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Plasma metabolomic characteristics of atrial fibrillation patients with spontaneous echo contrast

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Abstract

Background The spontaneous echo contrast (SEC) in patients with atrial fibrillation (AF) indicates a prethrombic state that ultimately progresses into thrombus formation. A comprehensive understanding of specific plasma metabolomics characteristics may protect AF patients from thrombus, particularly in the early stage.

Objectives Through the investigation of metabolic pathways, we endeavor to uncover the metabolomic characteristics associated with SEC states, and to examine the differential metabolites by which may exert their influence on thrombotic states.

Methods Patients with AF were enrolled, and the participants were divided into three groups based on the results of the echocardiogram: non-SEC, low-SEC and high-SEC group. Samples were collected and subjected to non-targeted metabolomics analysis. The analytical process included data quality control, metabolite difference analysis, component analysis, Kegg cluster analysis, etc.

Results Our metabolic phenotype revealed a clear differential metabolic pattern between the SEC and non-SEC. Specifically, we identified 35 and 142 significantly differential metabolites in venous and atrial plasma, respectively, suggesting that SEC may be involved in pervasive metabolic dysregulation and that the degree of metabolic dysregulation in atrial plasma is more severe than that in venous blood.

Conclusion Patients with SEC have a significantly different metabolic pattern compared to those without SEC. Our work promoted the understanding of mechanism of the occurrence and development of SEC, facilitated the screening of the target metabolites for its therapeutic intervention, and provided evidence for the prevention and treatment of SEC or thrombosis in AF. Our work also provided new directions for subsequent research in related fields. In conclusion, our study not only provides a theoretical basis for understanding the occurrence and development of

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SEC in AF, but also provides recommendations for the daily diet of AF patients with SEC, such as a balanced intake of essential amino acids, avoiding excessive intake of benzoic acid, and intake of appropriate inositol.

Clinical trial number Not applicable.

Keywords Atrial fibrillation, Spontaneous echo contrast, Differential metabolites, Plasma metabolomics

Introduction

Atrial fibrillation (AF), one of the most prevalent cardiac arrhythmias, affects approximately 2–4% of adults and steadily increases with age. AF is an independent risk factor for stroke, leading to significant economic and healthcare burdens in terms of thrombus-related deaths [1]. It has been recognized that spontaneous echo contrast (SEC) within the heart chambers indicates stagnant blood flow and is a precursor to thrombosis [2, 3]. However, the pathogenesis of SEC is complex, with multiple interrelated contributing factors. Therefore, in-depth exploration of the mechanism of SEC formation and attenuation of these causative factors may reduce SEC and thus prevent thrombosis to some extent. In light of these considerations, the prevention and treatment of AF centers around reducing thrombotic events [4]. Consequently, this study utilized non-targeted metabolomic profiling to examine metabolic pattern and biomarkers in the plasma of SEC in AF patients, providing insights for evaluating the risk of SEC and thrombus formation.

Materials and methods

Study populations

The study population consisted of patients with non-valvular AF who underwent radiofrequency ablation therapy at the Atrial Fibrillation Center of the Second Hospital of Tianjin Medical University between March and September 2023. The inclusion criteria involved all patients during the study period who sought medical care at the Atrial Fibrillation Center and were diagnosed with non-valvular AF by experienced experts in cardiac arrhythmias. Exclusion criteria were: valvular AF, poorly controlled diabetes, chronic obstructive pulmonary disease, severe liver dysfunction (alanine aminotransferase level > 135 U/l), heart failure, severe renal dysfunction, thyroid disease and malignant tumors. In addition, we also excluded patients who were unwilling to participate in the study, those who were unable to complete necessary examinations (such as transesophageal echocardiography), and those with incomplete clinical data. This study has been approved by the Ethics Committee of the Second Hospital of Tianjin Medical University (No: KY2023K058) and adheres to the principles of the Helsinki Declaration. All participants are included voluntarily and have signed informed consent forms.

Trans Esophageal echocardiography examination

Experienced experts in cardiac arrhythmias assessed and confirmed the suitability of trans esophageal echocardiography examination (TEE) for each patient. For patients in good condition, after fasting for at least 12 h, the examination was conducted by experienced sonographers in the Ultrasound Department using GE HealthCare Vivid E95 echocardiography equipment (**GE** Healthcare; Vingmed Ultrasound, Horten, Norway). GE HealthCare EchoPAC was used to analyze TEE test results. SEC refers to a smoky echo of swirling blood flow in the left atrial appendage and/or left atrial. Its presence is associated with slow blood flow velocity, abnormal blood cell aggregation, locally hypercoagulable state and can be considered a pre-thromboembolic condition [5]. The severity assessment methodology of SEC was proposed by Fatkin et al. [5], According to the evaluation criteria, three experienced sonographers categorize the observed SEC into three classes: none-SEC group, low-SEC group and high-SEC group [6]. Three experienced cardiac sonographers each independently evaluated the same TEE images, and patients were included in the study only if the reports from the three experts were the same.

Sample collection

For AF patients who require ablation surgery, we asked them to temporarily stop taking anticoagulants (NOACs or low molecular weight heparin) once on the day of surgery [7, 8]. In the atrial fibrillation ablation procedure at our Atrial Fibrillation Research Center, heparin is not used after femoral venous puncture, but only after successful transseptal puncture. For this study, in order to avoid the influence of heparin on the selection of SEC-related differential metabolites, we did not administer a standard dose of heparin until appropriate amounts of blood were collected immediately after transseptal puncture. During transseptal puncture, we used a conventional septal puncture needle, not a radiofrequency needle. After successful femoral vein puncture, an appropriate amount of peripheral venous blood was collected, and left atrial blood was collected immediately after successful transseptal puncture.

All blood samples obtained were placed in an EDTA-anticoagulated vacuum blood collection tube and temporarily stored in a 4℃ freezer for no more than 4 h. The blood samples were then centrifuged at 3000 rpm for

15 min, and the super-clarified plasma was aliquoted in cryovials and stored at -80℃.

Metabolomics testing

Plasma non-targeted metabolomic analysis was conducted by Novogene Co., Ltd. We prepared samples in strict accordance with the testing requirements before testing. Firstly, 400 μL of 80% methanol-water solution (V/V) was added to 100 μL of plasma, followed by vortex mixing. The mixture was then placed in an ice bath for 5 min and finally centrifuged at 15,000 g for 20 min at 4℃. An appropriate amount of supernatant was added to mass spectrometry grade water and diluted to a methanol content of 53% (V/V), then centrifuged at 15,000 g for 20 min at 4℃ and an appropriate amount of supernatant was taken for LC-MS analysis. Mass spectrometer: Q Exactive™ HF/Q Exactive™ HF-X(Thermo Fisher), Chromatograph: Vanquish UHPLC (Thermo Fisher), Columns: Hypesil Gold column (100×2.1 mm, 1.9 μm) (Thermo Fisher).

Statistical analysis

The KEGG database (https://www.genome.jp/kegg/pathway.html), HMDB database (httpshmdb.ca/metabolites), and LIPMaps database (http://wwwipidmaps.org/) were utilized for annotating the identified metabolites. For the multivariate statistical analysis, the metabolomics data processing software metaX was used to convert the data, and then the principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed to obtain the Variable important in projection (VIP) value of each metabolite. In the univariate analysis, the *student's t test* was used to calculate the statistical significance (*P* value) of each metabolite between the two groups, and the fold change (FC value) of the metabolite between the two groups was calculated to quantify the differences in metabolite levels between the two groups.

The default criteria for initial screening for differential metabolites are VIP-value > 1, *P*-value < 0.05 and FC ≥ 1.2 or FC ≤ 0.8. The differential metabolites were assigned different weight points according to different rules for further screening, and the metabolites that met the two or more rules were preferentially selected. Rule 1: Metabolites presented in 2 or 3 comparison groups were assigned a score of 0.5 and 1, respectively. Rule 2: The metabolites of VIP > 2 was given weight points of 1 point; Rule 3: Metabolites present in the KEGG-enrichment pathway were assigned a weight score of 1 point.

The volcano map was plotted using the R package ggplot2, which can be combined with the VIP value of metabolites, log2 (Fold Change) and -log10 (*P*-value) to screen metabolites. Clustering heatmap, plotted with the R package Pheatmap, normalized metabolite data using *z*-score. Correlation analysis between differential metabolites (Pearson correlation) was performed using R package cor(), statistical significance was calculated with R package cor.mtest(), *P*-value < 0.05 was considered statistically different, and correlation plots were plotted with R package corrplot. The bubble plot was plotted with the R package ggplot2. The KEGG database was used to study the function and metabolic pathways of metabolites, and when x/n > y/n, the metabolic pathways were considered to be enriched; When the P value of the metabolic pathway was less than 0.05, it was considered to be significantly enriched. GraphPad Prism 8 was used for histogram plotting and statistical analysis, the baseline data was analyzed by R package compare Groups, the one-way ANOVA was used for comparison between multiple groups, and the *student's t test* was used for comparison between two groups, and *P* < 0.05 was statistically significant.

Results

Study population characteristics

A total of 15 patients were included in this study (Fig. 1). Based the different TEE presentations, they were divided into three groups: non-SEC group (5 patients), low-SEC group (5 patients) and high-SEC group (5 patients). The clinical baseline data of these groups are shown in Table 1. We found that as the severity of SEC increased, the amount of LDLc gradually decreased and the LAD gradually increased. There were no significant differences in the remaining items.

Metabolomics data quality assessment

Constructing PCA models for quality control (QC) samples and plasma samples in positive and negative modes in LC-MS. In the study, the QC samples exhibited good clustering, and all plasma samples fell within the 95% confidence interval in the PCA model, indicating minimal systematic errors during sample handling and detection processes (Fig. 2A and B). The metabolic profile of the plasma sample aligns with a typical chromatogram (Supplementary Fig. 1A-D). A total of 913 distinct metabolites were identified under the positive mode in LC/MS analysis(Supplementary Fig. 2A), while the negative mode, 470 different metabolites were detected(Supplementary Fig. 2B).The metabolites encompass various classes, including lipids and like-lipid molecules, organic acids and their derivatives, aromatic compounds, nucleosides, nucleotides and analogs, heterocyclic organic compounds, organic oxides, phenylpropanoids and polyketides, organic halogen compounds, organic nitrogen compounds, alkaloids and their derivatives, organic sulfur compounds, and more(Supplementary Fig. 2).

