

A Pilot Study on Plasma lipidomic Profile of Giant Pulmonary Bulla

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Research

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Abstract

Background: The symptom of chronic obstructive pulmonary disease (COPD) and giant pulmonary bulla (GPB) also called giant emphysematous bulla (GEB) is too similar to distinguish, and the treatment of the two diseases are completely different because of the different pathology. We investigated the plasma lipid profiles from patients of COPD and GPB to find targeted lipid changes responsible for differentiation between two diseases.

Methods: Plasma was collected from 9 patients with COPD, and 10 patients with COPD and GPB. Extracted lipids were analyzed using high performance liquid chromatography-triple quadrupole mass spectrometry (HPLC-QqQ-MS) to characterize lipid profiles. Principal components analysis (PCA), orthogonal projection to latent structures-discriminant analysis (OPLS-DA) and variable importance in projection (VIP) scores were used to estimate the importance of each lipid variable.

Results: The HPLC-QqQ-MS method identified 582 kinds of lipids at negative mode, and 577 kinds of lipids at positive mode. Lipid profiles were significantly different between COPD and GPB. Principal discriminant phospholipids between COPD and GPB were LPC(16:1)+AcO, LPC(16:0)+AcO, LPC(18:2)+AcO, LPC(18:1)+AcO, PC(16:0/22:5)+AcO, PC(18:2/18:2)+AcO, LPE(18:0)-H, LPE(16:0)-H, LPE(18:2)-H, PE(18:1/18:2)-H and FFA(24:0)(GPB group were significantly decreased compared to COPD group, $VIP > 1$ and $P < 0.05$).

Conclusions: Our study provides insights into the alteration of the plasma lipid profile of GPB patients, commonly resulting from COPD, that may lead to improved GPB treatment and differentiation of this disease from COPD. Furthermore, LPC (16:0) +AcO was found to have a high potential to be a possible biomarker to distinguish the two diseases.

1. Background

According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, chronic obstructive pulmonary disease (COPD) is defined as a preventable and treatable disease with considerable extrapulmonary effects that may be directly related to disease severity[1]. It is associated with partially reversible airflow limitation, and is often characterized by parenchymal destruction and development of emphysema. However, COPD is a complex, heterogeneous disease with various phenotypes and syndromes, even among patients with a similar degree of airflow limitation [2].

With the deterioration of COPD, the emphysema becomes into an irreversible bulla. And giant pulmonary bulla (GPB), a rare symptom of emphysematous destruction of the lung parenchyma, was originally described by Burke in 1937[3]. It's an idiopathic clinical syndrome with progressive dyspnea caused by extensive, predominantly asymmetric upper lobe bullous emphysema, leading to respiratory failure eventually[4]. The radiographic criteria for GPB, as defined by Roberts et al.[5], is the presence of giant bullae in one or both upper lobes, occupying at least one third of the hemithorax and compressing the surrounding normal lung parenchyma. It can cause severe symptoms such as dyspnea, thoracic pain,

infection, pneumothorax and even slow progression to malignancy. Once the pulmonary capacity could not perform gas exchange effectively, these symptoms would occur with the enlargement of bullae.

It has been reported that dysfunction of pulmonary surfactant proteins may be included in pathophysiology of COPD[6]. Pulmonary surfactant, synthesized and secreted by alveolar type II epithelial cells (ATII), is a complex fluid that comprises phospholipids and four proteins (SP-A, SP-B, SP-C, and SP-D) with different biological functions. Phospholipid represents the major surface-active component of surfactant, the phospholipid composition is dominated by phosphatidylcholine (PC), with a high content of the disaturated species dipalmitoylphosphatidylcholine (DPPC; PC16:0/16:0) compared with typical mammalian cell membranes[7]. Stern et al. described the CT findings of GPB[8], which include multiple large bullae, ranging from 1 to 20 cm in diameter (usually 2–8 cm), without a single dominant giant bulla. Radiologically, bullae appear as avascular radiolucent areas with thin curvilinear walls[4]. CT scans are more sensitive than chest x-rays to detect bullae for the accurate assessment of the number, size, and position of bullae, especially when the bullae are obscured[8]. However, there were no any research on the identification of biomarkers of the GPB. We believed that identifying biological differences between COPD and GPB may provide a new marker to prevent the deterioration of COPD into GPB. And searching for potential and convenient biomarkers with high specificity and sensitivity to screen for GPB remain as major concerns for clinical practice. Therefore, we performed the study to investigate the plasma liposome profiles for GPB patients. The study not only identify potential lipid signatures associated with GPB, but also reveal the potential biomarkers that could play crucial role in the deterioration of COPD.

2. Materials And Methods

Samples

A total of 19 patients were recruited from Fujian Medical University Second Affiliated Hospital between April 28, 2018 and November 20, 2018, including 10 GPB patients and 9 COPD patients. GPB was diagnosed by a respiratory physician and by an imaging physician, and COPD was diagnosed by two respiratory physicians after undertaking a pulmonary function test. Each participant provided a one-time 10 mL of venous blood sample for further experiments. After a written informed consent was obtained, all subjects were interviewed to collect their demographic information.

The study was conducted according to the principles of Helsinki declaration. The bioethical committees at Fujian Medical University Second Affiliated Hospital, China, gave written approval for the study.

Materials and lipid standards

The Internal standards kit consisting of labelled internal standards for 13 lipid classes were purchased from Sciex (Framingham, MA); MTBE and ammonium acetate were from Sigma-Aldrich Chemie GmbH (Munich, Germany). LC-MS grade acetonitrile, 2-propanol, methanol and water were purchased from Merck (Darmstadt, Germany).

Sample preparation

All patients underwent collection of 10 ml of peripheral blood in tubes with ethylenediaminetetraacetic acid (EDTA) and the blood was centrifuged at $2000 \times g$ (4°C) to obtain plasma. After centrifugation, the plasma was stored at -80°C for further lipids analysis.

