Effect of Kechuanting Acupoint Sticking Therapy On Asthma Control And Serum Metabolite Characteristics In Asthma Patients Treated With Long-Term Inhaled Corticosteroid-Formoterol

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Abstract

Background: Despite the availability of inhaled corticosteroid-formoterol treatment, asthma in some patients is poorly controlled. Kechuanting acupoint sticking therapy may regulate immunological functions to improve asthma. In this study, we focused on the effect of Kechuanting acupoint sticking therapy on disease control and the characteristics of serum metabolites in asthma patients treated with long-term inhaled corticosteroid-formoterol.

Methods: We enrolled healthy controls (n=30) and asthma patients treated with inhaled corticosteroid-formoterol for at least 6 months (n=30) and evaluated asthma control, lung function, and airway inflammation after treatment with Kechuanting acupoint sticking therapy (in asthma patients at baseline and week 6). Gas chromatography-mass spectrometry was used to analyze the serum samples of the two groups.

Results: Asthma control test scores, forced expiratory volume in one second, and peak expiratory flow increased ($P<0.01$) at week 6 in the inhaled corticosteroid-formoterol+ Kechuanting acupoint sticking therapy group, while the level of fractional exhaled nitric oxide did not change significantly ($P=0.359$). Among the 46 significant metabolites in the asthma patients and healthy controls (at baseline), 12 were restored after 6 weeks of inhaled corticosteroid-formoterol+ Kechuanting acupoint sticking therapy treatment and 8 (e.g., glycine, sucrose, and glycerol) were correlated with the clinical characteristics.

Conclusions: Kechuanting acupoint sticking therapy improved asthma control in patients treated with long-term inhaled corticosteroid-formoterol, and the serum metabolomic pathway analysis demonstrated the association of Kechuanting acupoint sticking therapy with carbohydrate, glycerolipid, and amino acid metabolism.

Trial registration: https://www.chictr.org.cn, ChiCTR1800016644.

1. Background

Asthma is a complex and heterogeneous disease. Within the past few decades, the prevalence of asthma has increased, with more than 358 million people having asthma globally [1]. The Global Initiative for Asthma (GINA) recommends inhaled corticosteroid-formoterol (ICS-formoterol) for asthma control. It has a strong local anti-inflammatory effect and acts directly on the respiratory tract, which can effectively reduce asthma symptoms and improve lung function [2–3]. Despite the availability of ICS-formoterol treatment, many asthma patients do not attain optimal control of their condition. The control rate in Western Europe is 47% [4] and it is only 28.5% in China [5]. The long-term goals of asthma management, which include achieving and maintaining optimal control, have not been achieved [6].

Acupoint sticking therapy is one of the classic external treatments used in traditional Chinese medicine, which combines herbal medicine and acupoints. Acupoint sticking therapy has been widely used in China since the Qing Dynasty in “Zhang Shi Yi Tong,” proposed for treating asthma [7]. Based on this, as well as our experience of using prescriptions and acupoints at Jiangsu Province Hospital of Chinese Medicine, we developed Kechuanting acupoint sticking therapy (KAST) to treat patients with chronic asthma. Our previous study demonstrated that KAST could improve the systemic immune response by elevating the Th1/Th2 cell ratio and decreasing the levels of immunoglobulin E and interleukin-4, which effectively prevented the recurrence of asthma [8]. However, KAST is a typical compound preparation comprising multiple Chinese herbs with several targets and pathways. Therefore,

it is necessary to explore the mechanism of KAST in the treatment of patients with chronic asthma from several viewpoints.

Metabolomics has emerged as a powerful tool for clarifying biological mechanisms and drug actions, and it is consistent with the holistic thinking of Chinese medicine [9]. Systematic analysis of the metabolic pathways has been previously conducted by investigating the changes in metabolites in the biological system (cells, tissues, or organisms); these metabolites are endogenous small molecules (<1000 Da) such as carbohydrates, amino acids, nucleotides, and lipids that are present after external stimuli or disturbances [10, 11]. Gas chromatography-mass spectrometry (GC-MS) has been used extensively in metabolomics, with high sensitivity and large databases [12]. Based on GC-MS findings, several studies have demonstrated that metabolites undergo significant changes in asthma patients relative to healthy people [13, 14]. However, only a few studies have explored the correlations between clinical characteristics and differential metabolites in asthma patients treated with long-term and low-dose ICS-formoterol.

To the best of our knowledge, the present study is the first clinical trial to comprehensively analyze the effects of KAST on asthma control and the serum metabolites of asthma patients treated with long-term and low-dose ICS-formoterol. We explored the mechanism of KAST in the treatment of patients with chronic persistent asthma based on the clinical characteristics and changes in metabolites to demonstrate the pharmaceutical efficacy.

2. Methods

2.1 Trial Design and Study Population

This study was carried out at the Affiliated Hospital of Nanjing University of Chinese Medicine, Tiexinqiao Community Health Service Center and Qinhuai District Hospital of Chinese Medicine, China, from 2018 to 2020. The study consisted of a 1-week screening period (week -1), 6 weeks of treatment (weeks 1–6), and 1 week of follow-up (contact) (week 7) (Figure 1).

This study was approved by the Ethics Committee of the Affiliated Hospital of the Nanjing University of Chinese Medicine. All participants provided written informed consent (trial registration ChiCTR1800016644). The recruited participants were assigned to two groups: (a) healthy control group (n=30) and (b) ICS-formoterol+KAST group (n=30). Participants in the ICS-formoterol+KAST group (b) were eligible for inclusion if they: (i) were confirmed to have been diagnosed with asthma according to the 2018 revision GINA guideline [15] and had clinical remission; (ii) were aged between 18 and 75 years; and (iii) had at least a 6-month history of receiving low-dose ICS-formoterol (total daily dose of 160/4.5–320/9 μg or equivalent). Age-matched healthy participants without respiratory diseases were enrolled in the control group for metabolomic analysis.

