Targeted lipidomics reveals phospholipids and lysophospholipids as biomarkers for evaluating community-acquired pneumonia

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Background: Community-acquired pneumonia (CAP) is often accompanied by changes in lipid metabolism. This study aimed to examine the changes in serum phospholipids (PLs) that may be useful for early disease stratification and as potential therapeutic targets in patients with CAP.

Methods: Serum samples from 58 patients hospitalized with CAP and 11 control samples were collected during admission between January 2017 and October 2018. Targeted lipidomic analysis was used to determine the concentrations of phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), and lysophosphatidylethanolamine (LPE). The Gene Expression Omnibus (GEO) database was used to evaluate the gene expression levels of key enzymes in the Lands cycle, and quantitative real-time polymerase chain reaction (qRT-PCR) was used for further verification.

Results: A significant decrease in LPC levels and an increase in PE levels, PC/LPC and PE/LPE ratios were observed in patients with CAP ($P < 0.05$). The area under the curve (AUC) of PE serum concentrations combined with CURB-65 scores (confusion, uremia, respiratory rate, blood pressure, and age ≥ 65 years) was 0.848 for discriminating disease severity, which was significantly higher than the discriminating disease severity of CURB-65 ($P < 0.05$). The efficiency of predicting 30-day mortality using PC, LPC, or PC/LPC ratio combined with CURB-65 scores (AUC = 0.811, AUC = 0.854, AUC = 0.838, respectively) was better than CURB-65 alone ($P < 0.05$). Gene expression analysis revealed the upregulation of LPC acyltransferase 2.

Conclusions: LPC or PE serum levels as well as PC/LPC ratios combined with CURB-65 are effective biomarkers for predicting the disease severity and 30-day mortality of patients with CAP. Further investigations of phospholipid metabolism will improve our understanding and treatment of CAP.

Keywords: Community-acquired pneumonia (CAP); phospholipids (PLs); targeted lipidomic analysis

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Introduction

Community-acquired pneumonia (CAP) is highly heterogeneous, with numerous etiologies and various clinical manifestations. Therefore, early disease assessment of CAP patients will help to improve the prognosis (1,2). CAP involves various changes in the immunity and metabolism of the host, of which lipid metabolism is an important component. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are important glycerophospholipids that are remodeled between phospholipids (PLs) and lysophospholipids (LPLs) through Lands cycle (3,4). Our previous lipidomics studies have found that LPL levels were significantly downregulated in patients with severe CAP (SCAP), while PL levels were significantly upregulated (5,6). Our research on the key lipid enzyme lysophosphatidylethanolamine acyltransferase (LPEAT) also showed that serum LPEAT levels were increased in SCAP patients and associated with disease severity and 30-day mortality (7).

Based on previous studies, we hypothesized a metabolic imbalance between PLs and LPLs in CAP, which may involve the activation of the Lands cycle. Targeted lipidomic analysis allows accurate quantification of specific molecules. Here, we used targeted lipidomic analysis to determine the concentrations of PE, PC, lysophosphatidylethanolamine (LPE), and lysophosphatidylcholine (LPC) in the serum samples of patients with CAP and nondisease controls (NCs). Furthermore, we used a public database to explore the gene expression levels of key enzymes involved in the Lands cycle. This study aimed to identify reliable biomarkers to facilitate early disease stratification, improve prognosis, and develop new insights into the pathophysiological processes involved in CAP. We present the following article in accordance with the STARD reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-21-4008/rc>).

Methods

Study design and population

This prospective study was conducted at six tertiary hospitals in China (Peking University People's Hospital, The Second Hospital of Jilin University, Fujian Provincial Hospital, West China Hospital of Sichuan University, Shanghai Pulmonary Hospital, and People's Hospital of Tibet Autonomous Region) between January 2017 and October 2018. Patients with confirmed CAP (n=58, age ≥ 18 years) and an age- and sex-matched control group

(n=11) were recruited. The study was approved by the ethics committee of Peking University People's Hospital (No. 2016PHB202-01) and registered as a clinical study at ClinicalTrials.gov (Identifier: NCT03093220). This trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the participants prior to clinical data collection and sampling.

The inclusion criteria for CAP were the presence of a new radiologic pulmonary infiltrate and onset of symptoms in the community, with at least one of the following signs (8): presence of cough, sputum production, and dyspnea; core body temperature $>38.0^{\circ}\text{C}$; abnormal breath sounds and rales during auscultation; and peripheral white blood cell count (WBC) $>10 \times 10^9/\text{L}$ or $<4 \times 10^9/\text{L}$. The exclusion criteria were as follows: age <18 years, pregnancy or lactation, surgery performed within the past 3 months, long-term use of hormones or immunosuppressive agents, end-stage renal or liver disease, malignant tumor, and definite diagnosis or highly suspected active pulmonary tuberculosis or infection with human immunodeficiency virus.

According to the guidelines published by the American Thoracic Society (9) for the diagnosis of SCAP, one major criterion or at least three minor criteria must be met. The major criteria were invasive mechanical ventilation or septic shock with the need for vasopressors. The minor criteria were as follows: respiratory rate ≥ 30 breaths/min; $\text{PaO}_2/\text{FiO}_2$ ratio ≤ 250 ; multilobar infiltrates; confusion or disorientation; blood urea nitrogen level ≥ 20 mg/dL; white blood cell count $<4,000$ cells/mm³; platelet count (PLT) $<100 \times 10^9/\text{L}$; core temperature $<36^{\circ}\text{C}$; and hypotension requiring aggressive fluid resuscitation. Patients who met the diagnostic criteria for SCAP were enrolled in the SCAP group, and the other patients were enrolled in the nonsevere CAP (NSCAP) group.

The inclusion criteria for the control population were as follows: age ≥ 18 years old; no other respiratory diseases, such as chronic bronchitis, bronchiectasis, or chronic obstructive pulmonary disease; no combined hematological tumors, solid tumors, long-term use of immunosuppressive agents and/or hormones and other immunosuppressive states; no recent fever or respiratory symptoms, such as cough, sputum, chest tightness, and dyspnea; and no recent hospitalization history.

Collection of clinical data and specimens

Demographic and clinical data, including age, sex, history,

underlying diseases, signs and symptoms, laboratory findings, and clinical strategies, were collected using standard forms. Fasting peripheral venous blood samples (5 mL) were drawn from patients within 24 hours after admission into sterile procoagulation tubes, centrifuged at 3,000 rpm for 10 min at 4 °C, aliquoted in 2–3 Eppendorf tubes, and stored at –80 °C for further analysis.

Serum pretreatment for targeted lipidomic analysis was as follows: 98 µL of serum was transferred to a new tube, and 196 µL of methyl tert-butyl ether, 39.2 µL of methanol, and 10 µL of mixed isotope internal standard were added. The samples were vortexed for 5 min, allowed to stand at 4 °C for 30 min, centrifuged at 4,000 ×g for 5 min, and filtered, and 100 µL of supernatant was collected and freeze-dried. The dried samples were stored at –80 °C for testing. LPE (18:1), LPC (18:1), PE (15:0_18:1), and PC (15:0_18:1) were used as internal standards for analysis.

Targeted lipidomic analysis

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze the samples, and 500 µL of diluent (dichloromethane: isopropanol: methanol at a ratio of 2:1:1) was added to each freeze-dried sample before analysis (the above samples were diluted 10 times for PC analysis, and both diluted and undiluted samples were analyzed). All serum samples were analyzed using an LC System (LC-30AD; Shimadzu, Kyoto, Japan) combined with a QTQF 5600 mass spectrometer (AB SCIEX, Framingham, MA, USA). A Kinetex 1.7u EVO C18 column (50 mm × 2.1 mm, 100 Å) (Phenomenex, Torrance, CA, USA) was used for LC separation. The column temperature was 40 °C, the injection volume was 3 µL, and the flow rate was 0.5 mL/min. Mobile phase A was 50% water + 50% acetonitrile + 10 mM ammonium formate, and mobile phase B was 10% acetonitrile + 90% isopropanol + 10 mM ammonium formate. The gradient elution conditions for mobile phases A and B are provided in [Appendix 1](#). The QTOF 5600 instrument was configured with an electrospray ionization source that was used in positive ionization mode, and the data collection method was time-of-flight (TOF)-MS-information dependent acquisition (IDA)-TOF-MS/MS (n=8). The scanning range of the quadrupole was 50–1,500 m/z, and the details of the ion source conditions are described in [Appendix 1](#). MS-DIAL software was used to qualitatively identify lipids from the original .wiff file data, and the main parameter settings are shown in [Table S1](#).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from white blood cells with TRIzol reagent (Thermo Fisher, Carlsbad, CA, USA). According to the manufacturer's instructions, complement DNA was synthesized using the PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio, Otsu, Japan). The expression of lysophosphatidylcholine acyltransferase (LPCAT) was quantified using TB Green Premix Ex Taq (Takara) on the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). The specific primers used are provided in [Table S2](#). *GAPDH* was served as the internal reference. The $2^{-\Delta\Delta C_t}$ method was used to calculate the relative expression of the gene, and each sample was examined in duplicate.

Statistical analysis

Normally distributed variables are presented as the mean ± standard deviation, abnormally distributed continuous variables are presented as medians and interquartile ranges (25th–75th), and categorical variables are described as numbers (percentages). Normally distributed variables were compared using Student's *t*-test or analysis of variance with a post hoc least significant difference test. Abnormally distributed continuous variables were compared using the Mann-Whitney U test or Kruskal-Wallis H-test, with the Nemenyi test to determine the significantly different groups; P values were adjusted by Bonferroni correction. The chi-square test or Fisher's exact test was used for categorical variables. SPSS 25.0 (IBM Corp., Armonk, NY, USA) was used for the data analysis.