Lipid extraction recipes

Lipid extraction was conducted with methyl tert-butyl ether (MTBE). Liquid configuration: A phase (95% acetonitrile water): water/ acetonitrile (5:95, v/v) with 10 mM ammonium acetate; B phase (50% acetonitrile water): water/ acetonitrile (50:50, v/v) with 10 mM ammonium acetate. Pretreatment of lipid sample: Methanol (225 μl) was added to a 20 μl plasma sample aliquot, which was placed into a glass tube, and the tube was vortexed at maximum speed for 10s. Then, 750 μl of MTBE was added and the mixture was vortexed at maximum speed for 10s then incubated for 30min at room temperature. Next, another 188 μl of water was added and vortexed for 20s. Upon 10 min of incubation at room temperature, the sample was centrifuged at 15000rpm for 15 min at 4°C . The samples were divided into three layers from the top to the bottom, including lipid phase, water phase and solid residue. The upper (lipid) phase (700 μl) was collected. Combined lipid phases were blow-dried with termovap sample concentrator. Extracted lipids were dissolved with 100 μl mixture which consists of 2-propanol: acetonitrile: water (30:65:5, v/v/v).

Mass spectrometric analysis of lipids from human plasma extracts

In the present study, HPLC-QqQ-MS was used for lipids class separation (AB SCIEX QTRAP 4500 LC-MS/MS system). The Waters Acquity UPLC BEH HILIC Pore column (100 mm \times 2.1 mm \times 1.7 μm) was used as stationary phase. Mobile phase A is 95% acetonitrile solution containing 10mmol/L ammonium acetate, and mobile phase B is 50% acetonitrile solution containing 10mmol/L ammonium acetate. The solvent gradient was programmed as follows: the gradient started with 0.1% of B and increased to 20% of B during 10 min, then linearly increased to 98% of B during 10min to 11 min, and 98% of B was held for 2 mins, and returning to the initial conditions 0.1% of B in 13.1 min, finally stop the analyze in 16 min, the flow rate through the column was 0.5mL/min. The column temperature was 35°C , the positive electrospray ionization (ESI+) mode injection volume was 1 μL , and the negative electrospray ionization (ESI-) mode injection volume was 10 μL . The curtain gas (CUR) was set to 35 psi, the ion source gas1(GS1) was 50 psi, the ion source gas2(GS2) was 60psi, and the temperature (TEM) set at 500°C . The ion spray voltage in ESI+ and ESI- mode was set at -5500V and 5500V respectively, the declustering potential (DP) was 80V, the entrance potential (EP) was 10V, and collision cell exit potential (CXP) was 15V. Samples were added to a lipid standard mixture.

Statistical Analysis

Relative ion abundances from the two examined groups of plasma extracts (COPD and GPB) were obtained by HPLC-QqQ-MS. Peak areas were then corrected by the area of the selected internal standard by exporting integrated peak area values. After correction, the processed data set was subjected to multivariate analysis by SIMCA 14.1.

Principal components analysis (PCA) and orthogonal projection to latent structures-discriminant analysis (OPLS-DA) were applied to the data set. The PCA, an unsupervised method, was used to detect intrinsic clusters based on the lipid profile, and to maximize the identification of differences in the metabolic profiles between the groups, the OPLS-DA model was applied and performed using SIMCA 14.1 software. The variable importance in projection (VIP) value of each variable in the model was calculated to indicate its contribution to the classification. Based on obtained OPLS-DA models the VIP values of those variables greater than 1.0 are considered to be significantly different and combine with the P value of t-test ($P < 0.05$) allows insight into which lipid species are different between COPD and GPB.

3. Results

Demographic

10 GPB patients (all males) with an average age of 56 years (range 31–66 years), and 9 COPD patients (all males) with an average age of 62 years (range 44–69 years) were enrolled in this study.

Lipid Analysis

We used a lipidomic methodology to investigate changes of plasma lipid profiles between COPD and GPB. Ion ratios were established according with their correlations and those variables were used for the analysis. The negative mode of the HPLC-QqQ-MS method identified 582 kinds of lipids (Fig.1), and the positive mode identified 577 kinds of lipids (Fig.2). PCA analysis was able to separate the COPD and GPB. The PCA plot (Fig.3), on which each point represents an individual sample, showed two groups, i.e. COPD and GPB. The PCA score chart showed that all samples were within the 95% confidence interval Hotelling's T-squared ellipse. The PCA analysis showed a tendency of separation between the studied groups (Fig.3), while in the figure, the abscissa $t1[1]$ represents the predicted principal component score of the first principal component, the ordinate $t2[1]$ represents the orthogonal principal component score, and the scatter shape and color represent different experimental groups.

It showed that the OPLS-DA score chart between the two groups of samples displayed very significant, and all samples are within Hotelling's t-squared ellipse. The OPLS-DA was able to clearly distinguish the group of COPD from the group of GPB (Fig.4-5). While in the figure of the S-plot, the abscissa $p[1]$ represents the load of the first principal component, the ordinate $p(\text{corr})[1]$ represents the magnitude of the correlation coefficient (reliability) between each material and the first principal component (Fig.6).

By randomly changing the order of classification variable Y, permutation test establishes the corresponding OPLS-DA model several times (times n = 200) to obtain R2 and Q2 values of the random model, which plays an important role in avoiding overfitting of the model and evaluating the statistical significance of the model. The abscissa of the permutation test represents the correlation between random group Y and the original group Y, and the ordinate represents the scores of R2 and Q2. The best case is that R2 is close to 1 and Q2 is on the negative axis, we meet the standard. (Fig.7)

In the OPLS-DA models, the VIP scores reflects the contribution of each lipid, and VIP scores > 1 were regarded as significant and therefore considered for quantitative analysis of variation (Fig.8). Using this criterion, combined with the P value of t-test($P < 0.05$), 11 kinds of lipid species were identified (table 1) including LPC(16:1)+AcO, LPC(16:0)+AcO, LPC(18:2)+AcO, LPC(18:1)+AcO, PC(16:0/22:5)+AcO, LPE(18:0)-H, LPE(16:0)-H, LPE(18:2)-H, PE(18:1/18:2)-H, PC(18:2/18:2)+AcO, FFA(24:0). The difference lipids can be found, and it can be found that the main different type of component is lysophosphatidylcholine (LPC), especially the LPC (16:0) +AcO is significant.

