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Efficacy and metabolomic analysis of the pneumonia compound formulation against community-acquired pneumonia: an observational controlled before-after clinical trial

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Abstract

Background Pneumonia Compound Formulation (PCF) is a traditional Chinese medicine (TCM) formula used for the clinical treatment of novel coronavirus pneumonia. However, its efficacy and mechanism of action for community-acquired pneumonia (CAP) are unknown. Therefore, the aim of this study was to evaluate the efficacy of PCF combined with antibiotics in the treatment of CAP and to explore its mechanism based on metabolomics.

Patients and methods This prospective controlled study included 100 CAP patients from June to December 2023. Patients were randomized into an antibiotics-only group (NCM, $n=50$) and a combined antibiotics and PCF treatment group (CM, $n=50$). Clinical data were collected for all participants. The efficacy of the treatments was assessed by comparing traditional Chinese medicine syndrome scores and clinical parameters before and after treatment. Levels of inflammatory mediators (CRP, IL-6, TNF-α) and immunoglobulins (IgA, IgG, IgM) in the plasma were measured using ELISA. Plasma metabolomics analysis was conducted using ultra-performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS).

Results Both the NCM and CM group improved the clinical symptoms of CAP patients, with the CM group showing more significant improvements. Both groups effectively reduced the levels of the inflammatory mediators CRP, but had no significant impact on immunoglobulin levels. CM group using additional PCF significantly altered glycerophospholipid metabolism in patients, primarily characterized by increased levels of phosphatidylinositol, phosphatidylglycerol, and 1-acyl-sn-glycero-3-phosphoethanolamine, and decreased levels of phosphatidylcholine and phosphatidylethanolamine.

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Conclusions PCF is an effective adjunct therapy to antibiotics for the treatment of CAP, enhancing clinical symptom improvement. Its mechanism may involve the regulation of glycerophospholipid metabolism levels in patients, providing a new theoretical basis for the application of PCF in the treatment of CAP.

Trial registration ChiCTR2400086283 (2024-06-27).

Keywords Community-acquired pneumonia, Glycerophospholipids, Metabolomics, Traditional Chinese medicine

Introduction

Community-acquired pneumonia (CAP) is an infectious disease of the lung parenchyma acquired outside the hospital, typically characterized by symptoms such as fever, cough, expectoration, and chest pain [1]. CAP has a high incidence and mortality rate worldwide, making it one of the leading causes of hospitalization and imposing a significant economic burden on both individuals and society [2]. CAP is caused by a diverse range of pathogens, including bacteria, viruses, and fungi, with bacterial infections being the most common. Tailoring antibiotic therapy to the specific causative pathogen remains a cornerstone of effective CAP management [3]. However, the treatment of CAP largely relies on empirical antibiotic use due to the lack of rapid diagnostic methods for pathogens [4]. This empirical approach often leads to adverse reactions in patients and the emergence of antibiotic-resistant strains, posing significant challenges to CAP treatment [5, 6]. The inflammatory response triggered by pathogen invasion is a primary pathogenic mechanism of CAP, and excessive inflammation is closely linked to treatment failure and adverse clinical outcomes in some patients [7]. Therefore, it is particularly important to explore adjunctive therapies that modulate the inflammatory response to enhance antibiotic effectiveness or reduce antibiotic use.

In China, traditional Chinese medicine (TCM) has been used to treat pneumonia for centuries. Numerous clinical studies have confirmed that TCM can serve as an effective adjunctive treatment for CAP, reducing the use of antibiotics and enhancing their effectiveness [8–10]. The Pneumonia Compound Formulation (PCF), developed by the Tongde Hospital of Zhejiang Province, is a combination of TCM formulas designed for treating COVID-19. It consists of three different prescriptions targeted at treating syndromes identified by TCM diagnosis: cold-dampness obstructing the lung pattern, dampness-toxins stagnating in the lung pattern, and qi and yin deficiency pattern. A clinical study demonstrated that the combined use of PCF with western medicine could effectively alleviate clinical symptoms of COVID-19 patients, improve lung infection conditions, and increase clinical recovery rates [11]. However, by comparing the pathophysiology of CAP and COVID-19, we identified significant differences in their pathogens, infection mechanisms, immune responses, and treatment strategies [12, 13]. Therefore, whether PCF can alleviate CAP and the underlying mechanisms through which it may exert therapeutic effects warrant further investigation.

