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Research Paper

Early diagnosis of the Need for surgical drainage in chronic pancreatitis patients based on serum metabolomics

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ABSTRACT

Purpose: Chronic pancreatitis (CP) is a chronic inflammatory disease caused by multiple factors. Numerous studies have found that implementing surgical drainage in the early stages of CP can help alleviate pain and improve prognosis in patients. However, there is currently no consensus on clinical indications for surgical drainage in the early stages of CP, making it difficult to determine whether surgical drainage is necessary. This study aims to use metabolomics methods to identify potential biomarkers that can differentiate whether CP patients require surgical drainage.

Methods: This study included two cohorts. The training cohort consisted of 32 serum samples from CP patients and 31 serum samples from healthy controls. The validation cohort comprised 73 serum samples from CP patients and 27 serum samples from healthy controls. All serum samples from CP patients were collected within 24 h of hospital admission. Liquid chromatography-tandem mass spectrometry (LC-MS) was used to perform metabolomic analysis on all collected serum samples.

Results: Based on the validation cohort, 24 differential metabolites, including 1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-glycerophosphocholine, PE(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)), and PGP(16:0/20:4(5Z,8Z,11Z,14Z)), were identified as potential biomarkers for distinguishing whether CP patients require surgical drainage. Among these, the combination of 1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-glycerophosphocholine and PE(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)) demonstrated improved diagnostic value in joint ROC analysis, with an AUC value of 0.819 (95% CI: 0.691–0.924).

Conclusion: This study represents the first prospective cohort research to identify 24 differential metabolites in serum through metabolomic profiling, which can be used for the early diagnosis of whether CP patients require surgical drainage.

1. Introduction

Chronic pancreatitis (CP) is a chronic inflammatory disease caused by multiple factors. The persistent inflammation leads to extensive fibrosis of pancreatic tissue, resulting in irreversible damage to the pancreas.[1] The typical clinical features of CP patients include pancreatic exocrine and endocrine insufficiency, as well as abdominal pain, with both incidence and prevalence increasing annually.

Abbreviations: AUC, area under the curve; CP, chronic pancreatitis; PCA, principal component analysis; OPLS-DA, orthogonal partial least-squares discrimination analysis; PLS-DA, partial least-squares discrimination analysis; VIP, variable importance in projection; FC, fold change; ROC, receiver operating characteristic.

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Surgical approaches for CP patients include pancreatic resection, pancreatic duct drainage, and a combination of both.[2] Among these, pancreatic duct drainage is suitable for patients without inflammatory lesions in the pancreatic head and can better preserve pancreatic function.[2] Currently, in clinical practice, surgical drainage is considered for CP patients only when both drug therapy and endoscopic treatment have proven ineffective or are unsuitable. Additionally, this approach is typically pursued when there is no inflammatory mass in the pancreatic head, accompanied by severe pain, significant pancreatic duct dilation, or intense abdominal pain.[2] However, CP patients often endure significant suffering during the progression from initial hospitalization to reaching this stage, and the clinical outcomes tend to be less favorable. In a study on an experimental animal model of chronic obstructive pancreatitis, it was found that the early drainage group showed improvements in pancreatic tissue scores and exocrine pancreatic function.[3] Furthermore, some researchers have found that, compared to the endoscopy group, CP patients in the surgery group demonstrated better pain relief and preservation of pancreatic exocrine function during medium- to long-term follow-up (2–5 years).[4–7] Some observational studies have also indicated that early surgical intervention in CP patients can delay disease progression, while also improving pain management and protecting pancreatic function.[8,9] Compared to late-stage drainage, performing pancreatic duct drainage in the early stages of CP is more effective in alleviating pain.[10,11] Meanwhile, a randomized trial found that patients in the early surgical drainage group showed significant pain relief compared to the conservative treatment group. Moreover, the incidence of new-onset pancreatic endocrine and exocrine dysfunction was markedly reduced in the early surgical drainage cohort compared to the conservative management group.[12] Therefore, biomarkers are needed to early diagnose whether CP patients require surgical drainage. However, there is currently no consensus on clinical indications for surgical drainage in the early stages of CP, and relevant clinical biomarkers are lacking. If patients requiring pancreatic duct drainage can be screened early in the disease course and undergo timely surgical drainage, it will help alleviate pain and improve pancreatic endocrine and exocrine function,[13] this will contribute to the improvement of patients' conditions and their quality of life.

In the field of pancreatic disease research, metabolomics has been widely applied to study the metabolic characteristics of chronic pancreatitis. For example, in a research study designed to differentiate chronic pancreatitis patients from non-pancreatic disease patients, a group of eight metabolites was proposed for diagnosing CP patients. These metabolites include β-carotene, cryptoxanthin, C22:0 fatty acid, IAA, hippurate, D-mannose, C24:1 ceramide, and N-acetylcytidine.[14] Additionally, another study proposed a group of five metabolites and explored their feasibility for the early diagnosis of pancreatic cancer in chronic pancreatitis patients.[15] Furthermore, some researchers have further investigated the differences in metabolic profiles between whether chronic pancreatitis is acutely exacerbated.[16] Recent studies have revealed that patients with type 3c diabetes mellitus (T3cDM) secondary to CP exhibit significantly elevated levels of sphingosine, carnitine, bile acids, and most lipid species compared to those with type 2 diabetes mellitus (T2DM).[17] Currently, many biomarkers related to pancreatic diseases have been identified, but none have been able to determine whether CP patients require surgical drainage in the early stages of the disease. This study, based on metabolomics, aims to explore biomarkers that can diagnose whether patients with chronic pancreatitis require surgical drainage in the early stages of the disease. The goal is to provide more precise and effective evidence and guidance for the clinical diagnosis and treatment of CP patients.

2. Methods

2.1. Clinical samples

The research enrolled 163 participants in total, The training cohort consisted of 32 serum samples from CP patients (including 11 samples from patients who underwent surgical drainage and 21 samples from patients who did not require surgical drainage) and 31 serum samples from healthy controls, collected between February 2021 and May 2021. The validation cohort included 73 serum samples from CP patients (including 43 samples from patients who underwent surgical drainage and 30 samples from patients who did not require surgical drainage) and 27 serum samples from healthy controls, collected between September 2021 and March 2022 (Fig. 1.). All serum samples from CP patients were collected within 24 h of hospital admission.

The diagnosis of CP is based on the following criteria: (1) imaging studies revealing characteristic CP-related findings; (2) histopathological analyses revealing typical CP-associated alterations; (3) patients presenting with persistent epigastric pain or abdominal discomfort without identifiable etiology; (4) abnormal levels of blood or urinary pancreatic enzymes; (5) impaired pancreatic exocrine function; and (6) pancreatic endocrine dysfunction. A confirmed diagnosis can be made if one of the typical manifestations in (1) or (2) is present, or if suspected manifestations in (1) or (2) are accompanied by any two of (3), (4), (5), or (6).[2].