Table 1
11 kinds of lipid species were Screened and considered significant*.

Component Name	M2.VIPpred	Fold change**	<i>P</i> -value
LPC(16:1)+AcO	1.23767	0.584592451	0.003403258
PC(16:0/22:5)+AcO	1.81768	0.598292983	0.004402319
LPE(18:0)-H	1.21837	0.620818413	0.007552045
LPC(16:0)+AcO	7.69956	0.662816973	0.009149644
LPE(16:0)-H	1.09987	0.556932752	0.011510331
LPC(18:2)+AcO	5.00009	0.583803171	0.01569506
LPC(18:1)+AcO	3.55087	0.67014607	0.020440573
PE(18:1/18:2)-H	1.16461	0.591158213	0.026266559
PC(18:2/18:2)+AcO	2.16997	0.625028501	0.028301311
LPE(18:2)-H	1.27145	0.616650119	0.049035072
FFA(24:0)	7.62268	0.369329062	0.049983032
* Screening criteria: VIP > 1, T test results < 0.05; ** Bulla/COPD			

4. Discussion

In the present study, we investigated the changes of plasma lipid profiles of GPB and COPD using mass spectrometry analysis. The results showed that a total of 11 kinds of lipid species were identified,

including LPC(16:1)+AcO, LPC(16:0)+AcO, LPC(18:2)+AcO, LPC(18:1)+AcO, PC(16:0/22:5)+AcO, LPE(18:0)-H, LPE(16:0)-H, LPE(18:2)-H, PE(18:1/18:2)-H, PC(18:2/18:2)+AcO, FFA(24:0).

Very little information is available with regards to the lipidomic biomarkers of COPD, and even less information is available with regards to the difference of lipidosome biomarkers between COPD and GPB. Given the paucity of past studies, we expected that our study would be an excellent pilot investigation to provide important information and future direction in identifying an appropriate model(s) and methodology(s) to screen the different lipidomic biomarkers between COPD and GPB.

Our data demonstrates that there are a number of lipid combinations with which the individuals of COPD or GPB can be separated. These preliminary results provide us an operational lead as to how lipidomics can be applied to identify patients with COPD or GPB. Meanwhile our findings may also provide some clues to lipidomics changes during COPD progression to GPB. Through the OPLS-DA model, VIP scores > 1 and P value of t-test ($P < 0.05$), our research reveals 11 kinds of lipids, which could be potential new biomarker. The different components between the two diseases were lysophosphatidylcholine (LPC), phosphatidylcholine (PC), Lysophosphatidylethanolamine (LPE) and phosphatidylethanolamine (PE), free fatty acid (FFA). These 4 kinds of lipids were significantly higher expressed in patients with COPD than patients with GPB. Phospholipids are major components of cell membranes, and are classified in species according to their distinctive structure and functions. Lysophospholipids are membrane-derived phospholipids that can arise from homeostatic lipid metabolism or as a response to stimulus-induced cellular activation[9]. Lysophosphatidylcholine (LPC) is the main component of oxidatively damaged low-density lipoprotein (oxLDL)[10]. LPC can exert its biological function by inducing cell division, the release of inflammatory factors and oxidative stress[11]. LPC is a major lipid constituent of oxidized-LDL, which induces inflammation in endothelial cells[12], and is widely used to mimic oxidized-LDL-induced inflammatory responses in endothelial cells(EC. LPC has been demonstrated to induce pro-inflammatory effects relevant to the production of inflammatory factors, including IL-6, TNF- α , and COX-2[16, 17, 18]. Rice KL's[19] study demonstrated a correlation between increased solute permeability of the alveolar epithelium and the development of emphysema after intratracheal administration of porcine pancreatic elastase (PPE) and LPC in the hamster, combined exposure to the same amounts of both substances caused substantial increases in solute permeability, particularly with regard to the macromolecular tracer dextran 70, and in the subsequent development of severe, diffuse emphysema. Some reports suggested that LPC primes neutrophil NADPH oxidase activity[20, 21], whereas others found it inhibited fMLP- and PMA-induced superoxide generation[22]. Phoebe Lin found that LPC-mediated inhibition of neutrophil superoxide generation parallels a protective effect against neutrophil-mediated lung injury[23]. Several published studies have shown that LPC activated calcium signaling and induced proinflammatory cytokine expression, functions that require the PAF receptor[24, 25, 26]. The main pathological changes of CDPD are chronic bronchitis and emphysema, and emphysema is the most common reason transforming into GPB. LPC can be reacylated into PC by LPC acyltransferase enzymes (PC remodeling)[27] and remodeling is critical for lipid composition of pulmonary surfactant and to maintain lipid homeostasis to prevent toxic levels of cellular endoplasmic reticulum (ER) stress[28, 29]. Phosphatidylcholine (PC) is by far the most abundant phospholipid component in all the lipoprotein classes. PC is physiologically

important as the principal component of eukaryotic cellular membranes, as a precursor of signaling molecules[30, 31], and as a key element of lipoproteins[32], and lung surfactant[33, 34].

Phosphatidylcholine plays a major role in the transport of essential fatty acids, and is a substrate for cell signaling[35].

Our study also had a few shortcomings that are worth mentioning. First, our case subjects were those who already developed COPD or GPB, and the observed patterns of the lipid in these subjects may be different as compared to the subjects in the developing stages of the disease. Although our strategy was necessary as a pilot study, this is a typical pitfall of a cross-sectional, case-controlled study design. A larger prospective study is necessary to answer causal interaction between COPD and GPB. Second, our subjects were recruited from a single center, so multicenter research is necessary in the future.

5. Conclusion

In conclusion, lipids from the lysophosphatidylcholine (LPC) pathway are higher expressed in the COPD group compared with the GPB group, the LPC (16:0) +AcO was especially significant. Considering their potential biologic properties, and the correlation between the two diseases, these lipids may play a role in the pathogenesis of COPD progresses into GPB. Lipidome analysis may become an important research tool that can lead to new drug targets and possible new biomarkers in COPD and GPB.