Compared to healthy individuals, patients with CAP exhibit significant metabolic characteristic changes, including alterations in the concentrations of certain key metabolites. These metabolites not only hold promise as biomarkers for the disease but may also be considered potential therapeutic targets [14, 15]. Metabolomics is an emerging technique for identifying and quantifying small molecule metabolites, effectively detecting metabolic alterations during disease progression [16]. Additionally, metabolomics can reveal metabolic differences before and after drug treatment, and is thus widely used in the investigation of the mechanisms of action of traditional Chinese medicines [17, 18].

In summary, this study aims to explore the therapeutic effects of PCF combined with antibiotics on CAP through a clinical controlled trial. Furthermore, we have further investigated the therapeutic mechanism of PCF through plasma metabolomics analysis. Our research provides clinical evidence and theoretical basis for the use of PCF in treating CAP, contributing to the optimization of existing treatment strategies for CAP.

Materials and methods

Study participants and inclusion criteria

This study screened patients with CAP admitted to the respiratory department of our hospital from June 2023 to December 2023, and enrolled them within 24 h of presentation. The diagnostic criteria for CAP patients were based on the "Chinese Adult Community-Acquired Pneumonia Diagnosis and Treatment Guidelines" (2016 Revised Edition) [19]. The inclusion criteria are as follows: (1) age between 18 and 90 years; (2) meets the diagnostic criteria for CAP; (3) signed informed consent form. The exclusion criteria are as follows: (1) pneumonia acquired in environments other than the community; (2) non-infectious lung diseases, such as pulmonary embolism, tuberculosis, pulmonary fibrosis, lung cancer; (3) severe immunodeficiency, including HIV infection, renal failure, malignant tumors; (4) pregnant or lactating women; (5) non-compliance with the planned medication regimen. This study protocol was approved by the Ethics Committee of Tongde Hospital of Zhejiang Province (Approval No. 2023-045(K)), in accordance with the

declaration of Helsinki. This study was registered at the Chinese Clinical Trial Registry (ChiCTR2400086283). All study participants signed an informed consent form before participating in the study.

Research design

A total of 100 CAP patients were collected and randomly divided into non-Chinese medicine group (NCM) and Chinese medicine group (CM), each consisting of 50 patients. Patients diagnosed with CAP in the NCM group received exclusive antibiotic treatment, which consisted of either intravenous infusion of 0.4 g moxifloxacin hydrochloride combined with sodium chloride injection daily, or intravenous ceftriaxone (CTRX) plus oral azithromycin (AZM), administered over a period of 7 days. Antibiotics are administered in strict compliance with the Sanford Guide to Antimicrobial Therapy 2020 (50th edition) [20]. In contrast, patients in the CM group with CAP were treated with TCM in addition to the aforementioned antibiotic treatment. The TCM treatment involved a sequential administration of three different formulas: the Huashi Xuanfei Formula for the initial 3 days, followed by the Jiedu Xiefei Formula for the next 5 days, and finally, the Qingfei Tongluo Formula for 7 days. Each formula was administered in 125 ml doses per session, culminating in a comprehensive 15-day treatment course (see prescription details in supplementary material).

Clinical observation and efficacy evaluation

Baseline characteristics of the patients were collected, including age, height, weight, gender, body mass index (BMI), and duration of illness. The primary endpoint for assessing treatment effectiveness was the improvement rate of TCM syndrome scores post-treatment, with secondary endpoints being changes in laboratory test indicators (routine blood test) and safety indicators [21, 22]. The scoring standards for TCM syndrome scores are shown in Table S1. The treatment effectiveness index (TEI) was calculated using the Nimodipine method [18], with the formula as follows:

$$TEI = \frac{\begin{pmatrix} score\,before\,treatment \\ -score\,after\,treatment \end{pmatrix}}{score\,before\,treatment} \times 100\%$$

Plasma sample collection

Plasma samples were collected both at baseline and two weeks post-treatment in the morning, using ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes to draw 5 ml of fasting venous blood from patients. The samples were then centrifuged at 3000 rpm for 15 min at 4 °C, and the supernatant (plasma) was extracted and stored at -80 °C.

Enzyme-Linked immunosorbent assay (ELISA)

Given the close association of CAP with inflammatory and immune responses [23], the concentrations of C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) in the plasma of 30 CAP patients were randomly measured using an ELISA kit (MultiSciences, Hangzhou, China). The assays were performed according to the manufacturer's instructions.