Participants were selected based on the following criteria: (1) fulfilling the diagnostic requirements for chronic pancreatitis, (2) possession of complete medical records, and (3) submission of signed consent forms.

The inclusion standards for surgical drainage (pancreatic duct drainage) in this study are: (1) drug therapy and endoscopic treatment are either unsuitable or ineffective; (2) absence of inflammatory masses in the pancreatic head; (3) substantial widening of the main pancreatic duct (diameter $\geq$ 7 mm); (4) main pancreatic duct lithiasis; and (5) recurrent and severe abdominal pain.[2].

The study implemented these exclusion standards: (1) current pregnancy or nursing status; (2) presence of autoimmune diseases; and (3) malignant conditions, such as tumors.

All blood serum specimens were preserved at −80 degrees Celsius.

2.2. Sample processing

Following specimen thawing at ambient temperature, aliquots of 100 μL were prepared from each sample. L-2-chlorophenylalanine (20 μL) was introduced, and the solution was vortex-mixed for 10 s. Subsequently, 300 μL of protein precipitation reagent was incorporated, followed by vigorous vortexing for 60 s. The mixtures underwent ultrasonic treatment in an ice-water bath for 10 min, then were maintained at −20 °C for 30 min. Centrifugation was performed at 13,000 rpm (4 °C) for 10 min, after which 300 μL of the supernatant was collected and evaporated. The resulting residue was dissolved in 300 μL of methanol–water (1:4, v/v), vortexed for 30 s, and sonicated for 3 min. The solutions were stored at −20 °C for 2 h and centrifuged again under identical conditions. For LC-MS analysis, 150 μL of the final supernatant was transferred to injection vials.

2.3. LC-MS analysis

The analytical system comprised a Dionex UltiMate 3000 UHPLC unit (Thermo Fisher Scientific, USA) interfaced with a Q-Exactive HF Orbitrap mass analyzer (Thermo Fisher Scientific, USA). Mass spectral data acquisition was conducted using dual-polarity detection mode, encompassing both positive and negative electrospray ionization.

The acquisition of raw spectral data was performed utilizing Xcalibur 4.2 SP1 software (Thermo Fisher Scientific). Subsequent data preprocessing for multivariate analysis was executed through Progenesis QI v2.3 (Nonlinear Dynamics, UK), incorporating multiple processing steps: baseline correction, peak detection and integration, retention time adjustment, chromatographic alignment, and data normalization.

Compound identification was achieved through a multi-parameter approach, incorporating exact mass measurements, MS/MS

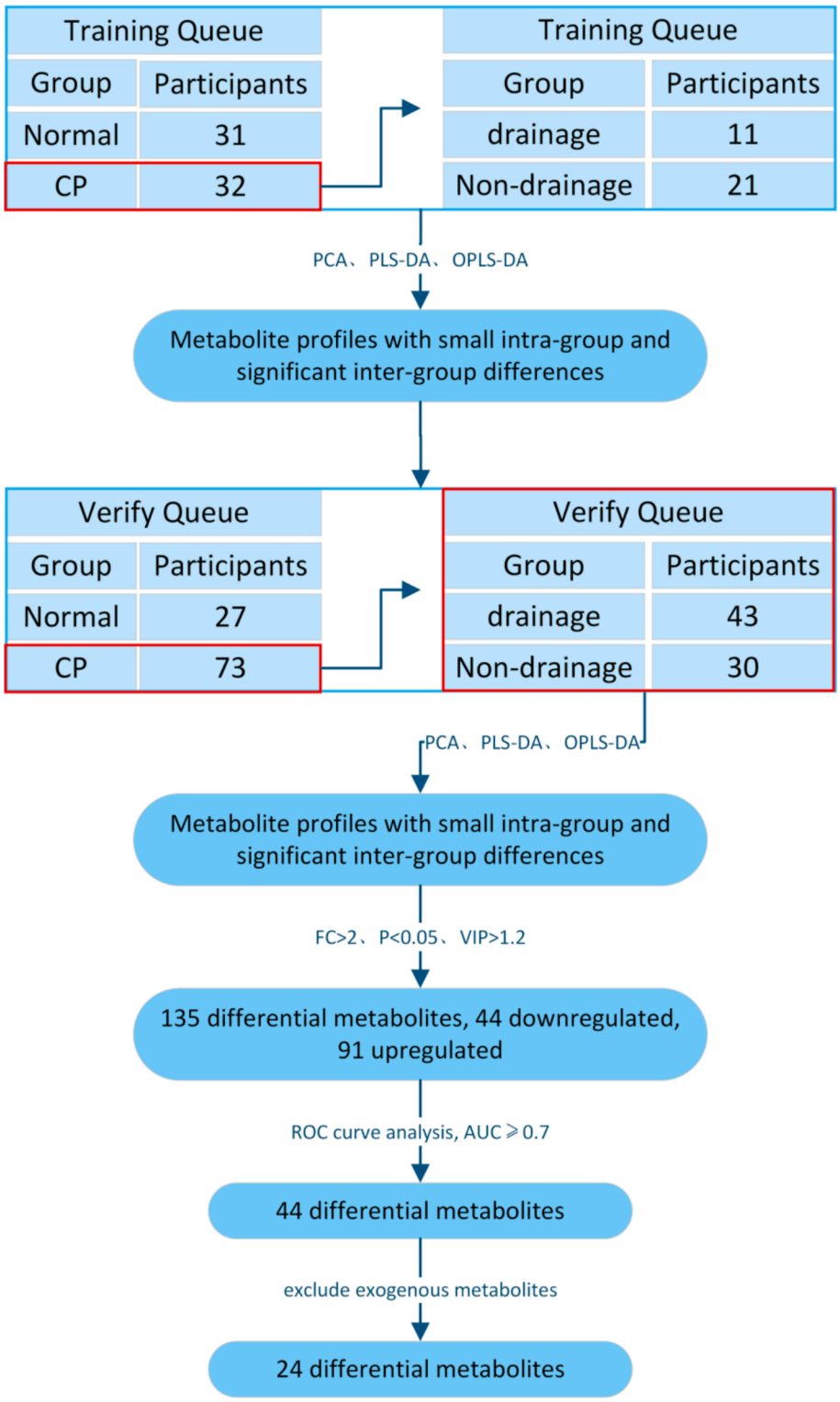

Fig. 1. Flowchart.

fragmentation patterns, and isotopic abundance ratios. Structural characterization was conducted by querying against three major metabolomics databases: HMDB (Human Metabolome Database), LipidMAPS (version 2.3), and METLIN.

Ion peaks exhibiting over 50 % missing data within groups were discarded from the dataset, and zero values were substituted with half the lowest detected value. Compounds characterized through structural identification were filtered using a scoring algorithm, with a passing threshold of 36 points (out of a maximum of 60 points). Scores < 36 were deemed unreliable. The final data integration step involved combining positive and negative ionization mode datasets into a unified data matrix, encompassing all relevant analytical information extracted from the raw spectral data for subsequent statistical analysis. All subsequent analyses were based on this data matrix.