Principal component analysis (PCA), volcano plots, and cluster analysis of lipidomics were performed using MetaboAnalyst 5.0 (Wishart Research Group, University of Alberta, AB, Canada). SIMCA 14.1 software (Umetrics, Umeå, Sweden) was used for orthogonal partial least squares discriminant analysis (OPLS-DA). Multivariate logistic regression data were used in receiver operating characteristic (ROC) analysis to distinguish patients with SCAP from patients with NSCAP and to separate nonsurvivors from survivors. The area under the curve (AUC), sensitivity, specificity, and 95% confidence interval were calculated. DeLong's test of MedCalc statistical software version 19.6 (MedCalc Software Ltd., Ostend, Belgium) was used to compare ROC curves. Furthermore, Youden's index was used to determine the optimal cutoff

value for the ROC analyses. The Kaplan–Meier method was used to establish 30-day survival curves, and the log-rank test was used to compare survival rates. Spearman's rank correlation analysis was used to evaluate associations between different values, and P values were adjusted using the Benjamini-Hochberg method.

Gene expression data of adult patients with CAP and the control group were selected from the Gene Expression Omnibus (GEO) database. Only datasets from the whole-blood leukocyte transcriptome were included in the study (Dataset: GSE65682) (10,11). Data conversion and grouping were performed using the R programming language. Differentially expressed genes (DEGs) between patients with SCAP and healthy controls were identified using multivariable linear models (limma) after adjusting for age (12); P values were adjusted using the Benjamini-Hochberg method. DEGs were screened with $|\log(\text{fold change})| > 1$ and false discovery rate (FDR) < 0.05 . Gene ontology (GO) enrichment analyses were performed using clusterProfiler (version v3.18.1) from the R packages (13). Inflammation-related genes were defined as genes with an “inflammatory response” in the GO term. All statistical tests were two-sided, and significance was defined as a P value or FDR < 0.05 .

Results

General characteristics of the study population

A total of 58 patients, including 30 with NSCAP and 28 with SCAP, and 11 NCs were enrolled in the study. Table 1 summarizes the demographic and selected clinical characteristics of the study population. Baseline characteristics were matched between the groups and revealed no significant differences in sex, age, smoking history, or underlying disease (Table 1, Table S3). Clinical laboratory results showed that inflammation-related indicators were significantly different between the SCAP and NSCAP groups. The neutrophil (NE) counts, neutrophil percentages (NE%), NE/lymphocyte (LY) ratio, and PLT/LY ratio were increased, while the LY counts and percentages of LY and monocytes (MO) were decreased in the SCAP group compared with those in the NSCAP group ($P < 0.05$). Serum C-reactive protein (CRP) and procalcitonin (PCT) levels were significantly increased in the SCAP group ($P < 0.05$). The CURB-65 score (confusion, uremia, respiratory rate, blood pressure, and age ≥ 65 years) and pneumonia severity index (PSI) in the SCAP group

were significantly higher than those parameters in the NSCAP group ($P < 0.05$). The mortality rate in the SCAP group was significantly higher than the mortality rate in the NSCAP group ($P < 0.05$).

Extensive changes in PL and LPL abundance in CAP patients

We utilized targeted lipidomic analysis to generate serum lipid profiles for 69 subjects. Sixty-seven lipids were detected, including 36 species of PC, 10 species of PE, 18 species of LPC, and 3 species of LPE. PCA was used to evaluate whether the differences in serum lipid profiles could effectively distinguish the three groups of subjects. The results showed differences in aggregation within and separation among the groups. PC1 explained 32.1% of the variance, followed by PC2 (12%) (Figure 1A). By comparing differences in the abundance of lipid groups between CAP patients and NCs, we found that LPC levels were mainly decreased, PE levels were mainly increased, and PC levels were both upregulated and downregulated, which may indicate involvement in different metabolic processes (Figure 1B, 1C). Analysis of the lipid groups showed that LPE and LPC were characterized mainly by decreased abundance in patients with CAP, while PE and PC were mainly increased. The differences in lipid abundance between PE, PC, and LPC among the three groups were statistically significant ($P < 0.05$; Table S4). PE abundance was found to be significantly upregulated between the SCAP and NSCAP groups (adjusted $P < 0.05$, Figure 1D, Table S4). PC/LPC and PE/LPE ratios were also significantly increased in patients with CAP ($P < 0.05$, Figure 1E, Table S5).

Levels of PLs and LPLs as predictors of disease severity

We evaluated the potential of PLs and LPLs to distinguish SCAP from NSCAP using binary ROC curve analysis. The results showed that the AUCs for PE and LPC were slightly lower than the AUCs for CURB-65 scores and the PSI, but the difference was not statistically significant (Figure 2A; Tables S6, S7). The prediction performances of the four lipid groups were significantly improved after combination with CURB-65 scores. The AUC of PE combined with CURB-65 was 0.848, which was higher than the AUC of CURB-65 alone ($P < 0.05$, Figure 2B; Tables S6, S7).

Lipid species were analyzed to build an OPLS-DA model to distinguish between NSCAP and SCAP, and the trend

Table 1 Demographic characteristics of the study population

Variable	Nonsevere (n=30)	Severe (n=28)	Control (n=11)	P value	
				Nonsevere vs. severe	All groups
Male sex	18 (60.0)	20 (71.4)	4 (36.4)	0.523	0.144
Age, years	65.7±18.37	65.64±13.2	61.91±9.14	0.989	0.755
BMI	21.97 (17.58, 25.39)	21.13±4.01		0.372	
Smoking history	7 (23.3)	11 (39.3)		0.304	
Underlying diseases					
Chronic lung disease	9 (30.0)	9 (32.1)	0	0.860	0.096
Hypertension	10 (33.3)	7 (25.0)	3 (27.3)	0.683	0.827
Diabetes	6 (20.0)	2 (7.1)	0	0.299	0.202
Cardiovascular disease	8 (26.7)	3 (10.7)	4 (36.4)	0.225	0.150
Autoimmune disease	1 (3.3)	2 (7.1)	0	0.951	0.768
Laboratory results					
WBC ($\times 10^9/L$)	6.05±2.8	5.95 (3.53, 13.56)		0.402	
NE ($\times 10^9/L$)	4.23±2.58	5.42 (3.07, 11.7)		0.046	
LY ($\times 10^9/L$)	1.23±0.53	0.55 (0.4, 0.7)		<0.001	
MO ($\times 10^9/L$)	0.47±0.25	0.26 (0.14, 0.68)		0.120	
NE percentages (%)	65.7±14.85	87 (78.58, 92.73)		<0.001	
LY percentages (%)	23.86±13.74	7.45 (4.73, 15.4)		<0.001	
MO percentages (%)	8.58±3.7	4.08±2.79		<0.001	
NLR (%)	3 (1.74, 5.73)	11.65 (4.72, 19.02)		<0.001	
PLR (%)	159.82±89.84	339.11±235.02		0.001	
PLT ($\times 10^9/L$)	174±86.93	192.93±128.88		0.520	
Alb (g/L)	35.84±5.05	27.7(24.05, 31.5)		<0.001	
PaO ₂ /FiO ₂	324.47±105.68	169.68±70.27		<0.001	
Total cholesterol (mmol/L)	3.59±0.89	3.15 (2.46, 4.13)		0.259	
Triglyceride (mmol/L)	0.97 (0.8, 1.56)	1.46 (1.05, 2.29)		0.012	
CRP (mg/L)	28.7 (5.78, 61.6)	130.27±110.68		<0.001	
PCT (μg/L)	0.16 (0.1, 1.6)	1.28 (0.34, 2.24)		0.035	
CURB-65	1 (0, 1)	2 (1, 2)		<0.001	
PSI	78 (59, 92)	98.5 (86.25, 126.25)		<0.001	
30-day mortality	0	18 (64.3)		<0.001	

Categorical variables are presented as numbers (percentages). Normally distributed variables are presented as the mean ± standard deviation, and abnormally distributed continuous variables are expressed as the median and interquartile ranges (25th-75th). BMI, body mass index; WBC, white blood cell; NE, neutrophil; LY, lymphocyte; MO, monocyte; NLR, neutrophil/lymphocyte ratio; PLR, platelet-lymphocyte ratio; PLT, blood platelet; Alb, albumin; PaO₂, partial pressure of oxygen; FiO₂, fraction of inspiration O₂; CRP, C-reactive protein; PCT, procalcitonin; CURB-65, confusion, uremia, respiratory rate, blood pressure, and age ≥65 years old; PSI, pneumonia severity index.

Figure 1 Extensive changes in PL and LPL abundance of patients with CAP. (A) PCA of lipidomic profiles in patients with CAP (including NSCAP and SCAP) and NCs. (B) Volcano plot of fold changes in lipid abundance between NC and patients with CAP. (C) Hierarchical clustering heatmap of the relative abundance of 67 lipids in patients with CAP (including NSCAP and SCAP) compared to NCs. Distance measure: Pearson; Clustering algorithm: ward. (D) Changes in the relative abundance of PC, LPC, PE, and LPE between the NSCAP group and NC group, SCAP group and NSCAP group. (E) Changes in the PC/LPC ratio and PE/LPE ratio between the NSCAP group and NC group, SCAP group and NSCAP group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. FC, Fold Changes; PL, phospholipid; LPL, lysophospholipid; CAP, community-acquired pneumonia; PCA, principal component analysis; NC, nondisease control; NSCAP, nonsevere community-acquired pneumonia; SCAP, severe community-acquired pneumonia; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine.