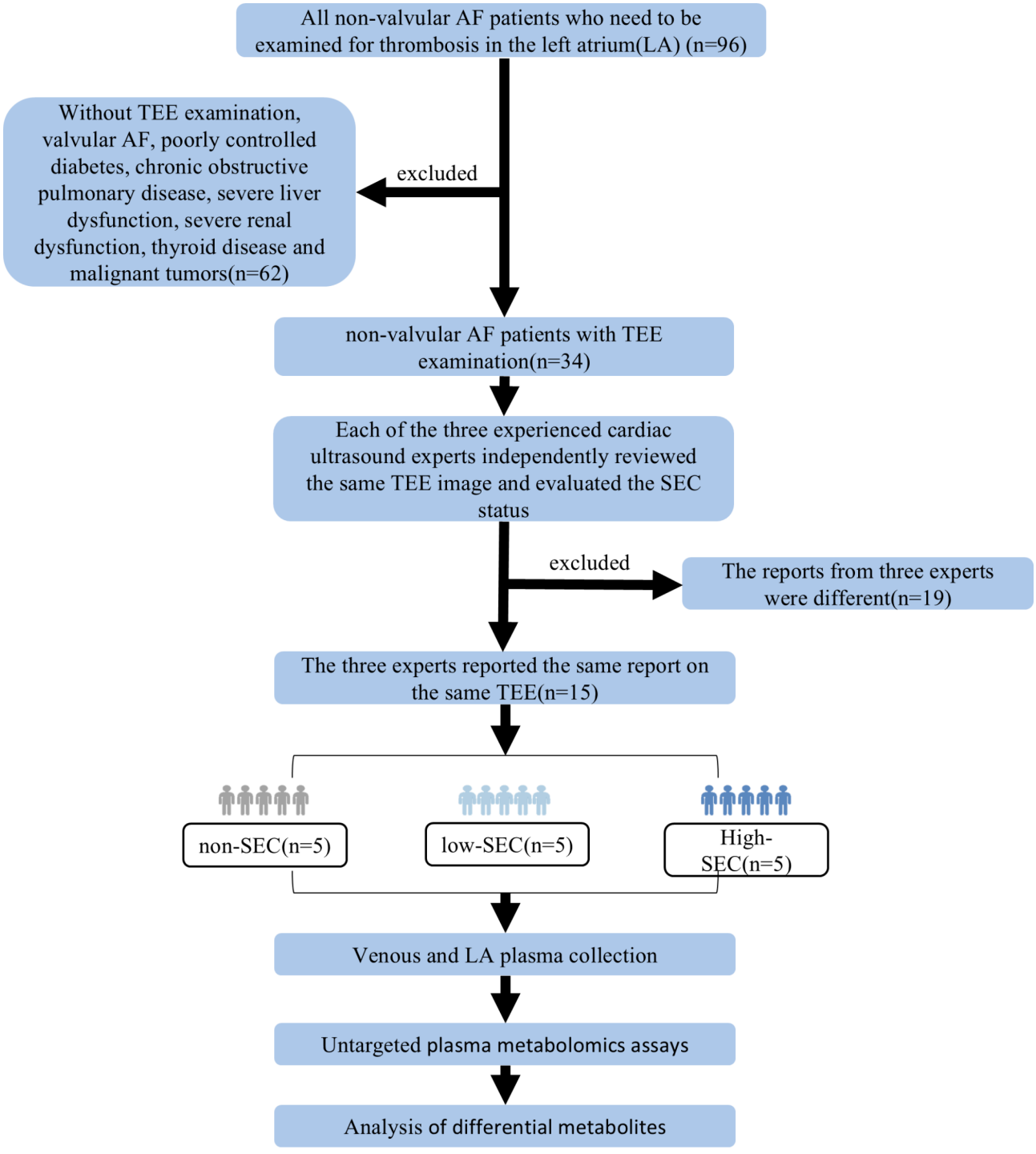

Fig. 1 Research flowcharts. TEE, Trans Esophageal echocardiography; SEC, spontaneous echo contrast

Pathway investigation of differential metabolites

First, the heat map showed that the venous and atrial plasma metabolism patterns of patients in the low-SEC group and high-SEC group were changed compared with the non-SEC group, and with the aggravation of the degree of SEC, the metabolomics changes were more and more, and the changes in atrial plasma were more obvious than those in venous(Fig. 3).This suggested that changes in metabolomics may play an important role in the occurrence and development of SEC, so we further explored the pathway analysis of differential metabolites. KEGG analysis revealed that the metabolic pathway was

Table 1 Baseline characteristics of participants

Variable	All (*N* = 15)	Non-SEC (*N* = 5)	low-SEC (*N* = 5)	high-SEC (*N* = 5)	*P* (low-SEC VS. Non-SEC)	*P* (high-SEC VS. Non-SEC)
Female (n, %)	8 (53.3%)	3 (60.0%)	3 (60.0%)	2 (40.0%)	1.000	0.549
Age(years)	69.0 [63.0;77.0]	66.0 [60.0;69.0]	74.0 [64.0;78.0]	70.0 [60.0;79.0]	0.12	0.349
Smoke (n, %)	4 (26.7%)	1 (20.0%)	1 (20.0%)	2 (40.0%)	1.000	0.513
Drink (n, %)	3 (20.0%)	0 (0.00%)	1 (20.0%)	2 (40.0%)	0.317	0.134
SBP (mmHg)	125[112.0;134.0]	127 [119.0;145.5]	115 [107.5;149.5]	121 [108.0;125.5]	0.686	0.118
DBP (mmHg)	78.0 [72.5;88.0]	93.0 [77.0;96.5]	75.0 [60.0;86.5]	77.0 [72.5;78.5]	0.118	0.066
HR (bpm)	79.0 [65.0;98.0]	70.0 [65.5;102.0]	67.0 [61.5;85.0]	85.0 [72.0;120]	0.408	0.393
Hypertension (n, %)	8 (53.3%)	2 (40.0%)	3 (60.0%)	3 (60.0%)	0.549	0.549
Diabetes (n, %)	2 (13.3%)	1 (20.0%)	1 (20.0%)	0 (0.00%)	1.000	0.317
Coronary heart disease (n, %)	7 (46.7%)	1 (20.0%)	3 (60.0%)	3 (60.0%)	0.221	0.221
HDLc (mmol/L)	1.22 [1.05;1.34]	1.26 [1.12;1.39]	1.26[1.17;1.35]	1.03 [0.84;1.22]	1.000	0.064
LDLc(mmol/L)	2.78 [2.04;3.34]	3.32 [2.89;3.84]	2.53 [2.00;3.06]	2.30 [1.76;2.97]	0.039	0.039
Prothrombin time (s)	13.7 [13.0;22.8]	12.9 [12.5;13.4]	13.9 [13.6;23.6]	14.0 [13.5;23.4]	0.09	0.09
Prothrombin percentage activity (%)	93.0[37;100]	100 [96.5;101.5]	88.0 [35.5;94.5]	90.0 [36.0;99.0]	0.069	0.109
INR	1.04 [1.00;2.03]	1.00 [1.00;1.02]	1.08 [1.03;2.08]	1.06 [1.01;2.07]	0.112	0.126
Partial thromboplastin time (s)	42.4 [33.5;44.2]	39.7 [30.5;43.7]	43.0 [35.3;64.2]	42.4 [34.6;43.5]	0.112	0.126
Fibrinogen(g/L)	3.06 [2.95;3.53]	3.01 [2.72;3.17]	4.16 [3.27;5.64]	2.95 [2.72;3.34]	0.059	0.792
Thrombin time (s)	18.1 [17.7;18.6]	18.1 [17.85;19.1]	18.6 [18.05;19.9]	18.0 [16.7;18.25]	0.473	0.188
BNP (pg/ml)	1029.8 [276.6;3795.35]	290.6 [71.4;2086.25]	375.9 [233.15;6456.1]	3795.35 [935;6655.7]	0.467	0.104
Thyrotropin (mIU/ml)	2.04 [0.98;2.8]	1.12 [0.49;3.345]	2.04 [0.775;3.265]	2.76 [2.1;3.425]	0.812	0.309
CHA_2DS_2_VASc	4 [2.0;4.0]	2 [1.0;3.5]	4 [2.5;4.0]	4 [3.5;4.5]	0.189	0.055
IVS (mm)	8.8[8;9.8]	8[6.95;9.25]	8.8[8.2;10.35]	9.8[8.7;15.3]	0.258	0.161
LVDd (mm)	49.1[43.3;50.3]	46.3[44.2;48.65]	42[40.8;49.55]	53.5[49.8;65.5]	0.457	0.075
LVDs(mm)	26.8[25.1;28.7]	26.5[25;29.35]	26[22.95;28.5]	27.5[26.4;55.1]	0.477	0.218
RA (mm)	49[45.8;56]	45.9[43.6;52.9]	46.9[46.1;62.55]	50.5[47.1;56.15]	0.343	0.281
LAD (mm)	42.1[39.2;48.2]	39.8[34.6;41.8]	40.1[38.7;49.65]	48.2[47.25;52.8]	0.229	0.001
RVAW (mm)	3.4[2.9;3.7]	2.9[2.5;3.45]	3.4[3.15;3.95]	3.6[2.95;3.95]	0.086	0.149
RVDd (mm)	21[20;23.6]	21.9[20.6;23.2]	20[19.45;26.25]	20.6[19.85;24.85]	0.868	0.944
AoD (mm)	22.1[21.8;25]	22.1[22.05;23.25]	22[20.85;23.75]	26.1[19.7;26.9]	0.753	0.487
PAD (mm)	21.2[19.5;24.2]	20.6[19.1;21.85]	21.6[19.4;34.55]	21.2[18.95;34]	0.301	0.281
LVEF (%)	59[55;68]	62[57.5;68.5]	59[57.5;71.5]	52[38;62.5]	0.899	0.12
IVC (mm)	15.3[14.1;18.3]	15.3[9.85;20.15]	17.3[15.65;19.2]	14.6[13.75;15.4]	0.373	0.845
Na^+(mmol/l)	141.5[138.6;142.5]	142.3[140.05;145]	141.5[139.5;142.45]	139.8[138.1;142.6]	0.429	0.273
K^+(mmol/l)	4[3.95;4.2]	4[3.725;4.15]	4.1[3.95;4.55]	4[3.9;4.2]	0.213	0.553
Cl^-(mmol/l)	107.4[104.7;109.5]	107.4[105.4;109.4]	109.1[104.3;114.4]	105.1[104.7;109.9]	0.544	0.745
CO_2CP (mmol/l)	24.1[23.3;25.6]	23.3[21.75;24.85]	25.1[16.4;26.1]	24.3[24.05;25.55]	0.705	0.143
glucose(mmol/l)	5.75[4.63;7.06]	4.9[4.235;8.035]	5.3[4.455;5.82]	6.71[5.685;8.855]	0.475	0.353
TP(g/l)	66.2[62.6;68.4]	68.9[61.35;69.9]	66.4[61.15;68.05]	64.3[62.35;66.15]	0.697	0.496
Albumin(g/l)	39.8[38.2;42]	41.7[38.3;44.15]	39.5[37.5;43.3]	39.2[37.75;40.65]	0.623	0.253
Globulin(g/l)	24.9[22.3;27.2]	26.9[21.05;27.9]	24.4[21.25;28.4]	25.1[23.5;26.6]	0.937	0.959
ALT(U/l)	16[10.4;22.7]	18.1[11.15;29.1]	16[8.7;27.9]	15.8[12.05;19.5]	0.788	0.488
IBIL (ummol/l)	12.8[11.7;14]	13.1[10.65;13.55]	12.3[7.65;13.4]	14.1[9.65;22.425]	0.521	0.401
CB (ummol/l)	2.3[2;3]	2.3[1.85;2.75]	2.3[1.95;6.35]	2.55[2.1;3.0]	0.383	0.457
AST(U/l)	16.8[14.1;24.8]	15.1[14;21.35]	24.8[14.7;27.5]	16.8[14.75;18.85]	0.273	0.902
TBIL (ummol/l)	15[14.1;16.1]	15[13.3;15.7]	14.4[13.85;15.6]	16.1[12.25;25.2]	0.947	0.376
ALP(U/l)	63.85[59.56;72.1]	79.5[59.3;118.9]	69.7[56.58;71.35]	63.3[60;67.975]	0.168	0.141
γ-GGT(U/l)	25.1[17.8;34.7]	20.7[19.05;31.95]	17.8[14.65;31.735]	30.675[19.75;41.6]	0.665	0.371
TG (mmol/l)	1.53[0.97;2.01]	1.53[0.64;2.71]	1.02[0.86;1.175]	2.01[1.74;2.38]	0.303	0.515
BUN (mmol/l)	6.6[5.7;8.5]	6.7[5.55;9.55]	6.3[5.5;16.65]	6.6[5.55;8.2]	0.533	0.655
Cr(mmol/l)	60.6[53.1;79.1]	55.4[48.4;73.45]	56.7[52.5;178.65]	75.2[66.05;85]	0.395	0.072