2.4. Results analysis

The integrated dataset was subsequently processed using MetaboAnalyst version 6.0, a web-based metabolomics analysis platform (accessible at https://www.metaboanalyst.ca/). Data were filtered based on the interquartile range (IQR), retaining 60 % of the data. Subsequently, the sample data were subjected to normalization: normalization using the constant sum method was applied to adjust for systematic differences between samples. Data transformation: logarithmic transformation with base 10 was performed to make the data more normally distributed. Data scaling: Pareto scaling was applied.

Multivariate statistical analyses, including PCA, PLS-DA, and OPLS-DA, were performed on the processed datasets to assess inter-group variations in metabolic profiles. These analytical approaches were employed to identify potential significant differences in metabolite composition among the study groups. A 100-iteration permutation test was performed through the OPLS-DA model to verify whether the model was overfitted. The Variable Importance in Projection (VIP) metric quantifies the relative contribution of each variable within the OPLS-DA model, reflecting its significance in the projection space.

Subsequently, the data were analyzed using T-tests and FC (Fold Change) analysis, and the results were imported into GraphPad Prism 10.1.2 to generate volcano plots. The differential metabolites identified were further analyzed using the biomarker analysis module on the MetaboAnalyst 6.0 website (https://www.metaboanalyst.ca/). The diagnostic performance of differential metabolites was assessed through ROC (Receiver Operating Characteristic) curve analysis, with AUC (Area Under the Curve) values calculated to quantify their discriminatory power and predictive accuracy. Finally, the obtained differential metabolites were matched with The Human Metabolome Database (HMDB) to rule out metabolites suggesting exogenous metabolites.[18].

After obtaining the potential biomarkers, a combined ROC analysis of the biomarkers was performed using the MetaboAnalyst 6.0 website (https://www.metaboanalyst.ca/) to determine the biomarker combination with the best diagnostic performance.

3. Results

3.1. Research cohort and clinical characteristics

The clinical features of both the training and validation cohorts are presented in Tables 1 and 2. Within the validation cohort, no significant differences were observed in age, gender, underlying diseases, or complication rates between the drainage group and the non-drainage group.

3.2. Metabolomic profiling

Within the training cohort, comparative analysis of serum metabolic profiles was performed between 32 chronic pancreatitis patients and 31 healthy control subjects, confirming variations in metabolite compositions between the CP and control groups (Fig. 2A, B, C). The CP cohort was further classified into a surgical drainage cohort and a non-surgical drainage cohort based on whether surgical drainage was performed during hospitalization, and variations in metabolic profiles were detected (Fig. 2D). For validation purposes, an additional set of serum samples comprising 73 CP patients and 27 healthy controls was collected to identify more precise biomarkers for differentiating between CP and control groups, as well as between surgical and non-surgical drainage subgroups. LC-MS analysis identified 56,409 spectral peaks corresponding to 18,126 distinct metabolites.

Table 1
Clinical characteristics of CP and Normal groups.

Training Cohort	CP(N = 32)	Normal(N = 31)	P value
Age, years (M (P_{25}, P_{75}))	56(46.25 ~ 64.50)	43(34 ~ 47)	0.127
Male	18(56, 2 %)	16(51.6 %)	0.712
Validation Cohort	CP(N = 73)	Normal(N = 27)	P value
Age, years (M (P_{25}, P_{75}))	52(39 ~ 64)	45(39 ~ 51)	0.053
Male	18(24.7 %)	15(55.6 %)	0.004

Table 2
Clinical characteristics of drainage and non-drainage groups.

Training Cohort	Drainage(N = 11)	Non-drainage(N = 21)	P value
Age, years (M (P_{25}, P_{75}))	55(46 ~ 62)	57(45.5 ~ 66)	0.349
Male	5(45.5 %)	13(61.9 %)	0.606
Diabetes	2(18.2 %)	10(47.6 %)	0.212
Hypertension	4(36.4 %)	4(19.0 %)	0.519
Validation Cohort	Drainage(N = 43)	Non-drainage(N = 30)	P value
Age, years (M (P_{25}, P_{75}))	55(42 ~ 64)	45.5(36 ~ 64)	0.252
Male	10(23.3 %)	8(26.7 %)	0.739
Diabetes	15(34.9 %)	11(36.7 %)	0.876
Hypertension	16(37.2 %)	14(46.7 %)	0.419
Pancreatic edema	3(7 %)	1(3.3 %)	0.880
Peripancreatic exudate	3(7 %)	6(20 %)	0.192
Pancreatic duct stones	12(27.9 %)	4(13.3 %)	0.139
Pancreatic duct dilation	8(18.6 %)	2(6.7 %)	0.265
Peripancreatic abscess	4(9.3 %)	1(3.3 %)	0.601
Pancreatic cyst	7(16.3 %)	2(6.7 %)	0.386
Pancreatic pseudocyst	11(25.6 %)	7(23.3 %)	0.826
Portal hypertension	3(7 %)	5(16.7 %)	0.356
Hyperlipidemia	4(9.3 %)	8(26.7 %)	0.099
Bile duct stones	10(23.3 %)	7(23.3 %)	0.994
Cholecystitis	11(25.6 %)	7(23.3 %)	0.826
Fatty liver	7(16.3 %)	4(13.3 %)	0.989
Hepatic cyst	5(11.6 %)	7(23.3 %)	0.314
Splenomegaly	5(11.6 %)	3(10 %)	1.000
Abdominal pain	42(97.7 %)	28(93.3 %)	0.749
Back pain	4(9.3 %)	4(13.3 %)	0.872
Total Bilirubin(μmol/L)	12.71(5.80 ~ 23.00)	11.06(8.10 ~ 14.14)	0.824
Direct Bilirubin(μmol/L)	5.65(2.60 ~ 11.76)	4.32(2.88 ~ 5.48)	0.222
Indirect Bilirubin(μmol/L)	4.83(3.34 ~ 7.75)	6.05(4.49 ~ 8.98)	0.132
Alkaline Phosphatase(U/L)	87.20(65.60 ~ 124.60)	73.25(57.58 ~ 102.05)	0.400
γ-GT(U/L)	41.60(25.00 ~ 96.10)	32.1(19.53 ~ 128.05)	0.711
Blood Urea Nitrogen (mmol/L)	4.52(3.55 ~ 5.55)	4.74(3.40 ~ 6.39)	0.695
Creatinine(μmol/L)	60.00(46.25 ~ 71.00)	64.68(51.75 ~ 76.75)	0.386

3.3. Differential metabolite analysis

In the training cohort, the serum metabolite profiles of the Normal and CP groups were subjected to Principal Component Analysis (PCA), revealing small differences in metabolites within groups but significant differences between groups. This indicates significant variations in metabolic profiles between healthy controls and chronic pancreatitis patients (Fig. 2E). Partial Least Squares Discriminant Analysis (PLS-DA) represents a supervised discriminant analysis method that enhances the separation between groups, reduces within-group variations while enhancing between-group distinctions. Orthogonal PLS-Discriminant Analysis (OPLS-DA) further removes classification-independent noise, improving the interpretability and visualization of the model. Using PLS-DA and Orthogonal PLS-DA methods to analyze the metabolite compositions of the Normal and CP groups, the results showed significant inter-group variations in metabolic profiles between healthy controls and CP patients (Fig. 2F, G). Similarly, OPLS-DA analysis of the metabolite profiles of the drainage and non-drainage groups demonstrated distinct separation and significant variations between the groups (Fig. 2H). The VIP scores obtained from the OPLS-DA models were employed to identify metabolites with VIP scores exceeding 1.2, which were considered more meaningful and thus selected for further analysis.