Figure 2 Levels of PL and LPL as predictors of disease severity. ROC curve analysis to discriminate SCAP from patients with CAP. (A) ROC curve analysis of lipid groups. (B) ROC curve analysis of lipid groups combined with CURB-65 and PSI. (C) OPLS-DA between SCAP and NSCAP. (D) ROC curve analysis of lipid species. (E) ROC curve analysis of lipid species combined with CURB-65. AUC, area under the curve; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; CURB-65, confusion, uremia, respiratory rate, blood pressure, and age ≥ 65 years old; PSI, pneumonia severity index; NSCAP, nonsevere community-acquired pneumonia; SCAP, severe community-acquired pneumonia; CAP, community-acquired pneumonia; VIP, variable importance in projection; FDR, false discovery rate; PL, phospholipid; LPL, lysophospholipid; ROC, receiver operating characteristic; OPLS-DA, orthogonal partial least squares discriminant analysis.

of differentiation is shown in Figure 2C. Lipid species were screened according to variable importance in projection (VIP) values. The primary screening criteria for lipid species were a VIP > 1 and lipid abundance that changed significantly between the NSCAP and SCAP groups (FDR < 0.05). A total of 12 lipid species were selected. Binary ROC analysis was performed on these lipid species, and lipid species with an AUC > 0.8 were selected for subsequent analysis (Table S8). Two lipid species were screened out, including PE (36:1) and PE (36:4). The predictive powers of the lipid species were slightly higher than the predictive power of the CURB-65 scores and the PSI, but the differences were not statistically significant (Figure 2D; Tables S6,S7). The AUCs for the lipid species combined with CURB-65 scores were significantly higher than the AUCs of CURB-65 alone ($P < 0.05$, Figure 2E; Tables S6,S7).

Levels of PLs and LPLs as predictors of 30-day mortality

Patients were divided into survivor and nonsurvivor groups according to 30-day survival. We evaluated the ability of lipids to distinguish between survivors and nonsurvivors using binary ROC curve analysis. The results showed that PE, LPC, PC/LPC ratio, and PE/LPE ratio appeared to be slightly more effective in distinguishing nonsurvivors and survivors than CURB-65 scores and the PSI, but the differences were not statistically significant (Figure 3A,3B; Tables S9,S10). The AUCs for PC, LPC, and the PC/LPC ratio were significantly higher when combined with CURB-65 scores than when compared with CURB-65 scores alone ($P < 0.05$, Figure 3C; Tables S9,S10).

The AUCs for LPC and PE were 0.789 and 0.725, respectively, to distinguish between survivor and

Figure 3 Levels of PL and LPL as predictors of 30-day mortality. ROC curve analysis was performed to discriminate nonsurvivors from patients with CAP. (A) ROC curve analysis of lipid groups. (B) ROC curve analysis of lipid ratios (PC/LPC, PE/LPE). (C) ROC curve analysis of lipid groups and lipid ratios combined with CURB-65. (D) Kaplan-Meier analysis of 30-day mortality in patients with CAP stratified by normalized LPC levels (0.3247). (E) Kaplan-Meier analysis of 30-day mortality in patients with CAP stratified by normalized PE levels (0.2669). (F) ROC curve analysis of lipid species. (G) ROC curve analysis of lipid species combined with CURB-65. (H) ROC curve analysis of lipid species combined with PSI. ROC, receiver operating characteristic; AUC, area under the curve; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; CAP, community-acquired pneumonia; CURB-65, confusion, uremia, respiratory rate, blood pressure, and age ≥ 65 years old; PSI, pneumonia severity index; VIP, variable importance in projection; FDR, false discovery rate; PL, phospholipid; LPL, lysophospholipid.

nonsurvivor patients with CAP. The optimal normalized relative abundance threshold for predicting mortality was 0.3247 for LPC and 0.2669 for PE. To further evaluate the relationship between LPC or PE and disease prognosis,

patients were divided into high- and low-abundance groups according to the threshold value determined by ROC analysis. Kaplan-Meier curves showed that patients with low LPC or high PE abundance had significantly higher 30-day

Figure 4 Correlation between phospholipid metabolism and clinical indicators. Colored circles represent Spearman correlation coefficients, and significant correlations are indicated by white asterisks. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; LY, lymphocyte; MO, monocytes; NE%, percentage of neutrophils; PLT, platelet count; LDH, lactate dehydrogenase; ALB, albumin; PSI, pneumonia severity index; CRP, C-reactive protein.

mortality ($P < 0.05$, Figure 3D,3E).

Subsequently, an OPLS-DA model was constructed based on lipid species to distinguish between survivors and nonsurvivors of CAP using supervised clustering. The preliminary screening criteria for lipid species were VIP > 1 and a significant difference in lipid abundance between the two groups ($FDR < 0.05$). A total of 25 lipid species were selected. We performed binary AUC analysis and selected lipids with an AUC > 0.8 for subsequent analysis (Tables S8). Two lipid species were screened out and included PC (36:4)∣PC (18:2_18:2) and LPC (18:2/0:0). Binary ROC analysis showed that the AUCs for these two lipid species were greater than those for the PSI ($P < 0.05$, Figure 3F; Tables S9,S10). The predictive performance of each lipid species was further improved after combination with CURB-65 scores and PSI. The AUCs of combined PC (36:4)∣PC (18:2_18:2) were 0.878 and 0.853, which were significantly better than those of CURB-65 and PSI alone

($P < 0.05$, Figure 3G,3H; Tables S9,S10).

Correlation between phospholipid metabolism and clinical indicators

We used Spearman's rank correlation test to calculate the correlations between clinical indicators and the relative levels of lipid groups, selected lipid species, and lipid ratios in patients with CAP (available online: <https://cdn.amegroups.cn/static/public/10.21037/atm-21-4008-1.xlsx>, <https://cdn.amegroups.cn/static/public/10.21037/atm-21-4008-2.xlsx>). The results showed that the relative abundances of PE and LPC were related to clinical indicators, including LY, monocytes (MO), NE%, PLT, lactate dehydrogenase (LDH), and albumin (Alb). Notably, PE was negatively correlated with LY, MO, and Alb and positively correlated with PSI and LDH. LPC was positively correlated with LY and Alb and negatively correlated with the PSI, NE% and CRP levels. Both PC/LPC and PE/LPE ratios were negatively correlated with LY and Alb and positively correlated with NE% and CRP ($FDR < 0.05$, Figure 4).

Activation of metabolic pathways and correlation with inflammation

We used the gene expression dataset (GSE65682) from the GEO database to further explore the relationship between phospholipid metabolism and inflammation in patients with CAP. The cohort included 108 patients with SCAP and 42 healthy controls. The median age of the SCAP group was higher than the median age of healthy controls (63.5 *vs.* 37 years, $P < 0.05$, Table S11). Whole-blood leukocyte transcriptional profiles were obtained by genome-wide microarrays. First, we analyzed the expression levels of key enzymes involved in the Lands cycle (Figure 5A). With age adjustment, the results showed that the gene expression of *LPCAT* 2-4 and *LPEAT1* was significantly upregulated in patients with SCAP, while the gene expression of *LPCAT1* was downregulated ($FDR < 0.05$, Figure 5B,5C, Figure S1). The gene expression of *LPCAT1* and *LPCAT2* was further confirmed in our study group using qRT-PCR (adjusted $P < 0.05$, Figure 5D,5E, Table S12).

GO enrichment analysis revealed significant enrichment of NE degranulation and NE activation involved in the immune response in CAP patients, which suggests extensive activation of NE-related inflammatory pathways in patients with CAP (Figure 6A). Table S13 summarizes DEGs involved in the inflammatory response ($|\log(\text{fold change})| > 1$ and $FDR < 0.05$). In addition, we

Figure 5 Upregulation of gene expression of lipid remodeling enzymes in patients with CAP. (A) Schematic diagram of Lands cycle. The Lands cycle maintains the balance between PL and LPL, and the key enzymes for these processes are PLA2 and lysophospholipid acyltransferase, mainly LPCAT1-4 and LPEAT1-2. (B,C) Gene expression levels of *LPCAT1* and *LPCAT2* in healthy controls and patients with severe CAP. (D,E) Gene expression levels of *LPCAT1* and *LPCAT2* were determined in the NC, NSCAP and SCAP groups using qRT-PCR. * adjusted $P < 0.05$. PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; LPCAT, lysophosphatidylcholine acyltransferase; LPEAT, lysophosphatidylethanolamine acyltransferase; PLA2, phospholipase A2; CAP, community-acquired pneumonia; NC, nondisease control; NSCAP, nonsevere community-acquired pneumonia; SCAP, severe community-acquired pneumonia; PL, phospholipid; LPL, lysophospholipid; qRT-PCR, quantitative real-time polymerase chain reaction.

explored the correlation between the gene expression of *LPCAT1/2* and inflammation-related genes. The results showed that *LPCAT2* was positively correlated with many inflammation-related upregulated genes in patients with CAP, while *LPCAT1* showed a negative correlation ($FDR < 0.05$; available online: <https://cdn.amegroups.cn/static/public/10.21037/atm-21-4008-3.xlsx>, <https://cdn.amegroups.cn/static/public/10.21037/atm-21-4008-4.xlsx>). The correlation between *LPCAT1/2* and the top five inflammation-related genes with the most significant differential expression is shown in Figure 6B, including upregulated genes and downregulated genes.

