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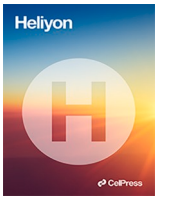

Research article

Differential serum metabolites in patients with pregnancy-associated venous thromboembolism analyzed using GC-MS/LC-MS untargeted metabolomics

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A B S T R A C T

Untargeted metabolomics can be used for the comprehensive analysis of metabolite profiles in biological samples without preset targets, making them particularly suitable for exploring metabolic characteristics and potential mechanisms in complex diseases. Therefore, in this study, we employed gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) techniques to analyze the serum metabolic characteristics of patients with pregnancy-associated venous thromboembolism (PA-VTE). In this study, 11 pregnant women with VTE and 11 healthy pregnant women were included in the experimental and control groups, respectively. Using GC-MS, we identified 325 metabolites, with the highest proportion being organic oxygen compounds. Using LC-MS, we identified 3104 metabolites, with the highest proportion being acylcarnitine. The results revealed significant differences in the levels of lipids, organic compounds, and other metabolites between patients compared to healthy pregnant women. Pathways such as pyrimidine metabolism, linoleic acid metabolism, and mineral absorption differed between patients with PA-VTE and controls. Furthermore, we identified biomarkers associated with metabolic processes, such as fatty acids and amino acids (2-hydroxyhexanedioic acid, hexadecenal, palmitoylethanolamide, glycerol-1-phosphate, and N-acetyl-beta-D-glucosamine). These findings revealed the metabolic characteristics of PA-VTE and provided important clues for further research on its pathophysiological mechanisms. Our findings may contribute to the development of new diagnostic markers and support early diagnosis and treatment of PA-VTE.

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Data availability statement

The datasets generated and/or analyzed during the current study are not publicly available due to ethical reason but are available from the corresponding author on reasonable request.

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Ethics approval and consent to participate

The informed consent was obtained from the participants and was approved by The Ethics Committee of the International Peace Maternity and Child Health Hospital ((GKLW)2020–03).

Consent for publication

All authors read and approved this manuscript for publication.

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Not applicable.

1. Introduction

Pregnancy-associated venous thromboembolism (PA-VTE) refers to the formation of blood clots during pregnancy or postpartum, typically involving deep vein thrombosis (DVT) and/or pulmonary embolism (PE) [1], [2]. This condition is a significant health concern during pregnancy and the postpartum period, as it poses serious risks not only to the health of the pregnant women but also to the health of the fetus. During pregnancy, coagulation factors increases in the female body, coupled with a slowing of venous return. These changes make pregnant women more prone to thrombosis than non-pregnant women. Additionally, as the fetus grows, uterine compression may affect blood circulation in the abdomen and pelvis, further increasing the risk of VTE. Certain factors may increase the risk of VTE during pregnancy, including genetic predisposition, history of VTE, advanced maternal age, multiple pregnancies, cesarean section, prolonged immobility, obesity, smoking, and pregnancy complications [3]. The typical symptoms of DVT include leg swelling, pain, redness, and warmth, whereas the symptoms of PE include acute chest pain, shortness of breath, rapid heart rate, and coughing up blood. However, the diagnosis of PA-VTE has certain limitations. The symptoms of PA-VTE, such as chest pain, shortness of breath, and coughing, are similar to those of other respiratory or cardiovascular diseases, making early diagnosis challenging [4]. Although CT pulmonary angiography (CTPA) is the gold standard for diagnosing PA-VTE, it carries risks in patients with renal insufficiency or allergy to contrast agents. Moreover, CTPA may have limitations in detecting small or distal clots. Metabolic abnormalities in PA-VTE are directly associated with thrombus formation. Metabolomic studies can reveal differences in the metabolic profiles between patients with PA-VTE and normal populations, including healthy pregnant women, thereby providing new insights for understanding the biological basis of the disease, early diagnosis, therapeutic interventions, and prognosis assessment.

Untargeted metabolomics is a method used to comprehensively analyze all detectable metabolites in biological samples, aiming to identify and quantify both known and unknown compounds. Owing to its independence from a predefined list of metabolites, untargeted metabolomics is commonly used in exploratory research to identify new metabolic biomarkers or pathways [5]. Common techniques include mass spectrometry (MS) and nuclear magnetic resonance (NMR). Gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) are two commonly used analytical methods [6].

GC-MS combines gas chromatography (GC) and mass spectrometry (MS), and is particularly suitable for analyzing volatile and semi-volatile small molecule metabolites, such as fatty acids, organic acids, amino acids, and sugars, owing to its high resolution and sensitivity [7], whereas LC-MS is suitable for analyzing various types of compounds, including non-volatile, polar, and large-molecular-weight compounds such as proteins, lipids, and complex organic molecules. LC-MS is particularly effective for analyzing metabolites in complex biological samples (such as serum, urine, and tissue extracts) [8]. LC-MS has extremely high sensitivity and diversity, can analyze a wide range of compound classes, and is suitable for large-scale metabolome analyses [9]. GC-MS and LC-MS play critical roles in untargeted metabolomics, driving the development of metabolomic research by providing comprehensive coverage of metabolites and in-depth chemical analysis. These two technologies are often used to achieve the most comprehensive analyses of a wide range of metabolites in biological samples [10].