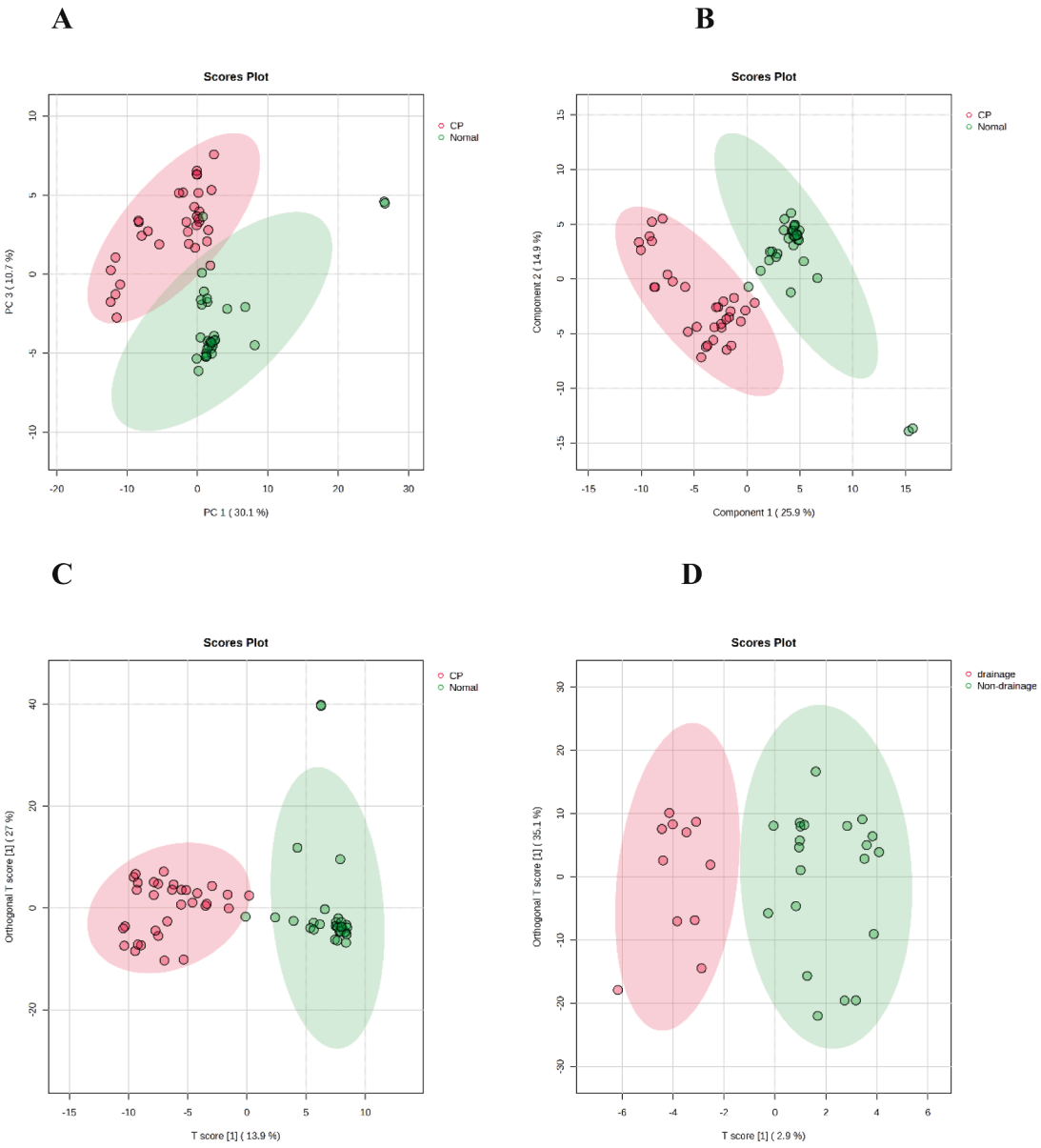

Fig. 2. Multivariate analysis of CP and Normal groups: (A, E) Principal Component Analysis (PCA), (A) training cohort, (E) validation cohort; (B, F) Partial Least Squares Discriminant Analysis (PLS-DA), (B) training cohort, (F) validation cohort; (C, G) Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), (C) training cohort, (G) validation cohort; multivariate statistical analysis of drainage and non-drainage groups: (D, H) Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), (D) training cohort, (H) validation cohort.

In the validation cohort, Student's t-tests and Fold Change (FC) analysis were used to statistically analyze the metabolic data of the Normal and CP groups, as well as the drainage and non-drainage groups, and volcano plots were generated. Differential metabolites between the two groups were identified. With FC $>$ 2 and P $<$ 0.05 as thresholds, 958 differential metabolites were found between control and CP groups, of which 468 were significantly downregulated and 490 were significantly upregulated (Fig. 3A). For the drainage and non-drainage groups, 137 differential metabolites were identified, with 44 significantly downregulated and 93 significantly upregulated (Fig. 3B).

The identification of differential metabolites was achieved through an integrated approach combining both multivariate and univariate statistical analyses. Specifically, based on FC $>$ 2.0, P $<$ 0.05, and VIP $>$ 1.2, 533 differential metabolites were identified in the validation cohort distinguishing CP and Normal groups, of which 231 were significantly downregulated and 302 were significantly upregulated. In the validation cohort distinguishing drainage and non-drainage groups, 135 differential metabolites were identified, with 44 significantly downregulated and 91 significantly upregulated. Subsequently, ROC analysis was utilized to evaluate the diagnostic performance of these differential metabolites for the disease. Among the 533 differential metabolites screened in the validation study distinguishing CP and Normal groups, 468 had AUC values $\geq$ 0.7, indicating that these differential metabolites possess strong diagnostic value. Examples include Acetylsalvipisone, Ethosuximide, 5alpha-Pregnan-3beta,20beta-diol 3-sulfate, N-Methyl-1H-indole-3-propanamide, (E,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl octanoate, and N6-Acetyl-L-lysine. In the validation study distinguishing the drainage and non-drainage groups, 135 differential metabolites were screened, of which 44 had AUC values $\geq$ 0.7. After excluding exogenous metabolites such as Cefpimizole, Azithromycin, and Clofop, 24 potential biomarkers with good diagnostic value were identified. These include 1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-

