulated in the NEG mode (figure 2d). KEGG analysis showed that in the POS mode (figure 1e), thiamine, purine and nicotinate metabolism were the predominantly altered pathways, whereas pathways such as riboflavin and caffeine metabolism were significantly changed in the NEG mode (figure 2e).

Clinical outcomes and correlations with differential metabolites in patients with SLE after MSCT

The clinical efficacy of MSCT before and after administration was evaluated in 17 patients with SLE (table 2). Notably, there was a significant reduction in the SLEDAI Score (p=0.001), uPCR (p=0.027) and erythrocyte sedimentation rate (p=0.020) post-treatment. Additionally, levels of complements C3 and C4, haemoglobin (Hb) and platelets (PLT) were observed to increase after MSCT.

To clarify whether changes in metabolites after MSC transplantation could predict clinical outcomes, especially improvements in SLEDAI Scores and uPCR, we performed a correlation analysis, and 13 metabolites were found to be significantly associated with changes in SLEDAI Scores (table 3), and 4 metabolites were found to be significantly associated with changes in uPCR (table 4). Specifically, metabolites such as thiamine monophosphate (TMP) and asiaticoside had a positive $\log_2$ fold change ($\log_2$FC) and were negatively correlated with SLEDAI, indicating that an increase in these metabolites post-MSCT was associated with a decrease in SLEDAI Score. In contrast, metabolites including 12(R)-HETE, d-erythro-imidazolylglycerol phosphate, defluoroatorvastatin, glycodeoxycholic acid and prop-2-enoic acid had a negative $\log_2$FC, which were positively correlated with SLEDAI, suggesting that reductions in these metabolites were associated with lower SLEDAI Scores post-treatment (figure 3). Regarding uPCR, the $\log_2$FC for TMP was positive and the correlation was negative, indicating that