This study aimed to explore the metabolic differences between patients with pregnancy-associated venous thromboembolism (PA-VTE) and healthy pregnant women through systematic metabolomic analysis with the goal of identifying potential biomarkers. We employed advanced untargeted metabolomic techniques, including GC-MS and LC-MS, to comprehensively identify the metabolites in the serum samples of patients with PA-VTE and healthy pregnant women. We used multivariate statistical analysis, employing principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), to assess the overall differences in metabolites between the patients with PA-VTE and healthy pregnant groups. These analytical methods effectively identify the group differences in high-dimensional data and provide a global overview of the disparities in metabolic characteristics. To further identify specific differential metabolites, we conducted univariate analysis to screen for significantly upregulated or downregulated metabolites. Metabolites with variable importance in projection (VIP) values greater than 1 and p-values less than 0.05 in the OPLS-DA model were defined as differential metabolites. These differential metabolites were then subjected to pathway enrichment analysis to identify the key metabolic pathways related to the pathogenesis of PA-VTE. The uniqueness of this study lies in its multilayered analytical approach, which not only accurately identifies differential metabolites through both multivariate and univariate methods,

but also further screens for biomarkers with high diagnostic potential using receiver operating characteristic (ROC) analysis. This comprehensive approach, which integrates multiple analytical techniques, allows effective identification of metabolite biomarkers that are crucial for disease diagnosis and prognosis in complex datasets. Through this study, we systematically revealed specific metabolic alterations in patients with PA-VTE, particularly abnormalities in key pathways such as lipid metabolism, pyrimidine metabolism, linoleic acid metabolism, and mineral absorption. This research not only offers new insights into the metabolic mechanisms of PA-VTE, but also provides robust scientific evidence for early diagnosis and personalized treatment strategies.

2. Materials and methods

2.1. Sample collection and grouping

Eleven patients with PA-VTE who were diagnosed and treated at the International Peace Maternity and Child Healthcare Hospital affiliated with Shanghai Jiaotong University School of Medicine between January and December 2022, and 11 healthy controls during the same period were included in the experimental and control groups, respectively. Basic clinical data, vital signs, routine blood tests, and blood biochemical indices were recorded for all patients. Venous plasma samples were collected.

2.2. Sample preprocessing

For LC-MS samples: Samples stored at −80 °C were thawed at room temperature, and 150 μL of the sample was transferred to a 1.5 mL EP tube. Then, 450 μL of a protein precipitation solution (methanol-acetonitrile, V:V = 2:1, containing L-2-chlorophenylalanine at 2 μg/mL) was added, and the mixture was vortexed for 1 min. The sample was then subjected to ultrasonic extraction in an ice bath for 10 min and left to stand at −40 °C for 2 h. After centrifugation for 10 min (13000 rpm, 4 °C), 150 μL of the supernatant was aspirated with a syringe, filtered through a 0.22 μm organic phase needle filter, transferred to an LC injection vial, and stored at −80 °C until LC-MS analysis (all extraction reagents were pre-cooled at −20 °C before use).

For GC-MS samples: Samples stored at −80 °C were thawed at room temperature, and 150 μL of the sample was transferred to a 1.5 mL centrifuge tube. Then, 450 μL of a methanol-acetonitrile solution (V:V = 2:1, containing L-2-chlorophenylalanine at 2 μg/mL) was added, and the mixture was vortexed for 1 min. The sample was then subjected to ultrasonic extraction for 10 min in an ice bath, followed by standing at −40 °C for 30 min. After centrifugation for 10 min (13000 rpm, 4 °C), 150 μL of the supernatant was transferred to a glass derivatization vial. The sample was dried in a cold centrifugal concentrator, and then 80 μL of methoxyamine hydrochloride pyridine solution (15 mg/mL) was added. After vortexing for 2 min, the vials were incubated at 37 °C for 60 min for oxidation. The sample was then removed, and 50 μL of BSTFA derivatization reagent and 20 μL of n-hexane were added. Ten internal standards (C8/C9/C10/C12/C14/C16/C18/C20/C22/C24, all chloroform-d) were added at 10 μL each. After vortexing for 2 min, the vial was reacted at 70 °C for 60 min. The samples were then incubated at room temperature for 30 min for GC-MS metabolomic analysis.

2.3. LC-MS and GC-MS analysis conditions

2.3.1. LC-MS conditions

Column: ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm); Column temperature: 45 °C; Mobile phase: A - water (containing 0.1 % formic acid), B - acetonitrile (containing 0.1 % formic acid); Flow rate: 0.35 mL/min; Injection volume: 2 μL; Gradient elution: 0.01–2 min, 5 % B; 2–4 min, 5%–30 % B; 4–8 min, 30%–50 % B; 8–10 min, 50%–80 % B; 10–14 min, 80%–100 % B; 14–15 min, 100 % B; 15–15.1 min, 100%–5% B; 15.1–16 min, 5 % B; MS conditions: Ion source: ESI; sample mass spectrometry signal acquisition in positive and negative ion scan modes.

The specific parameter settings are shown in Table 1.

2.3.2. GC-MS conditions

Chromatography Conditions: DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent J&W Scientific, Folsom, CA, USA) was used with high purity helium carrier gas (purity not less than 99.999 %) at a flow rate of 1.0 mL/min. The injection port temperature was set to 260 °C. A 1 μL sample was injected without splitting, and a solvent delay of 5 min was observed. The program ramped as follows: the initial temperature of the column oven was set at 60 °C and held for 0.5 min; then ramped at 8 °C/min to 125 °C; further

Table 1
LC-MS mass spectrometry parameters.