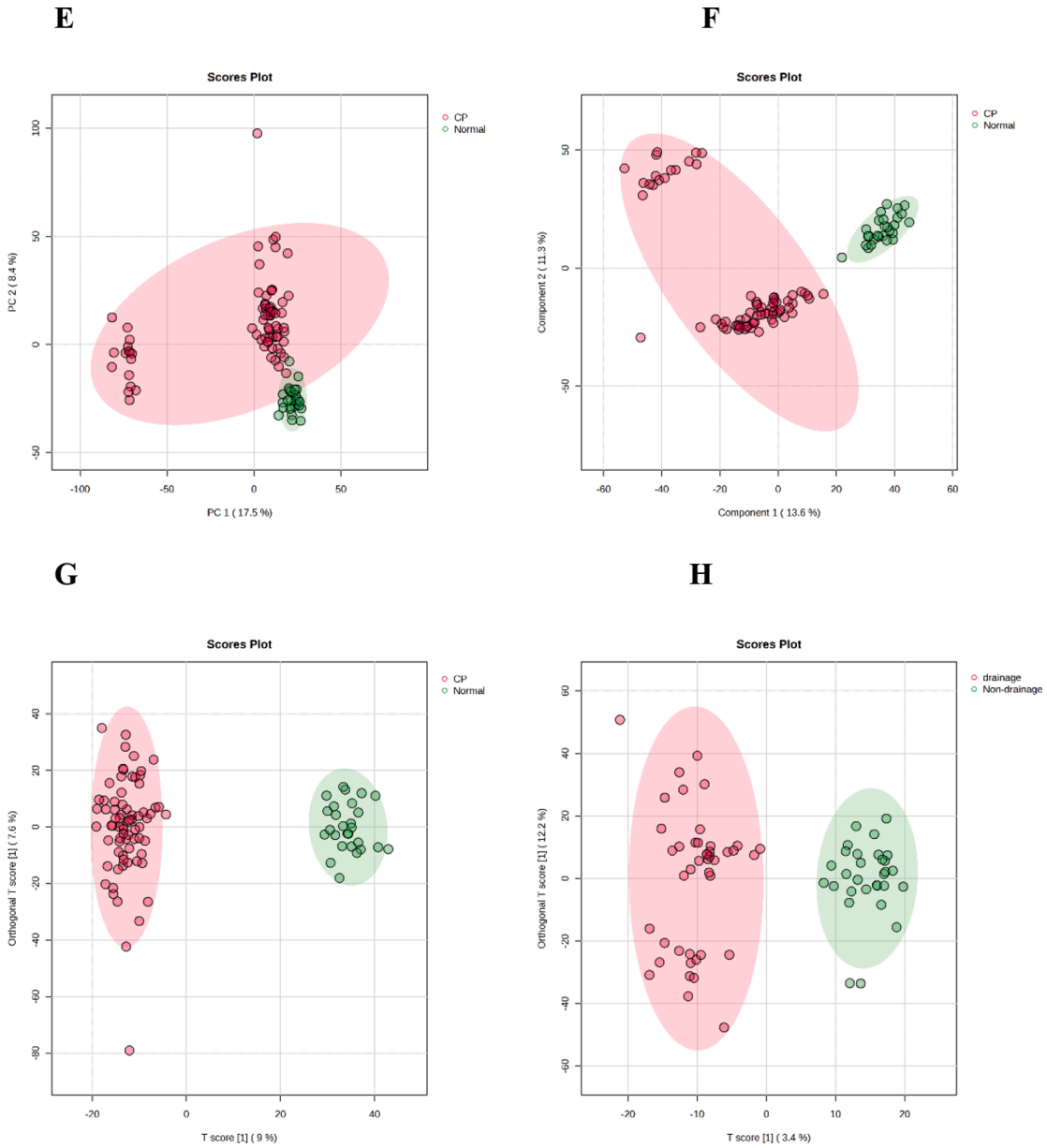

Fig. 2. (*continued*).

glycerophosphocholine, PE(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)), PGP(16:0/20:4(5Z,8Z,11Z,14Z)), CerP(d18:1/18:0), PI(12:0/17:2(9Z,12Z)), 1-(2-methoxy-tetracosanyl)-*sn*-glycero-3-phosphoserine, Deoxycholic acid, PE(18:1(11Z)/18:2(9Z,12Z)), PE(17:0/18:2(9Z,12Z)), PI(12:0/15:1(9Z)), TG(10:0/8:0/8:0), PS(20:0/16:1(9Z)), Thiamine aldehyde, Presqualene diphosphate, lithocholic acid sulfate, PE(16:0/20:5(5Z,8Z,11Z,14Z,17Z)), PtdIns-(1,2-dihexanoyl), PS(O-20:0/18:4(6Z,9Z,12Z,15Z)), GlcCer(d15:1/18:0), PE(20:4(5Z,8Z,11Z,14Z)/P-18:1(11Z)), PE(18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)), PS(17:1(9Z)/12:0), 2-Hydroxy-4-methoxyacetophenone 5-sulfate, and Sphingosylphosphoryl choline (VIP $>$ 1.2, FC $>$ 2, P $<$ 0.05, and AUC $\geq$ 0.7) (Table 3, Fig. 4). Among these, the combination of 1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-glycerophosphocholine and PE(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)) was subjected to joint ROC analysis, achieving higher diagnostic performance exhibiting an AUC value of 0.819 (95 % confidence interval: 0.691–0.924) (Fig. 5).

4. Discussion

According to medical guidelines, there is currently no consensus on clinical indications for surgical drainage in the early stages of CP, and relevant clinical biomarkers are lacking. This study is the first to demonstrate that 24 metabolites in serum samples can differentiate whether CP patients require surgical drainage in the early stages of the disease with acceptable diagnostic performance (AUC $\geq$ 0.7).

In this study, metabolomics research successfully constructed PCA, PLS-DA, and OPLS-DA models, confirming considerable inter-group variations between the drainage and non-drainage groups. Subsequently, volcano plots were generated using T-tests and FC analysis, and ROC curve analysis was performed on the differential metabolites to screen for reliable biomarkers. Ultimately, 24 potential biomarkers were identified in the drainage and non-drainage groups, including 1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-glycerophosphocholine, PE(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)), PGP(16:0/20:4(5Z,8Z,11Z,14Z)), CerP(d18:1/18:0), PI(12:0/17:2(9Z,12Z)), 1-(2-methoxy-tetracosanyl)-*sn*-glycero-3-phosphoserine, Deoxycholic acid,