Sample processing and metabolomic analysis

The plasma of the above selected 30 patients was subsequently analyzed for metabolome using UPLC-HRMS. A 100 µl aliquot of plasma was mixed with 400 µl of cold methanol in a 2.0 ml centrifuge tube, thoroughly mixed, and then left to stand at -20 °C for 30 min to precipitate proteins. After centrifugation at 20,000 g for 15 min, 400 µl of the supernatant was transferred to a new eppendorf tube, dried under freezing conditions, and reconstituted in 100 µl of 50% cold methanol. Following another centrifugation at 20,000 g for 15 min, the supernatant was transferred to a sample vial. Additionally, 10 µl of the extract from each sample was pooled to create a quality control (QC) sample to assess instrument stability and correct for batch effects.

Chromatographic separation was achieved using a Vanquish Flex UPLC system (Thermo Fisher Scientific, Bremen, Germany) equipped with an ACQUITY UPLC T3 column (100 mm ×2.1 mm, 1.8 µm, Waters, UK) maintained at 40 °C. The injection volume for each sample was 10 µl with a flow rate of 0.35 ml/min. The mobile phase consisted of 5 mM ammonium acetate and 5 mM acetic acid in water (A) and acetonitrile (B). The gradient elution profile was as follows: 0–0.8 min, 2% B; 0.8–2.8 min, 2-70% B; 2.8–5.0 min, 70-90% B; 5.0–5.5 min, 90-100% B; 5.5–7.5 min, 100% B. Mass spectrometric data were acquired in both positive and negative ion modes using an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). QC samples were analyzed every ten samples to correct for systemic errors across the batch.

Metabolomics data processing

Raw data were converted to mzXML format using ProteoWizard software and then analyzed using the R package XCMS for peak identification, peak extraction, peak alignment, and integration [24]. Metabolites were annotated using online databases including KEGG (Kyoto Encyclopedia of Genes and Genomes) and HMDB. Principal component analysis (PCA) and analysis of

Table 1 Demographic characteristics of the CAP patients

	NCM ($n=50$)	CM ($n=50$)	*p*-value
Male, n (%)	30 (60%)	31 (62%)	0.838
Age (years)	56.9 ± 20.1	58.5 ± 28.8	0.508
Height (cm)	165.6 ± 8.3	167.2 ± 7.8	0.314
Weight (kg)	65.0 (57.0, 71.6)	65.9 (55.3, 78.1)	0.715
BMI (kg/m^2)	24.0 (21.5, 25.9)	23.1 (21.0, 26.4)	0.756
Course of disease (day)	7 (6, 8)	7 (5, 9)	0.889

CAP, community acquired pneumonia; NCM, non-Chinese medicine; CM, Chinese medicine; BMI, body mass index

significantly different metabolites were performed using the R package metaX, while partial least squares discriminant analysis (PLS-DA) was conducted using the ropls package, which also computed the variable importance in projection (VIP) scores [25]. Correlation analysis was performed using Pearson correlation coefficients in the R package cor. Metabolites meeting the criteria of *P*-value < 0.05, fold change > 1.2, and VIP ≥ 1 were defined as significantly different metabolites [26]. Differential enrichment analysis of KEGG pathways was conducted using hypergeometric testing, with pathways showing a *P*-value < 0.05 considered significantly enriched.

Statistical analysis

Clinical data and laboratory test results were statistically analyzed using SPSS 27.0 (Chicago, IL, USA). All continuous variables were tested for normal distribution. Those conforming to a normal distribution were analyzed using the t-test and described as mean ± standard deviation. Non-normally distributed data were presented as median (interquartile range), with the Wilcoxon signed-rank test and the Mann-Whitney U test applied for within-group and between-group comparisons, respectively. Categorical variables were presented as frequencies and percentages and compared using the chi-square test. The *p*-value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics of CAP patients

Based on the inclusion and exclusion criteria, 100 patients suffering from CAP were divided into two groups: antibiotics-only treatment group (NCM) and combined treatment group with PCF and antibiotics (CM). There was no significant difference in the demographic characteristics of patients between the groups (Table 1), ensuring a balanced comparison and enhancing the reliability of the subsequent findings.