Parameter	Positive ion	Negative ion
Mass Scan Range	100–1200	100–1200
Resolution (full scan)	70000	70000
Resolution (HCD MS/MS scans)	17500	17500
Spray Voltage (V)	3800	−3000
Sheath Gas Flow Rate (Arb)	35	35
Aux Gas Flow Rate (Arb)	8	8
Capillary Temperature (°C)	320	320

ramped at 8 °C/min to 210 °C; then ramped at 15 °C/min to 270 °C; finally, ramped at 20 °C/min to 305 °C and held for 5 min. MS conditions: electron impact ionization source was used with an ion source temperature of 230 °C, quadrupole temperature of 150 °C, and electron energy of 70 eV. The scanning mode was set to full scan with a mass scan range of 50–500 *m/z*.

2.4. Data processing and statistical analysis

Raw LC-MS data were processed using Progenesis QI V2.3 software (Nonlinear Dynamics, Newcastle, UK) for baseline filtering, peak identification, integration, retention time correction, peak alignment, and normalization. The main parameters included 5 ppm precursor tolerance, 10 ppm product tolerance, and 5 % product threshold. Compound identification was based on accurate mass (*m/z*), secondary fragments, and isotopic distribution using the Human Metabolome Database [11], Lipidmaps V2.3, Metlin, EMDB, PMDB, and a custom database for qualitative analysis. The extracted data were further processed to remove any peaks with missing values (ion intensity = 0) in more than 50 % of the groups, by replacing zero values with half the minimum value and filtering based on the qualitative results of the compounds. Compounds with scores lower than 36 (out of 60) were considered inaccurate and removed. Positive and negative ion data were merged into a single data matrix. This matrix was imported into R for PCA to observe the overall distribution between samples and the stability of the entire analysis process. OPLS-DA was used to differentiate metabolites between the different groups [12]. To prevent overfitting, a 7-fold cross-validation and 200 response permutation tests were used to assess the quality of the model. The VIP values obtained from the OPLS-DA model were used to rank the overall contribution of each variable to group differentiation. Furthermore, a two-tailed Student's t-test was used to validate the significance of intergroup differentiating metabolites. Metabolites with VIP values greater than 1.0 and p-values less than 0.05 were selected as differentiating metabolites. Metabolites with an AUC value greater than 0.8 and a p-value less than 0.01 were selected as metabolic biomarkers.

3. Results

3.1. Metabolite identification

We identified and characterized 3104 metabolites using LC-MS, with 1702 in positive-ion mode and 1402 in negative-ion mode. Fatty acyls accounted for the highest proportion at 13.53 %, followed by carboxylic acids and derivatives at 11.47 %, and polyketides had the lowest proportion at 2.03 % (Fig. 1A). Using GC-MS, we identified 325 metabolites, with organooxygen compounds accounting for the highest proportion at 24.04 %, followed by carboxylic acids and derivatives at 19.13 %, and imidazopyrimidines had the lowest proportion at 2.19 % (Fig. 1B).

3.2. Multivariate statistical analysis

PCA is a statistical method that transforms original data into a set of linearly uncorrelated components, called principal components, which are arranged in order of decreasing variance explained by the original dataset [13]. The goal of the PCA is to determine the underlying structure of the data, reduce the dimensionality of the dataset, and retain as much variability as possible. OPLS-DA is an advanced multivariate statistical analytical method [14]. Its core advantage lies in its ability to separate the variation related to the response variable (predictive variation) from the variation unrelated to the response variable (non-predictive variation), thereby enhancing the interpretability and predictability of the model [15]. PCA results showed significant differences in the principal components between the diseased and normal groups (Fig. 2A and D). The distribution of samples from the diseased and normal groups in the OPLS-DA score plot showed a certain degree of separation, indicating that the model could distinguish between these two types of samples within a 95 % confidence interval (Fig. 2B and E). To eliminate overfitting, permutation tests were used to assess whether the OPLS-DA model could predict the results better than the randomly allocated data [16]. In this study, 200 permutation tests were performed, and the results showed that the R2 value was significantly higher than any Q2 value in the permutation test, whereas the Q2 value was slightly lower than zero, indicating that the OPLS-DA model had high validity and predictability (Fig. 2C and F).

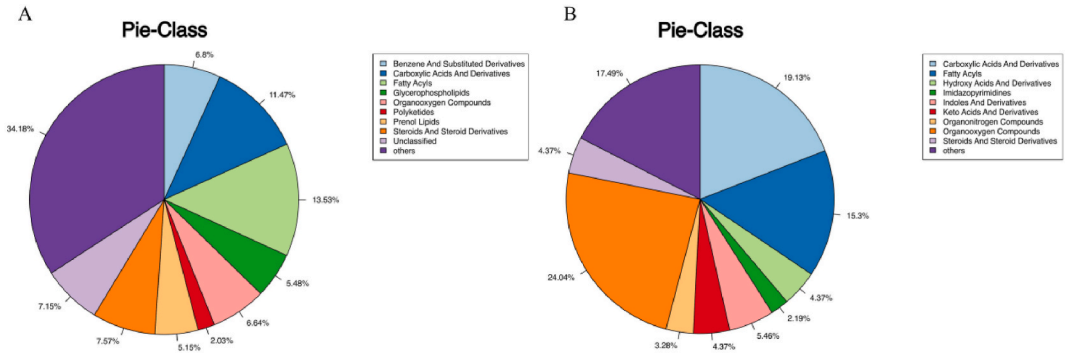

Fig. 1. (A) Pie chart of metabolite classification identified by LC-MS and (B) GC-MS.