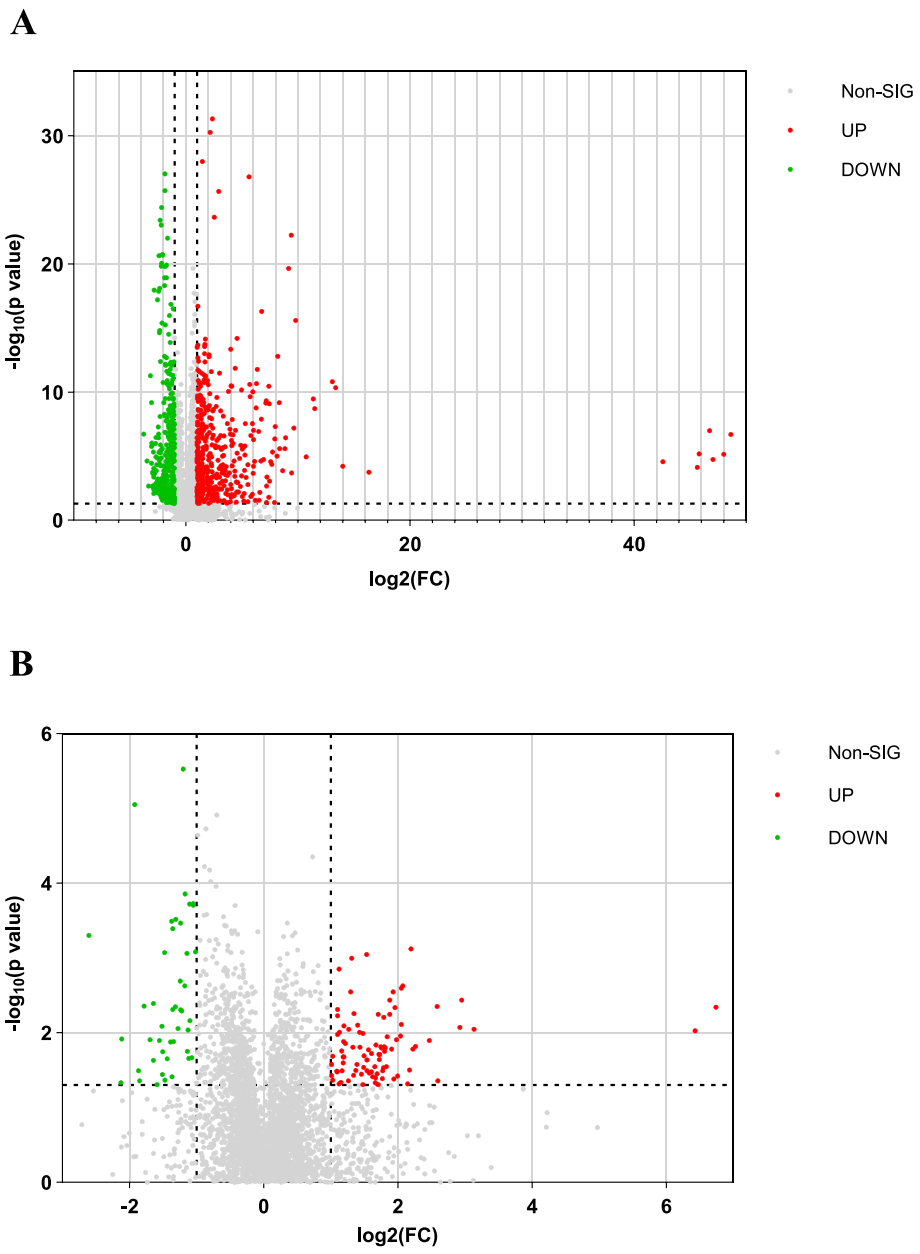

Fig. 3. Volcano plots of CP and Normal groups in the validation study (A), and drainage and non-drainage groups in the validation study (B); gray dots indicate no significant differences, red dots on the right represent upregulated metabolites, and green dots on the left represent downregulated metabolites. The X-axis corresponds to log2(FC), and the Y-axis corresponds to −log10(P value).

PE(18:1(11Z)/18:2(9Z,12Z)), PE(17:0/18:2(9Z,12Z)), PI(12:0/15:1(9Z)), TG(10:0/8:0/8:0), PS(20:0/16:1(9Z)), Thiamine aldehyde, Presqualene diphosphate, lithocholic acid sulfate, PE(16:0/20:5(5Z,8Z,11Z,14Z,17Z)), PtdIns-(1,2-dihexanoyl), PS(O-20:0/18:4(6Z,9Z,12Z,15Z)), GlcCer(d15:1/18:0), PE(20:4(5Z,8Z,11Z,14Z)/P-18:1(11Z)), PE(18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)), PS(17:1(9Z)/12:0), 2-Hydroxy-4-methoxyacetophenone 5-sulfate, and Sphingosylphosphoryl choline.

1-(6-[3]-Ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-glycerophosphocholine is a derivative of glycerophosphocholine. Researchers have found that in nude mice implanted with human pancreatic cancer, the levels of glycerophosphocholine in the pancreas are significantly reduced.[19] Additionally, in an animal study, researchers observed elevated levels of glycerophosphocholine in the pancreas of rats with chronic pancreatitis.[20] However, some scholars have found that in a porcine obstructive CP model, the levels of glycerophosphocholine were reduced in the pancreas of mild CP.[21] In our study, 1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-glycerophosphocholine also exhibited a corresponding downward trend. The mechanism of action of glycerophosphocholine in CP patients requires further investigation.

PE(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)) and five other metabolites belong to phosphatidylethanolamine (PE). In mice with hypertriglyceridemic acute pancreatitis (HTG-AP), glycerophospholipid (GPL) metabolism is excessively activated, and the transcriptional expression of GPL enzymes involved in the synthesis of phosphocholine and

Table 3
24 potential biomarkers screened in the drainage and non-drainage groups.

NAME	P value	Log2FC	VIP	AUC
1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-glycerophosphocholine	8.88×10^{-6}	−1.92	2.94	0.75
PE(18:1(9Z)/20:4(5Z,8Z,11Z,14Z))	2.96×10^{-6}	−1.19	2.88	0.74
PGP(16:0/20:4(5Z, 8Z, 11Z, 14Z))	4.60×10^{-3}	1.96	1.97	0.74
CerP(d18:1/18:0)	2.20×10^{-2}	−1.11	1.42	0.74
PI(12:0/17:2(9Z,12Z))	3.16×10^{-2}	2.18	1.50	0.73
1-(2-methoxy-tetracosanyl)-*sn*-glycero-3-phosphoserine	3.65×10^{-3}	1.86	2.09	0.73
Deoxycholic acid	3.62×10^{-2}	−1.54	1.36	0.73
PE(18:1(11Z)/18:2(9Z,12Z))	3.41×10^{-4}	−1.35	2.25	0.73
PE(17:0/18:2(9Z,12Z))	2.03×10^{-3}	−1.33	1.92	0.72
PI(12:0/15:1(9Z))	2.35×10^{-3}	2.07	2.21	0.72
TG(10:0/8:0/8:0)	9.43×10^{-3}	6.88	1.73	0.72
PS(20:0/16:1(9Z))	4.39×10^{-2}	−1.97	1.31	0.72
Thiamine aldehyde	8.17×10^{-3}	−1.74	1.79	0.72
Presqualene diphosphate	1.13×10^{-2}	1.84	1.91	0.71
lithocholic acid sulfate	1.21×10^{-2}	−2.30	1.78	0.71
PE(16:0/20:5(5Z,8Z,11Z,14Z,17Z))	8.64×10^{-4}	−1.31	2.16	0.71
PtdIns-(1,2-dihexanoyl)	8.93×10^{-3}	3.01	1.74	0.71
PS(O-20:0/18:4(6Z,9Z,12Z,15Z))	1.39×10^{-4}	−1.34	2.29	0.71
GlcCer(d15:1/18:0)	4.06×10^{-4}	−1.48	2.30	0.71
PE(20:4(5Z,8Z,11Z,14Z)/P-18:1(11Z))	8.29×10^{-4}	−1.14	1.97	0.71
PE(18:2(9Z,12Z)/22:6 (4Z,7Z,10Z,13Z,16Z,19Z))	1.97×10^{-4}	−1.18	2.42	0.71
PS(17:1(9Z)/12:0)	1.54×10^{-2}	1.79	1.59	0.70
2-Hydroxy-4-methoxyacetophenone 5-sulfate	2.33×10^{-2}	−1.89	1.51	0.70
Sphingosylphosphoryl choline	1.69×10^{-2}	1.54	1.81	0.70