Discussion

This study systematically investigated the metabolic remodeling of PLs and LPLs in patients with CAP. Serum-targeted lipidomic analysis revealed extensive variations in PL and LPL levels in these patients. LPC decreased upon aggravation of the disease, while the PC, PE, PC/LPC ratio, and PE/LPE ratio increased. Changes in lipid abundance can be used to predict disease severity and 30-day mortality in patients. Compared with CURB-65 scoring and the PSI alone, the AUCs of the selected lipids and lipid ratios were significantly increased after combining

Figure 6 Correlation between lipid remodeling enzymes and the inflammatory response. (A) Gene ontology enrichment analysis of differentially expressed genes between patients with severe CAP and healthy controls. (B) Correlation of *LPCAT1* and *LPCAT2* with inflammation-related genes. Upregulated genes: *HP*, haptoglobin; *MMP8*, matrix metalloproteinase 8 (neutrophil collagenase); *LCN2*, lipocalin-2 (neutrophil gelatinase-associated lipocalin); *IL1R2*, interleukin-1 receptor type 2; *VNN1*, vascular noninflammatory molecule 1 (pantetheinase). Downregulated genes: *CCR3*, C-C chemokine receptor type 3; *KLRG1*, killer cell lectin-like receptor subfamily G member 1; *CX3CR1*, CX3C chemokine receptor 1; *CCR7*, C-C chemokine receptor type 7; *CHI3L1*, chitinase-3-like protein 1. Colored circles represent Spearman correlation coefficients, and significant correlations are indicated by white asterisks. *, $P < 0.05$; ***, $P < 0.001$. LPCAT, lysophosphatidylcholine acyltransferase; LPEAT, lysophosphatidylethanolamine acyltransferase; CAP, community-acquired pneumonia.

them with CURB-65 scores and PSI. Correlation analysis showed a significant correlation between phospholipid metabolism and inflammatory indicators such as CRP ($P < 0.05$). Additionally, we used a public database to further explore the PL and LPL metabolic imbalance and its relationship with inflammation in patients with CAP at the gene expression level.

Biomarkers in body fluids are increasingly being used to diagnose and predict the prognosis of pulmonary infectious diseases (14). Our results showed that the lipid concentrations (PE, LPC) and lipid ratios (PC/LPC, PE/LPE) were equally effective to CURB-65 and the PSI in predicting mortality in patients with CAP. Moreover, the combination with CURB-65 can effectively improve

the predictive performance. Previous studies have also evaluated the efficacy of LPC and the PC/LPC ratio to predict the mortality of patients with CAP and sepsis, which was comparable to CURB-65 and the PSI (15-20). Dynamic changes in LPC were more closely related to patient prognosis (19-22); LPC concentrations in survivors increased with disease improvement, while nonsurvivors showed a continuous decrease in LPC levels (17-19). Each lipid group includes many lipid species according to the degree of saturation of their side chains. Because different lipid species may play different or even opposite roles in the metabolic process (23), the prediction efficiency of lipid species may be better than the prediction efficiency of lipid groups. The predictive performance of lipid species in

infectious diseases has been reported in previous lipidomic studies (5,16,24). However, owing to the complexity of the detection methods, there are some obstacles to the clinical application of lipid species.

Lipids are essential building blocks of cells, and inflammation can induce a variety of alterations in lipid metabolism (25). The relationship between PL metabolism and inflammatory diseases has attracted increasing attention in recent years. Our study found that serum PE and PC concentrations and PC/LPC and PE/LPE ratios were significantly increased in CAP, and LPC was significantly decreased. Furthermore, similar results have also been observed in patients with pneumonia and sepsis (15-22). PC and PE are the most abundant PLs in mammals, acting as the major structural components of cell membranes and playing an important role in regulating lipids, lipoproteins, and systemic energy metabolism (26). PE mainly affects membrane topology, promotes cell and organelle membrane fusion, and maintains mitochondrial membrane homeostasis (27). Elevated PE concentrations in disease states may lead to associated metabolic and immunological imbalances that affect an individual's ability to cope with infections. LPC and LPE are mainly generated from phospholipase A2 (PLA2) hydrolysis and act as signaling mediators with wide-ranging biological effects (28). LPC can exert anti-inflammatory effects by increasing the chemotaxis and phagocytosis of macrophages and enhancing the antibacterial activity of NEs (29,30). Yan *et al.* showed that LPC injection could effectively protect against sepsis-induced lethality and enhance bacterial clearance in mice. In addition, an *in vitro* study showed that LPC significantly improved the bactericidal activity of NEs (31). The decrease in LPC levels in patients with CAP may indicate an impaired resolution of inflammation. Furthermore, the strong negative correlation between LPC and CRP reported in our study is consistent with the findings of other studies (19,32). We also reported significant positive correlations of the PC/LPC ratio and the PE/LPE ratio with CRP, further indicating their role as inflammation markers. The majority of LPC in plasma is carried by Alb (33), which may partly explain the positive correlation between LPC and Alb. While LPC concentrations are reduced in most infectious diseases, studies on coronavirus disease have shown contradictory results (34-37), which suggests that different pathogens may induce different phospholipid metabolism alterations.

The metabolism of PLs and LPLs involves multiple pathways, and a common source of PC and PE in the body

is Lands cycle, a deacylation/acylation process centered on glycerophospholipids. The Lands cycle maintains the balance between PLs and LPLs, and the key enzymes for these processes are PLA2 and lysophospholipid acyltransferase (LPLAT). Different LPLATs are distributed throughout tissues and play various roles; LPCAT1 and LPCAT2 were first cloned and have been particularly well studied. *LPCAT1* is primarily expressed in lung alveolar type II cells for pulmonary surfactant biosynthesis (38,39), while *LPCAT2* is highly expressed in inflammatory cells, including resident macrophages and casein-induced NEs (40). We hypothesized that changes in phospholipid levels in patients with CAP may be related to the preprogramming of phospholipase activity in infected cells (21). Therefore, we explored the gene expression of key enzymes involved in the Lands cycle, especially *LPCAT1* and *LPCAT2*. The results showed that *LPCAT1* was significantly downregulated in patients with CAP, while *LPCAT2* was significantly upregulated. These findings were further validated using qRT-PCR. *LPCAT1* is a constitutively expressed enzyme, while *LPCAT2* is inducible (41,42), which may explain their different regulations of gene expression in patients with CAP. GO enrichment analysis of differentially expressed upregulated genes in CAP patients showed significant enrichment of genes associated with NE activation, suggesting that NEs play a crucial role in the disease course of CAP. NEs are critical inflammatory cells, and lipid remodeling in NEs is important in lipidomics data in serum samples. The positive correlation between PC/LPC ratios and NE%, as well as the upregulation of gene expression levels of *LPCAT2* in whole-blood leukocytes, further validated the association between NEs and serum lipidomics.

This study has some limitations. First, although our results were consistent with published evidence, the small sample size and lack of an external validation cohort remained an issue in this study. The predictive performance of these lipids, their usefulness as biomarkers, and the activation status of the Lands cycle require further verification. Second, serum samples were collected from patients only within 24 h after admission without longitudinal comparison, and dynamic monitoring of lipid abundance may be more helpful in predicting the prognosis of patients.

This study systematically evaluated the metabolism of key PLs and LPLs in patients with CAP and further validated the predictive power of LPC in patients with CAP and proposed the potential of PE as a biomarker for the first time. Additionally, we explored the metabolic disorders of

PLs and LPLs from the perspective of gene expression and found that activation of the Lands cycle could be a possible explanation for changes in phospholipid metabolism in patients with CAP. The role of PL and LPL metabolic disorders in different infectious diseases requires further study.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-21-4008/rc>

Data Sharing Statement: Available at <https://atm.amegroups.com/article/view/10.21037/atm-21-4008/dss>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-21-4008/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the ethics committee of Peking University People's Hospital (No. 2016PHB202-01) and registered as a clinical study at ClinicalTrials.gov (Identifier: NCT03093220). The study was performed in line with the principles of the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patients prior to clinical data collection and sampling.

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Appendix 1 Targeted lipidomic analysis

The detailed gradient elution conditions for mobile phases A and B were as follows: flow rate: 0.5 mL/min. 0 min—90% (A):10% (B); 0.5 min—90% (A):10% (B);11 min—100% (B); 17 min—100% (B); 17.1 min—90% (A):10% (B); 20 min—90% (A):10% (B).

The ion source conditions were set as follows: temperature, 550 °C; curtain gas, 30 psi; ion source gas 1, 50 psi; ion source gas 2, 60 psi; ion spray voltage, 5,500 V in positive mode; declustering potential, 80 V; collision energy, 10 V for time-of-flight mass spectrometry (TOF-MS), and 35±15 V for TOF-MS/MS.

Figure S1 Gene expression levels of *LPEAT1-2* and *LPCAT3-4* in healthy controls and patients with severe CAP. LPCAT, lysophosphatidylcholine acyltransferase; LPEAT, lysophosphatidylethanolamine acyltransferase.

Table S1 Search conditions

Parameter	Condition
Retention time tolerance	100 min
Accurate mass tolerance (MS1)	0.01 Da
Accurate mass tolerance (MS2)	0.05 Da
Identification score cut off	90%
Use retention time for scoring	√

Table S2 Primers used for qRT-PCR analysis

Gene	Forward sequence	Reverse sequence
<i>LPCAT1</i>	5'-ACCTATTCCGAGCCATTGACC-3'	5'-CCTAATCCAGCTTCTTGCGAAC-3'
<i>LPCAT2</i>	5'-TTGTTGCTGCCCCTCATTCA-3'	5'-GGGAATCCGGATCTACACGG-3'

Table S3 Demographic characteristics of the study population (CAP vs. Control)

Characteristic	CAP (n=58)	Control (n=11)	P value
Male sex	38 (65.5)	4 (36.4)	0.095
Age years	68 (59.25,78.25)	61.91±9.14	0.261
Underlying diseases			
Chronic lung disease	18 (31.0)	0	0.055
Hypertension	17 (29.3)	3 (27.3)	1.000
Diabetes	8 (13.8)	0	0.338
Cardiovascular disease	11 (19.0)	4 (36.4)	0.237
Autoimmune disease	3 (5.2)	0	1.000

Categorical variables are presented as numbers (percentages). Normally distributed variables are presented as the mean ± standard deviation, and abnormally distributed continuous variables are expressed as the median and interquartile ranges (25th-75th). CAP, community-acquired pneumonia.