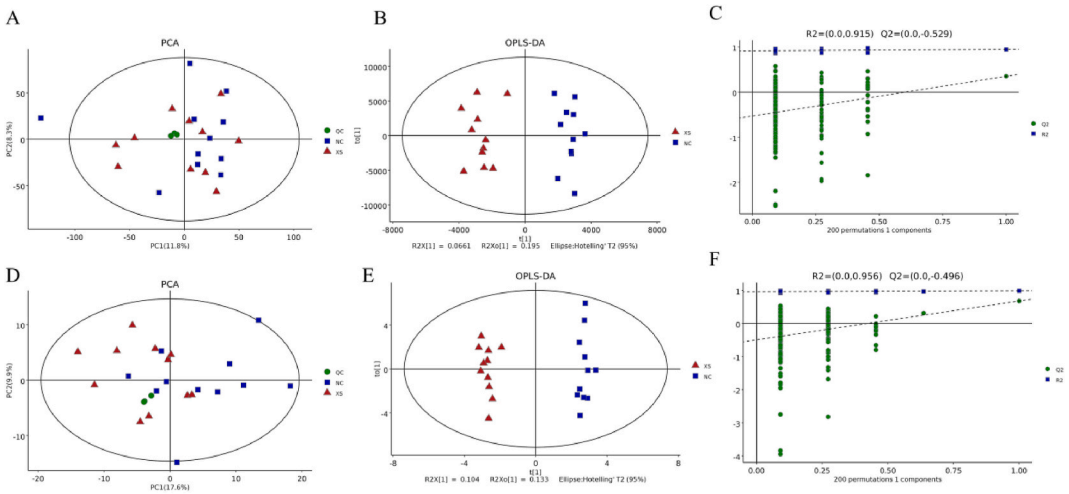

Fig. 2. (A) PCA results of the LC-MS platform. (B) OPLS-DA results of the LC-MS platform. (C) Model validity assessment of the OPLS-DA model on the LC-MS platform. (D) PCA results of the GC-MS platform. (E) OPLS-DA results of the GC-MS platform. (F) Model validity assessment of the OPLS-DA model on the GC-MS platform.

3.3. Univariate analysis

We defined metabolites with p-values less than 0.05 and fold change greater or less than 1 as significantly upregulated or downregulated metabolites, respectively. On the LC-MS platform, 128 metabolites were significantly upregulated, whereas 108 were significantly downregulated (Fig. 3A). Similarly, on the GC-MS platform, 23 metabolites were significantly upregulated, and 34 were significantly downregulated (Fig. 3B)

3.4. Differential metabolite selection and pathway enrichment analysis

The criteria for selecting differential metabolites were a VIP value greater than 1 for the first principal component of the OPLS-DA model and a p-value less than 0.05 for the *t*-test. A total of 71 differential metabolites were identified using LC-MS, including 45 upregulated and 26 downregulated metabolites. The upregulated metabolites included 27 lipids and lipid-like molecules, 5 organic oxygen compounds, 4 benzenoids, 3 organic acids and derivatives, and 3 organoheterocyclic compounds. In contrast, the downregulated metabolites included 10 organoheterocyclic compounds, 6 lipids and lipid-like molecules, 3 organic acids and derivatives, 2 benzenoids, 2 organic oxygen compounds, 1 organosulfur compound, and 1 nucleoside, nucleotide, or analog (Fig. 4A and C, Supplementary Table 1).

A total of 48 differential metabolites were identified using GC-MS, including 18 upregulated and 30 downregulated metabolites. The upregulated metabolites included 3 organic acids and their derivatives, 2 lipids and lipid-like molecules, 2 organic oxygen compounds, 1 organoheterocyclic compound, and 1 phenylpropanoid and polyketide. In contrast, the downregulated metabolites included 6 organic acids and derivatives, 6 organoheterocyclic compounds, 2 organic oxygen compounds, 2 organic nitrogen

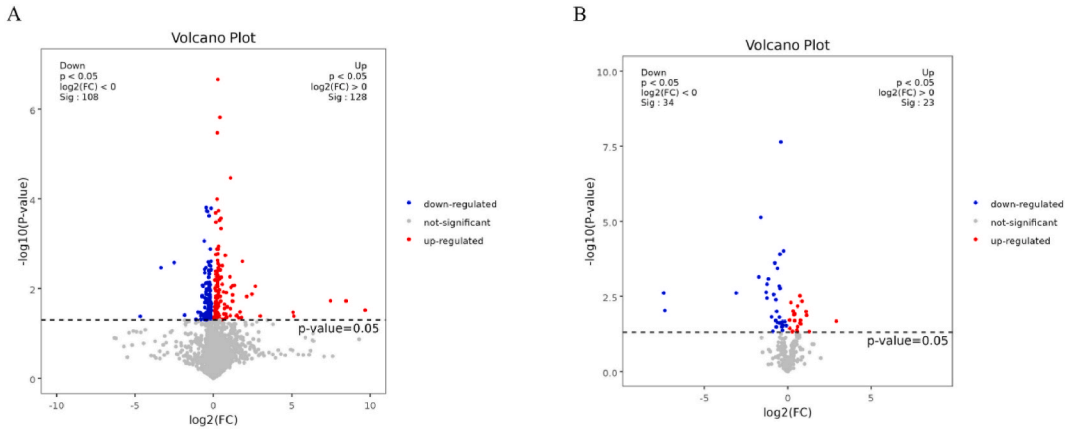

Fig. 3. (A) Volcano plot of the LC-MS platform and (B) the GC-MS platform.