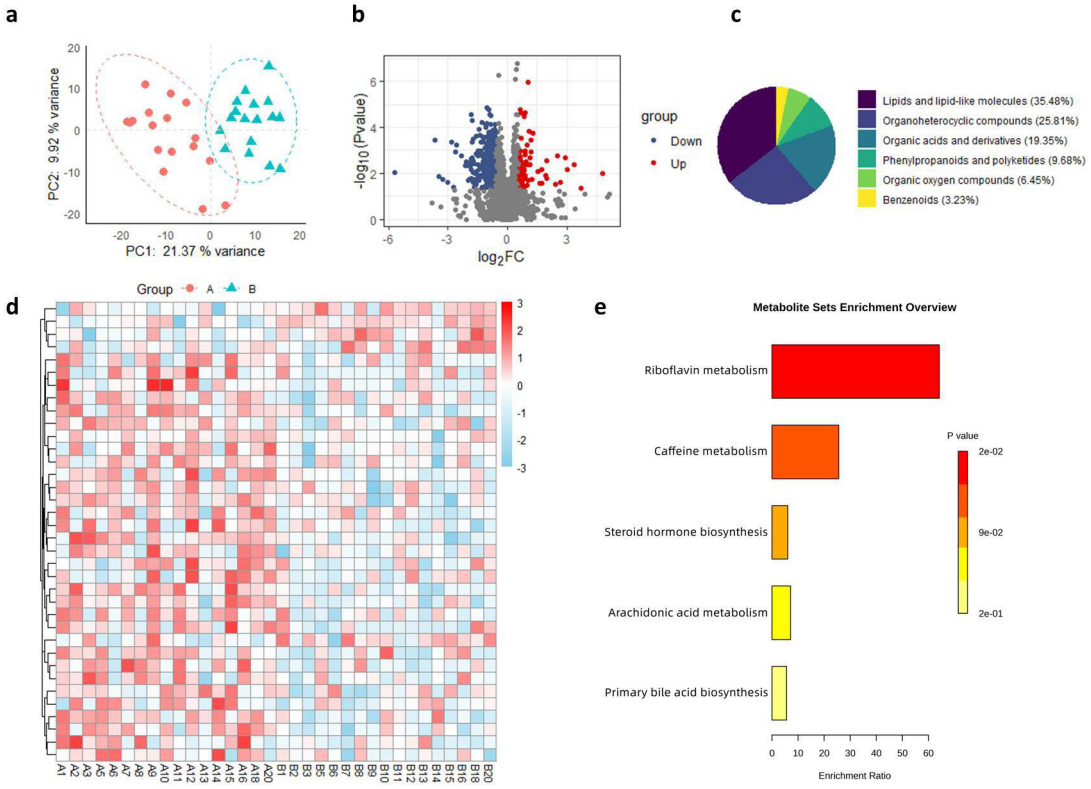

Figure 2 Differential metabolites associated with MSCT in anionic mode. (a) Principal component analysis of the metabolomics before and after MSCT. (b) Differential metabolites identified by volcano plots. (c) Categories of recognisable differential metabolites depicted in pie charts. (d) Heatmap of recognisable differential metabolites. (e) KEGG pathway analysis of differential metabolites. KEGG, Kyoto Encyclopedia of Genes and Genome; MSCT, mesenchymal stem cell transplantation.

the elevation of these metabolites after MSCT was associated with a decrease in uPCR. In contrast, the $\log_2$FC for metabolites such as 12(R)-HETE and D-erythro-imidazole glycerophosphate was negative and positively correlated, suggesting that a decrease in these metabolites after treatment was associated with a decrease in uPCR (figure 4).

Combining both SLEDAI Score and uPCR, a total of seven different metabolites were found to be associated with clinical efficacy. Specifically, TMP and asiaticoside levels increased following MSCT, with a higher increase associated with lower SLEDAI Scores and uPCR. Conversely, levels of 12(R)-HETE, d-erythro-imidazolylglycerol phosphate, defluoroatorvastatin, glycodeoxycholic acid and prop-2-enoic acid levels decreased after MSCT, where a greater reduction was linked to lower SLEDAI Scores and uPCR.

Table 2 Clinical efficacy of MSCT

	Pre-MSCT	Post-MSCT	P value
SLEDAI Score	11 (5–13)	5 (4–8)	0.001
uPCR (mg/mmol)	4.22 (1.92–5.77)	1.38 (0.85–2.00)	0.027
C3 (g/L)	0.78 (0.7–1.2)	0.80 (0.75–1.23)	0.014
C4 (g/L)	0.13 (0.11–0.19)	0.17 (0.13–0.19)	0.004
ESR (mm/h)	40 (28–62)	32 (16–48)	0.020
Hb (g/L)	103 (88–112)	106 (98–116)	0.028
PLT ($\times10^9$/L)	144 (120–176)	163 (140–208)	0.036
WBC ($\times10^9$/L)	4.5 (2.7–6.3)	4.7 (3.2–6)	0.390

The median follow-up time was 180 days (IQR: 164–241 days).
C3, complement 3; C4, complement 4; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; MSCT, mesenchymal stem cell transplantation; PLT, platelet; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; uPCR, urine protein/creatinine ratios; WBC, white blood cells.

The value of TMP changes in assessing the prognosis of MSCT

Based on the change in SLEDAI Score and uPCR at follow-up, a total of 12 out of 17 patients were categorised in the effective group and 5 in the ineffective group; of these, 4 out of 5 non-renal patients with SLE were in the effective group and 1 was in the ineffective group, whereas 8 out of 12 patients with LN were in the effective group and four were in the ineffective group.

To determine the prognostic value of the changes in seven differential metabolites in predicting the clinical efficacy of MSCT, ROC analysis was conducted on the differences in metabolite levels before and after treatment. Among these, TMP exhibited the highest area under the ROC curve (AUC) at 0.817 (95% CI 0.601 to 1.000), with a value of $p<0.05$. At a cut-off value of 0.451, the sensitivity and specificity were 80% and 75%, respectively (figure 5a). As mentioned earlier, metabolite levels were log-transformed to better process the data. Thus, a cut-off value of 0.4513 represented a change in TMP levels of approximately 1.35 times, which means that

Table 3 Pearson correlation analysis of changes in differential metabolites and SLEDAI Scores