2.2 Intervention

The ICS-formoterol+KAST group received the following treatment: (a) ICS-formoterol: budesonide-formoterol (Symbicort Turbuhaler, AstraZeneca, Cambridge, UK), 160 μg of budesonide, and 4.5 μg of formoterol, with 1–2 inhalations from a pressurized metered-dose inhaler for 6 weeks; (b) KAST: the detailed composition and preparation process for KAST were the same as that in our previous study [8]. Briefly, the KAST prescription comprises Baijiezi (*Sinapis alba L.*), Yanhusuo (*Corydalis yanhuosuo W.T. Wang*), Gansui *(radix Euphorbia kansui T.N. Liou ex T.P. Wang*), Xixin (*Asarum sieboldii Miq.*), Mahuang (*Ephedra sinica Stapf*), Tinglizi (*Descurainia Sophia (L.) Webb. ex Prantl*), Rougui (*Cinnamomum cassia Presl*), Dingxiang (*Eugenia caryophμllata Thunb.*), and

Zaojia (*Gleditsia sinensis Lam.*) in a ratio of 2:2:1:1:1:1:1:1:1. All the herbs were ground into powder provided by the pharmacy department of the Affiliated Hospital of the Nanjing University of Chinese Medicine; 110 g of the powder was mixed with 70 mL of ginger juice and 30 mL of liquid Vaseline to make a patch with a diameter of 2 cm, a thickness of 1 mm, and a weight of approximately 1.5 g. KAST was applied bilaterally at the *Dingchuan* (EX-B1), *Feishu* (BL13), *Geshu* (BL17), *Pishu* (BL20), and *Shenshu* (BL23) points once a week for 8 h each time for 6 weeks.

Participants in the healthy control group did not receive any treatment.

2.3 Outcomes

The primary efficacy outcome was the change in the asthma control test (ACT) score from baseline to week 6. According to the 2020 GINA guidelines, ACT score ranges from 5 to 25 (higher is better), with a score of 20–25, 16–19, 5–15 indicating well-controlled asthma, not well-controlled asthma, and poorly controlled asthma, respectively [2]. The secondary efficacy outcomes were forced expiratory volume in 1 second (FEV_1), peak expiratory flow (PEF), and the level of fractional exhaled nitric oxide (FeNO) from baseline to week 6, which were assessed by professional doctors who provided a pulmonary function report. The safety endpoints included routine blood test results, kidney and liver function, the incidence of adverse events and severe adverse events, as well as vital signs (body mass index [BMI], temperature, blood pressure, and heart rate).

2.4 Collection of Blood Samples

Five milliliters of venous blood were collected from participants at baseline and week 6. The samples were incubated at 25°C or 30 min and centrifuged at 1500 × g at 4°C for 15 min. The serum fraction was separated and stored immediately at −80°C until further use. All samples were collected in the morning after a minimum of 12 h of overnight fasting.

2.5 Metabolomics

2.5.1 Quality Control Samples

The preparation involved mixing equal aliquots of 5 μl from each sample. Three quality control (QC) samples were injected before the samples, whereas one QC injection was performed regularly after injections of 20 samples.

2.5.2 Sample Preparation

After thawing, 50 μl of serum from each sample was mixed with 200 μl of ice-cold methanol containing 12.5 μg of the internal standard. 1,2-13C-myristic acid was added, vortexed for 3 min, and centrifuged at 18,000 rpm for 10 min at 4°C. Thereafter, 100 μl of the supernatant was evaporated in a centrifugal concentrator at 45°C and mixed with 30 μl of methoxyamine hydrochloride in pyridine (10 mg/mL). Next, 30 μl N, O-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane was added, and the mixture was vortexed for 1 min and agitated at 300 r/min for 30 min at 37°C. After derivatization, the sample was centrifuged at 18,000 rpm for 10 min at 4°C, and 50 μl of the supernatant was transferred into a sample vial with a glass insert for GC-MS analysis [14]

2.5.3 GC-MS Conditions

According to the procedure described in the previously published metabolomic profiling methods [16], GC-MS analysis was performed using the Thermo Trace 1310-TSQ 8000 gas chromatography system coupled with a mass spectrometer. One microliter of each derivatized sample was injected and separated with a TG-5MS GC column (0.25 mm*30 m*0.25 μm, Thermo Fisher, San Jose, CA, USA) in split mode at a 20:1 ratio. The GC temperature method was as follows: 60°C for 1 min; 60–320°C for 1–14 min; and 320°C for 14–19 min. The electron energy was 70 eV, and MS data were acquired in full-scan mode with a mass range of 50–500 m/z. Helium was used as the carrier gas and maintained at a constant flow of 1.2 mL/min.

2.6 Statistical Analysis

All values were expressed as means ± standard deviations. The paired-samples t-test and two independent samples t-test were used to compare the parametric data; Wilcoxon's signed-rank tests and Mann–Whitney U tests were used to compare the nonparametric data. Pearson's (parametric data) or Spearman's (non-parametric) correlation was used to describe the specific correlation between the concentrations of the differential metabolites and the clinical characteristics. P-values of <0.05 denoted statistical significance. Statistical analyses were performed using SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) for Windows. The plot was drawn with GraphPad Prism version 8.0 software (GraphPad Software, San Diego, CA, USA).

The GC-MS data of all the samples were converted to Axon Binary File (ABF) format by using ABF Converter, and all these data were imported into an MS-DIAL software program for peak detection, identification, and alignment; a three-dimensional matrix data set was obtained [15]. The data were normalized, and MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) was used for principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis. After pairwise comparison of the two groups, significant metabolites were selected based on a fold change of >1.2 or <0.83, as well as P-values of <0.05. MetaboAnalyst 5.0 was used for data enrichment analysis and pathway analysis.

3. Results

Of the 60 participants enrolled in the trial, 56 completed the study. Among the enrolled participants, four in the ICS-formoterol+KAST group stopped participating in the study (two for not taking the drugs regularly, one for discontinuing KAST, and one for unwillingness). The flow diagram illustrates the allocation of participants in the study (Figure 1).

3.1 Study Population

The mean age of the 26 asthma patients was 51.73 years; 80.77% were women, and the mean BMI was 24.72 kg/m^2. There were no differences in the demographic characteristics between the ICS-formoterol+KAST and healthy control groups at baseline (P>0.05); however, there was a significant difference in the eosinophil percentage (P=0.001), as indicated in Table 1. During the study, there were no liver and kidney dysfunctions or severe adverse events. There were few cases of minor burning sensation and itching in the ICS-formoterol+KAST group, but they did not lead to the discontinuation of the study.