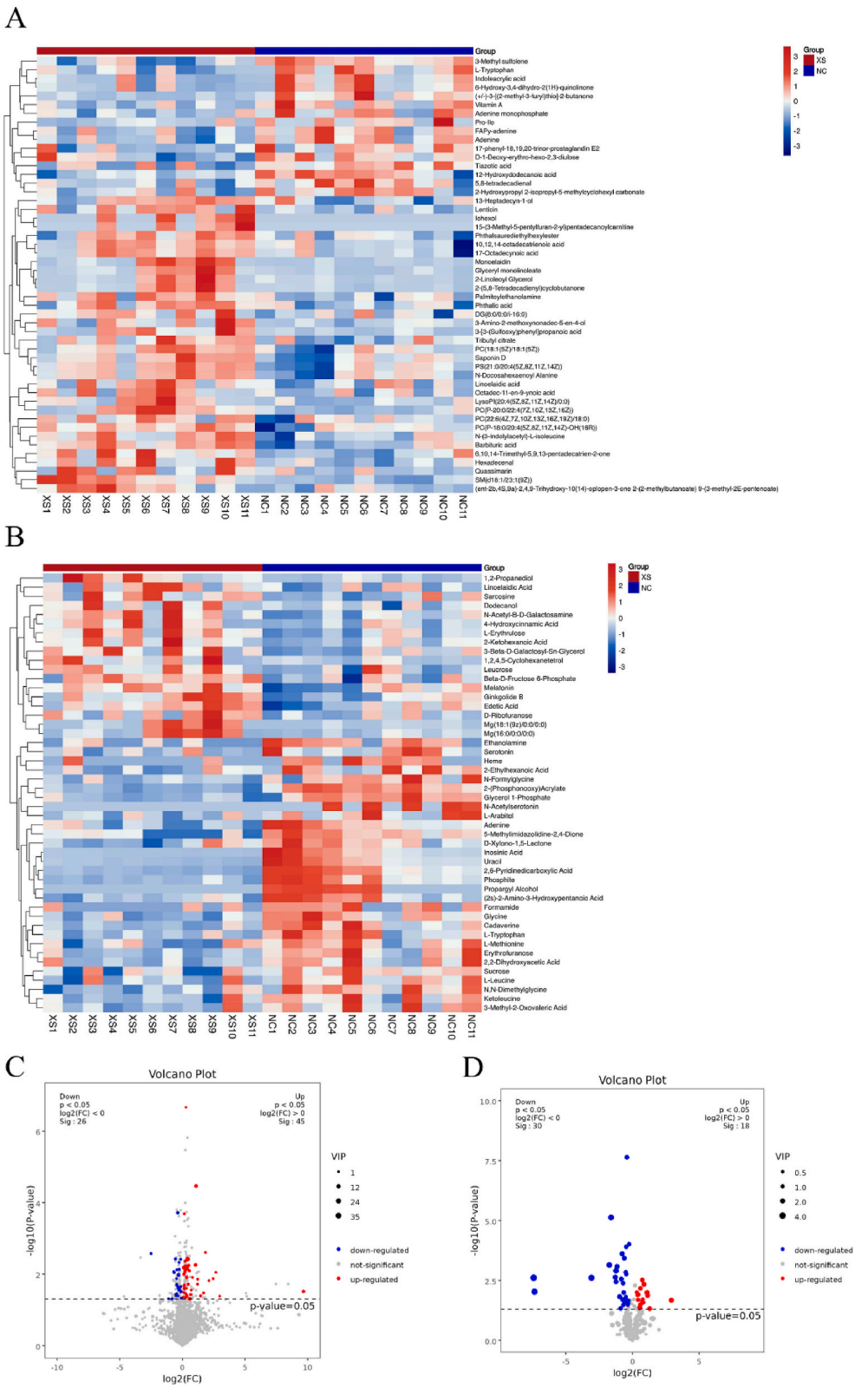

Fig. 4. (A) Heatmap of differential metabolites identified by LC-MS and (B) GC-MS. (C) Volcano plot of differential metabolites identified by LC-MS and (D) GC-MS.

compounds, 1 lipid and lipid-like molecule, and 1 homogeneous nonmetal compound (Fig. 4B and D, Supplementary Table 2).

To elucidate the pathways and biological functions associated with these differential metabolites, we conducted pathway enrichment analysis using the KEGG database to study the mechanisms of metabolic pathway changes between the disease and normal groups. The results indicated significant enrichment in the pyrimidine metabolism, linoleic acid metabolism, mineral absorption, protein digestion and absorption pathways (Fig. 5A and B).

3.5. Selection of potential metabolic biomarkers

We identified the metabolites that showed significant differences between the diseased and normal groups as potential metabolic biomarkers. These biomarkers were evaluated using ROC curve analysis, which provides a method for assessing classifier performance without the need to determine a classification threshold. ROC curves illustrate the performance of a classifier across all possible thresholds, and comparison of the ROC curves and the area under the curve (AUC) of different metabolites allows for an intuitive comparison of their discriminatory abilities. Generally, a larger AUC indicates a better predictive performance of the metabolite. From the differentially identified metabolites, we further screened biomarkers based on a p-value less than 0.01 and an AUC greater than 0.8. The selected biomarkers were 2-hydroxyadipic acid (Fig. 6A, AUC = 0.843), hexadecenal (Fig. 6B, AUC = 0.843), palmitoyl