	Pre-MSCT	Post-MSCT	r	p1	log_2FC	p2
TMP	650.4 (445.6–821.3)	1167.4 (745.2–1578.6)	–0.682	0.003	0.597	0.036
Asiaticoside	1180.3 (823.5–2149.8)	1914.6 (1346.9–3252.5)	–0.573	0.016	0.680	0.006
12(R)-HETE	2242.1 (1757.0–3767.4)	1803.1 (1329.8–2061.8)	0.490	0.046	–0.599	0.005
D-erythro-imidazolylglycerol phosphate	90 101.0 (61 150.8–139139.0)	67 651.7 (51 540.9–74 946.1)	0.575	0.016	–0.633	0.035
Defluoroatorvastatin	1133.0 (641.8–2355.2)	493.3 (219.2–638.3)	0.684	0.003	–0.845	0.018
Glycodeoxycholic acid	147 922.6 (57 415.9–233955.0)	53 864.1 (41 528.1–60 784.8)	0.603	0.010	–1.056	0.001
Prop-2-enoic acid	4635.9 (3401.8–4879.8)	3933.0 (1679.5–4354.7)	0.515	0.035	–0.664	0.001
L-Ascorbic acid	29 433.5 (15 731.1–38 208.4)	49 768.8 (40 113.4–67 732.5)	0.699	0.002	0.594	0.036
1h-indole-3-propanoic acid	16 112.5 (920.6–23 909.5)	14 416.3 (4573.8–212577.2)	0.580	0.015	1.554	0.008
Arcaine	30 353.8 (19 156.0–30 923.3)	15 180.9 (11 526.8–20 401.0)	–0.569	0.017	–0.628	0.001
Clopidogrel carboxylic acid	2692.0 (2163.1–5032.7)	1713.4 (1177.7–2198.6)	–0.484	0.049	–0.812	0.003
Diethyl phthalate	22 664.6 (16 456.0–36 664.1)	12 906.0 (10 211.9–23 249.4)	–0.517	0.033	–0.614	0.005
Fructoselysine	56 853.5 (43 425.5–115218.3)	37 755.4 (33 093.9–37 972.1)	–0.508	0.037	–0.758	0.002

Pre and post columns are the median and IQR of the metabolites pretreatment and post-treatment.
p1: Value of p for Pearson correlation. p2: Value of p from the paired t-test comparing pretreatment and post-treatment. Note that for 1h-indole-3-propanoic acid, $log_2FC>0$ shows that the mean value increased after treatment, but the median value decreased. Such a discrepancy could happen when the distributions are not normal, which is why we need to do the logarithmic transform.
log_2FC, logarithm to base 2 of the fold change; 12(R)-HETE, 12(R)-hydroxyeicosatetraenoic acid; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; TMP, thiamine monophosphate.

Table 4 Pearson correlation analysis of changes in differential metabolites and uPCR

	Pre-MSCT	Post-MSCT	R	p1	log_2FC	p2
12(R)-HETE	2242.1 (1757.0–3767.4)	1803.1 (1329.8–2061.8)	0.715	0.009	−0.599	0.005
TMP	650.4 (445.6–821.3)	1167.4 (745.2–1578.6)	−0.612	0.035	0.597	0.036
D-erythro-imidazolylglycerol phosphate	90101.0 (61150.8–139139.0)	67651.7 (51540.9–74946.1)	0.589	0.044	−0.633	0.035
Diethyl phthalate	22664.6 (16456.0–36664.1)	12906.0 (10211.9–23249.4)	−0.681	0.015	−0.614	0.005

p1: Value of p for Pearson correlation. p2: Value of p from the paired t-test comparing pretreatment and post-treatment.
log_2FC, logarithm to base 2 of the fold change; 12(R)-HETE, D-erythro-imidazolylglycerol phosphate; TMP, thiamine monophosphate; uPCR, urine protein/creatinine ratio.

patients may respond well to treatment when their TMP levels increase by at least 35% after MSCT. Meanwhile, the log_2FC values of TMP in the effective group were higher than those in the ineffective group (p=0.044, figure 5b). ROC analyses for the other six metabolites did not yield statistically significant results (table 5).

DISCUSSION

Our study found that patients with SLE treated with MSCT showed significant decreases in post-treatment SLEDAI Scores, uPCR and erythrocyte sedimentation rate, as well as increases in levels of complement components C3 and C4, Hb and PLTs, along with significant changes in their metabolic profiles. Ninety-three significantly differentially expressed metabolites were identified through this study, among which changes in specific metabolites, especially TMP, may be useful in determining the efficacy of MSCs in patients with SLE.