Table 1
Baseline demographics and clinical characteristics

	Healthy control group (N=30)	ICS-formoterol + KAST group(N=26)	χ2/Z/t	P value
Sex				
Male, N (%)	13 (43.33)	5 (19.23)	3.710	0.054
Female, N (%)	17 (56.66)	21 (80.77)		
BMI, mean (SD), kg/m^2	23.09 (2.60)	24.72 (3.71)	-1.388	0.165
Age, mean (SD), years	50.30 (11.03)	51.73 (14.37)	-0.839	0.402
Duration of asthma, mean (SD), years	–	12.66 (12.65)	–	–
ACT score, mean (SD)	–	17.77 (3.31)	–	–
FEV1, mean (SD), %	–	81.19 (16.88)	–	–
PEF, mean (SD), %	–	76.51 (19.22)	–	–
FeNO, mean (SD), ppb	–	33.29 (21.80)	–	–
Peripheral blood leukocyte classification				
Eosinophils percentage, mean (SD), %	2.22(1.19)	3.63 (1.89)	-3.207	0.001
Neutrophils percentage, mean (SD), %	57.72(5.34)	60.29 (8.48)	1.374	0.175
Lymphocytes percentage, mean (SD), %	32.80(5.84)	29.30 (8.40)	-1.812	0.076
Monocytes percentage, mean (SD), %	6.65(1.15)	6.27 (1.49)	-1.088	0.281

Abbreviations: ICS, inhaled corticosteroid; KAST, Kechuanting acupoint sticking therapy; BMI: body mass index; ACT: asthma control test; FEV_1, forced expiratory volume in one second; PEF, peak expiratory flow; FeNO, fractional exhaled nitric oxide; SD, standard deviation; N, number

3.2 Symptom Control, Lung Function, and Airway Inflammation

Compared with the baseline, the ACT score for clinical asthma symptoms and patient self-assessed control increased at week 6 in the ICS-formoterol+KAST group (P<0.01) (Figure 2A).

In terms of lung function, the FEV_1 and PEF also increased at week 6 in the ICS-formoterol+KAST group (P<0.01) (Figure 2B-C).

Regarding airway inflammation, FeNO is usually measured in the clinic, and its level in the ICS-formoterol+KAST group did not change significantly at week 6 (P=0.359) (Figure 2D).

3.3. Serum Metabolomics

3.3.1 Untargeted GC-MS-Based Metabolomic Profile

A total of 657 characteristic peaks were identified for 206 known and 451 unknown metabolites. The total ion current chromatograms of the QC group, healthy control group, ICS-formoterol+KAST group (baseline), and ICS-formoterol+KAST group (week 6) in the serum are shown in Figure 3.

After normalization, the unsupervised segregation was assessed using PCA to confirm the associations between the groups, and a separation between the ICS-formoterol+KAST (baseline) and healthy control groups was observed (Figure 4A). PCA showed that metabolic changes occurred in the serum of asthma patients. To obtain an improved separation and maximize the differences between the groups, a supervised clustering orthogonal partial least squares-discriminant analysis was conducted, and a clear separation was observed between the characteristics at baseline and week 6 in the ICS-formoterol+KAST group (Figure 4B). These results demonstrate that metabolic components in the serum of asthma patients were significantly altered after KAST. Moreover, PCA showed separation between the ICS-formoterol+KAST at week 6 and healthy control groups (Figure 4C).

3.3.2 Differentially Expressed Metabolites in the Serum Samples

Using linear matching of retention indexes and times of the 206 identified metabolites, 131 metabolites were chosen for further screening. Forty-six significant metabolites above the threshold ($P<0.05$ and fold change >1.2 or <0.83) in the asthma patients and healthy controls are presented in Supplementary Table 1 (see Additional File 1).

Twelve significant metabolites, including beta-gentiobiose, glycine, hydroxylamine, dehydroabietic acid, glycerol, guanine, hexadecane, isofucostanol, m-cresol, octadecanol, stigmasterol, and sucrose, were restored in the ICS-formoterol+KAST group ($P<0.05$) (Table 2).

Table 2. Differentially expressed metabolites that were restored after treatment

Abbreviations: ICS, inhaled corticosteroid; KAST, Kechuanting acupoint sticking therapy; HMDB, Human Metabolome Database; KEGG, Kyoto Encyclopedia of Genes and Genomes; FC, fold change; NA, not applicable

3.3.3 Metabolic Pathway Analysis

Pathway enrichment analysis and the pathway impact values from the pathway topology analysis were used to determine the most relevant pathways after identifying the differential metabolites. The pathway analysis indicated 30 potential metabolic pathways involved in the ICS-formoterol+KAST group (baseline) (Figure 5A). For the treatment with ICS-formoterol+KAST, 10 pathways were identified; the most important were the glycine, serine, and threonine metabolism (impact: 0.246); glycerolipid metabolism (impact: 0.237); glyoxylate and dicarboxylate metabolism (impact: 0.106); glutathione metabolism (impact: 0.089); starch and sucrose metabolism (impact: 0.050); and galactose metabolism (impact: 0.039) (Figure 5B).

3.3.4 Correlations between Differential Metabolites and Clinical Characteristics

We further explored the associations of differential metabolites with clinical characteristics in the treatment with ICS-formoterol+KAST (Figure 6). Glycine was positively correlated with BMI ($r=0.431$, $P=0.028$), and sucrose was

No.	ICS-formoterol+KAST group vs. healthy control group (baseline)					ICS-formoterol+ KAST group (week 6 vs. baseline)	
	Metabolites	HMDB	KEGG	FC	P	FC	P
1	Beta-Gentiobiose	NA	NA	0.566	0.00000360540	1.385	0.02679824800
2	Dehydroabietic acid	NA	NA	3.916	0.00000000015	0.452	0.00000000062
3	Glycerol	HMDB0000131	C00116	1.402	0.00019198346	0.721	0.00000048330
4	Glycine	HMDB0000123	C00037	0.492	0.00201092000	8.587	0.00000000528
5	Guanine	HMDB0000132	C00242	2.925	0.00000000015	0.722	0.00000015010
6	Hexadecane	HMDB0033792	C14499	7.498	0.00000000015	0.480	0.00000000173
7	Hydroxylamine	HMDB0003338	C00192	0.537	0.00000013374	1.636	0.00164501530
8	Isofucostanol	NA	NA	1.374	0.00000045675	0.752	0.00000279817
9	m-Cresol	HMDB0002048	C01467	2.365	0.00425499500	0.577	0.00639367940
10	Octadecanol	HMDB0002350	D01924	3.898	0.00000000015	0.569	0.00000000087
11	Stigmasterol	HMDB0000937	C05442	1.279	0.00028258216	0.686	0.00025198100
12	Sucrose	HMDB0000258	C00089	1.764	0.04682440700	0.466	0.00000733283

correlated with the ACT score (r=0.424, P=0.031). Glycine and hexadecane were positively correlated with PEF, which reflected airway obstruction (r>0.45, P<0.05). Finally, we observed the relationships between differential metabolites and inflammatory cells, and isofucostanol was positively correlated with the percentage of eosinophils (r=0.478, P=0.013), while m-cresol showed a negative correlation (r=-0.473, P=0.015). Hydroxylamine was correlated with the percentage of neutrophils (r=0.394, P=0.047), and glycerol and hydroxylamine were inversely associated with the percentage of lymphocytes (r>-0.39, P<0.05).