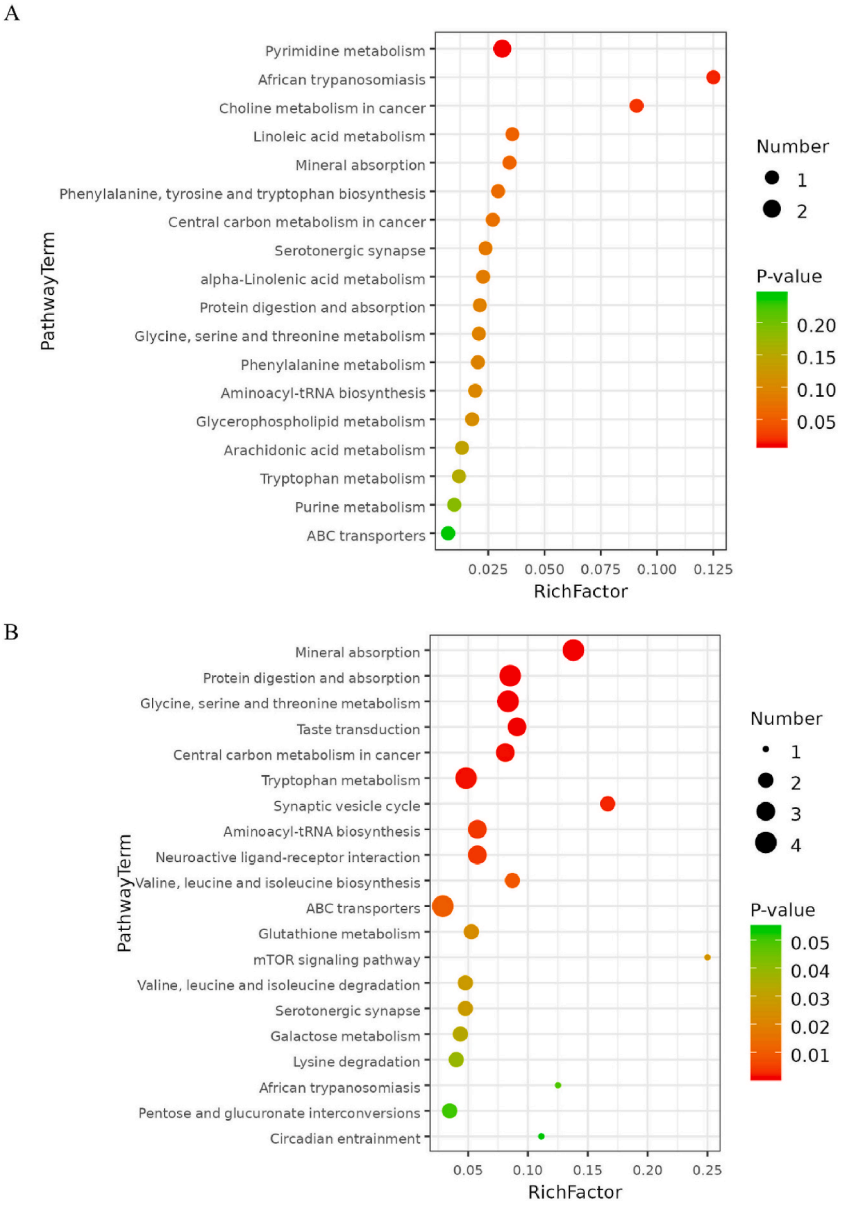

Fig. 5. (A) KEGG bubble plot of differential metabolites identified by LC-MS, and (B) GC-MS.

ethanolamide (Fig. 6C, AUC = 0.835), glycerol-1-phosphate (Fig. 6D, AUC = 0.917), and N-acetyl-β-D-glucosamine (Fig. 6E, AUC = 0.843).

4. Discussion

This study primarily utilized LC-MS and GC-MS to identify and screen for differential metabolites in the serum of patients with PA-VTE and healthy pregnant women. Both PCA and OPLS-DA revealed significant differences in metabolites between these groups. LC-MS identified more metabolites than GC-MS, with a higher number of differentially upregulated metabolites than downregulated metabolites in LC-MS, whereas the opposite was observed in GC-MS. This difference may be due to the fact that LC-MS is more suitable