Among the discernible differentially expressed metabolites, lipids and lipid-like molecules were predominant. A strong connection between SLE and lipid metabolism has been established according to previous studies.[14–16] However, despite this discovery, lipid metabolism was not significantly enriched in our KEGG enrichment analysis. KEGG pathway analysis revealed that most enriched pathways were associated with the metabolism of specific vitamins, including thiamine metabolism, riboflavin metabolism, and nicotinate and nicotinamide metabolism. Thiamine (vitamin B1), riboflavin (vitamin B2) and niacin (vitamin B3) are essential nutrients involved in various physiological processes, playing a vital role in cellular energy metabolism.[17] Reports have shown a negative correlation between riboflavin and thiamine and the left atherosclerotic plaques in patients with SLE.[18] Additionally, research has suggested that nicotinamide can alleviate kidney injury and pregnancy outcomes in lupus-prone MRL/lpr mice treated with lipopolysaccharide.[19]

The significantly differentially expressed metabolite TMP is a phosphorylated form of thiamine, and an intermediate to facilitate the synthesis of free thiamine to thiamine diphosphate (ThDP) and triphosphate. ThDP, also known as thiamine pyrophosphate (TPP) or

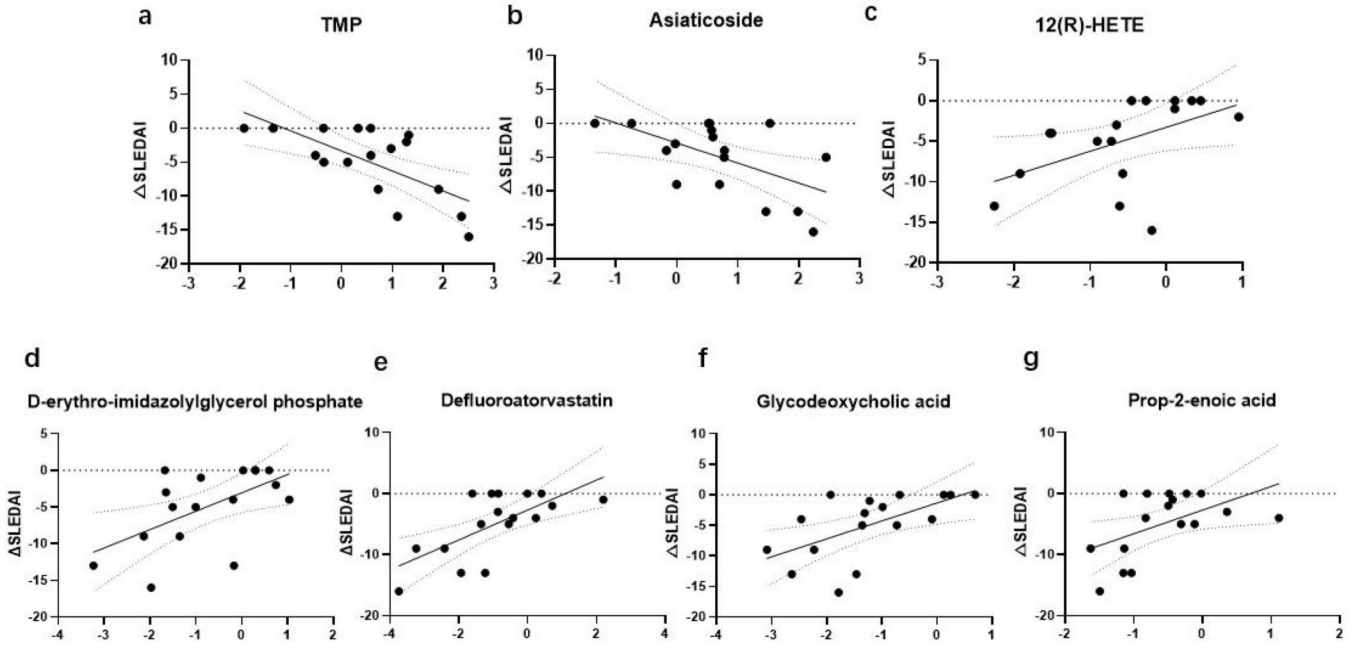

Figure 3 Pearson correlation of changes in specific metabolites and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (a–g): TMP, Asiaticoside, 12(R)-HETE, D-erythro-imidazolylglycerol phosphate, Defluoroatorvastatin, Glycodeoxycholic acid and Prop-2-enoic acid. 12(R)-HETE, D-erythro-imidazolylglycerol phosphate; TMP, thiamine monophosphate.