Abbreviations

COPD:chronic obstructive pulmonary disease; GPB:giant pulmonary bulla; GEB:giant emphysematous bulla; HPLC-QqQ-MS:high performance liquid chromatography-triple quadrupole mass spectrometry; PCA:principal components analysis; OPLS-DA:orthogonal projection to latent structures-discriminant analysis ; VIP:variable importance in projection; GOLD:Global Initiative for Chronic Obstructive Lung Disease; ATⅡ:alveolar type Ⅱ epithelial cells; PC:phosphatidylcholine; DPPC:dipalmitoylphosphatidylcholine; EDTA:ethylenediaminetetraacetic acid; MTBE:methyl tert-butyl ether; LPC:lysophosphatidylcholine; LPE:lysophosphatidylethanolamine; PE:phosphatidylethanolamine; FFA:free fatty acid; oxLDL:oxidatively damaged low-density lipoprotein; PPE:porcine pancreatic elastase; ER:endoplasmic reticulum.

Declarations

Ethics approval and consent to participate

The study was conducted according to the principles of Helsinki declaration. The bioethical committees at Fujian Medical University Second Affiliated Hospital, China, gave written approval for the study.

Consent for publication

Not applicable.

Availability of data and materials

Data are available upon reasonable request.

Competing interests

The authors declare that there are no Competing interests.

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Authors' contributions

All the authors contributed to the writing of this article. All the authors read and approved the final manuscript.