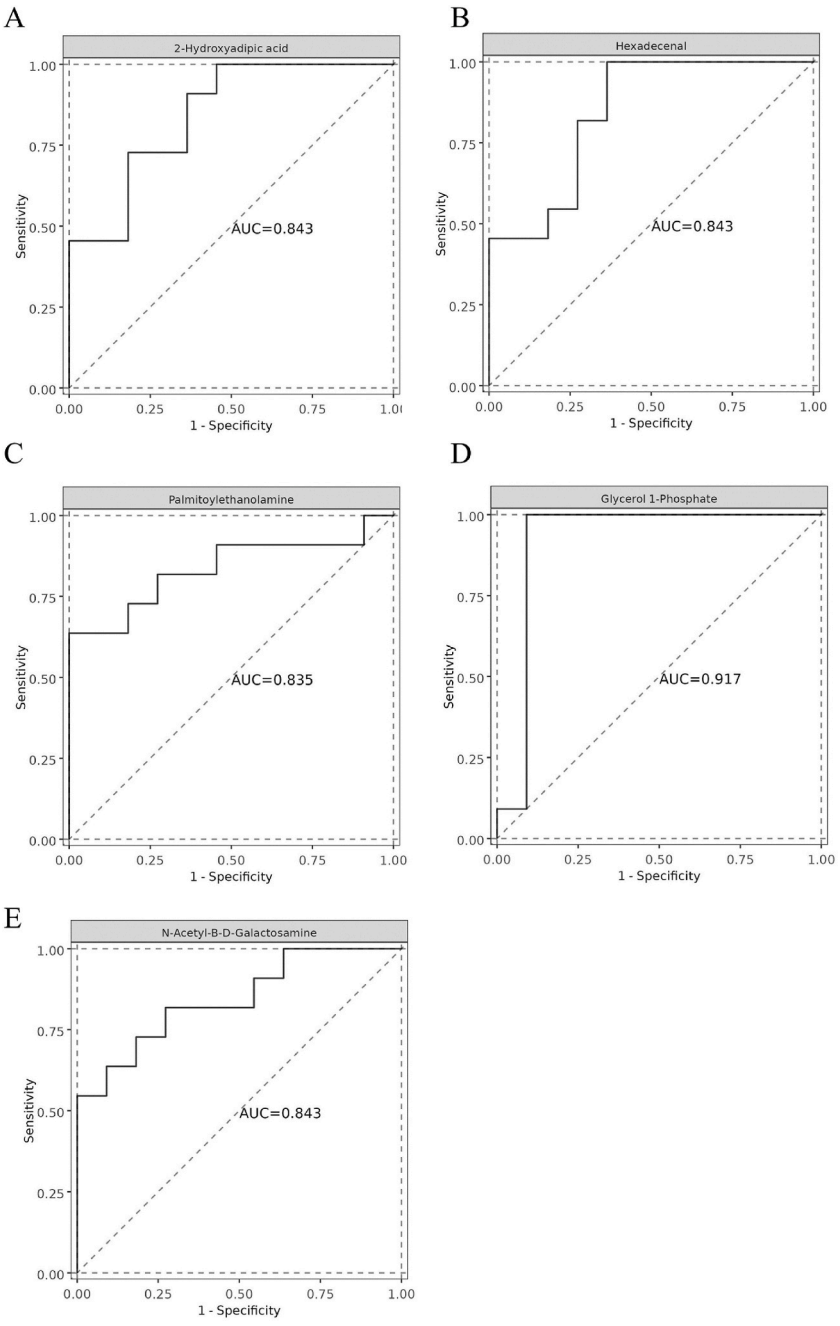

Fig. 6. (A) ROC curves of 2-hydroxyadipic acid, (B) hexadecenal, (C) palmitoyl ethanolamide, (D) glycerol-1-phosphate, and (E) N-Acetyl-B-D-galactosamine.

for analyzing polar and non-volatile compounds, whereas GC-MS is more suitable for analyzing small and volatile compounds [17]. Therefore, LC-MS can detect a wide range of compound types, especially those that are difficult to volatilize or decompose [18].

Pathway analysis revealed differences in the pyrimidine metabolic pathway between patients with PA-VTE and healthy pregnant women, which may have been influenced by genetic factors. Certain genetic variations in the pyrimidine metabolism pathway, such as those affecting enzymes like methylenetetrahydrofolate reductase [19], are associated with an increased risk of thrombosis. These genetic variations may lead to elevated homocysteine levels and increased risk of thrombosis. Mineral absorption abnormalities may also be associated with PA-VTE. Minerals, such as iron, calcium, and magnesium, play crucial roles in blood clotting and vascular health. Therefore, absorption and metabolism may indirectly influence the risk of developing VTE. For example, iron is a key component of red blood cell production, and iron deficiency may lead to anemia, which can affect blood flow. However, excess iron may increase oxidative stress and inflammation, potentially contributing to the risk of thrombosis [20].

Thus, the metabolic biomarkers identified in this study are important. By monitoring changes in these biomarkers, abnormalities can be detected early in the development of PA-VTE, enabling early diagnosis and intervention. Metabolic biomarkers can indicate the pathogenesis of PA-VTE. For example, analyzing the changes in metabolites can elucidate how PA-VTE affects metabolic pathways in the body. The susceptibility to and response to disease treatment may vary among individuals, and metabolic biomarkers can help tailor treatment plans based on individual metabolic characteristics [21]. The identified metabolic biomarkers exhibit various physiological functions and are involved in multiple metabolic processes in humans. 2-hydroxyadipic acid is an organic compound that is a hydroxy derivative of adipic acid. It is involved in several metabolic pathways in the body, the main pathway being lysine degradation. In the lysine degradation pathway, lysine is first converted to saccharopine, which then undergoes several steps to convert it into 2-hydroxyadipic acid [22]. Subsequently, 2-hydroxyadipic acid can be further degraded to acetyl-CoA, which is an intermediate in many important metabolic pathways, including the tricarboxylic acid cycle and the synthesis and degradation of fatty acids. The abnormal accumulation of this substance may be associated with several metabolic diseases. This may be caused by mutations in DHTKD1, which encodes an enzyme involved in the lysine metabolism pathway that converts 2-ketoadipate to glutaryl-CoA. Loss of function of DHTKD1 may lead to the accumulation of 2-ketoadipate and 2-aminoadipate, affecting mitochondrial function and may be associated with certain forms of neurodegenerative diseases [23].

Hexadecenal, also known as hexenal, is a long-chain unsaturated aldehyde comprising 16 carbon atoms. In organisms, long-chain aldehydes are typically intermediate products of fatty acid metabolism, especially in the beta-oxidation of unsaturated fatty acids. During this metabolic process, fatty acids are gradually broken down to form aldehydes and other small molecules, which can be further metabolized into energy or serve as starting materials for other biosynthetic pathways. The 16-carbon aldehyde may also be involved in cellular signal transduction processes. Unsaturated fatty aldehydes such as this compound sometimes serve as signaling molecules involved in regulating inflammatory responses or cell apoptosis, among other biological processes [24]. Unsaturated aldehydes can act as stress signals that affect antioxidant defense mechanisms and apoptotic pathways. Reactive aldehydes such as 4-HNE can interact with transcription factors inside the cells, thereby influencing gene expression [25]. Long-chain unsaturated aldehydes may also participate in intercellular communication, such as in the immune system and in regulating the production and release of cytokines [26].