Table 1 (continued)

Variable	All (*N* = 15)	Non-SEC (*N* = 5)	low-SEC (*N* = 5)	high-SEC (*N* = 5)	*P* (low-SEC VS. Non-SEC)	*P* (high-SEC VS. Non-SEC)
Ua (mmol/l)	355.9[245.2;422.4]	256.3[221.4;524.4]	292.4[210.35;459.9]	391.1[347.25;422.9]	0.832	0.672
WBC(*10^9/L)	6.58[5.4;7.55]	7.39[6.42;8.385]	5.96[4.91;9.015]	5.52[4.965;7.225]	0.591	0.095
NEUT(*10^9/L)	4.12[3.39;5.39]	4.41[3.945;5.68]	4.12[3.41;5.5]	3.39[2.605;4.81]	0.64	0.182
LY(*10^9/L)	1.93[1.54;2.12]	2.12[1.715;2.245]	1.67[0.915;2.8]	1.69[1.585;2.02]	0.729	0.23
Mono(*10^9/L)	0.42[0.38;0.51]	0.51[0.445;0.595]	0.41[0.33;0.48]	0.38[0.36;0.48]	0.085	0.053
PLT(*10^9/L)	209[174;240]	209[179;252.5]	225[166;271]	201[127;227.5]	0.866	0.301
FT3(pmol/l)	4.32[3.99;4.82]	4.34[4.175;5.7425]	3.99[3.515;4.245]	4.72[3.60;5.2]	0.110	0.591
FT4(pmol/l)	15.7[13.94;16.3]	15.9[12.7;19.66]	15.8[15.3;17.6]	14.3[11.45;16.25]	0.926	0.383

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; INR, international normalized ratio; BNP, Brain Natriuretic Peptide. IVS, Interventricular septum thickness; LVDd, Left ventricular end diastolic diameter; LVDs, Left ventricular end systolic diameter; RA, right atrium diameter; LAD, left atrial diameter; RVAW, right ventricular anterior wall thickness; RVDd, right ventricular end diastolic diameter; AoD, aortic diameter; PAD, pulmonary artery diameter; LVEF, Left Ventricular Ejection Fraction; IVC, Inferior caval vein diameter; CO_2CP, carbon dioxide combining power; TP, total protein; ALT, alanine aminotransferase; IBIL, indirect bilirubin; CB, conjugated bilirubin; AST, aspartate aminotransferase; TBIL, total bilirubin; ALP, Alkaline phosphatase; γ-GGT, γ-glutamyl transpeptidase; TG, triglyceride; BUN, urea nitrogen; Cr, creatinine; Ua, uric acid; WBC, White blood cell count; NEUT, Neutrophil count; LY, Lymphocyte count; Mono, Monocyte count; PLT, platelet count; FT3, Free T3; FT4, Free T4

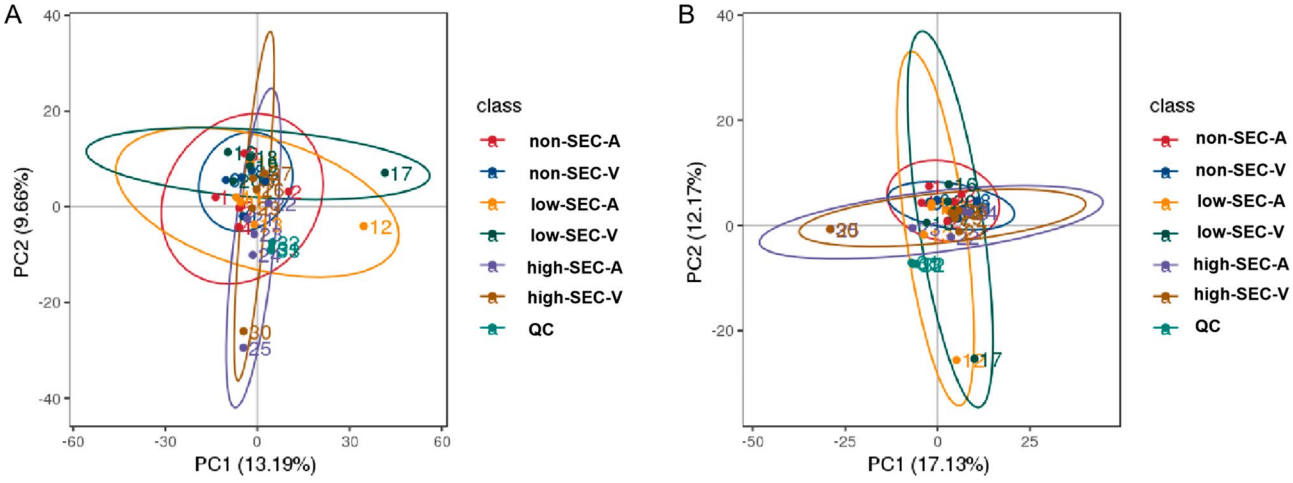

Fig. 2 PCA plots of all plasma samples and QC samples in positive mode (**A**)and negative mode (**B**). The QC samples exhibited good clustering, and all plasma samples fell within the 95% confidence interval in the PCA model. non-SEC-A, atrial plasma of non-SEC; non-SEC-V, venous plasma of non-SEC; low-SEC-A, atrial plasma of low-SEC; low-SEC-V, venous plasma of low-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-V, venous plasma of high-SEC

most altered in the high-SEC group compared to the non-SEC group, regardless of venous or atrial plasma(P<0.05) (Fig. 4). In the comparison between the high-SEC group and the non-SEC group, both venous and atrial plasma metabolic pathways were enriched in Phenylalanine metabolism and Phenylalanine, Tyrosine, and Tryptophan biosynthesis(Fig. 4A and D).This suggested that the metabolism of Phenylalanine, Tyrosine, and Tryptophan is markedly influential in the pathogenesis and development of SEC. Additionally, in the venous plasma metabolism, significant changes were also observed in the Pentose phosphate pathway, Amyotrophic Lateral Sclerosis (ALS), Salmonella infection, and Chagas disease (American trypanosomiasis) pathways, while in atrial plasma, notable alterations were detected in Fatty acid biosynthesis(Fig. 4A and D). In the low-SEC group vs. the non-SEC group, the metabolic pathways related to fatty acid metabolism were the most significantly changed in the venous and atrial plasma(Fig. 4B and E) .For the SEC group (low-SEC group plus high-SEC group) VS. non-SEC group, both venous and atrial plasma metabolic pathways were enriched in the Biosynthesis of unsaturated fatty acids and Ferroptosis, with the atrial plasma exhibiting the most substantial changes (Fig. 4C and F).The findings above indicated that distinct metabolic dysregulation patterns were presented at different stages of SEC.

Inter-group comparison and screening of differential metabolites

We created a Venn diagram to represent the changes in differential metabolites between the different comparison

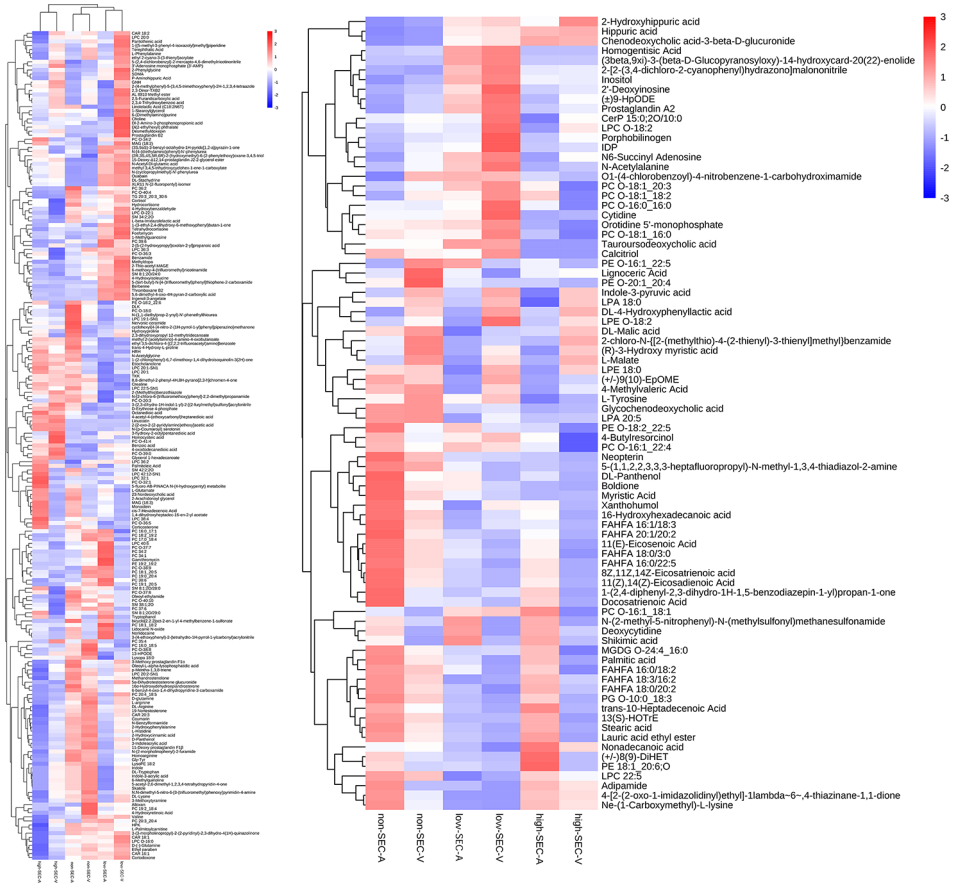

Fig. 3 Heatmap of metabolic patterns in venous and atrial plasma in positive(left) and negative(right) modes. The metabolism patterns of patients in the low-SEC group and high-SEC group were changed compared with the non-SEC group, and with the aggravation of the degree of SEC, the metabolomics changes were more and more, but the changes in atrial plasma were more obvious than those in venous. non-SEC-A, atrial plasma of non-SEC; non-SEC-V, venous plasma of non-SEC; low-SEC-A, atrial plasma of low-SEC; low-SEC-V, venous plasma of low-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-V, venous plasma of high-SEC

groups (Fig. 5). Based on the results of statistical analysis and the criteria for the initial screening of metabolites that have been specified, in venous plasma samples, a total of 46 differential metabolites were identified from the low-SEC group vs. non-SEC group(Fig. 6A, Supplementary Table 1), including 29 elevated metabolites(Fig. 5A) and 17 decreased metabolites(Fig. 5B), 48 differential metabolites were identified from the high-SEC group vs. non-SEC group(Fig. 6B, Supplementary Table 2), including 15 elevated metabolites(Fig. 5A) and 33 decreased metabolites(Fig. 5B), and 74 differential metabolites were identified from the SEC group vs. non-SEC group(Fig. 6C, Supplementary Table 3), including 28 elevated metabolites(Fig. 5A) and 46 decreased metabolites(Fig. 5B).In the atrial plasma samples, 69 differential metabolites were identified from the low-SEC group vs. non-SEC group(Fig. 6D, Supplementary Table 4), including 16 elevated metabolites(Fig. 5C) and 53 decreased metabolites(Fig. 5D), 51 differential metabolites were identified from the high-SEC group vs. non-SEC group(Fig. 6E, Supplementary Table 5), including 9 elevated metabolites(Fig. 5C) and 42 decreased metabolites(Fig. 5D), and 84 differential metabolites were identified from the SEC group vs. non-SEC group(Fig. 6F, Supplementary Table 6), including 17 elevated metabolites(Fig. 5C) and 67 decreased metabolites(Fig. 5D).