4. Discussion

Asthma is a common chronic respiratory disease affecting 1–18% of the population in different countries, and is usually triggered by factors such as exercise, allergen or irritant exposure, and change in weather [17]. Many patients have chronic persistent asthma, which has a long course, frequently relapses, and requires long-term treatment. Based on the feedback on clinical treatment and recommendations by GINA, ICS-formoterol may be the preferred controller. Therefore, it is common for patients with chronic persistent asthma to be treated with long-term ICS-formoterol in clinical practice. In this study, we focused on the differences between asthma patients and normal people and explored how to further improve their asthma control.

Acupoint sticking therapy is popular in China because it is considered “through the skin” rather than being invasive. KAST, which is an improved form of acupoint sticking therapy, has been applied clinically for more than 10 years. By treating asthma with KAST, lung and immunological functions may be regulated through the absorption of herbs and stimulation of the meridians [7, 18]. This study confirmed that ICS-formoterol+KAST treatment increased

the ACT score, FEV_1, and PEF, thereby improving symptom control and lung function. For airway inflammation, only 14 patients completed two examinations, and there was no significant change in the level of FeNO. We speculate that these asthma patients had already been treated with ICS-formoterol for at least half a year. Their airway inflammation was mild (the level of FeNO was 33.29 ppb at baseline), and therefore the benefits of short-term acupoint sticking therapy treatment were limited.

Most metabolomic studies have focused on distinguishing asthma patients from healthy people for diagnostic purposes [19]. This study investigated the advantages of integrating traditional Chinese and Western medicine in the treatment of asthma using metabolomics. We monitored the characteristics of serum metabolites in asthma patients who had used ICS-formoterol for a long time to explore the changes after KAST. Based on the metabolomic profiles, asthma patients could be discriminated from the healthy control group at baseline and after 6 weeks of KAST. The results show that 12 of 46 serum differential metabolites were restored after ICS-formoterol+KAST treatment in asthma patients. The asthma patients and the healthy controls had different metabolic profiles even after regular treatment with ICS-formoterol+KAST, which is consistent with the findings of Ferraro et al. [20]. However, the degree of disparity between the two groups decreased.

Pathway analysis showed that mechanisms of KAST might involve carbohydrate, glycerolipid, and amino acid metabolism. Carbohydrates are among the main energy sources for humans, and glycolysis and gluconeogenesis are indispensable in carbohydrate metabolism [21]. In our study, the three major carbohydrate metabolic pathways were starch and sucrose metabolism, galactose metabolism, and glyoxylate and dicarboxylate metabolism. Increased sucrose, glycerol, citric acid, and glutamate as well as reduced glycine were associated with alternations in glycolysis and gluconeogenesis. Previous studies reported that starch and sucrose metabolism, galactose metabolism, an increase in citric acid in ovalbumin, and ice water-induced cold asthma were induced in Sprague-Dawley rats [22], and they speculated that the changes in energy metabolism might have been related to the increase in respiratory burden and energy use to recruit inflammatory cells, which is consistent with our results. With the treatment of ICS-formoterol+KAST, the serum levels of sucrose, glycerol, and glycine were restored; however, there were no significant changes in other metabolites.

Glycerol is a common cellular metabolite mainly derived from lipolysis in adipose tissues. It has a relatively stable level and is mainly disposed of through oxidation and gluconeogenesis [23, 24]. Our study found that glycerol was also involved in glycerolipid metabolism. As the skeletal component of the triglyceride molecule, glycerol may participate in the formation of mast cell lipid droplets after hydrolysis. Lipid droplets can release a large amount of arachidonic acid and cause airway inflammation [25, 26]. In a clinical study on the effects of electronic cigarette vaping on lung inflammation and pulmonary function, glycerol aerosol at high wattage induced airway epithelial injury and sustained the decrease in transcutaneous oxygen tension in young tobacco smokers [27]. In this study, glycerol increased in asthma patients; however, its serum level was restored with the ICS-formoterol+KAST treatment, which may be the therapeutic mechanism of KAST.

Our study found further associations between differential metabolites and selected clinical characteristics. As the results indicated, some metabolic signatures were associated with airway obstruction, which was reflected in the PEF. Glycine contributes to the relaxation of airway smooth muscle and alleviates bronchoconstriction, mainly through its receptor chloride channels [28]. Its cotransport with sodium and chlorine through the glycine transporter might be the mechanism of changes in the electrophysiological properties of airway smooth muscle [29]. In our study, the measured level of glycine was significantly lower in asthma patients than in healthy people, an

observation consistent with the findings of a previous study [22]. With the treatment of ICS-formoterol+KAST, the serum level of glycine was upregulated.

Furthermore, pathway analysis showed that glycine participates in glycine, serine, and threonine metabolism, and glutathione metabolism may be associated with airway inflammation. Glycine inhibits the lipopolysaccharide-induced increase in Ca^{2+} and the subsequent production of superoxide in alveolar macrophages through a glycine-gated chloride channel to reduce airway inflammation [22]. Previous studies have also indicated that glycine can significantly reduce the release of reactive oxygen species by neutrophils [30, 31]. Interestingly, glycine also inhibits oxidative stress by regulating intracellular glutathione concentrations [31, 32]. Therefore, we speculate that the decrease in the level of glycine in asthma patients may have a bearing on peroxidation, and ICS-formoterol+KAST improves the situation; however, this has not been validated.

Asthma has been characterized by airway inflammation. In this study, no significant association between the FeNO level and asthma has been established; however, we observed correlations between differential metabolites and inflammatory cells. m-Cresol can be absorbed through the respiratory tract and excreted as glucuronide and sulfate metabolites. In a 28-day study involving rats and mice, excessive m-cresol caused irritation in the gastrointestinal tract and nasal epithelia [33]. Weber et al. found that m-cresol could activate P38 and JNK signaling pathways and promote the chemokine MCP-1 to induce a pro-inflammatory response [34]. Nitrogen is of central importance to pulmonary physiology, and hydroxylamine is one of its reduced forms. Hydroxylamine has vasodilating properties similar to those of endothelium-derived relaxing factors and is likely involved in the downstream signaling of nitric oxide synthase activity. It is also an intermediate in the oxidative conversion of L-arginine to nitric oxide [35]. In the present study, the levels of m-cresol and hydroxylamine were restored with ICS-formoterol+KAST treatment. Moreover, m-cresol was associated with the percentage of eosinophils, and hydroxylamine was associated with neutrophils and lymphocytes.