Table S4 Between-group comparisons of lipid groups

Variable	PE	LPE	PC	LPC
Relative abundances of four lipid groups				
NC	-1.128 (-1.417, -0.723)	0.233 (-0.477, 1.007)	-0.888 (-2.116, -0.060)	1.292 (0.794, 1.894)
CAP	0.215 (-0.648, 0.840)	-0.218 (-0.604, 0.375)	0.250 (-0.218, 0.672)	-0.270 (-0.901, 0.327)
NSCAP	-0.350 (-0.722, 0.419)	-0.334 (-0.464, 0.344)	0.136 (-0.134, 0.679)	-0.019 (-0.394, 0.369)
SCAP	0.728 (-0.217, 1.362)	-0.135 (-1.024, 0.461)	0.284 (-0.298, 0.665)	-0.723 (-1.108, -0.133)
Results of comparisons				
All groups	P<0.001	P=0.518	P=0.004	P<0.001
NC vs. NSCAP	P=0.017	–	P=0.009	P=0.006
NSCAP vs. SCAP	P=0.026	–	P=1.000	P=0.064
NC vs. CAP	P<0.001	P=0.003	P<0.001	P<0.001

Variables are presented as medians and interquartile ranges (25th-75th). PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; CAP, community-acquired pneumonia; NC, nondisease control; NSCAP, nonsevere community-acquired pneumonia; SCAP, severe community-acquired pneumonia.

Table S5 Between-group comparisons of lipid ratios

Variable	PC/LPC	PE/LPE
Lipid ratios		
NC	16.11 (13.49, 19.10)	14.83 (13.37, 19.45)
CAP	30.54 (22.77, 46.37)	24.29 (18.82, 33.54)
NSCAP	26.43 (22.29, 32.73)	22.49 (18.30, 28.53)
SCAP	40.43 (28.20, 55.80)	28.77 (19.76, 45.58)
Results of comparisons		
All groups	P<0.001	P<0.001
NC vs. NSCAP	P=0.005	P=0.024
NSCAP vs. SCAP	P=0.073	P=0.199
NC vs. CAP	P<0.001	P<0.001

Variables are presented as medians and interquartile ranges (25th-75th). PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; CAP, community-acquired pneumonia; NC, nondisease control; NSCAP, nonsevere community-acquired pneumonia; SCAP, severe community-acquired pneumonia.

Table S6 Areas under the curves (AUCs) and thresholds for ROC analysis (NSCAP *vs.* SCAP)

Variable		Threshold	Sensitivity (%)	Specificity (%)	AUC	P value	95% CI
CURB65		>1	53.57	93.33	0.782	<0.0001	0.654 to 0.880
PSI		>92	67.86	80.00	0.781	<0.0001	0.653 to 0.879
Lipid groups	PE	>0.2669	60.71	80.00	0.726	0.0010	0.593 to 0.835
	PC	>0.0746	57.14	60.00	0.530	0.7028	0.394 to 0.662
	LPE	≤-0.5284	35.71	83.33	0.504	0.9644	0.369 to 0.638
	LPC	≤-0.8148	50.00	96.67	0.708	0.0044	0.574 to 0.820
	PE_CURB65	>0.5517	71.43	86.67	0.848	<0.0001	0.729 to 0.928
	PC_CURB65	>0.3961	53.57	93.33	0.782	<0.0001	0.654 to 0.880
	LPE_CURB65	>0.3961	53.57	93.33	0.782	<0.0001	0.654 to 0.880
	LPC_CURB65	>0.3961	53.57	93.33	0.782	<0.0001	0.654 to 0.880
	PE_PSI	>0.6124	60.71	86.67	0.799	<0.0001	0.673 to 0.893
	PC_PSI	>0.4907	67.86	80.00	0.781	<0.0001	0.653 to 0.879
	LPE_PSI	>0.4907	67.86	80.00	0.781	<0.0001	0.653 to 0.879
	LPC_PSI	>0.4907	67.86	80.00	0.781	<0.0001	0.653 to 0.879
Lipid ratios	PC/LPC	>40.4475	50.00	93.33	0.704	0.0056	0.569 to 0.816
	PE/LPE	>33.6364	39.29	96.67	0.661	0.0323	0.525 to 0.780
	PC/LPC_CURB65	>0.6420	71.43	96.67	0.843	<0.0001	0.723 to 0.925
	PE/LPE_CURB65	>0.5121	67.86	93.33	0.824	<0.0001	0.701 to 0.911
	PC/LPC_PSI	>0.6023	60.71	86.67	0.821	<0.0001	0.699 to 0.910
	PE/LPE_PSI	>0.3445	92.86	60.00	0.811	<0.0001	0.686 to 0.902
Lipid species	PE 36:1	>0.39	67.86	93.33	0.836	<0.0001	0.715 to 0.920
	PE 36:4	>0.55	89.29	70.00	0.811	<0.0001	0.686 to 0.902
	PE 36:1_CURB65	>0.3610	92.86	86.67	0.928	<0.0001	0.829 to 0.979
	PE 36:4_CURB65	>0.3070	89.29	76.67	0.905	<0.0001	0.799 to 0.966
	PE 36:1_PSI	>0.2955	92.86	73.33	0.886	<0.0001	0.775 to 0.954
	PE 36:4_PSI	>0.3742	89.29	76.67	0.858	<0.0001	0.742 to 0.936

ROC analysis, receiver operating characteristic analysis; NSCAP, nonsevere community-acquired pneumonia; SCAP, severe community-acquired pneumonia; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; CURB-65, confusion, uremia, respiratory rate, blood pressure, and age ≥65 years old; PSI, pneumonia severity index.

Table S7 Comparisons of ROC curves (NSCAP *vs.* SCAP)

Comparison	Standard Error ^a	95% CI	Significance level
Lipid groups			
PE_CURB65 <i>vs.</i> CURB65	0.0325	0.00180 to 0.129	P=0.0439
PE_CURB65 <i>vs.</i> PSI	0.0678	−0.0662 to 0.200	P=0.3253
PE_PSI <i>vs.</i> CURB65	0.0693	−0.119 to 0.152	P=0.8099
PE_PSI <i>vs.</i> PSI	0.0417	−0.0638 to 0.0995	P=0.6683
Lipid ratios			
PC/LPC_CURB65 <i>vs.</i> CURB65	0.0414	−0.0205 to 0.142	P=0.1427
PC/LPC_CURB65 <i>vs.</i> PSI	0.0758	−0.0867 to 0.210	P=0.4142
PE/LPE_CURB65 <i>vs.</i> CURB65	0.0370	−0.0308 to 0.114	P=0.2596
PE/LPE_CURB65 <i>vs.</i> PSI	0.0728	−0.0999 to 0.186	P=0.5562
PC/LPC_PSI <i>vs.</i> CURB65	0.0670	−0.0919 to 0.171	P=0.5574
PC/LPC_PSI <i>vs.</i> PSI	0.0399	−0.0377 to 0.119	P=0.3101
PE/LPE_PSI <i>vs.</i> CURB65	0.0671	−0.103 to 0.160	P=0.6703
PE/LPE_PSI <i>vs.</i> PSI	0.0335	−0.0359 to 0.0954	P=0.3742
Lipid species			
PE 36:1 <i>vs.</i> CURB65	0.0802	−0.104 to 0.211	P=0.5041
PE 36:1 <i>vs.</i> PSI	0.0873	−0.116 to 0.226	P=0.5304
PE 36:4 <i>vs.</i> CURB65	0.0840	−0.136 to 0.193	P=0.7338
PE 36:4 <i>vs.</i> PSI	0.0879	−0.142 to 0.202	P=0.7348
PE 36:1_CURB65 <i>vs.</i> CURB65	0.0467	0.0543 to 0.237	P=0.0018
PE 36:1_CURB65 <i>vs.</i> PSI	0.0705	0.00886 to 0.285	P=0.0370
PE 36:4_CURB65 <i>vs.</i> CURB65	0.0447	0.0357 to 0.211	P=0.0058
PE 36:4_CURB65 <i>vs.</i> PSI	0.0697	−0.0121 to 0.261	P=0.0741
PE 36:1_PSI <i>vs.</i> CURB65	0.0671	−0.0280 to 0.235	P=0.1230
PE 36:1_PSI <i>vs.</i> PSI	0.0566	−0.00616 to 0.216	P=0.0641
PE 36:4_PSI <i>vs.</i> CURB65	0.0716	−0.0642 to 0.217	P=0.2876
PE 36:4_PSI <i>vs.</i> PSI	0.0514	−0.0233 to 0.178	P=0.1319

^a, DeLong *et al.*, 1988. ROC curves, receiver operating characteristic curves; NSCAP, nonsevere community-acquired pneumonia; SCAP, severe community-acquired pneumonia; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; CURB-65, confusion, uremia, respiratory rate, blood pressure, and age ≥65 years old; PSI, pneumonia severity index.