Palmitoylethanolamide (PEA), an endogenous fatty acid amide, belongs to a class of endocannabinoid-like compounds. It is naturally produced by cells and primarily acts on the endocannabinoid system, an important biological regulatory system involved in various physiological processes. Once synthesized within cells, PEA is released into the extracellular space where it can affect other cells. Its main mechanism of action is through binding to specific receptors, such as peroxisome proliferator-activated receptor-alpha (PPAR-α), thereby regulating inflammation and pain responses [27]. PEA synthesis and degradation are influenced by various physiological and pathological conditions including inflammatory reactions, pain, and cellular stress responses. This indicates that PEA can act as a natural modulator and help to maintain physiological balance [28]. Although PEA does not directly activate cannabinoid receptors, it is associated with the endocannabinoid system and can enhance the effects of endocannabinoids, in part by inhibiting their metabolizing enzymes, such as fatty acid amide hydrolase, thereby indirectly regulating the activity of cannabinoid receptors.

Glycerol 1-phosphate is a biochemical compound formed by the esterification of one hydroxyl group of glycerol with a phosphate group. As a major component of cell membrane phospholipids, glycerol 1-phosphate plays a crucial role in phospholipid synthesis by providing the necessary glycerol backbone for phospholipid production [29]. In glycerolipid metabolism, glycerol 1-phosphate may be involved in methylation reactions, which are ways in which cells regulate their lipid composition. In the biosynthesis of triacylglycerol (fat), glycerol 1-phosphate serves as a glycerol backbone and combines with fatty acids to form lipid molecules. Glycerol 1-phosphate plays a role in the breakdown of fats and the release of energy, particularly in the adipose tissue and the liver.

N- Acetyl-beta-d-glucosamine (GlcNAc) is a monosaccharide derivative that belongs to a class of amino sugars. It is an N-acetylated derivative of glucosamine, in which the amino group of glucosamine is acetylated. GlcNAc is an important component of many biomolecules, including certain types of glycoproteins and glycopeptides, as well as the exoskeleton chitin found in bacteria and insects [30]. In the human body, GlcNAc is a critical component of proteoglycans and glycoproteins in connective tissue, mucin, and other tissues. It participates in various important metabolic processes. GlcNAc plays a role in glycosylation processes on the cell surface, affecting cell signaling [31] and also influences certain aspects of the immune system, particularly in cell surface recognition [32].

5. Limitations

First, the sample size was small, potentially limiting the generalizability of the results. Secondly, the study relied solely on metabolomics, which may have overlooked other factors that could contribute to PA-VTE, such as genetic susceptibility and

environmental factors. Finally, the cross-sectional design of the study may not have captured the changes in metabolite levels over time, thereby limiting the ability to establish causal relationships. Future research should involve larger longitudinal designs and comprehensive assessments of genetic and environmental factors to address these limitations and further elucidate the role of metabolites in PA-VTE.

With a larger sample size, it will be possible to identify additional biomarkers. As the sample size increases, the statistical power improves, allowing for more reliable detection of true metabolic differences while reducing the risk of false positives and false negatives. A larger sample size could make small but important metabolic differences more apparent, allowing them to be identified as potential biomarkers. In a small sample size, the biological variability between individuals may obscure the signals of certain biomarkers. Increasing the sample size can better reflect the metabolic characteristics of the overall population, thereby reducing the impact of random variation and making the true differences more pronounced. Additionally, a larger sample size usually includes more individual differences, such as variations in age, sex, and lifestyle. These individual differences may reveal biomarkers related to specific subgroups, thus providing a more comprehensive and detailed biomarker profile.

6. Conclusion

In this study, we used untargeted metabolomics to analyze the differences in serum metabolites of patients to help develop new biomarkers, thereby improving the early diagnosis and risk assessment of PA-VTE. The downregulation of 2-hydroxyhexanedioic acid, upregulation of 16-carbon aldehyde, upregulation of palmitoylethanolamide, downregulation of glycerol-1-phosphate, and upregulation of N-acetyl-beta-d-glucosamine in both groups indicated an increased risk of thrombosis after childbirth. Among these biomarkers, PEA possesses anti-inflammatory and neuroprotective properties and is commonly used to treat chronic pain and inflammation, especially in cases of neuropathic pain. N-acetyl-B-D-galactosamine is a component of glycosaminoglycans, and owing to its role in glycoprotein and glycolipid synthesis, is typically used in research related to diabetes and other metabolic diseases. Analysis of metabolic changes can provide a deeper understanding of the biological basis and pathogenesis of PA-VTE during pregnancy. Metabolomic analysis can reveal metabolic differences between individuals, helping to tailor personalized prevention and treatment strategies. New treatment targets may be identified by determining the key metabolic pathways related to the disease. Changes in specific metabolites may be related to disease severity and prognosis, aiding in the subsequent treatment of patients.

CRediT authorship contribution statement

Yao Lv: Writing – original draft, Data curation, Conceptualization. **Xianjing Xie:** Writing – review & editing, Data curation. **Hong Shi:** Writing – review & editing, Supervision. **Yuna Guo:** Supervision, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yuna Guo reports financial support was provided by The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China. Yuna Guo reports a relationship with The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38788.

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