To identify the pivotal metabolites may involve in the onset and progression of SEC, we assigned different weight points to the differential metabolites based on diverse criteria, ultimately selecting those with a cumulative weight score of two or higher. Rule 1: Metabolites present in 2 or 3 comparison groups were assigned a score of 0.5 and 1, respectively. In venous plasma, five metabolites were concurrently present across the three groups (high-SEC vs. non-SEC, low-SEC vs. non-SEC, SEC vs. non-SEC), while 35 metabolites were found in two of the groups. In atrial, 6 metabolites were concurrently identified in the three of groups (high-SEC vs. non-SEC, low-SEC vs. non-SEC, SEC vs. non-SEC), with 50 metabolites present in two groups concurrently. Rule 2: The metabolites of VIP > 2 was given weight points of 1; In both

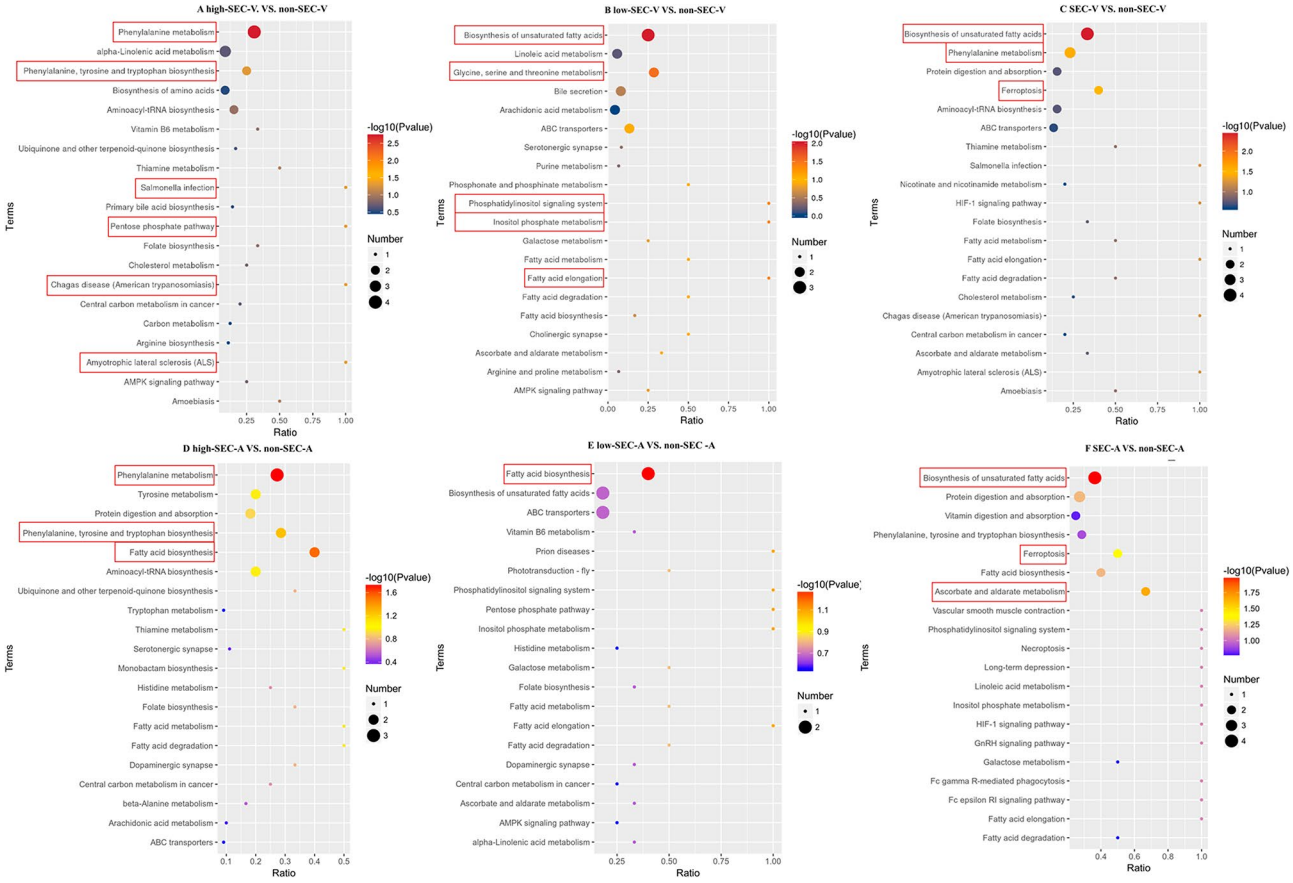

Fig. 4 KEGG analysis of differential metabolites. KEGG pathways that are significantly different are selected in the box. non-SEC-A, atrial plasma of non-SEC; non-SEC-V, venous plasma of non-SEC; low-SEC-A, atrial plasma of low-SEC; low-SEC-V, venous plasma of low-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-V, venous plasma of high-SEC

venous and atrial plasma, 55 and 49 metabolites, respectively, were filtered out in accordance with the established criteria. Rule 3: Metabolites present in the KEGG-enrichment pathway were assigned a weight score of 1. Within the venous and atrial plasma, respectively, 12 and 27 metabolites conformed to the specified criteria. Based on a cumulative total of weight scores of ≥2, 13 key metabolites were respectively selected from both the venous and atrial plasma (Tables 2 and 3). The expression levels were depicted in the figures (Figs. 7 and 8). As indicated by the Venn diagram(Fig. 9), three metabolites: Benzoic acid、Inositol and 5-(1,1,2,2,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine are all present in both the venous and atrial plasma.

Discussion

Spontaneous echo contrast (SEC) is considered to be a pre-thrombotic state in the atrial of patients with AF, and it is likely to progress to thrombosis eventually, which is very harmful [9]. The occurrence and development of SEC may require the participation of many metabolites, so exploring the metabolomic changes of SEC can protect the AF patients from thrombosis and provide ideas for the development of new antithrombotic drugs. But so far, few studies have systematically reported SEC-related metabolomic changes in patients with AF, and our study explored not only venous plasma metabolomic changes in patients with SEC atrial fibrillation, but also atrial plasma. This paper attempts to explore the characteristics of SEC-related metabolic changes from different perspectives, so as to provide a basis for the prevention and treatment of SEC.

In this study, patients with AF who completed TEE testing were divided into three groups: non-SEC group, low-SEC group, and high-SEC group according to the different degrees of SEC, and a comprehensive analysis of venous and atrial plasma metabolomics was performed. Our metabolic phenotype revealed a clear differential metabolic pattern among the three groups (Fig. 3). Specifically, we identified 35 and 142 significantly differential metabolites in venous and atrial plasma, respectively (Fig. 5, Supplementary Tables 1–6), suggesting that SEC may be involved in pervasive metabolic dysregulation and that the degree of metabolic dysregulation in atrial

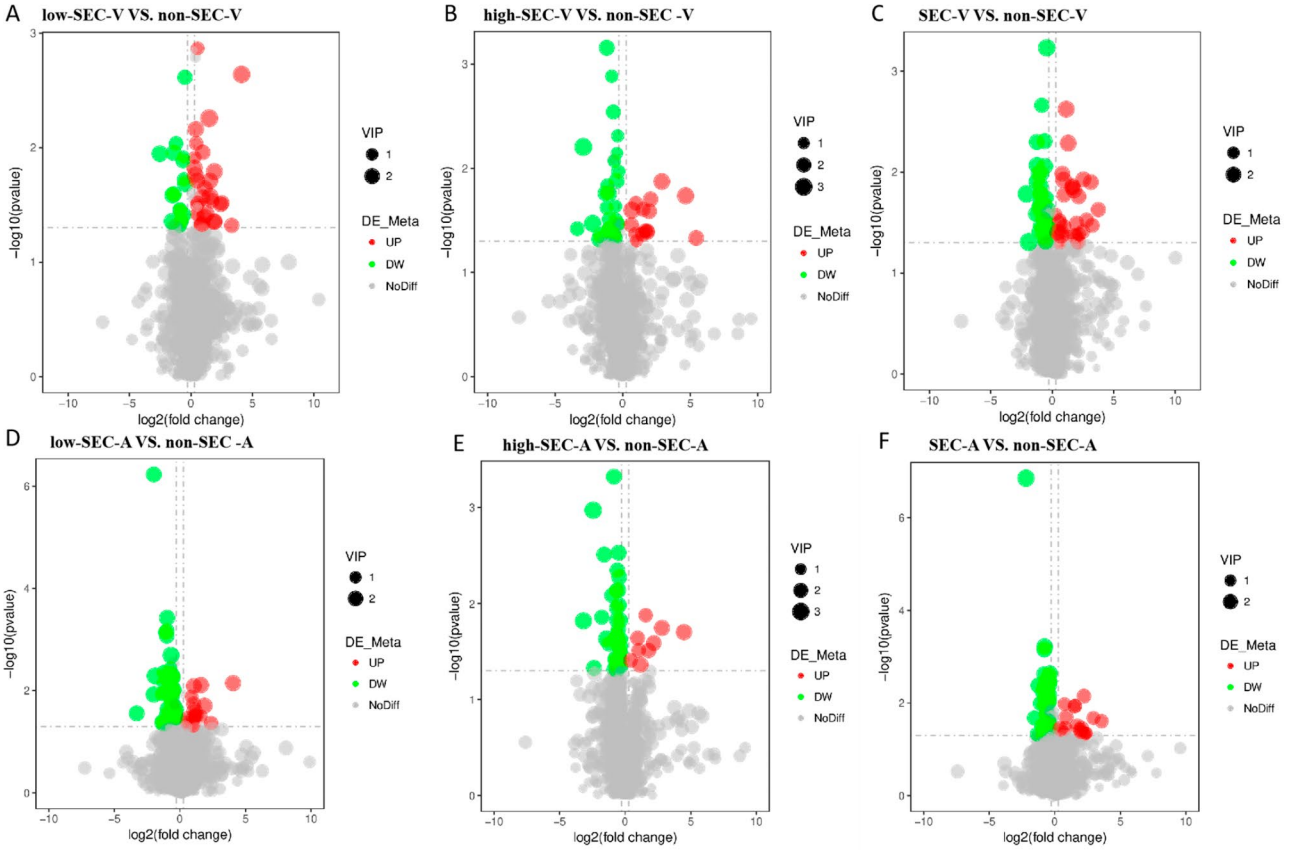

Fig. 5 Volcano diagram of differential metabolites. The abscissa indicates the expression times of metabolites in different groups number change (log-2FC), the ordinate represents the difference significance level (-log10(p-value)), and each point in the plot represents one metabolite, the size of the dots represent VIP values, metabolites that are significantly upregulated are indicated by red dots, and metabolites that are significantly downregulated objects are indicated by green dots. In venous plasma samples, a total of 46 differential metabolites were identified from the low-SEC group vs. non-SEC group including 29 elevated metabolites and 17 decreased metabolites (**A**), 48 differential metabolites were identified from the high-SEC group vs. non-SEC group, including 15 elevated metabolites and 33 decreased metabolites (**B**), and 74 differential metabolites were identified from the SEC group vs. non-SEC group, including 28 elevated metabolites and 46 decreased metabolites (**C**).In the atrial plasma samples, 69 differential metabolites were identified from the low-SEC group vs. non-SEC group including 16 elevated metabolites and 53 decreased metabolites (**D**), 51 differential metabolites were identified from the high-SEC group vs. non-SEC group, including 9 elevated metabolites and 42 decreased metabolites (**E**), and 84 differential metabolites were identified from the SEC group vs. non-SEC group, including 17 elevated metabolites and 67 decreased metabolites (**F**). non-SEC-A, atrial plasma of non-SEC; non-SEC-V, venous plasma of non-SEC; low-SEC-A, atrial plasma of low-SEC; low-SEC-V, venous plasma of low-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-V, venous plasma of high-SEC

plasma is more severe than that in venous blood. Then, according to the rule of VIP≥2, we screened 13 significant differential metabolites from atrial and venous plasma, respectively, and it is worth noting that Benzoic acid, Inositol and 5-(1,1,2,2,3,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine displayed significant alterations in both venous and atrial plasma, potentially indicating their pivotal role in the onset and progression of SEC.