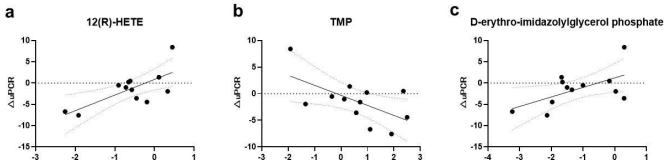

Figure 4 Pearson correlation of changes in specific metabolites and uPCR (a–c): 12(R)-HETE, TMP and D-erythro-imidazolylglycerol phosphate. 12(R)-HETE, D-erythro-imidazolylglycerol phosphate; TMP, thiamine monophosphate; uPCR, urine protein/creatinine ratio.

Table 5 ROC analysis of seven metabolites that predicted the clinical efficacy

	AUC	P value
TMP	0.817	0.045
12(R)-HETE	0.767	0.092
Glycodeoxycholic acid	0.617	0.460
D-erythro-imidazolylglycerol phosphate	0.567	0.673
Defluoroatorvastatin	0.517	0.916
Prop-2-enoic acid	0.517	0.916
Asiaticoside	0.500	>0.999

AUC, area under the ROC curve; 12(R)-HETE2, 12(R)-hydroxyeicosatetraenoic acid; ROC, receiver operating characteristics; TMP, thiamine monophosphate.

cocarboxylase, is a cofactor for several key enzymes in energy metabolism, playing a crucial role in glycolysis, the citric acid cycle, the pentose phosphate pathway, among others.[20] Previous studies have highlighted a connection between the development of SLE and energy metabolism.[21 22] Our findings indicate a significant increase in TMP levels in patients with SLE post-MSCT, with an increase associated with lower SLEDAI Scores and uPCR. Moreover, the $\log_2$FC of TMP in the effective group was higher than in the ineffective group, and ROC analysis suggested that an increase in TMP levels of at least 35% post-treatment compared with pretreatment levels indicates a favourable response to therapy. Additionally, past research using faecal metabolomics analysis discovered that thiamine content was lower in patients with SLE compared with healthy controls.[23] These findings may indicate a disturbance in energy metabolism in the state of SLE, which could improve after MSC therapy.

In addition, the synthesis of TPP is catalysed by an enzyme called thiamine diphosphokinase. TPP activates the decarboxylation of pyruvate in the pyruvate dehydrogenase complex. This complex is a group of enzymes and cofactors that form acetyl Coenzyme A (CoA), which condenses with oxaloacetate to form citrate, the first component of the citric acid cycle. It has been reported that acetyl CoA may enhance Treg function through Peroxisome Proliferator-Activated Receptor Gamma (PPARγ).[24] This means that the significant increase in TMP levels in patients with SLE post-MSC treatment might lead to increased acetyl CoA through TPP, thereby enhancing Treg function.

Asiaticoside, a triterpenoid derived from the medicinal plant *Centella asiatica*, exhibits a wide range of biological activities.[25] Our findings indicate a significant increase in siaticoside levels in patients with SLE post-MSCT, with an increase associated with lower SLEDAI Scores. Extensive research indicates that asiaticoside provides neuroprotection,[26] antiulceration,[27] anti-inflammation and antioxidation.[28 29] It significantly impacts diseases such