Several limitations of our study need to be addressed. On one hand, the results may have been biased because of the limited sample size and the short period of observation. On the other hand, some of the detected metabolites are unknown, which to an extent limits the exploration of the potential metabolic characteristics. Moreover, we simply analyzed the associations between differential metabolites and clinical characteristics, and a causal relationship could not be established. Therefore, further studies and validation are needed.

5. Conclusions

In summary, our present study confirmed that ICS-formoterol+KAST treatment had effects on asthma patients, improved the clinical symptoms, and ameliorated lung dysfunction. Moreover, significant abnormalities of 46 serum metabolites were observed in asthma patients treated with long-term ICS-formoterol. After a 6-week course of ICS-formoterol+KAST treatment, 12 significant metabolites were restored, and eight of them were correlated with clinical characteristics. Pathway analysis demonstrated that the therapeutic mechanisms involved carbohydrate, glycerolipid, and amino acid metabolism. These results provide new directions for our future research, and we will replicate and verify them. Therefore, KAST can be a convenient and effective complement for treating asthma. The study findings also highlight the value of integrating traditional Chinese and Western medicine therapies.

6. Abbreviations

ABF, axon binary File; ACT, asthma control test; BMI, body mass index; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; GC-MS, gas chromatography-mass spectrometry; GINA, Global Initiative for Asthma; ICS-formoterol, inhaled corticosteroid-formoterol; KAST, Kechuanting acupoint sticking therapy; PCA, principal component analysis; PEF, peak expiratory flow; QC, quality control.

7. Declarations

7.1 Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Affiliated Hospital of the Nanjing University of Chinese Medicine (approval number 2017NL-11-02). All participants provided written informed consent (trial registration ChiCTR1800016644).

7.2 Consent for Publication

Not applicable.

7.3 Availability of Data and Materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

7.4 Competing Interests

The authors declare that they have no competing interests.

7.5 Funding

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7.6 Authors' contributions

S.Z. and C.Z recruited the patients, collected the samples, and drafted the manuscript. K.D., J.H., H.L., and K.G. collected the samples and contributed to data interpretation. P.Z., and Q.H. performed the analytical measurements. X.G. performed the statistical data analysis. J.S., H.W., and L.L. contributed to data interpretation and critically revised the manuscript for important intellectual content. All authors have read and agreed to the published version of the manuscript. S.Z. and C.Z contributed equally to this work. All studies were supervised by L.L.

7.7 Acknowledgements

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Figures

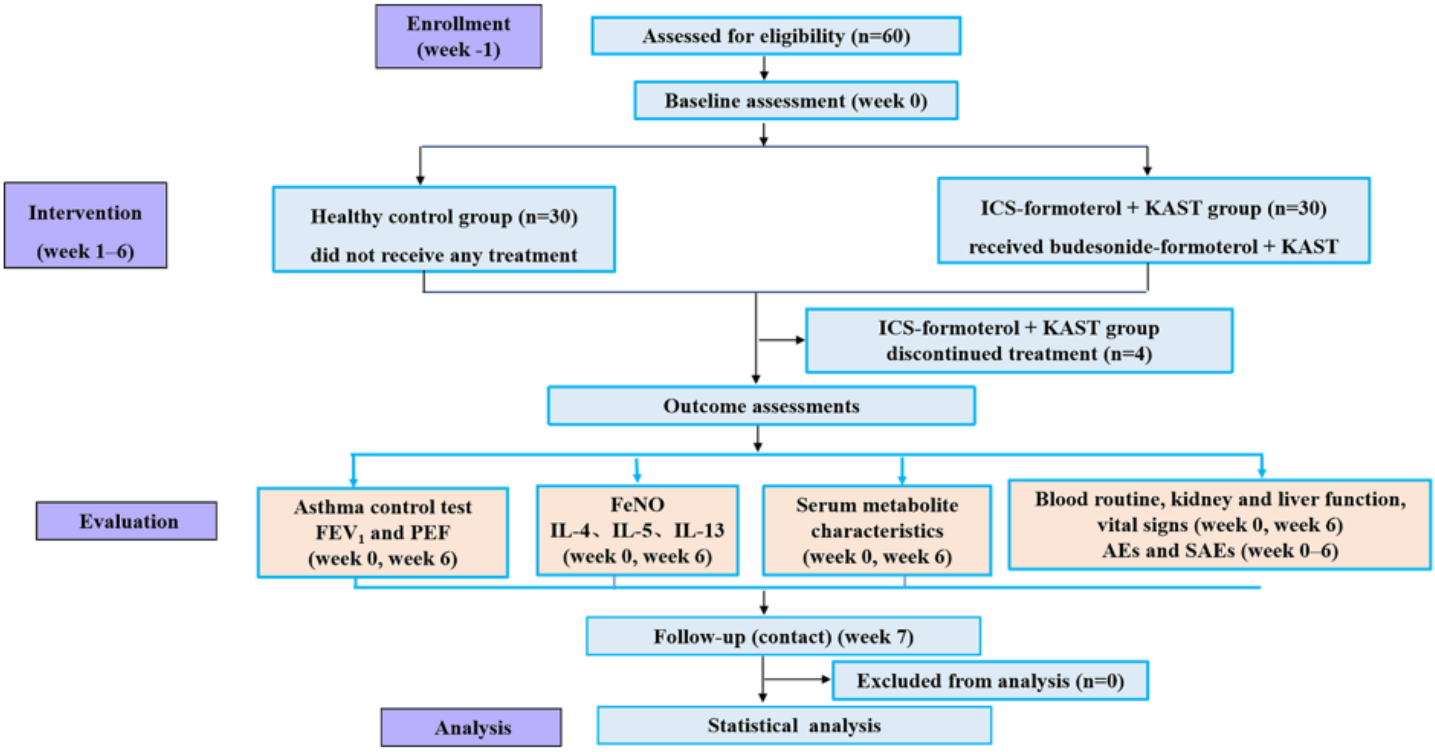

Figure 1

Flow diagram. Abbreviations: ICS, inhaled corticosteroid; KAST, Kechuanting acupoint sticking therapy; FEV1, forced expiratory volume in one second; PEF, peak expiratory flow; FeNO, fractional exhaled nitric oxide; IL, interleukin; AEs, adverse events; SAEs, serious adverse events