In view of the large number of differential metabolites identified, we will focus on some substances that are most likely to play an important role in the development of the SEC and AF, so as to provide theoretical basis for subsequent research and related drug development. Benzoic acid ($C_7H_6O_2$, BA), an organic acidulant, is an colorless crystalline solid and constitutes the simplest aromatic carboxylic acid, frequently utilized as a food and pharmaceutical additive [10]. Its absorption and transport predominantly occur in the small intestine [11]. Following oral administration, BA is entirely metabolized to hippuric acid in humans and an additional twenty species of animals, and is subsequently excreted via urine [12]. It has been reported that excessive intake of BA can lead to dysfunction and damage to various organs [13]. In consonance with previous research findings, our study demonstrates that BA and hippuric acid levels are elevated in the SEC group (low-SEC group plus high-SEC group) compared to the non-SEC group, with the most pronounced increase observed in the high-SEC group, suggesting that BA levels may influence the onset and progression of SEC. On the other hand, as an acidulant, BA can lower the pH of body fluids [14–16]. Studies have

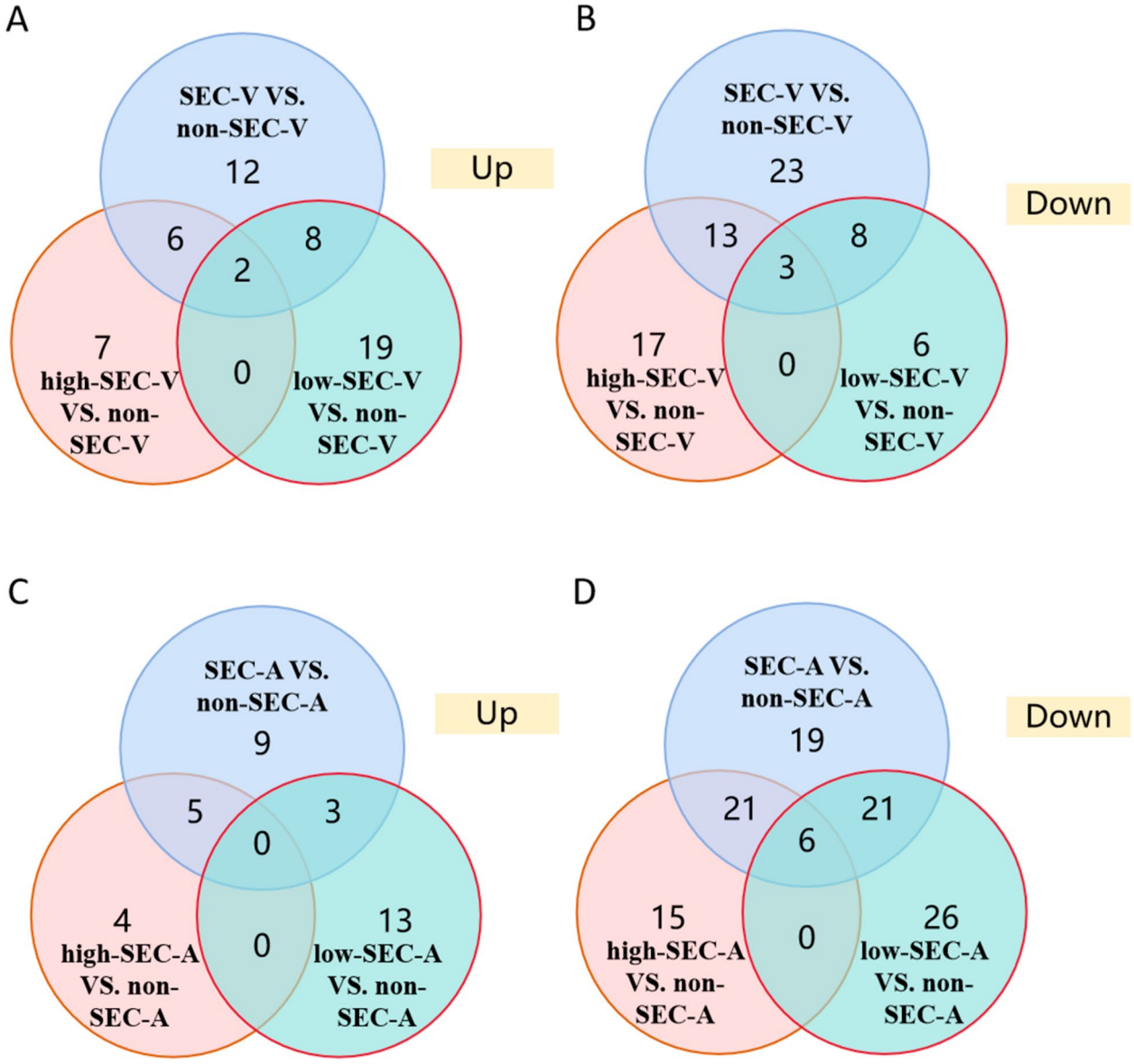

Fig. 6 The Venn plots to reflect the changes in differential metabolites between different comparison groups. non-SEC-A, atrial plasma of non-SEC; non-SEC-V, venous plasma of non-SEC; low-SEC-A, atrial plasma of low-SEC; low-SEC-V, venous plasma of low-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-A, atrial plasma of high-SEC; high-SEC-V, venous plasma of high-SEC

proved that a reduced pH can affect coagulation system function, potentially leading to a hypercoagulable state [17]. Consequently, it is plausible to infer from our findings that the increased content of BA in the SEC group may lead to a decrease in blood pH, thereby promoting the occurrence and development of SEC. However, it is regrettable that routine clinical examinations for patients do not include pH level assessments. In addition, it has been shown that excess BA can impair normal cell function, which may be related to impaired redox status regulated by the Nrf2 pathway [18], which also plays an important role in the occurrence and development of AF [19, 20]. Furthermore, it has been reported that BA and its derivatives can inhibit the activity of acetylcholinesterase to a certain extent, thereby reducing the degradation of acetylcholine [21–23], and the elevated content of acetylcholine can induce or aggravate atrial fibrillation [24, 25], resulting in a series of pathophysiological effects. From the above evidence, we can speculate that BA may have an impact on the occurrence and development of SEC and AF through multiple pathways.

In particular, it is important to note that dietary intake is one of the primary sources of BA within the human body [26]. Therefore, our results showed that the increase of BA in the SEC group was most likely due to excessive dietary intake, indicating that attention should be paid

Table 2 Venous plasma differential metabolite screening

Metabolites_ID	Name	Venn	VIP > 2	KEGG	weight point	Up_Down[#]
Com_6047_neg	Lignoceric Acid	○	◆	■	2.5	down
Com_192_neg	8Z,11Z,14Z-Eicosatrienoic acid	○	◆	■	2.5	down
Com_1152_neg	2-Hydroxyhippuric acid	○	◆	■	2.5	up
Com_11014_neg	5-(1,1,2,2,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine	●	◆		2	down
Com_24259_pos	N-Acetyl-Dl-glutamic acid	●	◆		2	up
Com_10599_pos	3′-Adenosine monophosphate (3′-AMP)	●	◆		2	up
Com_889_neg	Lauric acid ethyl ester	●	◆		2	down
Com_20378_pos	PE 19:2_19:2	●	◆		2	down
Com_12898_pos	D-Erythrose 4-phosphate		◆	■	2	up
Com_360_pos	Benzoic acid		◆	■	2	up
Com_779_pos	Choline		◆	■	2	up
Com_100_pos	Creatine		◆	■	2	down
Com_20691_neg	Inositol		◆	■	2	up
Com_11053_pos	PC 18:1_20:5	○	◆		1.5	down
Com_22568_pos	PC O-38:8	○	◆		1.5	down
Com_801_neg	FAHFA 16:0/18:2	○	◆		1.5	down
Com_6959_pos	Linolelaidic Acid (C18:2N6T)	○	◆		1.5	up
Com_350_neg	11(E)-Eicosenoic Acid	○	◆		1.5	down
Com_953_pos	methyl 3,4,5-trihydroxycyclohex-1-ene-1-carboxylate	○	◆		1.5	up
Com_8383_neg	PE O-20:1_20:4	○	◆		1.5	down
Com_9351_neg	Neopterin	○	◆		1.5	down
Com_4402_neg	2-[2-(3,4-dichloro-2-cyanophenyl)hydrazono]malononitrile	○	◆		1.5	up
Com_41_neg	Hippuric acid	○	◆		1.5	up
Com_24272_pos	Glycerol 1-hexadecanoate	○	◆		1.5	up
Com_1318_pos	PC 16:0_17:1	○	◆		1.5	down
Com_5086_pos	Linustatin	○	◆		1.5	up
Com_1356_neg	L-Tyrosine	○	◆		1.5	down
Com_3598_pos	LPC 36:3	○	◆		1.5	down
Com_1316_neg	(3beta,9xi)-3-(beta-D-Glucopyranosyloxy)-14-hydroxycard-20(22)-enolide	○	◆		1.5	up
Com_13931_pos	Dl-2-Amino-3-phosphonopropionic acid	○	◆		1.5	up
Com_11652_pos	Di(2-ethylhexyl) phthalate	○	◆		1.5	up
Com_292_neg	DL-Malic acid	○	◆		1.5	down
Com_5249_neg	FAHFA 18:3/16:2	○	◆		1.5	down
Com_25_neg	Palmitic acid	○		■	1.5	down
Com_25968_pos	L-arginine	○		■	1.5	down
Com_24425_pos	ethyl 2-[4,6-dimethyl-3-oxoisothiazolo[5,4-b]pyridin-2(3 H)-yl]acetate		◆		1	up
Com_7366_neg	FAHFA 16:0/22:5		◆		1	down
Com_890_pos	5-(2,4-dichlorobenzyl)-2-mercapto-4,6-dimethylnicotinonitrile		◆		1	up
Com_2780_pos	PC 17:0_18:4		◆		1	down
Com_2181_neg	PG O-10:0_18:3		◆		1	down
Com_9698_pos	LPC 19:0		◆		1	down
Com_16106_pos	PC 17:0_18:3		◆		1	down
Com_3742_pos	(2R,3 S,4 S,5R,6R)-2-(hydroxymethyl)-6-(2-phenylethoxy)oxane-3,4,5-triol		◆		1	up
Com_11066_pos	PC 19:0_20:4		◆		1	down
Com_27_neg	Glycochenodeoxycholic acid		◆		1	down
Com_10664_pos	PC 37:6		◆		1	down
Com_19801_pos	PC O-39:0		◆		1	up
Com_1380_pos	SDMA		◆		1	up
Com_6191_neg	LPC O-18:2		◆		1	up
Com_3425_pos	5,6-dimethyl-4-oxo-4 H-pyran-2-carboxylic acid		◆		1	up
Com_4653_pos	MAG (18:2)		◆		1	up
Com_20804_pos	Fosfomycin		◆		1	up
Com_7640_pos	PC 35:4		◆		1	down

Table 2 (continued)

Metabolites_ID	Name	Venn	VIP > 2	KEGG	weight point	Up_Down[#]
Com_9830_pos	PC 16:0_18:5		◆		1	down
Com_21545_pos	1-Methylguanosine		◆		1	up
Com_2850_pos	Tetrahydrocortisone		◆		1	up
Com_7374_pos	2-Phenylglycine		◆		1	up
Com_142_neg	Adrenic acid			■	1	down
Com_3270_pos	L-Cystine			■	1	up
Com_4999_pos	15-Deoxy-Δ12,14-prostaglandin J2-2-glycerol ester	○			0.5	up
Com_5823_pos	N-(cyclopropylmethyl)-N′-phenylurea	○			0.5	up
Com_8882_pos	Ouabain	○			0.5	up
Com_45_pos	DL-Stachydrine	○			0.5	up
Com_4409_pos	PC 34:1	○			0.5	down
Com_9692_pos	PC O-37:6	○			0.5	down
Com_331_pos	2-Hydroxyphenylalanine	○			0.5	down
Com_9156_pos	LPC 40:6	○			0.5	down
Com_17518_neg	PE O-16:1_22:5	○			0.5	down
Com_260_pos	2-Hydroxycinnamic acid	○			0.5	down

○represented the metabolites that are present between the two comparison groups and was given a weighted score of 0.5. ●represented the metabolites that are present between the three comparison groups and was given a weighted score of 1. ◆represents the metabolite of VIP ≥ 2 and was given a weighted score of 1. ■represents the metabolites enriched in the KEGG pathway and was given a weighted score of 1. #,compared to the non-SEC group

to the BA content in food, particularly providing crucial dietary recommendations for patients with AF. Our discovery affords evidence for the prevention of SEC and atrial thrombosis in patients with AF through dietary means.