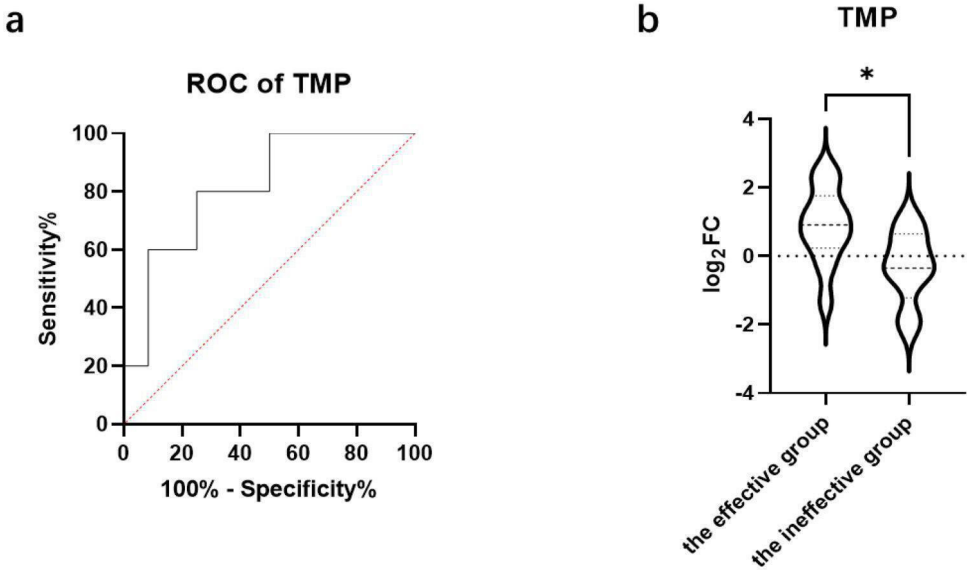

Figure 5 ROC curve and violin plots of TMP 33 (a) ROC curve for evaluating the prognosis of MSCT based on changes in TMP levels, with an AUC of 0.8167. (b) Violin plot comparison of TMP $\log_2$FC between the effective and ineffective treatment groups, showing a higher $\log_2$FC in the effective group. *P<0.05, t-test. AUC, area under the ROC curve; $\log_2$FC, logarithm to base 2 of the fold change; MSCT, mesenchymal stem cell transplantation; ROC, receiver operating characteristics; TMP, thiamine monophosphate.

as osteolytic bone diseases,[30] multiple sclerosis,[31] Alzheimer's disease[28] and pulmonary hypertension.[32] Recent studies have found that asiaticoside notably decreases the protein levels of Toll-Like Receptor 4 (TLR4) and interleukin (IL) 18, which subsequently inhibits the phosphorylation of Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-κB).[33] This inhibition reduces the levels of Tumor Necrosis Factor Alpha (TNF-α) and IL-6, leading to the suppression of Signal Transducer and Activator of Transcription 3 (STAT3) phosphorylation. These findings suggest that asiaticoside obstructs the TLR4/NF-κB/STAT3 pathway.

Diethyl phthalate is thought to be a hazardous substance. However, it would appear to be protective in both SLE and LN. It has been reported in the literature that the analogue of diethyl phthalate has glucocorticoid-like activity.[34] However, we currently do not know what role this metabolite plays on the immune system, and further studies are needed.

We recognise that there are still shortcomings in this study. First, our sample size was relatively small, comprising only 17 patients, possibly limiting the statistical power of our findings. Second, our collection of samples at pre-MSC treatment and 24 hours post-MSC treatment may not adequately reflect the full effect of MSCs on the patients' metabolome. Additionally, although we revealed the relationship between TMP and MSC therapeutic efficacy, these results still require validation in larger cohorts. Lastly, our study did not fully explore the precise correlation of these metabolic changes with SLE pathophysiology. It is also worth noting that in this study, none of the participants smoked or consumed alcohol. Future studies will have to determine whether metabolomics findings are reproducible in patients who smoke or consume alcohol.

In conclusion, our study provides new insights into the mechanism of action of MSCs in patients with SLE by revealing the metabolic changes induced by MSCT. We also identified potential efficacy markers such as TMP. Nevertheless, these findings require further experimental validation to fully understand how MSCs affect the metabolic profile of patients with SLE.

Contributors XF, XT and LS designed the study protocol. XJ, ZJ and BY collected and analysed clinical data of the patients. XJ conducted data analysis and drafted the manuscript. XF and XT revised the manuscript critically for important intellectual content. All authors approved the final version of the article. All authors had access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants. The research protocol was approved by the Ethics Committee of the Affiliated Drum Tower Hospital of Nanjing University Medical School (No. 2008017). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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