phosphatidylethanolamine in the liver are significantly upregulated.[22] However, our study found that the six phosphatidylethanolamines (PE) in the drainage group showed a significant downward trend, which may be associated with impaired glycerophospholipid metabolic pathways in chronic pancreatitis patients necessitating surgical drainage intervention.

PGP(16:0/20:4(5Z,8Z,11Z,14Z)) is a phosphatidylglycerophosphate (PGP), a type of glycerophospholipid. PI(12:0/17:2(9Z,12Z)) and PI (12:0/15:1(9Z)) both belong to phosphatidylinositol (PI). To our knowledge, no studies have linked phosphatidylglycerophosphate (PGP) or phosphatidylinositol (PI) to pancreatitis.

PS(20:0/16:1(9Z)), PS(O-20:0/18:4(6Z,9Z,12Z,15Z)), and PS(17:1 (9Z)/12:0) all belong to phosphatidylserine (PS). In CP patients, the mRNA levels of PS receptors in pancreatic tissue are significantly upregulated. These PS receptors are not only expressed on macrophages but also on pancreatic acinar cells, ductal cells, and islet cells, and are closely related to apoptosis.[23] This provides a theoretical basis for further research into the regulatory role of phosphatidylserine (PS) in chronic pancreatitis.

In addition, glycerophosphocholine, phosphatidylethanolamine (PE), phosphatidylglycerophosphate (PGP), phosphatidylinositol (PI), phosphatidylserine (PS), and 1-(2-methoxy-tetracosanyl)-*sn*-glycero-3-phosphoserine are all important phospholipids and key components of cell membrane structures. They help maintain the integrity and fluidity of cellular membranes and play a significant role in cell signaling processes.[24].

CerP(d18:1/18:0) is a ceramide phosphate and an important metabolite of ceramide. Currently, research has demonstrated that ceramide phosphate (CerP) plays a crucial role in regulating cancer cell proliferation, motility, and viability.[25] Some researchers have found that ceramide levels in pancreatic tissue from acute pancreatitis rat models are significantly reduced.[26] In this research, CerP(d18:1/18:0) exhibited a similar downward trend. In addition, Ceramide (d18:1, C24:0) has been identified as a potential biomarker for distinguishing between chronic pancreatitis and pancreatic cancer patients.[27] GlcCer (d15:1/18:0) is a type of glucosylceramide. Both glucosylceramide and ceramide belong to sphingolipids and are closely associated with inflammation.[28,29] This suggests that these two metabolites are involved in the pathogenesis of chronic pancreatitis. Sphingosylphosphoryl choline is also a bioactive sphingolipid that influences pancreatic carcinoma cell proliferation and motility.[30] The role of Sphingosylphosphoryl choline in chronic pancreatitis still requires further investigation.

Deoxycholic acid is a secondary bile acid and a metabolite of intestinal bacteria.[31] Previously, researchers have induced acute pancreatitis in animals by injecting Deoxycholic acid[32] or three forms of Deoxycholic acid, including Sodium deoxycholate [33,34], Taurodeoxycholate[35,36], and Glycodeoxycholic acid[37,38]. A case report documented that a patient with recurrent acute pancreatitis over seven years had significantly elevated levels of Deoxycholic acid in their stool.[39] Additionally, a high-fat diet can promote the secretion of Deoxycholic acid by gut microbiota, thereby exacerbating colon inflammation through ferroptosis.[40] At the same time, long-term high-fat diets can also stimulate the liver to secrete bile, leading to the production of more Deoxycholic acid.[41] Prior research has demonstrated that Deoxycholic acid plays an significant role in constructing animal or cellular models of acute pancreatitis, and excessive levels of Deoxycholic acid can promote the formation and exacerbation of inflammation. In this study, the Deoxycholic acid content in the drainage group showed a significant downward trend. Additionally, Lithocholic acid sulfate is a sulfated product of lithocholic acid, which also belongs to the category of secondary bile acids.

TG(10:0/8:0/8:0) is a representation of triglyceride. Hypertriglyceridemia is one of the significant causes of acute pancreatitis,[42] and when serum triglyceride levels exceed 1000 mg/dL, every 100 mg/dL increase raises the incidence of acute pancreatitis by approximately 4 %.[43–45] Hypertriglyceridemia is also one of the important factors contributing to the onset of chronic pancreatitis.[2] We found that the TG(10:0/8:0/8:0) content in the drainage group was markedly higher compared to the non-drainage group, which might be an important reason why CP patients require surgical drainage.

Presqualene diphosphate is an important intermediate in the cholesterol biosynthesis pathway. Its precursor, squalene, undergoes the addition of two phosphate groups through enzymatic action to form presqualene diphosphate, which is then converted into cholesterol through a series of reactions.[46] A retrospective study has suggested that total cholesterol may be a risk factor for chronic pancreatitis.[47] Additionally, in CP patients, the total cholesterol levels in the group with pancreatic diabetes were substantially lower compared to the non-pancreatic diabetes group. Total cholesterol levels can be used to assess pancreatic insufficiency caused by chronic pancreatitis.[48] In this study, the blood levels of Presqualene diphosphate were markedly elevated in the drainage group, providing a foundation for further research into the role of cholesterol in CP.