Inositol, apart from regulating intracellular osmolarity in the brain [27], is also a structural component in the formation of phosphatidylinositol and phosphatidylinositides, which act as second messengers. Phosphatidylinositides participate in cellular signal transduction through pathways involving phosphatidylinositol kinases [28, 29], protein kinase C, and nuclear factor κB (NFκB) [30, 31]. NFκB signaling plays a role in the regulation of adhesion molecules, cytokines, chemokines, growth factors and matrix metalloproteinases (MMPs) [32, 33]. Inflammatory cytokines are considered to be associated with AF-related Sect. [34]. Our results similarly support this inference, with elevated levels of inositol observed in the SEC group compared to the control group, which may lead to increased inflammation and subsequently affect the occurrence of SEC. Interestingly, our results showed that while the SEC group had higher levels of inositol than the control group, the increase was significant in the low-SEC group and less in the high-SEC group. This phenomenon reflected that the degree of inositol metabolic disorder was different between the low-SEC group and high-SEC group. Studies have shown that inositol can exert antioxidant properties by scavenging free radicals and increasing the activity of antioxidant enzymes [35, 36]. Oxidative stress is closely related to coagulation, and it plays a role at different aspects of the coagulation process [37]. Besides, oxidative stress contributes to the development of AF [38, 39]. In general, atrial fibrillation, oxidative stress, and coagulation interact with each other, and once the balance is broken, it will lead to the development of pathological states. Therefore, based on our results, it can be inferred that the metabolic disorders of the antioxidant system in the low-SEC group were still in the compensated stage, and can still produce enough inositol to exert antioxidant effects and avoid thrombosis. The antioxidant system of patients in the high group was in the decompensated stage and cannot produce enough inositol, resulting in excessive accumulation of oxidized substances affecting the coagulation system and even thrombus.

Furthermore, the increased inositol can exacerbate the imbalance of MMPs activity and tissue inhibitor of matrix metalloproteinase (TIMP) by upregulating growth factors and inflammatory cytokines, thereby intensifying the structural remodeling of atrial fibrillation [40]. This structural remodeling, in turn, can promote the onset and progression of SEC, creating a vicious cycle [41]. Hence, the metabolic dysregulation of inositol is of significant concern, as its disturbance can simultaneously impact SEC, thrombosis, and atrial structural remodeling.

Inositol can be ingested from food or synthesized in the body [42]. Based on our findings, dietary inositol should be considered due to the growing problems posed by unhealthy diets and living conditions, as they have antioxidant effects, especially in AF patients with SEC.

5-(1,1,2,2,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine, an amine derivative, exhibits an undefined mechanism in the pathogenesis and progression of SEC in AF, although literature suggests it possesses antioxidant properties [43]. The role of 5-(1,1,2,2,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-

Table 3 Atrial plasma differential metabolite screening

Metabolites_ID	Name	Venn	VIP > 2	KEGG	weight point	Up_Down[#]
Com_6944_pos	3-Methoxytyramine	●	◆	■	3	down
Com_9351_neg	Neopterin	●	◆	■	3	down
Com_14927_neg	Myristic Acid	○	◆	■	2.5	down
Com_360_pos	Benzoic acid	○	◆	■	2.5	up
Com_41_neg	Hippuric acid	○	◆	■	2.5	up
Com_534_pos	L-Histidine	○	◆	■	2.5	down
Com_20691_neg	Inositol	○	◆	■	2.5	down
Com_11014_neg	5-(1,1,2,2,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine	●	◆		2	down
Com_14291_pos	N-(1,1-diethylprop-2-ynyl)-N′-phenethylthiourea	●	◆		2	down
Com_1493_pos	3-Methoxy prostaglandin F1α	●	◆		2	down
Com_16682_pos	PC 20:4_18:5	●	◆		2	down
Com_21879_pos	L-Palmitoylcarnitine		◆	■	2	down
Com_4640_pos	Corticosterone		◆	■	2	up
Com_11492_pos	PC O-40:4	○	◆		1.5	down
Com_19303_pos	Methandrostenolone	○	◆		1.5	down
Com_2074_pos	Homoarginine	○	◆		1.5	down
Com_209_pos	Indole	○		■	1.5	down
Com_24272_pos	Glycerol 1-hexadecanoate	○	◆		1.5	up
Com_331_pos	2-Hydroxyphenylalanine	○	◆		1.5	down
Com_4008_pos	Coumarin	○	◆		1.5	down
Com_467_pos	LPC 20:2-SN1	○	◆		1.5	down
Com_4674_pos	3-Indoleacrylic acid	○	◆		1.5	down
Com_5086_pos	Linustatin	○	◆		1.5	up
Com_795_pos	N-Benzylformamide	○	◆		1.5	down
Com_1104_pos	N-[2-chloro-6-(trifluoromethoxy)phenyl]-2,2-dimethylpropanamide	○	◆		1.5	down
Com_192_neg	8Z,11Z,14Z-Eicosatrienoic acid	○	◆		1.5	up
Com_2475_pos	PC O-20:3	○	◆		1.5	down
Com_25_neg	Palmitic acid	○		■	1.5	down
Com_2782_neg	FAHFA 16:1/18:3	○	◆		1.5	down
Com_292_neg	DL-Malic acid	○	◆		1.5	down
Com_5249_neg	FAHFA 18:3/16:2	○	◆		1.5	down
Com_7267_pos	ethyl 3,5-dichloro-4-[(2,2,2-trifluoroacetyl)amino]benzoate	○	◆		1.5	up
Com_733_pos	DLK	○	◆		1.5	down
Com_99_pos	PC O-18:0	○	◆		1.5	down
Com_10007_pos	16α-Hydroxydehydroepiandrosterone		◆		1	down
Com_10132_pos	PC O-32:1		◆		1	up
Com_11786_neg	Boldione		◆		1	down
Com_1356_neg	L-Tyrosine			■	1	down
Com_1360_pos	LPC 36:2		◆		1	up
Com_14791_pos	19-Nortestosterone		◆		1	down
Com_20021_pos	Prostaglandin B2			■	1	up
Com_24505_pos	Ethyl paraben		◆		1	down
Com_3204_pos	bicyclo[2.2.2]oct-2-en-1-yl 4-methylbenzene-1-sulfonate		◆		1	down
Com_370_pos	LPC O-16:0		◆		1	down
Com_3961_pos	HPK		◆		1	down
Com_4455_pos	3-(3-morpholinopropyl)-2-(2-pyridinyl)-2,3-dihydro-4(1 H)-quinazolinone		◆		1	down
Com_9328_pos	Palmitoleic Acid			■	1	up
Com_12898_pos	D-Erythrose 4-phosphate			■	1	up
Com_1316_neg	(3beta,9xi)-3-(beta-D-Glucopyranosyloxy)-14-hydroxycard-20(22)-enolide		◆		1	down
Com_13482_pos	Gamithromycin		◆		1	up
Com_20378_pos	PE 19:2_19:2		◆		1	up
Com_21545_pos	1-Methylguanosine		◆		1	down
Com_3610_neg	13(S)-HOTrE			■	1	down

Table 3 (continued)

Metabolites_ID	Name	Venn	VIP > 2	KEGG	weight point	Up_Down[#]
Com_3615_neg	Porphobilinogen			■	1	down
Com_4424_pos	TKK		◆		1	down
Com_761_neg	Prostaglandin A2			■	1	up
Com_7677_pos	Etiocholanolone			■	1	down
Com_8878_pos	Berberine			■	1	up
Com_90_neg	Stearic acid			■	1	down
Com_141_neg	Docosapentaenoic acid			■	1	down
Com_142_neg	Adrenic acid			■	1	down
Com_20146_pos	2-hydroxy-3,6-diphenylcyclohexyl acetate		◆		1	down
Com_24889_pos	L-Ascorbate			■	1	down
Com_2696_pos	Pyridoxamine 5-phosphate			■	1	down
Com_46_neg	Arachidonic acid			■	1	down
Com_8635_pos	Papaverine		◆		1	down
Com_9058_neg	L-Tryptophan			■	1	down
Com_10_pos	DL-Tryptophan	○			0.5	down
Com_11165_pos	Indole-3-acrylic acid	○			0.5	down
Com_1446_neg	(+/-)9(10)-EpOME	○			0.5	down
Com_167_pos	6-Methylquinoline	○			0.5	down
Com_17576_pos	LysoPE 18:2	○			0.5	down
Com_260_pos	2-Hydroxycinnamic acid	○			0.5	down
Com_542_pos	5-acetyl-2,6-dimethyl-1,2,3,4-tetrahydropyridin-4-one	○			0.5	down
Com_6300_pos	Gly-Tyr	○			0.5	down
Com_7994_pos	N-(2-morpholinophenyl)-2-furamide	○			0.5	down
Com_824_pos	Skatole	○			0.5	down
Com_890_pos	5-(2,4-dichlorobenzyl)-2-mercapto-4,6-dimethylnicotinonitrile	○			0.5	up
Com_12314_neg	FAHFA 20:1/20:2	○			0.5	down
Com_16790_neg	2′-Deoxyinosine	○			0.5	up
Com_1698_neg	Xanthohumol	○			0.5	down
Com_212_neg	16-Hydroxyhexadecanoic acid	○			0.5	down
Com_2181_neg	PG O-10:0_18:3	○			0.5	down
Com_350_neg	11(E)-Eicosenoic Acid	○			0.5	down
Com_3504_pos	LPC 19:1-SN1	○			0.5	up
Com_400_neg	11(Z),14(Z)-Eicosadienoic Acid	○			0.5	down
Com_4402_neg	2-[2-(3,4-dichloro-2-cyanophenyl)hydrazono]malononitrile	○			0.5	down
Com_6872_neg	Docosatrienoic Acid	○			0.5	up
Com_889_neg	Lauric acid ethyl ester	○			0.5	down
Com_9476_neg	FAHFA 18:0/3:0	○			0.5	down
Com_7366_neg	FAHFA 16:0/22:5	○			0.5	up
Com_15726_pos	4-Hydroxyretinoic Acid				0	down
Com_16455_neg	LPA 18:0				0	down
Com_2179_neg	CerP 15:0;2O/10:0				0	down
Com_2593_pos	CAR 20:3				0	down
Com_298_pos	DL-Lysine				0	down
Com_10125_pos	cyclohexyl{4-[4-nitro-2-(1 H-pyrrol-1-yl)phenyl]piperazino}methanone				0	down
Com_1027_pos	13-HPODE				0	down
Com_1063_neg	trans-10-Heptadecenoic Acid				0	down
Com_11354_pos	LPC 42:12-SN1				0	up
Com_1265_pos	LPC 20:1				0	up
Com_13598_pos	PC O-38:9				0	down
Com_157_pos	MAG (18:3)				0	down
Com_19370_pos	Lysopa 18:0				0	up
Com_1951_neg	FAHFA 18:0/20:2				0	down
Com_20671_neg	IDP				0	down