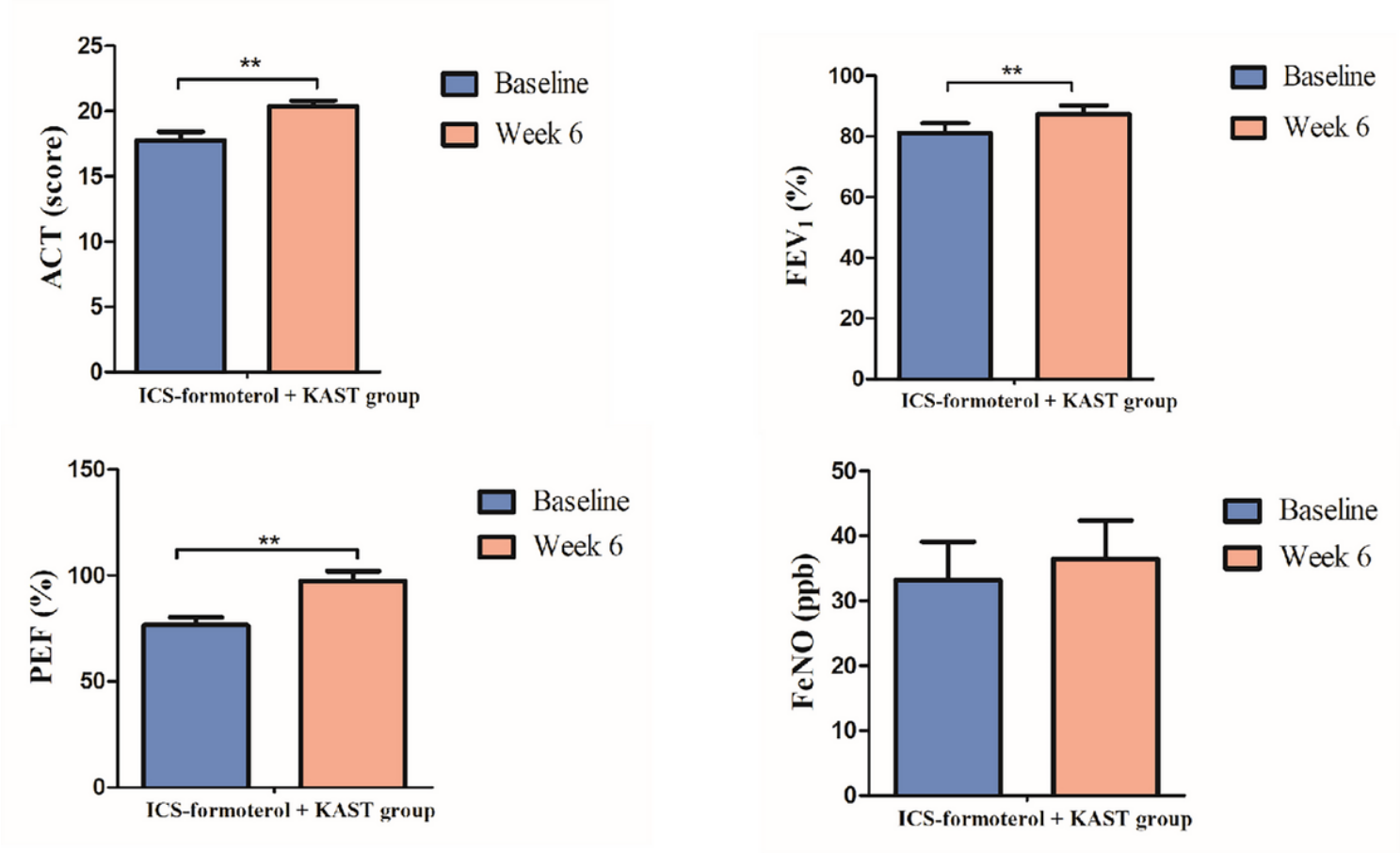

Figure 2

Effect of ICS-formoterol + KAST on ACT, FEV1, PEF, and FeNO of participants. (A) Mean change in ACT from baseline to week 6; (B) Mean change in FEV1 from baseline to week 6; (C) Mean change in PEF from baseline to week 6; (D) Mean change in FeNO from baseline to week 6. Compared with baseline: **P<0.01. Abbreviations: ICS, inhaled corticosteroid; KAST,