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Figures

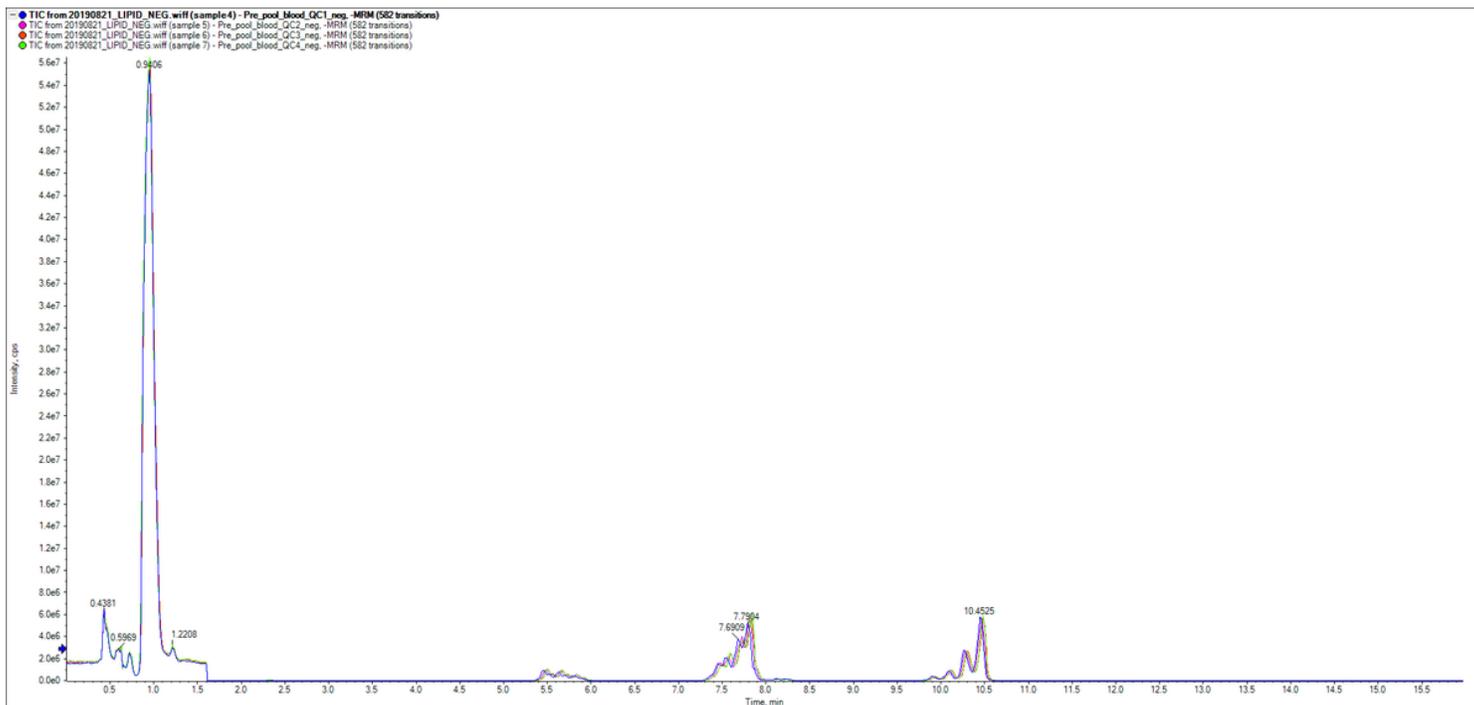


Figure 1

The negative mode of the HPLC-QqQ-MS method identified 582 kinds of lipids

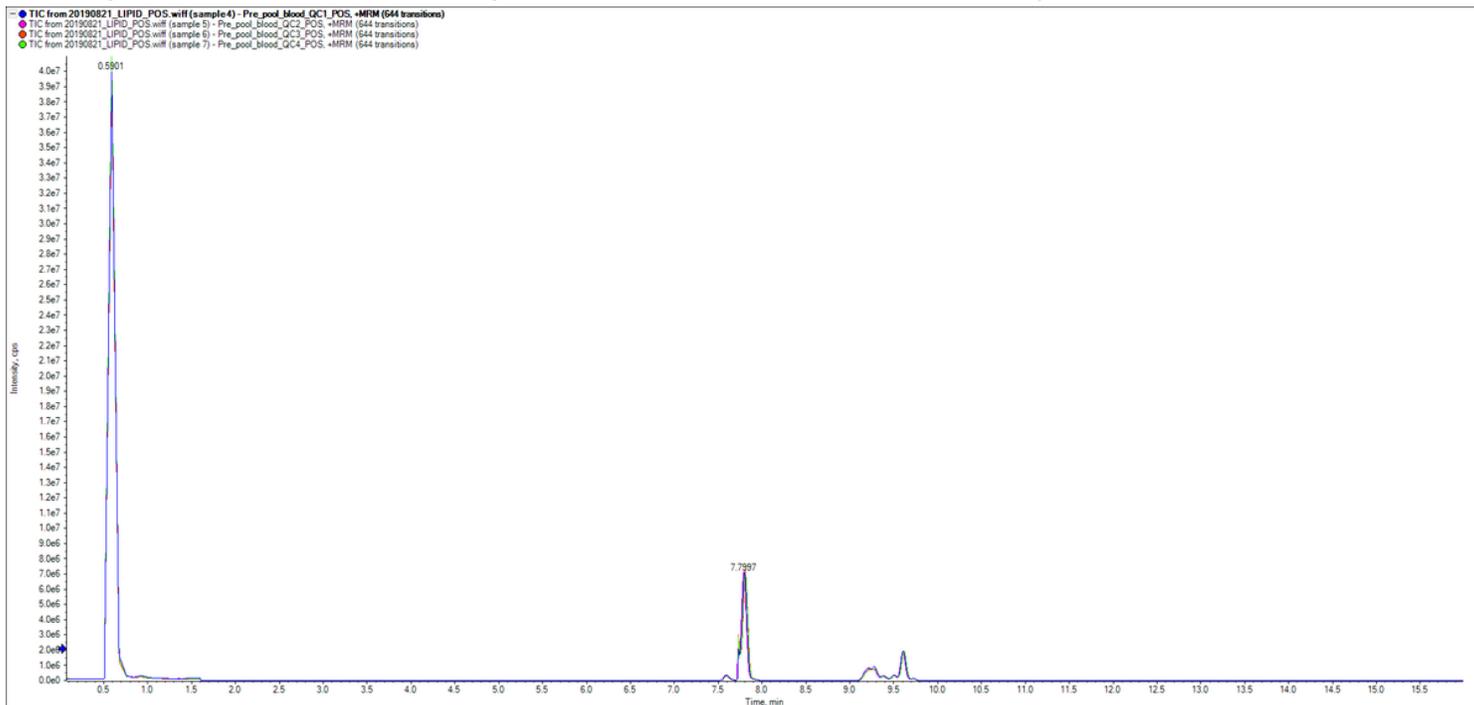


Figure 2

The positive mode of the HPLC-QqQ-MS method identified 577 kinds of lipids.

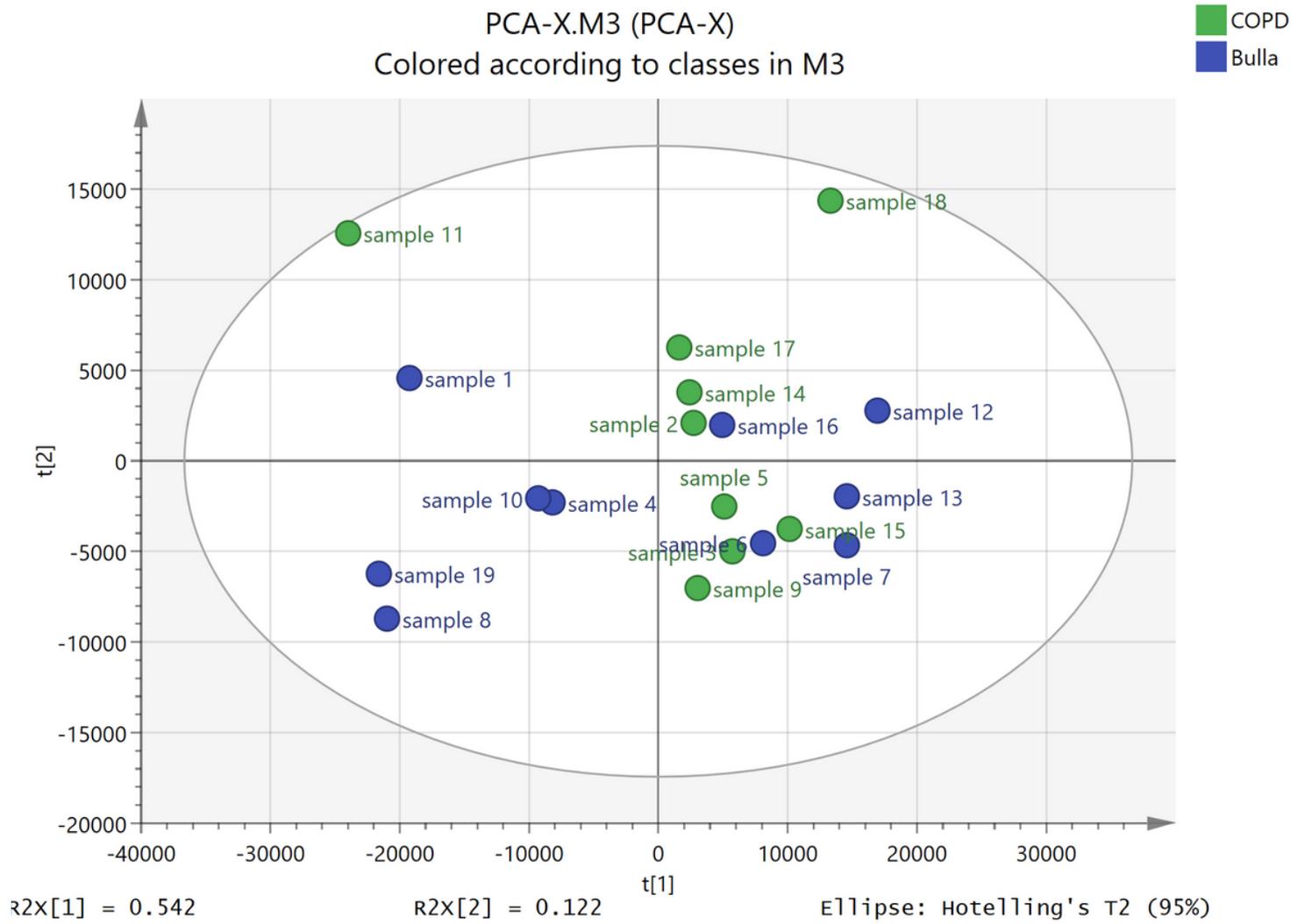


Figure 3

Score scatter plot of PCA* model for group COPD** vs GPB***

COPD VS Bulla.M2 (OPLS-DA)
 Scaled proportionally to R2X
 Colored according to classes in M2