Table S8 67 lipid species as predictors of disease severity and mortality

No.	Lipid	Formula	Reference m/z	Ontology	NSCAP vs. SCAP			Non-Survivors vs. Survivors		
					FDR	VIP	ROC	FDR	VIP	ROC
1	LPC 14:0/0:0	C22H46NO7P	468.3085	LPC	0.83563	0.793598	0.612	0.022973	1.38158	0.749
2	LPC 15:0	C23H48NO7P	482.3241	LPC	0.19621	0.923794	0.643	0.011075	1.39135	0.783
3	LPC 16:0	C24H50NO7P	496.33981	LPC	0.075358	0.935215	0.654	0.011075	1.33066	0.740
4	LPC 16:1	C24H48NO7P	494.3241	LPC	0.48261	0.77831	0.614	0.015777	1.33353	0.733
5	LPC 17:0	C25H52NO7P	510.35541	LPC	0.049975	1.2005	0.673	0.0081951	1.54735	0.773
6	LPC 17:1	C25H50NO7P	508.33981	LPC	0.33353	0.769032	0.602	0.023985	1.15304	0.696
7	LPC 18:0	C26H54NO7P	524.37109	LPC	0.049975	1.24612	0.689	0.0061942	1.53301	0.775
8	LPC 18:1	C26H52NO7P	522.35541	LPC	0.24738	0.858686	0.640	0.015777	1.32437	0.738
9	LPC 18:2/0:0	C26H50NO7P	520.33978	LPC	0.03284	0.981038	0.686	0.00055093	1.52466	0.819
10	LPC 18:3/0:0	C26H48NO7P	518.3241	LPC	0.83563	0.530427	0.565	0.023985	1.25328	0.738
11	LPC 20:0	C28H58NO7P	552.4024	LPC	0.03284	1.572	0.723	0.011075	1.66979	0.788
12	LPC 20:1	C28H56NO7P	550.38672	LPC	0.39817	0.974651	0.650	0.17646	1.1212	0.686
13	LPC 20:2	C28H54NO7P	548.37109	LPC	0.10697	1.2062	0.651	0.014959	1.52131	0.741
14	LPC 20:3	C28H52NO7P	546.35541	LPC	0.45934	1.04493	0.604	0.034766	1.48429	0.729
15	LPC 20:4	C28H50NO7P	544.33978	LPC	0.083065	1.11815	0.652	0.023913	1.37936	0.726
16	LPC 20:5	C28H48NO7P	542.3241	LPC	0.1865	1.09937	0.588	0.26601	1.18821	0.635
17	LPC 22:5	C30H52NO7P	570.35541	LPC	0.049975	0.860263	0.684	0.15529	1.11783	0.709
18	LPC 22:6/0:0	C30H50NO7P	568.33978	LPC	0.049975	0.988376	0.625	0.046584	1.20703	0.717
19	LPE 16:0	C21H44NO7P	454.29279	LPE	0.85856	0.178465	0.508	0.25502	0.474754	0.623
20	LPE 18:0	C23H48NO7P	482.3241	LPE	0.82696	0.0551849	0.515	0.30499	0.52997	0.619
21	LPE 18:1	C23H46NO7P	480.3085	LPE	0.83563	0.203683	0.527	0.30499	0.562532	0.628
22	PC 28:0	C36H72NO8P	678.50677	PC	0.97368	0.534154	0.511	0.5295	0.249578	0.612
23	PC 30:0	C38H76NO8P	706.53809	PC	0.6713	0.536389	0.515	0.70858	0.00560963	0.536
24	PC 32:0 PC 16:0_16:0	C40H80NO8P	734.5694	PC	0.83563	0.611099	0.531	0.15529	0.442061	0.564
25	PC 32:1 PC 16:0_16:1	C40H78NO8P	732.55377	PC	0.61017	1.03207	0.602	0.57029	0.417093	0.526
26	PC 32:2	C40H76NO8P	730.53809	PC	0.71146	0.14117	0.568	0.040949	0.958506	0.711
27	PC 33:1	C41H80NO8P	746.5694	PC	0.6653	1.21991	0.711	0.70858	0.583084	0.638
28	PC 33:2	C41H78NO8P	744.55377	PC	0.79718	0.167913	0.517	0.20101	0.616444	0.665
29	PC 34:0	C42H84NO8P	762.60071	PC	0.002639	0.8537	0.666	0.023913	1.14555	0.717
30	PC 34:1 PC 16:0_18:1	C42H82NO8P	760.58508	PC	0.0013469	1.88253	0.796	0.0038959	1.07796	0.669
31	PC 34:2 PC 16:0_18:2	C42H80NO8P	758.5694	PC	0.37919	0.766746	0.598	0.57195	0.0450517	0.515
32	PC 34:3	C42H78NO8P	756.55377	PC	0.83665	0.448726	0.539	0.42663	0.468133	0.605
33	PC 34:4	C42H76NO8P	754.53809	PC	0.72028	0.517448	0.581	0.065522	1.15962	0.707
34	PC 35:1 PC 16:0_19:1	C43H84NO8P	774.60071	PC	0.78764	0.749997	0.671	0.47621	0.559	0.660
35	PC 35:2 PC 17:0_18:2	C43H82NO8P	772.58508	PC	0.48366	0.0767404	0.528	0.96948	0.323908	0.678
36	PC 35:4	C43H78NO8P	768.55377	PC	0.35556	0.491759	0.589	0.26601	0.822216	0.660
37	PC 36:1	C44H86NO8P	810.59833	PC	0.049975	1.37514	0.710	0.47621	0.402855	0.549
38	PC 36:1 PC 18:0_18:1	C44H86NO8P	788.61639	PC	0.049975	1.37514	0.710	0.47621	0.402855	0.549
39	PC 36:2 PC 18:0_18:2	C44H84NO8P	786.60071	PC	0.049975	0.129804	0.517	0.036849	0.603332	0.667
40	PC 36:3 PC 16:0_20:3	C44H82NO8P	784.58508	PC	0.25822	0.256825	0.544	0.12114	0.94292	0.654
41	PC 36:4 PC 16:0_20:4	C44H80NO8P	782.5694	PC	0.51212	0.0189675	0.502	0.377	0.049348	0.514
42	PC 36:4 PC 18:2_18:2	C44H80NO8P	782.5694	PC	0.034486	0.688094	0.664	0.000042994	1.34042	0.854
43	PC 36:5 PC 16:0_20:5	C44H78NO8P	780.55377	PC	0.72028	0.474037	0.546	0.65071	0.484758	0.514
44	PC 36:6	C44H76NO8P	778.53809	PC	0.21652	0.798896	0.604	0.036411	1.24251	0.692
45	PC 37:2 PC 18:1_19:1	C45H86NO8P	800.61639	PC	0.45559	0.374756	0.558	0.65957	0.141989	0.517
46	PC 37:6	C45H78NO8P	792.55377	PC	0.10697	0.885707	0.639	0.04828	1.0758	0.710
47	PC 38:2	C46H88NO8P	836.61401	PC	0.93273	0.20337	0.561	0.49707	0.166121	0.504
48	PC 38:3 PC 18:0_20:3	C46H86NO8P	812.61639	PC	0.61017	0.537317	0.556	0.28466	1.01933	0.644
49	PC 38:4 PC 18:0_20:4	C46H84NO8P	810.60071	PC	0.057973	0.604517	0.619	0.7559	0.464577	0.586
50	PC 38:5 PC 16:0_22:5	C46H82NO8P	808.58508	PC	0.027012	0.885151	0.643	0.70858	0.839523	0.619
51	PC 38:6 PC 16:0_22:6	C46H80NO8P	806.5694	PC	0.10697	0.393498	0.608	0.44478	0.404371	0.647
53	PC 38:7	C46H78NO8P	804.55377	PC	0.10751	0.6012	0.607	0.023985	0.973503	0.716
54	PC 40:3	C48H90NO8P	840.64771	PC	0.03284	0.518565	0.659	0.15529	0.712402	0.656
55	PC 40:5	C48H86NO8P	836.61639	PC	0.012156	0.773788	0.649	0.2959	0.817348	0.649
56	PC 40:6 PC 18:0_22:6	C48H84NO8P	834.60071	PC	0.03284	0.732863	0.663	0.16367	0.719783	0.703
57	PC 40:7	C48H82NO8P	832.58508	PC	0.049975	1.07401	0.600	0.12659	1.29611	0.672
59	PC 42:8	C50H84NO8P	858.60071	PC	0.0033254	1.47184	0.747	0.049302	1.46965	0.732
60	PE 34:1 PE 16:0_18:1	C39H76NO8P	718.53809	PE	0.1865	1.98995	0.816	0.023985	1.50987	0.785
61	PE 34:2 PE 16:0_18:2	C39H74NO8P	716.52252	PE	0.0021878	2.13097	0.789	0.023985	1.24452	0.649
62	PE 35:1	C40H78NO8P	732.55377	PE	0.72028	0.00401754	0.576	0.44478	0.26545	0.622
63	PE 36:1	C41H80NO8P	746.5694	PE	0.0013469	2.13882	0.836	0.014959	1.25587	0.689
64	PE 36:2 PE 18:1_18:1	C41H78NO8P	744.55377	PE	0.0094713	2.11736	0.780	0.11525	1.12754	0.598
65	PE 36:4	C41H74NO8P	740.52252	PE	0.0044169	2.06769	0.811	0.015777	1.36878	0.698
66	PE 37:2	C42H80NO8P	758.5694	PE	0.23914	0.0564084	0.532	0.87882	0.352906	0.581
67	PE 38:2	C43H82NO8P	772.58508	PE	0.45934	0.383227	0.522	0.65957	0.298693	0.571
68	PE 38:6	C43H74NO8P	764.52252	PE	0.12449	1.38928	0.668	0.29655	0.854962	0.563
69	PE 40:6	C45H78NO8P	792.55377	PE	0.1502	1.3421	0.648	0.28466	0.861305	0.561

PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; NSCAP, non-severe community acquired pneumonia; SCAP, severe community acquired pneumonia; VIP, variable importance on projection value; FDR, false discovery rate.