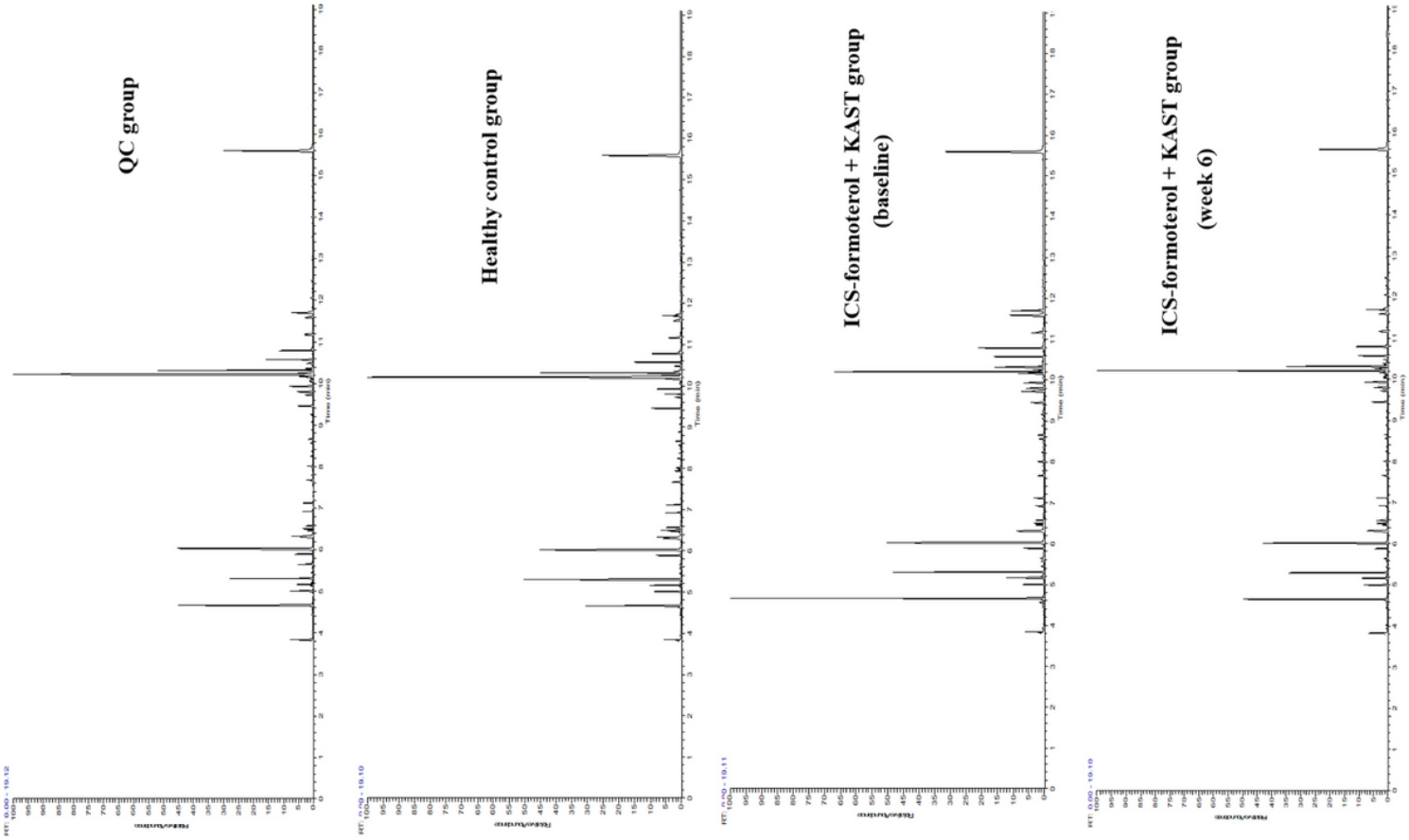

Figure 3

Representative GC-MS total ion current chromatograms. Abbreviations: GC-MS: gas chromatography-mass spectrometry; ICS, inhaled corticosteroid; KAST, Kechuanting acupoint sticking therapy; QC, quality control

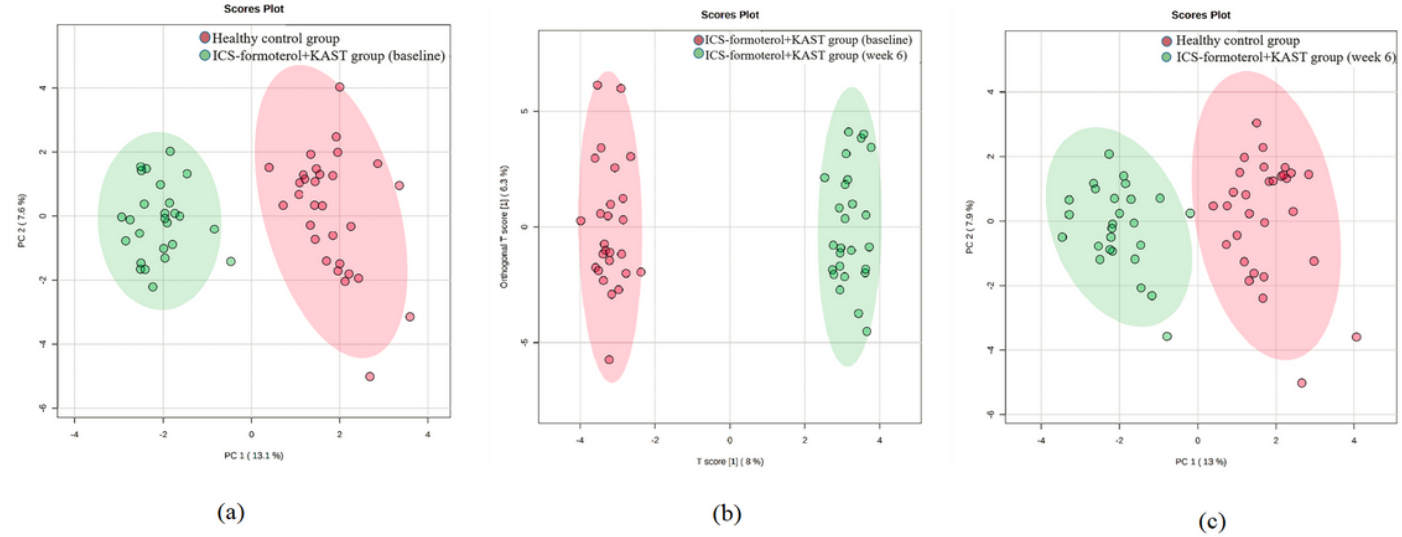

Figure 4

Untargeted GC-MS-based metabolomic profile. (A) PCA scores plot of the ICS-formoterol+KAST group (baseline) and healthy control group; (B) OPLS-DA scores plot of the ICS-formoterol+KAST group between baseline and week 6. R2Y = 0.989 and Q2 = 0.905, p<0.001; (C) PCA scores plot of the ICS-formoterol+KAST group (week 6) and

healthy control group. Abbreviations: GC-MS: gas chromatography-mass spectrometry; ICS, inhaled corticosteroid; KAST, Kechuanting acupoint sticking therapy; PCA: principal component analysis; OPLS-DA, orthogonal partial least squares-discriminate analysis