COPD
 Bulla

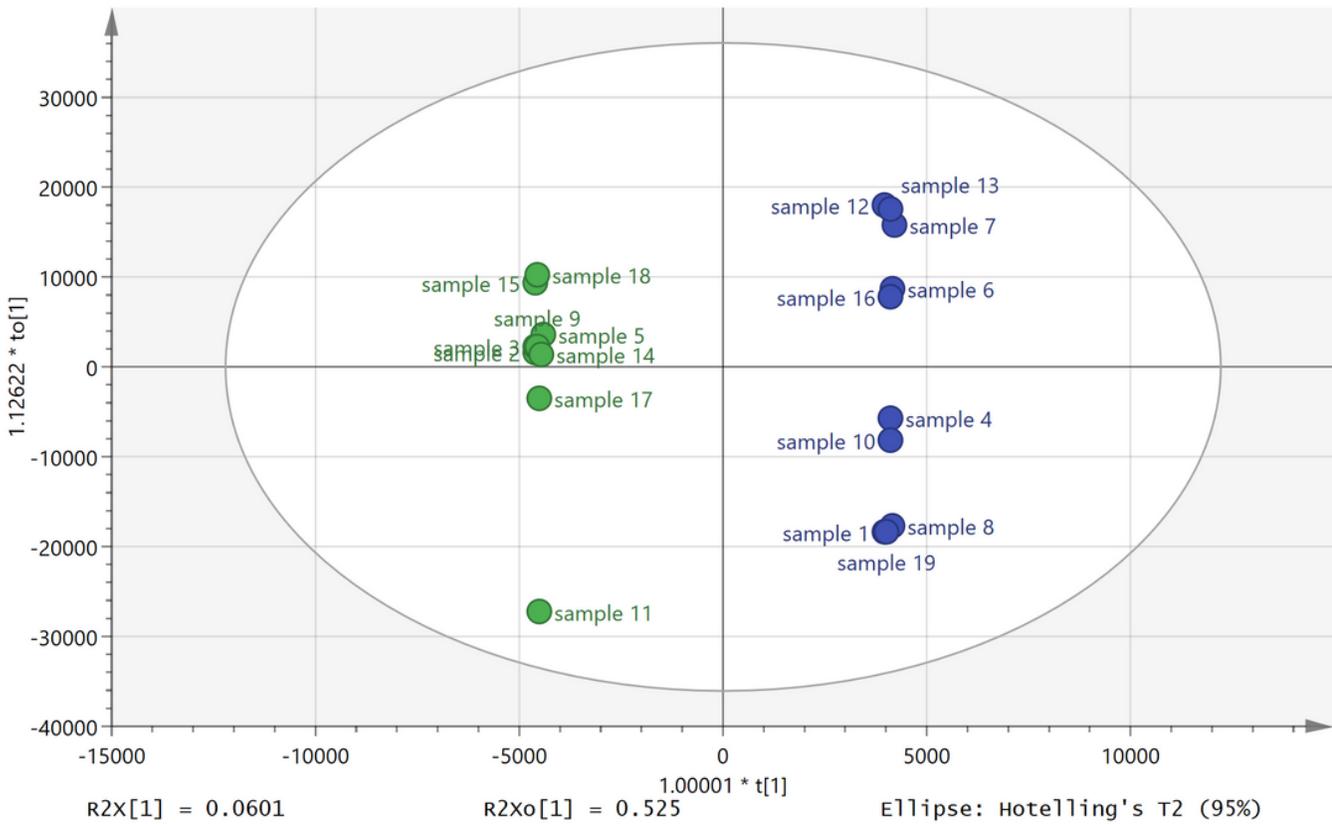


Figure 4

Score scatter plot of OPLS-DA* model for group COPD vs Bulla.