Table S9 Areas under the curves (AUCs) and thresholds for ROC analysis (30-day mortality)

Variable		Threshold	Sensitivity (%)	Specificity (%)	AUC	P value	95% CI
CURB65		>1	55.56	82.50	0.722	0.0013	0.588 to 0.831
PSI		>75	94.44	40.00	0.649	0.0402	0.513 to 0.770
Lipid groups	PE	>0.2669	72.22	75.00	0.725	0.0035	0.592 to 0.834
	PC	>-0.2332	83.33	45.00	0.656	0.0429	0.519 to 0.775
	LPE	≤0.9095	100.00	20.00	0.560	0.4861	0.423 to 0.690
	LPC	≤0.3247	100.00	50.00	0.789	<0.0001	0.662 to 0.885
	PE_CURB65	>0.2321	55.56	82.50	0.722	0.0013	0.588 to 0.831
	PC_CURB65	>0.2469	83.33	70.00	0.811	<0.0001	0.687 to 0.902
	LPE_CURB65	>0.2321	55.56	82.50	0.722	0.0013	0.588 to 0.831
	LPC_CURB65	>0.3984	77.78	90.00	0.854	<0.0001	0.737 to 0.933
	PE_PSI	>0.3337	72.22	75.00	0.725	0.0035	0.592 to 0.834
	PC_PSI	>0.2597	83.33	45.00	0.656	0.0429	0.519 to 0.775
	LPE_PSI	>0	0.00	100	0.500	1.0000	0.366 to 0.634
	LPC_PSI	>0.1682	100.00	50.00	0.789	<0.0001	0.662 to 0.885
Lipid ratios	PC/LPC	>26.1709	100.00	50.00	0.788	<0.0001	0.660 to 0.884
	PE/LPE	>20.2685	94.44	47.50	0.742	0.0004	0.610 to 0.848
	PC/LPC_CURB65	>0.3601	77.78	90.00	0.838	<0.0001	0.717 to 0.921
	PE/LPE_CURB65	>0.2586	77.78	75.00	0.797	<0.0001	0.671 to 0.891
	PC/LPC_PSI	>0.1914	100.00	50.00	0.788	<0.0001	0.660 to 0.884
	PE/LPE_PSI	>0.2125	94.44	47.50	0.742	0.0004	0.610 to 0.848
Lipid species	PC 36:4 PC 18:2_18:2	≤1.636	66.67	95.00	0.854	<0.0001	0.737 to 0.933
	LPC 18:2/0:0	≤0.8014	88.89	67.50	0.819	<0.0001	0.696 to 0.908
	PC 36:4 PC 18:2_18:2_CURB65	>0.2796	94.44	77.50	0.878	<0.0001	0.765 to 0.949
	LPC 18:2/0:0_CURB65	>0.4318	77.78	90.00	0.856	<0.0001	0.738 to 0.934
	PC 36:4 PC 18:2_18:2_PSI	>0.5103	66.67	90.00	0.853	<0.0001	0.735 to 0.932
	LPC 18:2/0:0_PSI	>0.3704	83.33	75.00	0.822	<0.0001	0.700 to 0.910

ROC analysis, receiver operating characteristic analysis; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; CURB-65, confusion, uremia, respiratory rate, blood pressure, and age ≥65 years old; PSI, pneumonia severity index.

Table S10 Comparisons of ROC curves (30-day mortality)

Comparison	Standard Error ^a	95% Confidence Interval	Significance level
Lipid groups			
LPC vs. CURB65	0.0834	−0.0961 to 0.231	P=0.4193
LPC vs. PSI	0.0794	−0.0161 to 0.295	P=0.0788
PE vs. CURB65	0.102	−0.197 to 0.204	P=0.9729
PE vs. PSI	0.0937	−0.108 to 0.259	P=0.4191
PC vs. PSI	0.0996	−0.189 to 0.201	P=0.9500
LPC_CURB65 vs. CURB65	0.0498	0.0350 to 0.230	P=0.0078
LPC_CURB65 vs. PSI	0.0747	0.0585 to 0.351	P=0.0061
PC_CURB65 vs. CURB65	0.0412	0.00882 to 0.170	P=0.0297
PC_CURB65 vs. PSI	0.0815	0.00199 to 0.322	P=0.0472
LPC_PSI vs. CURB65	0.0834	−0.0961 to 0.231	P=0.4193
LPC_PSI vs. PSI	0.0794	−0.0161 to 0.295	P=0.0788
PE_PSI vs. CURB65	0.102	−0.197 to 0.204	P=0.9729
PE_PSI vs. PSI	0.0937	−0.108 to 0.259	P=0.4191
Lipid ratios			
PC/LPC_CURB65 vs. CURB65	0.0484	0.0211 to 0.211	P=0.0166
PC/LPC_CURB65 vs. PSI	0.0787	0.0340 to 0.342	P=0.0167
PE/LPE_CURB65 vs. CURB65	0.0407	−0.00404 to 0.155	P=0.0628
PE/LPE_CURB65 vs. PSI	0.0791	0.00712 to 0.303	P=0.0615
PC/LPC_PSI vs. CURB65	0.0843	−0.0993 to 0.231	P=0.4340
PC/LPC_PSI vs. PSI	0.0794	−0.0173 to 0.294	P=0.0816
PE/LPE_PSI vs. CURB65	0.0849	−0.146 to 0.187	P=0.8125
PE/LPE_PSI vs. PSI	0.0867	−0.0775 to 0.262	P=0.2865
Lipid species			
PC 36:4 PC 18:2_18:2 vs. CURB65	0.0775	−0.0193 to 0.285	P=0.0870
PC 36:4 PC 18:2_18:2 vs. PSI	0.0824	0.0433 to 0.366	P=0.0130
LPC 18:2/0:0 vs. CURB65	0.0724	−0.0440 to 0.240	P=0.1764
LPC 18:2/0:0 vs. PSI	0.0794	0.0145 to 0.326	P=0.0321
PC 36:4 PC 18:2_18:2_CURB65 vs. CURB65	0.0528	0.0527 to 0.260	P=0.0031
PC 36:4 PC 18:2_18:2_CURB65 vs. PSI	0.0787	0.0743 to 0.383	P=0.0037
LPC 18:2/0:0_CURB65 vs. CURB65	0.0464	0.0431 to 0.225	P=0.0039
LPC 18:2/0:0_CURB65 vs. PSI	0.0783	0.0528 to 0.360	P=0.0084
PC 36:4 PC 18:2_18:2_PSI vs. CURB65	0.0760	−0.0176 to 0.280	P=0.0840
PC 36:4 PC 18:2_18:2_PSI vs. PSI	0.0751	0.0562 to 0.351	P=0.0068
LPC 18:2/0:0_PSI vs. CURB65	0.0692	−0.0349 to 0.236	P=0.1457
LPC 18:2/0:0_PSI vs. PSI	0.0720	0.0318 to 0.314	P=0.0163

^a, DeLong *et al.*, 1988. ROC curves, receiver operating characteristic curves; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; CURB-65, confusion, uremia, respiratory rate, blood pressure, and age ≥65 years old; PSI, pneumonia severity index.

Table S11 Demographic characteristics of the study population (GEO database)

Characteristic	Severe CAP (n=108)	Healthy Control (n=42)	P value
Male sex-no. (%)	63 (58.3)	24 (57.1)	1.000
Age years-no. (%)	63.5 (52,73)	37 (30, 63.25)	<0.001
18-29	10 (9.3)	9 (21.4)	<0.001
30-44	5 (4.6)	15 (35.7)	
45-59	21 (19.4)	4 (9.5)	
60-74	48 (44.4)	10 (23.8)	
≥75	24 (22.2)	4 (9.5)	
Diabetes	25/105 (23.8)	0	
28-day mortality-no. (%)	23/106 (21.7)	0	

Categorical variables are presented as numbers (percentages). Abnormally distributed continuous variables are expressed as medians and interquartile ranges (25th-75th). CAP, community-acquired pneumonia.

Table S12 Demographic characteristics of the study population (qRT-PCR)

Characteristic	NSCAP (n=5)	SCAP (n=5)	Control (n=5)	P value
Male sex-no. (%)	2 (40)	2 (40)	3 (60)	1.000
Age years	66.00 (52.50, 72.00)	51.00 (43.00, 71.50)	35.00 (34.50, 56.50)	0.079
Underlying diseases no. (%)				
Chronic lung disease	0	1 (20)	0	1.000
Hypertension	0	2 (40)	0	0.286
Diabetes	1 (20)	0	0	1.000
Cardiovascular disease	1 (20)	0	0	1.000
Autoimmune disease	0	0	0	

Categorical variables are presented as numbers (percentages). Abnormally distributed continuous variables are presented as medians and interquartile ranges (25th-75th). NSCAP, nonsevere community-acquired pneumonia; SCAP, severe community-acquired pneumonia; qRT-PCR, quantitative real-time polymerase chain reaction.