Table 3 (continued)

Metabolites_ID	Name	Venn	VIP > 2	KEGG	weight point	Up_Down[#]
Com_21937_neg	O1-(4-chlorobenzoyl)-4-nitrobenzene-1-carbohydroximamide				0	down
Com_225_neg	4-[2-(2-oxo-1-imidazolidinyl)ethyl]-1lambda ~ 6~,4-thiazinane-1,1-dione				0	down
Com_232_pos	LPC 20:1-SN1				0	up
Com_2674_pos	2-(Methylthio)benzothiazole				0	up
Com_2768_neg	(+/-)8(9)-DiHET				0	down
Com_309_neg	(±)9-HpODE				0	down
Com_4634_neg	Chenodeoxycholic acid-3-beta-D-glucuronide				0	up
Com_5197_neg	1-(2,4-diphenyl-2,3-dihydro-1 H-1,5-benzodiazepin-1-yl)propan-1-one				0	up
Com_5207_pos	Ingenol-3-angelate				0	up
Com_5619_neg	MGDG O-24:4_16:0				0	up
Com_577_neg	LPC 22:5				0	down
Com_597_pos	2-Arachidonoyl glycerol				0	up
Com_6468_pos	Nervonic ceramide				0	down
Com_683_pos	methyl 2-(acetylamino)-4-amino-4-oxobutanoate				0	down
Com_698_pos	LPC 22:5-SN1				0	up
Com_869_neg	Ne-(1-Carboxymethyl)-L-lysine				0	down
Com_1078_pos	2-methyl-2,3,4,5-tetrahydro-1,5-benzoxazepin-4-one				0	down
Com_12312_neg	beta-Nicotinamide mononucleotide				0	down
Com_12460_pos	N1-[1-(2,4-dichlorobenzoyl)-4-piperidyl]cyclohexane-1-carboxamide				0	up
Com_15605_neg	N3-cyclohexyl-3-azabicyclo[3.2.2]nonane-3-carbothioamide				0	down
Com_20327_pos	4-Pregnen-17alpha,20alpha-Diol-3-One				0	up
Com_2178_pos	CAR 26:0				0	down
Com_26805_pos	Alloxan				0	up
Com_2773_neg	ST 28:1;O; Hex; FA 18:2				0	down
Com_3881_pos	CAR 26:2				0	down
Com_4240_pos	1,7-bis(4-hydroxyphenyl)heptan-3-one				0	down
Com_437_pos	LPC 20:3-SN1				0	down
Com_4829_pos	Chlortetracycline				0	down
Com_4888_pos	Maslinic acid				0	down
Com_4999_pos	15-Deoxy-Δ12,14-prostaglandin J2-2-glycerol ester				0	up
Com_5823_pos	N-(cyclopropylmethyl)-N'-phenylurea				0	up
Com_6671_pos	Asp-Phe				0	up
Com_7400_neg	FAHFA 4:0/17:2				0	up
Com_8329_pos	N-[1-(4-methoxy-2-oxo-2 H-pyran-6-yl)-2-methylbutyl]acetamide				0	down
Com_8882_pos	Ouabain				0	up
Com_953_pos	methyl 3,4,5-trihydroxycyclohex-1-ene-1-carboxylate				0	up

○ represented the metabolites that are present between the two comparison groups and was given a weighted score of 0.5. ● represented the metabolites that are present between the three comparison groups and was given a weighted score of 1. ◆ represents the metabolite of VIP ≥ 2 and was given a weighted score of 1. ■ represents the metabolites enriched in the KEGG pathway and was given a weighted score of 1. #,compared to the non-SEC group

thiadiazol-2-amine in medicine is very little, but we can speculate its function based on its chemical structure. Compounds containing 1,3,4-thiadiazol usually have a wide range of biological activities, including anti-inflammatory, anti-tumor, etc [44, 45]. , and 5-(1,1,2,2,3,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine contains the same chemical structure. Therefore, we speculate that it may also have anti-inflammatory effects in addition to antioxidant effects. Importantly, we found that the content of 5-(1,1,2,2,3,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine in the SEC group was significantly reduced, suggesting that the occurrence and development of SEC may be affected by it. Based on the above conjectures about the possible effects of 5-(1,1,2,2,3,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine, we believed that low concentration of it may lead to decreased antioxidant capacity, increased levels of oxidative stress, and possibly increased levels of inflammation, which in turn affected the pathophysiology of SEC and AF. Reports have associated increased oxidative stress in AF patients with SEC, contributing to the development of SEC and thrombosis [46], which supports our hypothesis. There are very few studies about this substance in the field of AF-related treatments, and our research provided a theoretical basis for its feasibility.

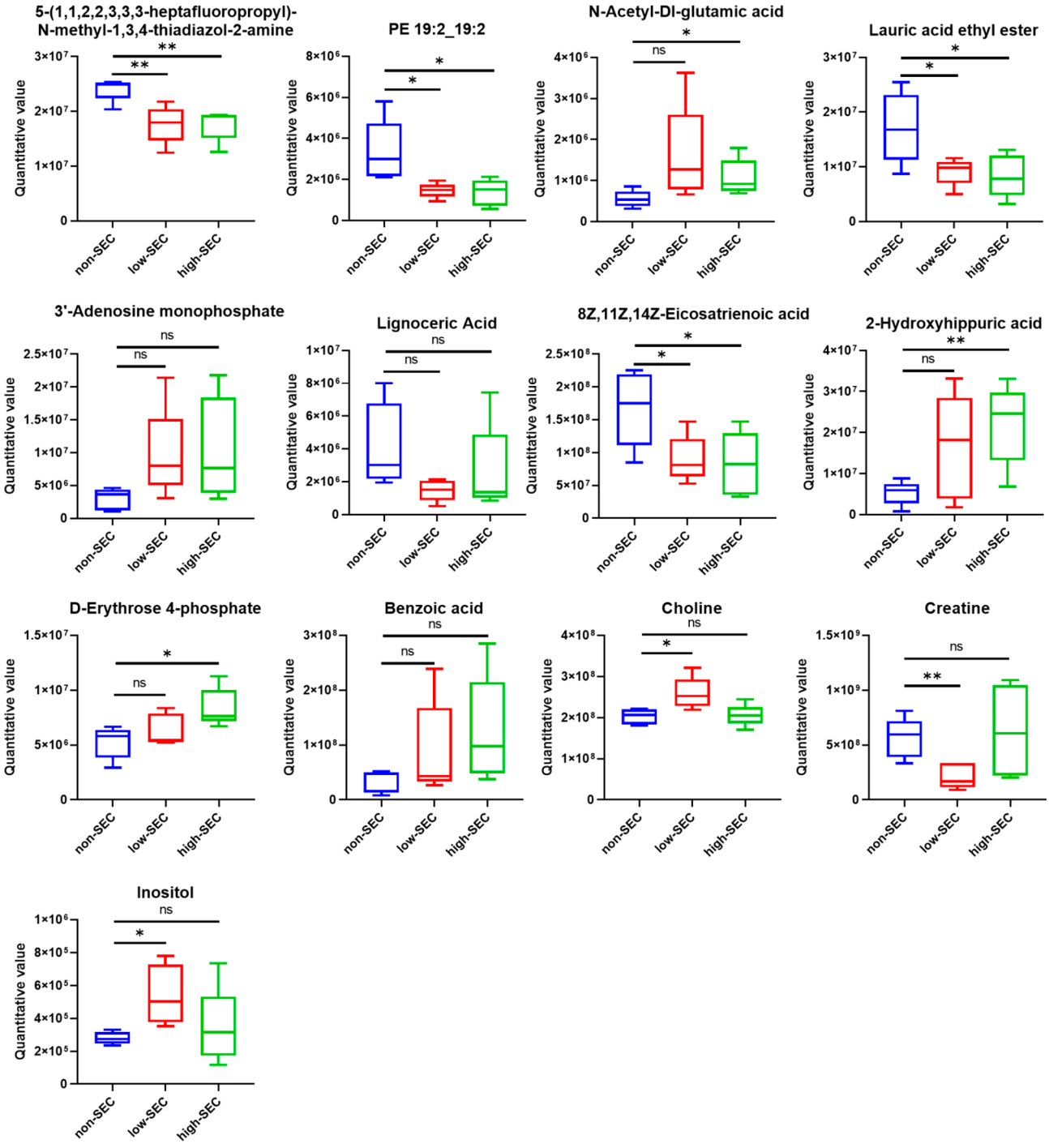

Fig. 7 Relative expression levels of differential metabolites among groups. Based on a cumulative total of weight scores of ≥ 2, 13 key metabolites were selected from the venous plasma. The expression levels are depicted in the figure

Moreover, within the venous plasma metabolites, we identified a group previously implicated as potential metabolites associated with thrombosis in AF, including Lignoceric acid [47], 8Z,11Z,14Z-Eicosatrienoic acid [48, 49], Lauric acid ethyl ester [50, 51], PE 19:2_19:2 [52], Choline [53, 54], Creatine [55], N-Acetyl-DL-glutamic acid [56, 57], D-Erythrose 4-phosphate [58], and 2-Hydroxyhippuric acid, which is a metabolite of salicylic acid and may be related to the administration of drugs such as aspirin.