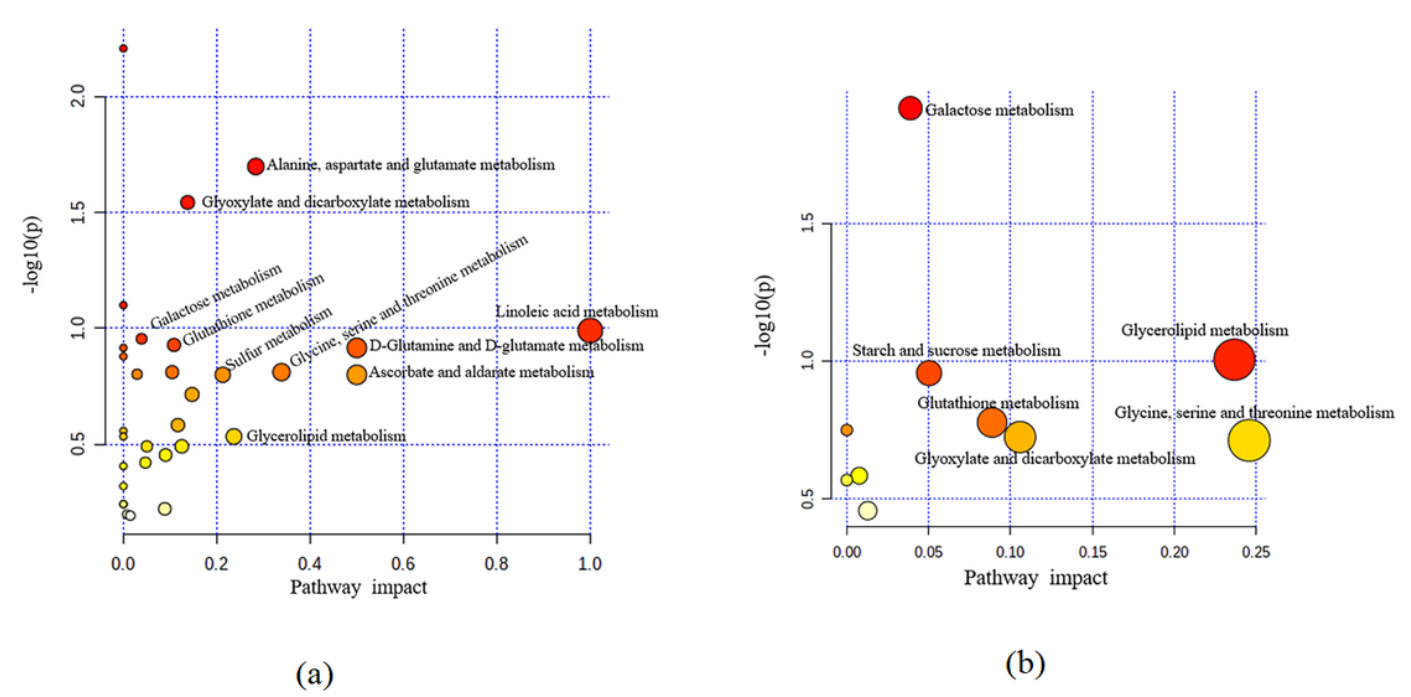

Figure 5

MetaboAnalyst pathway impact based on differentially expressed metabolites. (A) Metabolic pathways between the ICS-formoterol+KAST group (baseline) and healthy control group; (B) Metabolic pathways for the treatment of asthma with ICS-formoterol+KAST. Abbreviations: ICS, inhaled corticosteroid; KAST, Kechuanting acupoint sticking therapy

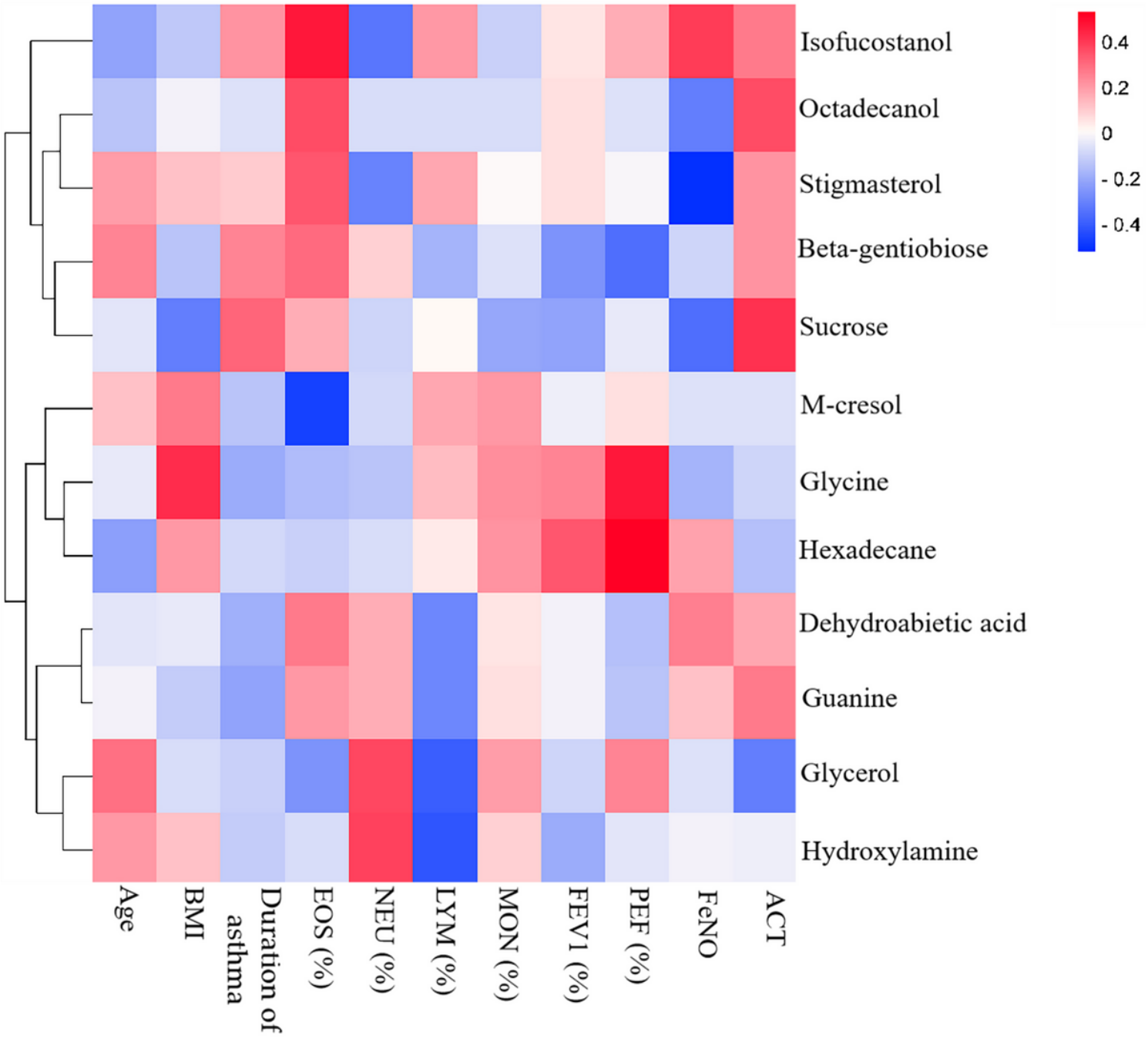

Figure 6

Heatmap of correlations between differential metabolites and clinical characteristics after treatment with ICS-formoterol+KAST. Blue squares indicate significant negative correlations, white squares indicate non-significant correlations, and red squares indicate significant positive correlations. Abbreviations: BMI: body mass index; ACT: asthma control test; FEV1, forced expiratory volume in one second; PEF, peak expiratory flow; FeNO, fractional exhaled nitric oxide; EOS: eosinophils; NEU: neutrophils; LYM: lymphocytes; MON: monocytes

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx
- DatasetS1.Originaldatasetclinicalcharacteristics.xlsx
- DatasetS2.Originaldatasetofmetabolites.xlsx
- DatasetS3.Originaldataset.xlsx