LC-MS is a commonly used method in clinical practice and is widely used in medical laboratories for routine testing of vitamin D and

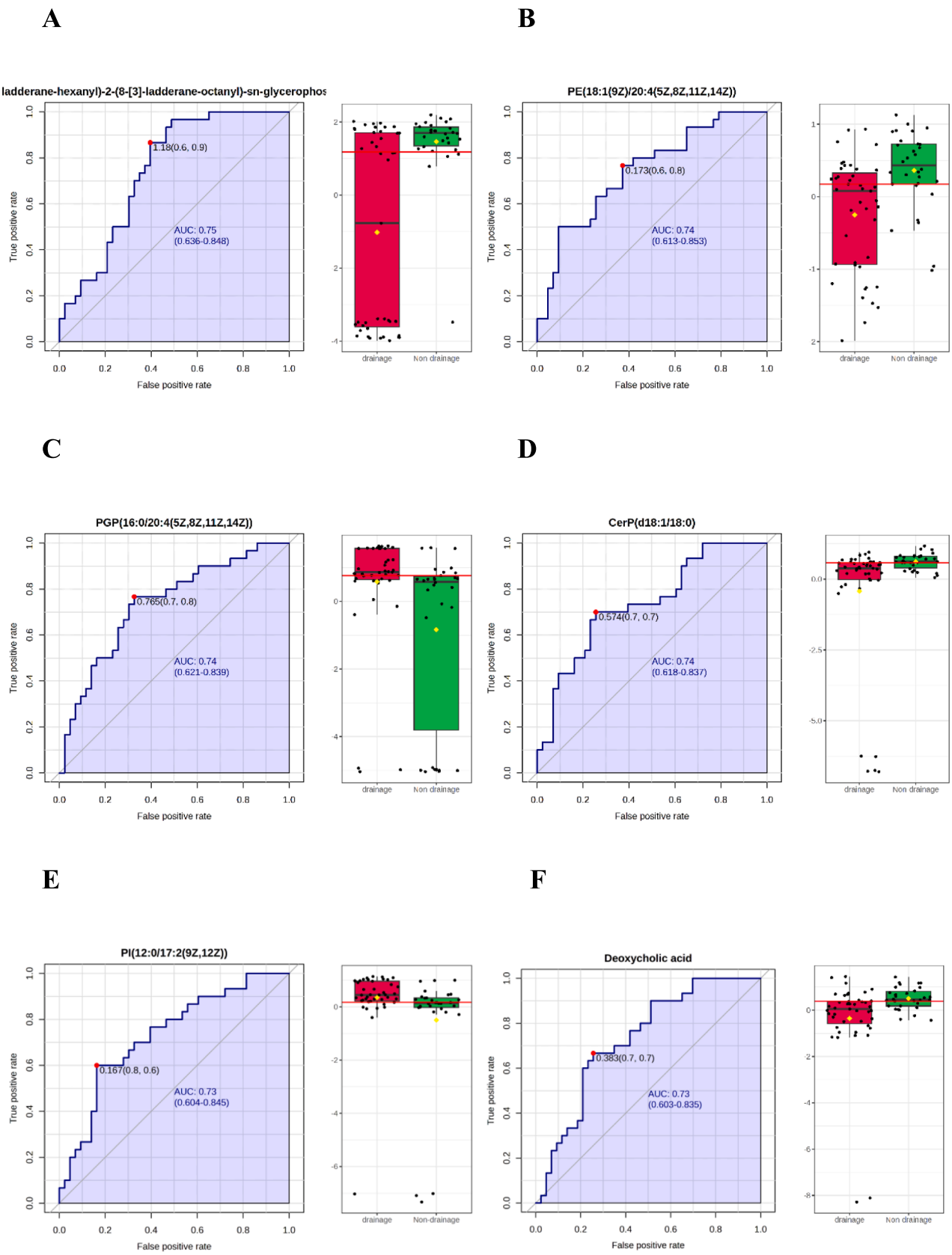

Fig. 4. ROC curves of some potential biomarkers in the drainage and non-drainage groups: (A) 1-(6-[3]-ladderane-hexanyl)-2-(8-[3]-ladderane-octanyl)-*sn*-glycerophosphocholine, (B) PE(18:1(9Z)/20:4(5Z,8Z,11Z,14Z)), (C) PGP(16:0/20:4(5Z,8Z,11Z,14Z)), (D) CerP(d18:1/18:0), (E) PI(12:0/17:2(9Z,12Z)), (F) Deoxycholic acid; the diagnostic value of different metabolites in disease screening was evaluated through ROC curve analysis. The x and y axes represent the false positive rate and true positive rate, respectively.

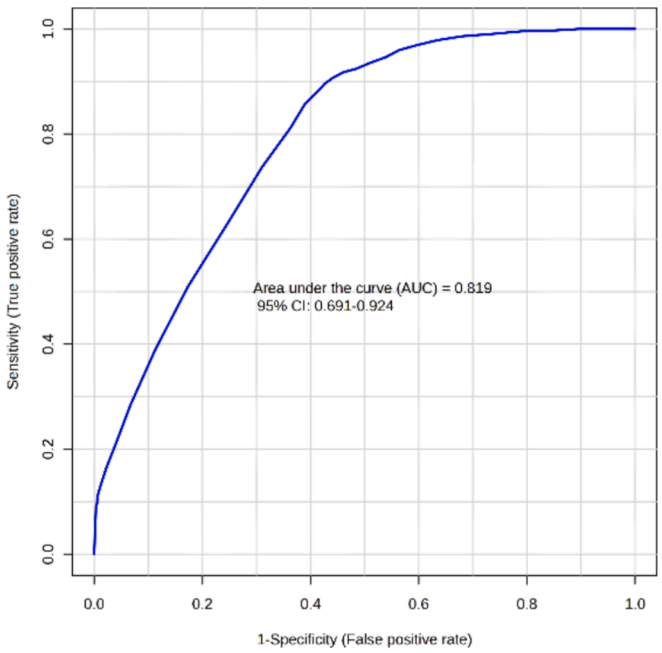

Fig. 5. Joint ROC curve of a group of two metabolites screened in the drainage and non-drainage groups in the validation cohort.

therapeutic drug monitoring.[49,50] The cost of LC-MS analysis for specific metabolites is about 10 dollar/sample, which is inexpensive. At the same time, the turnaround cycle of plasma samples in LC-MS is 4–6 h, which can provide support for clinical decision-making in time. Shows great potential for transformation.

The limitation of this study lies in its focus on a single metabolomics approach rather than a multicenter clinical study. The research concentrated on the metabolite level, exploring differences in metabolite profiles to determine whether CP patients require surgical drainage, and identified potential biomarkers with good diagnostic value. This study lays the groundwork for future research, which could further delve into genomics and proteomics to expand on these findings.

In summary, we have identified LC-MS-based human serum metabolomic profiles that successfully distinguish CP patients requiring surgical drainage from those who do not. The clinical utility of these biomarkers for disease monitoring and progression assessment warrants further prospective investigation.

CRediT authorship contribution statement

Xu Xu: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Sixiang Liu:** Writing – original draft, Visualization, Software, Data curation. **Min Shao:** Supervision, Resources, Funding acquisition. **Ling Wu:** Investigation, Conceptualization. **Qianhui Ouyang:** Data curation. **Qi Yi:** Conceptualization. **Ying Huang:** Supervision, Resources. **Jia Wang:** Writing – original draft, Supervision, Resources, Methodology. **Chaochao Tan:** Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Hunan Provincial People's Hospital in Changsha, Hunan Province, China ([2020]-20). All study participants provided written informed consent to participate in this study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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