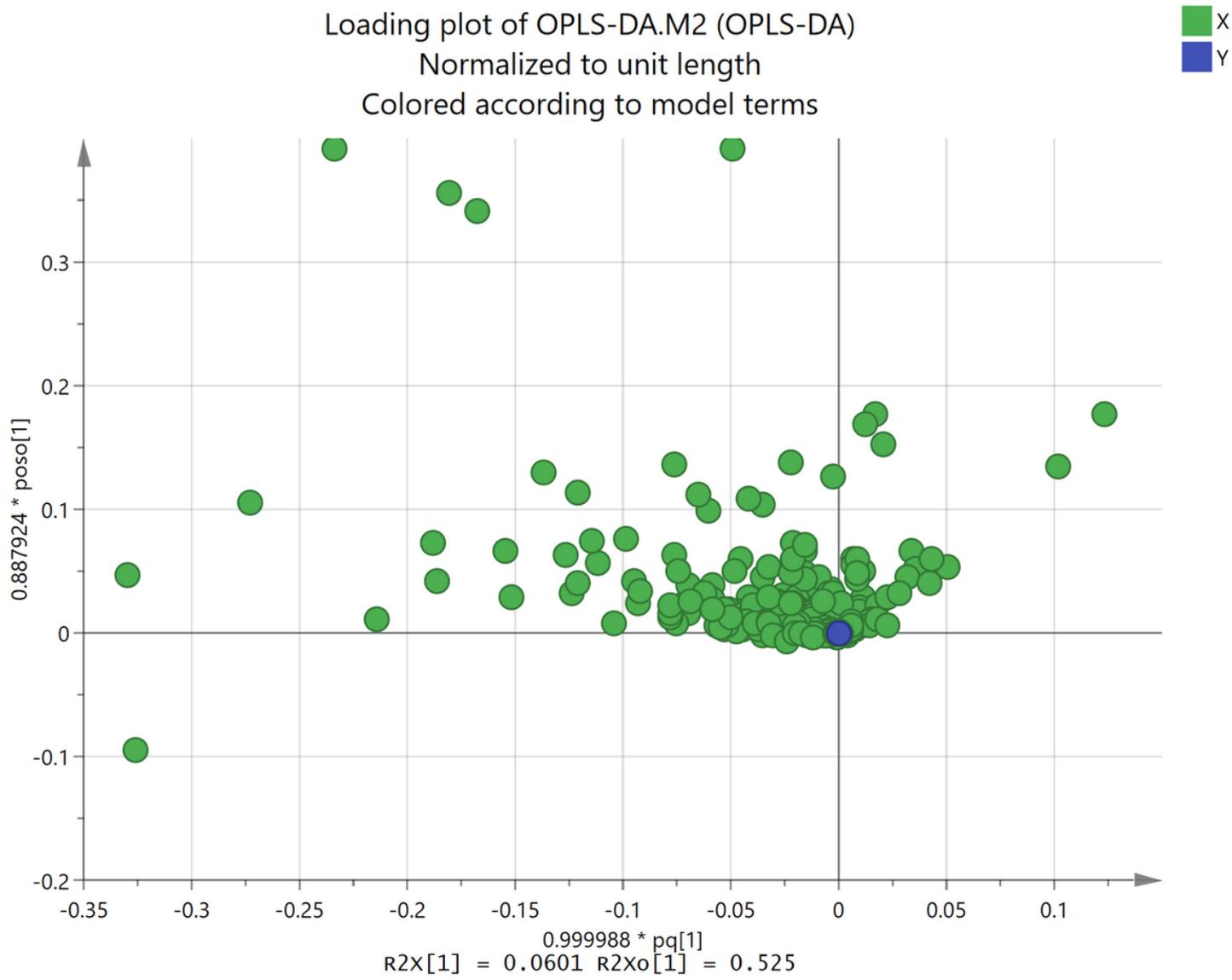


Figure 5

Loading plot of OPLS-DA

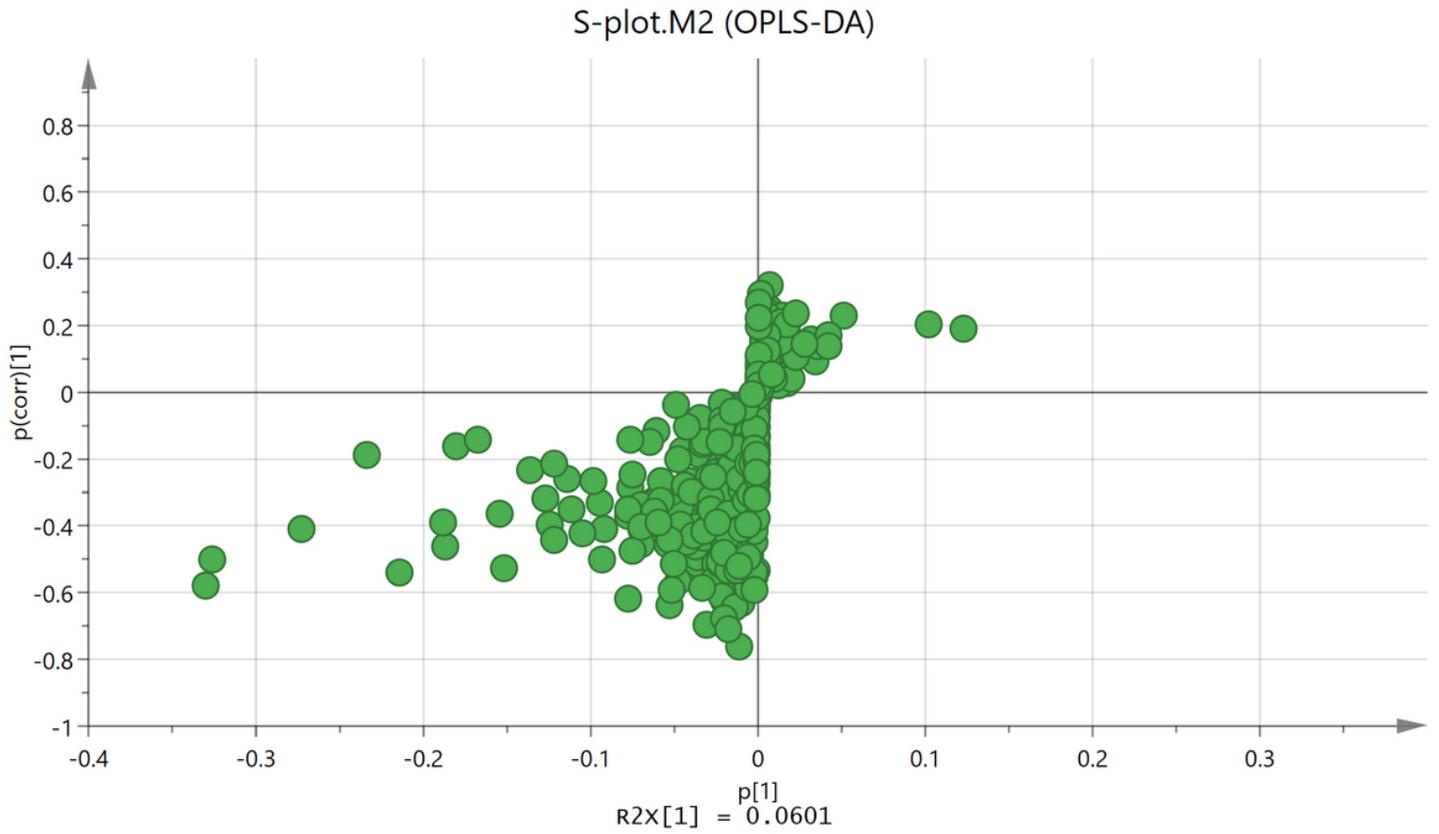


Figure 6

S-plots.