Table S13 Differentially expressed genes involved in the inflammatory response

SYMBOL	logFC	AveExpr	adj.PVal	Description
<i>HP</i>	5.082	7.159	1.95E-15	Haptoglobin
<i>MMP8</i>	4.307	5.515	5.90E-07	Neutrophil collagenase
<i>LCN2</i>	3.534	8.584	2.58E-07	Neutrophil gelatinase-associated lipocalin
<i>IL1R2</i>	3.338	10.014	2.04E-15	Interleukin-1 receptor type 2
<i>VNN1</i>	3.002	5.433	6.49E-14	Pantetheinase
<i>S100A12</i>	2.964	10.996	5.27E-22	Protein S100-A12
<i>MMP9</i>	2.815	10.337	5.38E-11	Matrix metalloproteinase-9
<i>TLR5</i>	2.795	7.041	1.24E-15	Toll-like receptor 5
<i>IL18R1</i>	2.611	5.293	7.33E-13	Interleukin-18 receptor 1
<i>PGLYRP1</i>	2.56	5.917	4.68E-12	Peptidoglycan recognition protein 1
<i>LILRA5</i>	2.506	8.654	2.27E-18	Leukocyte immunoglobulin-like receptor subfamily A member 5
<i>LY96</i>	2.415	6.824	1.28E-09	Lymphocyte antigen 96
<i>AIM2</i>	2.282	6.7	1.87E-09	Interferon-inducible protein AIM2
<i>IL18RAP</i>	2.158	6.807	1.11E-14	Interleukin-18 receptor accessory protein
<i>PLSCR1</i>	2.117	7.354	4.92E-12	Phospholipid scramblase 1
<i>MAPK14</i>	2.092	7.327	1.35E-17	Mitogen-activated protein kinase 14
<i>CD163</i>	2.075	6.413	3.09E-06	Scavenger receptor cysteine-rich type 1 protein M130
<i>TNFAIP6</i>	1.998	8.617	1.77E-08	Tumor necrosis factor-inducible gene 6 protein
<i>IL1R1</i>	1.966	6.456	1.46E-11	Interleukin-1 receptor type 1
<i>NAIP</i>	1.946	7.067	9.38E-10	Baculoviral IAP repeat-containing protein 1
<i>SOCS3</i>	1.919	4.262	1.38E-10	Suppressor of cytokine signaling 3
<i>ADM</i>	1.907	7.33	4.90E-12	Pro-adrenomedullin
<i>CST7</i>	1.893	10.08	2.42E-13	Cystatin-F
<i>S100A8</i>	1.738	12.405	8.30E-25	Protein S100-A8
<i>C3AR1</i>	1.699	8.244	2.71E-08	C3a anaphylatoxin chemotactic receptor
<i>HMGB2</i>	1.6	5.182	1.79E-14	High mobility group protein B2
<i>CASP5</i>	1.582	3.847	0.00011	Caspase-5
<i>ORM1</i>	1.554	4.89	1.54E-05	Alpha-1-acid glycoprotein 1
<i>BCL6</i>	1.527	9.359	1.05E-08	B-cell lymphoma 6 protein
<i>LTB4R</i>	1.519	6.496	1.21E-12	Leukotriene B4 receptor 1
<i>BST1</i>	1.485	8.365	3.37E-10	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2
<i>PROK2</i>	1.483	8.874	2.71E-05	Prokineticin-2
<i>FUT7</i>	1.473	5.539	7.06E-09	Fucosyltransferase 7
<i>NLRC4</i>	1.443	4.248	3.55E-12	NLR family CARD domain-containing protein 4
<i>JAK2</i>	1.441	5.047	6.77E-07	Tyrosine-protein kinase JAK2
<i>ANXA1</i>	1.42	9.231	1.27E-08	Annexin A1
<i>IL1RN</i>	1.414	6.831	1.38E-07	Interleukin-1 receptor antagonist protein
<i>ELANE</i>	1.375	4.41	0.00755	Neutrophil elastase
<i>SLC11A1</i>	1.364	5.577	1.84E-09	Natural resistance-associated macrophage protein 1
<i>IL4R</i>	1.345	4.481	4.27E-09	Interleukin-4 receptor subunit alpha
<i>MAC1R</i>	1.27	3.717	0.000125	Macrophage immunometabolism regulator
<i>ALOX5</i>	1.227	7.746	6.66E-07	Polyunsaturated fatty acid 5-lipoxygenase
<i>TPST1</i>	1.213	4.649	6.73E-05	Protein-tyrosine sulfotransferase 1
<i>GBA</i>	1.192	5.133	4.41E-08	Lysosomal acid glucosylceramidase
<i>PTX3</i>	1.19	3.208	0.000501	Pentraxin-related protein PTX3
<i>THBS1</i>	1.181	3.983	0.000128	Thrombospondin-1
<i>TSPAN2</i>	1.154	5.74	0.000181	Tetraspanin-2
<i>TLR4</i>	1.128	6.574	3.49E-06	Toll-like receptor 4
<i>PPBP</i>	1.097	10.106	0.000378	Platelet basic protein
<i>TLR8</i>	1.089	7.601	3.96E-05	Toll-like receptor 8
<i>ACER3</i>	1.076	3.619	2.80E-06	Alkaline ceramidase 3
<i>TLR2</i>	1.064	7.785	2.05E-05	Toll-like receptor 2
<i>NMI</i>	1.063	7.506	7.38E-07	N-myc-interactor
<i>ORM2</i>	1.053	3.882	0.000421	Alpha-1-acid glycoprotein 2
<i>HGF</i>	1.027	3.071	0.001437	Hepatocyte growth factor
<i>P2RX1</i>	1.02	6.049	7.90E-06	P2X purinoceptor 1
<i>PIK3AP1</i>	1.009	6.315	2.61E-05	Phosphoinositide 3-kinase adapter protein 1
<i>OSM</i>	1	3.715	1.57E-08	Oncostatin-M
<i>PTGDR</i>	-1.006	2.44	1.12E-12	Prostaglandin D2 receptor
<i>DROSHA</i>	-1.011	3.208	3.95E-16	Ribonuclease 3
<i>LIPA</i>	-1.028	5.283	5.53E-05	Lysosomal acid lipase/cholesteryl ester hydrolase
<i>GPR68</i>	-1.05	3.673	1.82E-11	Ovarian cancer G-protein coupled receptor 1
<i>LY75</i>	-1.119	5.141	0.00287	Lymphocyte antigen 75
<i>MEF2C</i>	-1.135	4.538	2.53E-07	Myocyte-specific enhancer Factor 2C
<i>PLCG1</i>	-1.151	2.937	3.68E-18	1-Phosphatidylinositol 4
<i>CCN3</i>	-1.187	2.751	1.26E-17	CCN family member 3
<i>ABCF1</i>	-1.199	4.64	1.34E-14	ATP-binding cassette subfamily F member 1
<i>LY86</i>	-1.206	5.908	0.001072	Lymphocyte antigen 86
<i>STK39</i>	-1.236	4.548	2.75E-12	STE20/SPS1-related proline-alanine-rich protein kinase
<i>NLRP1</i>	-1.247	4.439	8.56E-07	NLR Family Pyrin Domain Containing 1
<i>NRROS</i>	-1.248	4.257	1.66E-12	Transforming growth factor beta activator LRRC33
<i>NR1D2</i>	-1.287	2.895	1.91E-15	Nuclear receptor subfamily 1 Group D member 2
<i>CCR5</i>	-1.289	3.275	1.37E-11	C-C Chemokine receptor type 5
<i>CCR6</i>	-1.331	2.878	3.20E-21	C-C Chemokine receptor type 6
<i>HNRNPA0</i>	-1.372	5.555	6.04E-15	Heterogeneous nuclear ribonucleoprotein A0
<i>SMAD3</i>	-1.415	3.832	1.65E-17	Mothers against decapentaplegic homolog 3
<i>NFATC3</i>	-1.484	5.639	4.90E-12	Nuclear factor of activated T cells
<i>ADA</i>	-1.506	4.742	2.50E-10	Adenosine deaminase
<i>GATA3</i>	-1.509	3.574	9.10E-25	Trans-acting T cell-specific transcription factor GATA-3
<i>APOL3</i>	-1.526	4.545	7.28E-14	Apolipoprotein L3
<i>CD81</i>	-1.574	5.757	2.66E-11	CD81 antigen
<i>ETS1</i>	-1.576	5.657	1.58E-13	Protein C-ets-1
<i>BIRC3</i>	-1.589	4.344	2.31E-09	Baculoviral IAP repeat-containing protein 3
<i>RPS6KA5</i>	-1.599	4.207	4.54E-12	Ribosomal protein S6 kinase alpha-5
<i>CCL5</i>	-1.672	8.963	1.40E-08	C-C Motif chemokine 5
<i>FYN</i>	-1.701	6.277	6.67E-11	Tyrosine-protein kinase Fyn
<i>BLNK</i>	-1.707	3.796	1.03E-09	B cell linker protein
<i>LAT</i>	-1.717	4.776	9.72E-17	Linker for activation of T cells family member 1
<i>PTGER4</i>	-1.735	6.358	2.40E-06	Prostaglandin E2 receptor EP4 subtype
<i>CD96</i>	-1.737	3.368	1.07E-24	T cell surface protein tactile
<i>RASGRP1</i>	-1.754	4.195	2.67E-21	RAS guanyl-releasing protein 1
<i>PRKCQ</i>	-1.853	3.659	4.69E-20	Protein kinase C theta type
<i>CD6</i>	-1.915	3.483	9.56E-22	T cell differentiation antigen CD6
<i>IL23A</i>	-1.989	6.113	1.61E-14	Interleukin-23 subunit alpha
<i>HLA-DRB1</i>	-2.259	8.675	5.65E-11	HLA class II histocompatibility antigen
<i>RORA</i>	-2.262	4.003	6.67E-16	Nuclear receptor ROR-alpha
<i>NLRC3</i>	-2.392	5.203	4.23E-20	NLR family CARD domain-containing protein 3
<i>CHI3L1</i>	-2.44	6.44	2.12E-05	Chitinase-3-like protein 1
<i>CCR7</i>	-2.463	6.686	6.14E-11	C-C Chemokine receptor type 7
<i>CX3CR1</i>	-2.494	8.461	1.14E-09	CX3C Chemokine receptor 1
<i>KLRG1</i>	-2.644	4.079	2.43E-16	Killer cell lectin-like receptor subfamily G member 1
<i>CCR3</i>	-3.268	4.553	1.03E-17	C-C Chemokine receptor type 3