Additionally, 3'-Adenosine monophosphate (3'-AMP) is derived from the hydrolysis of 2',3'-cAMP, a compound recently discovered to regulate immune function [59]. Normally, there is a dynamic equilibrium between

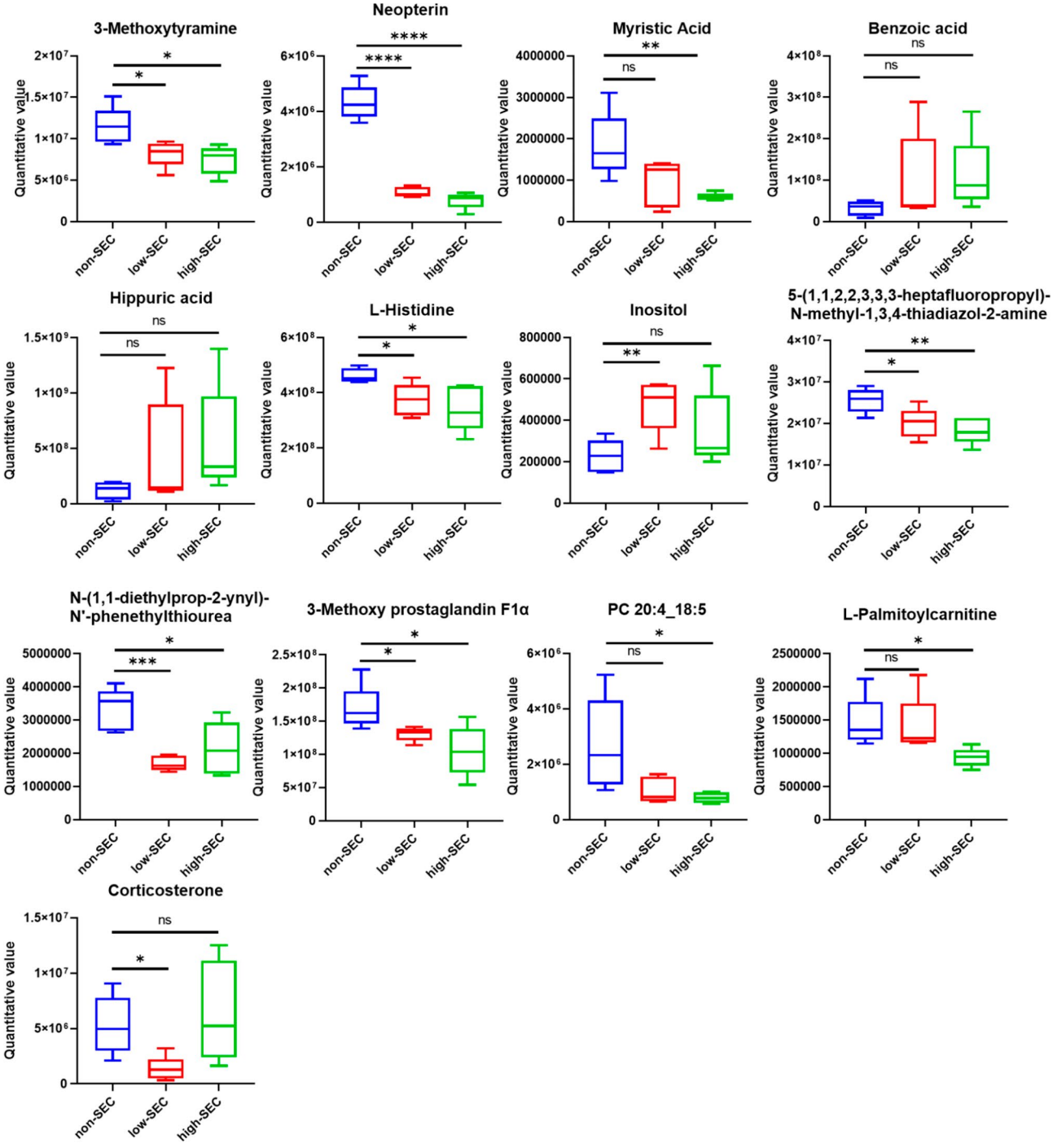

Fig. 8 Relative expression levels of differential metabolites among groups. Based on a cumulative total of weight scores of ≥ 2, 13 key metabolites were selected from the atrial plasma. The expression levels are depicted in the figure

the metabolism of 2',3'-cAMP and 3'-AMP. Studies have shown that 3'-AMP has a pro-inflammatory and vasoconstrictive effect [60]. Our study revealed an increase in its concentration within the SEC group, suggesting a possible dysregulation of 3'-AMP metabolism. Because it has a pro-inflammatory effect, an abnormally high level of 3'-AMP in the SEC group may lead to an inflammatory state in the body, which promotes the onset and progression of SEC. This provides a new perspective for the prevention and treatment of SEC in AF patients.

It is worth noting that we also studied the metabolic changes in the atrial plasma that is spatially closer to the left atrium. Their changes may have a more direct impact on the development of SEC. In addition to

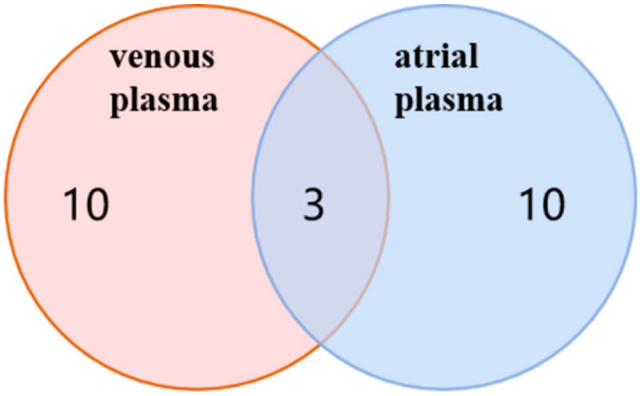

Fig. 9 Metabolites selected from the venous and atrial plasma. Three metabolites: Benzoic acid、Inositol and 5-(1,1,2,2,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine are all present in both the venous and atrial plasma

the previously discussed Benzoic acid, Inositol, and 5-(1,1,2,2,3,3,3-heptafluoropropyl)-N-methyl-1,3,4-thiadiazol-2-amine, the level of 3-Methoxytyramine (3-MT) significantly decreased in the SEC group (as depicted in the Fig. 8). 3-MT is not only a metabolite of dopamine, but also a neuromodulator [61]. Neuroelectrophysiological experiments showed that 3-MT could affect the activity of the dopaminergic nervous system through the trace amine associated receptor 1 (TAAR1), which showed that 3-MT could activate TAAR1 to reduce the spontaneous electrical rate of dopaminergic neurons, otherwise, it could increase the activity of the dopaminergic nervous system [62]. Studies have shown that excessive activity of the dopaminergic nervous system can promote the occurrence of SEC by activating platelets [63]. Combined with the above results, we can surmise that the reduction of 3-MT in the SEC group may increase the activity of the dopaminergic nervous system through TAAR1, which may affect the progression of SEC and AF. Thus, our findings implied that AF patients with SEC may have dopaminergic nervous system dysfunction mediated by 3-MT, and it also provided a basis for the cross-study of nervous system and heart diseases. Patients with AF should pay attention to the regulation of nervous system function.

Neopterin is a pterin analogue produced by the oxidation of 7,8-dihydroneopterin, which is a potent free radical scavenger and antioxidant [64–66]. Therefore, the content of neopterin is negatively correlated with the level of oxidative stress in the body, that is, the level of oxidative stress in the body is high when the neopterin content is low. Our results showed a significant decrease in neopterin content in the SEC group compared to the control group, and combined with previous discussions, we believed that the decrease in neopterin content in the SEC group may be involved in the process of increased oxidative stress levels, which led to the progression of SEC and AF. Over all, our study showed that the reduced neopterin content of AF patients with SEC may means that the body's antioxidant capacity is reduced, which affected the pathophysiology of SEC and AF.

L-Histidine, an essential amino acid, possesses unique physicochemical properties that make it with acid-base buffering capabilities, thus preventing organ damage from drastic fluctuations in pH [67]. Moreover, it can exert antioxidant effects through chelation of iron ions and singlet oxygen, and it also reduces the viscosity of solutions, enhancing fluidity [68]. The metabolic disorder of histidine in the SEC group, characterized by a significant decrease in its content, may consequently lower the patient's antioxidant capacity and increase blood viscosity, contributing to the onset and progression of SEC and AF. This suggested a balanced intake of essential amino acids, as they are not only nutrients but also affect the pathophysiological processes of the body.

L-Myristic Acid, with its anti-inflammatory effects [69], was found to be reduced in the SEC group in this study, potentially leading to a decrease in anti-inflammatory action and thus contributing to the onset and progression of SEC. L-Palmitoylcarnitine (L-PC), an important long-chain acylcarnitine, has been shown in previous studies to exert antithrombotic effects by enhancing plasmin and tissue-type plasminogen activator (tPA) activity. Moreover, reduced levels of plasma long-chain acylcarnitines have been associated with venous thrombosis [70]. Our results support these findings, with the change in L-PC levels indicating that carnitine metabolism dysregulation may aid in the occurrence and progression of SEC. The reduction in L-PC content in the SEC group may, like previously reported cases, promote SEC by affecting plasmin and tPA activity. Additionally, we identified significant metabolic abnormalities in atrial plasma for compounds such as N-(1,1-diethylprop-2-ynyl)-N'-phenethylthiourea, 3-Methoxy prostaglandin F1α, PC 20:4_18:5, and Corticosterone, although their specific impact on SEC is yet to be further investigated. These findings suggest that there are multiple metabolic pathways and mediators that

may be involved in the pathophysiology of SEC, and that a comprehensive understanding of these mechanisms could lead to better diagnostic and therapeutic strategies for AF patients with SEC.

Conclusion

We reported a comprehensive metabolomics assessment to characterize the metabolic derangement of SEC associated with AF. This study showed a significant change in metabolic patterns in SEC compared to non-SEC. Our work promoted the understanding of mechanism of the occurrence and development of SEC, facilitated the screening of the target metabolites for its therapeutic intervention, and provided evidence support for the prevention and treatment of SEC and thrombosis associated with AF. At the same time, our work also provided a direction for subsequent research in related fields. In conclusion, our study not only provides a theoretical basis for understanding the occurrence and development of SEC in AF, but also provides recommendations for the daily diet of AF patients with SEC, such as a balanced intake of essential amino acids, avoiding excessive intake of benzoic acid, and intake of appropriate inositol.

Limitations

First, our study sample size was relatively small. TEE is the traditional test to determine whether there is atrial thrombosis before AF ablation, but it also has obvious drawbacks, such as esophageal damage and unbearable nausea, etc. Therefore, according to the patient's preference and for the patient's safety, most of them chose the more acceptable and less invasive examination—Left Atrium Computed Tomography Angiography test, which led to a sharp decrease in the number of patients eligible for our study. In addition, in order to minimise the impact of diagnostic bias on the results of the study, we rigorously reviewed the results of TEE. Three experienced cardiac sonographers each independently evaluated the same TEE images, and patients were included in the research only if the reports from the three experts were the same, which further limited the number of people included in the research. However, the rigorous selection process made our findings more credible and representative.

The small sample size may restrict the generalizability of the findings, suggesting future studies with larger cohorts. To assess whether the results of our research were representative, we made cross-sectional comparisons with other similar studies. Since there is no research on the characteristics of plasma metabolomics in AF patients with SEC, which is also the innovation of our project, we compared it with other metabolomics studies related to coagulation and found that our sample size is similar to that of most studies [71–79], which indicates that although the small sample size limits the generalizability of the results, it can also reflect the scientific problems to a certain extent and provide a theoretical basis and direction for subsequent research.

Furthermore, we did not employ alternative metabolomic platforms to detect a broader spectrum of metabolites, nor did we quantitate the potential biomarkers absolutely. Lastly, due to the absence of in-depth exploration through molecular biology, cellular biology, and pharmacological methodologies, the metabolic mechanisms underlying disease onset and progression remain undetermined.

Abbreviations

SEC	Spontaneous echo contrast
AF	Atrial fibrillation
TEE	Trans esophageal echocardiography examination
PCA	Principal component analysis
PLS-DA	Partial least squares discriminant analysis
VIP	Variable important in projection
FC	Fold change
QC	Quality control
ALS	Amyotrophic Lateral Sclerosis
BA	Benzoic acid
NFκB	Nuclear factor κB
MMPs	Matrix metalloproteinases
TIMP	Tissue inhibitor of matrix metalloproteinase
3'-AMP	3'-Adenosine monophosphate
3-MT	3-Methoxytyramine
L-PC	L-Palmitoylcarnitine
tPA	Tissue-type plasminogen activator

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12872-024-04306-y.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

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Not applicable.

Author contributions

B.s. , R. S. and X. L. designed the study; B.s. ,X.w. , D.q. , X.y.,R.n.,H.p. and W.h. collected and analyzed the data; B.s. writed the manuscript; G.p., X. L., T. L. and X. L. corrected the initial manuscript; G.p., X. L. and T. L. reviewed and edited the final manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study has been approved by the Ethics Committee of the Second Hospital of Tianjin Medical University (No: KY2023K058) and adheres to the principles of the Helsinki Declaration. All participants are included voluntarily and have signed informed consent forms.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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