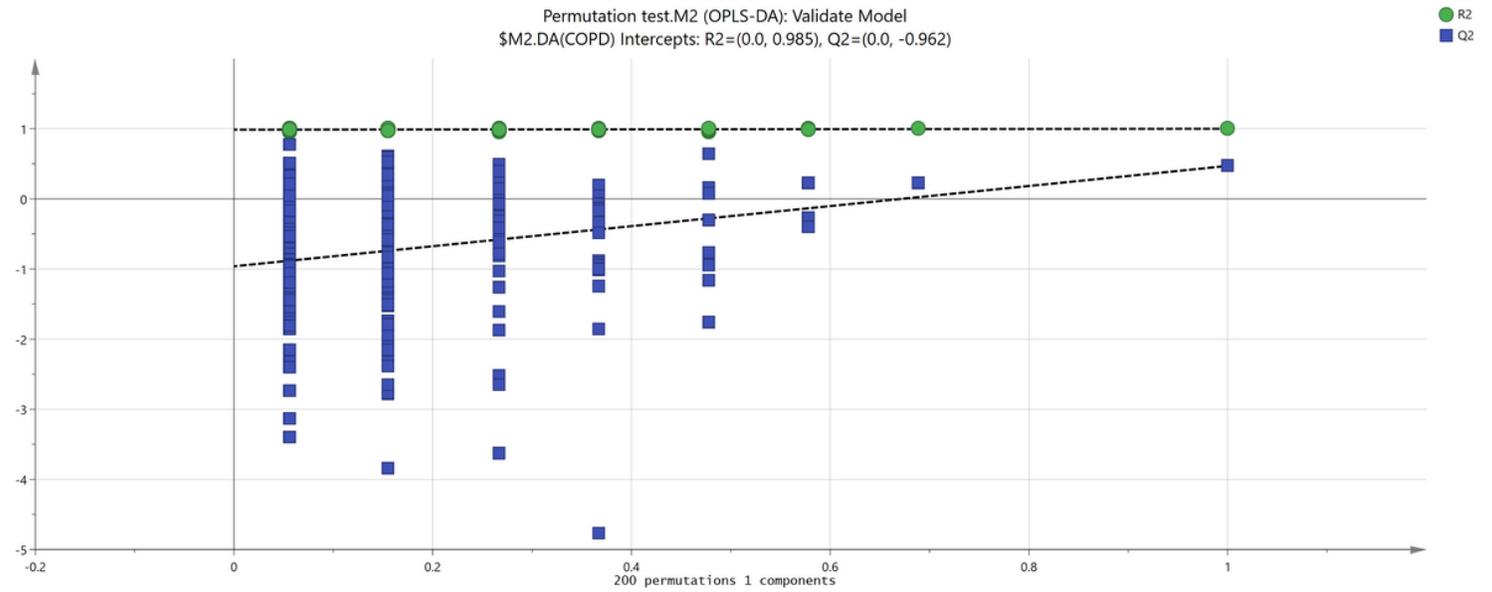


Figure 7

Substitution test (hypothesis test number n=200)

VIP.M2 (OPLS-DA)

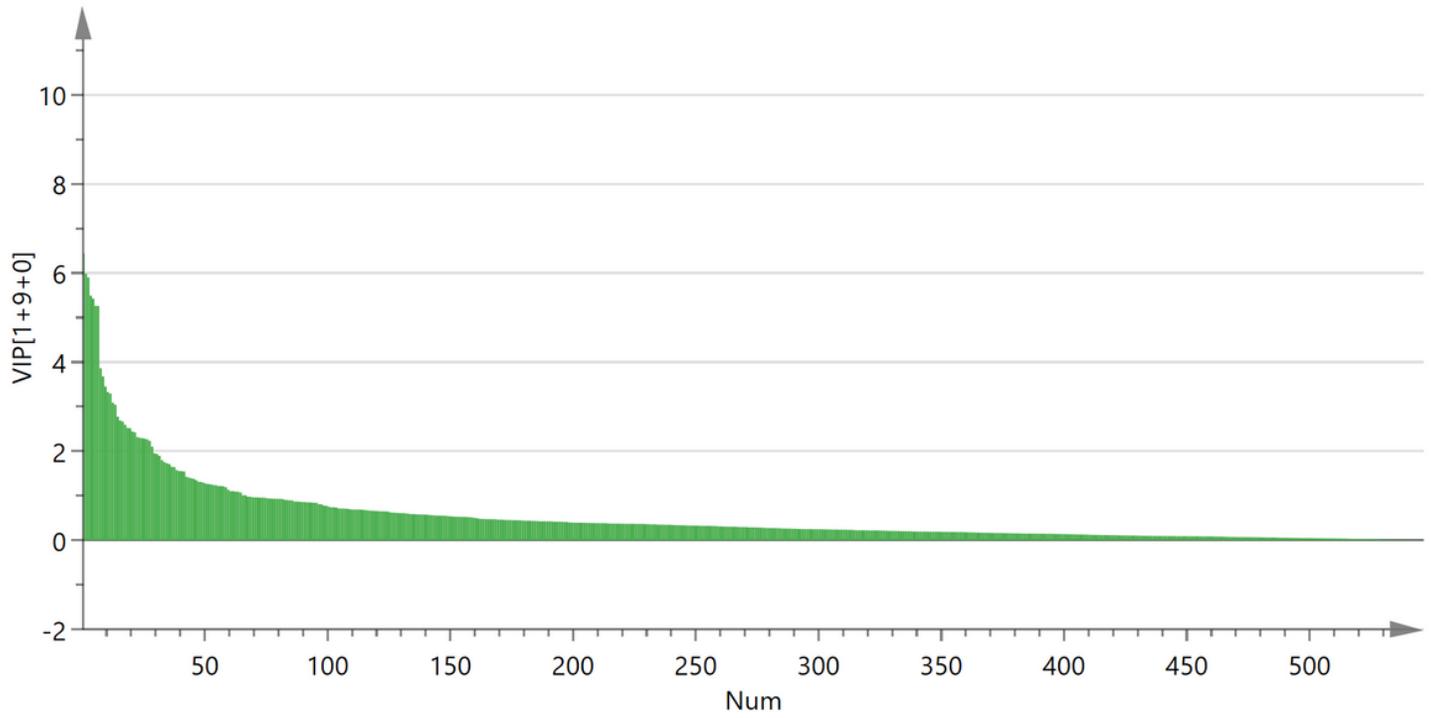


Figure 8

VIP* scores > 1 were regarded as significant. (variable importance in projection (VIP))