Comparison of efficacy

As shown in Fig. 1A, both antibiotics-only treatment and the combination of PCF with antibiotics significantly improved the clinical symptoms of CAP patients, with the combined treatment of PCF and antibiotics showing even better results. Importantly, the efficacy index was higher and statistically significant in the combination of PCF with antibiotics group than in the antibiotics group (Fig. 1B). In addition, the main results for the secondary endpoints are shown in Table 2. Both PCF and antibiotics significantly reduced inflammation markers, particularly neutrophil (NEU) levels, with no significant difference in efficacy between the treatments. Liver injury markers, such as AST and CRE, also showed a decrease post-treatment, indicating effective management of CAP without adverse liver effects. However, an increase in plasma creatinine (CRE) levels was noted post-treatment, specifically in the combination of PCF with antibiotics group, which was statistically significant but still within safe limits. The above evidence suggests that PCF is an effective adjuvant for the treatment of CAP.

Inflammatory mediators and Immunoglobulin levels

A targeted analysis of inflammatory and immune response markers among 30 randomly selected patients from each group revealed that antibiotics significantly reduced plasma CRP and IL-6 levels (Fig. 2A). The

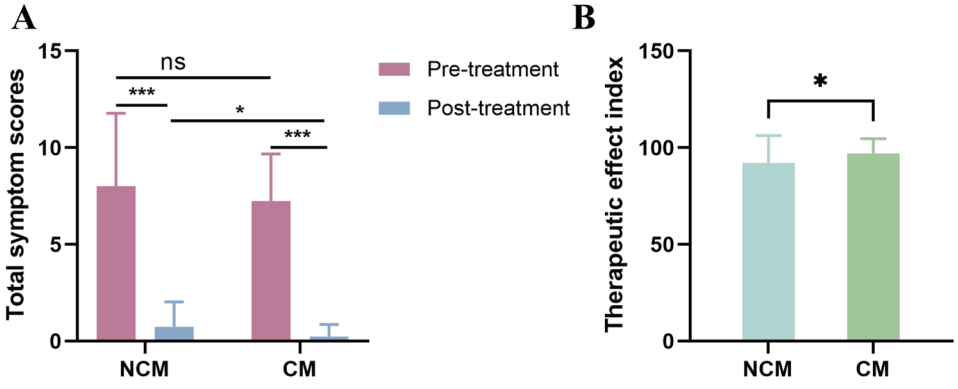

Fig. 1 Primary endpoints of the CAP Patients in NCM and CM groups. (**A**) Total TCM symptom scores before and after treatment. (**B**) Difference in therapeutic effect index between NCM and CM groups. NCM, non-Chinese medicine; CM, Chinese medicine. "*", $p < 0.05$; "***", $p < 0.001$; "ns", no statistical significance

Table 2 Secondary endpoints of the CAP patients

	NCM ($n=50$)		CM ($n=50$)		Intergroup p-value	
	Pre	Post	Pre	Post	Pre	Post
WBC ($\times10^9$/L)	7.0 ± 2.3	6.1 ± 1.9 *	6.8 ± 2.3	6.1 ± 2.3	0.718	0.809
NEU ($\times10^9$/L)	4.9 ± 2.1	3.3 ± 1.5***	4.6 ± 2.2	3.4 ± 2.0*	0.465	0.560
NEU (%)	67.8 ± 11.1	59.0 ± 9.0***	64.5 ± 12.1	59.2 ± 9.4**	0.158	0.914
ALT (U/L)	21.5(12.3, 30.0)	18(13, 34.8)	21(13.3, 33.8)	20.5(13.3, 31.5)	0.833	0.627
AST (U/L)	22(18.3, 30.8)	22(18.0, 29.5)	23(19.0, 32.0)	22(19.0, 27.8)*	0.727	0.817
TBIL (μmol/L)	13.4(10.4, 17.8)	10.9(8.3, 15.3)***	11.5(8.9, 13.4)	9.9(8.2, 12.7)	0.018	0.285
BUN (mmol/L)	4.3 ± 1.8	4.7 ± 1.5	4.5(3.8, 5.8)	4.9(3.9, 5.5)	0.126	0.379
CRE (μmol/L)	68.5 ± 16.0	70.4 ± 16.5	64.8 ± 16.0	68.1 ± 14.3***	0.244	0.450

CAP, community acquired pneumonia; NCM, non-Chinese medicine; CM, Chinese medicine; Pre, pre-treatment; Post, post-treatment; WBC, white blood cells; NEU, neutrophils; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; BUN, blood urea nitrogen; CRE, creatinine. "*" ($p<0.05$), "**" ($p<0.01$) "***" ($p<0.001$), pre vs. post between NCM or CM group. Intergroup p value, NCM vs. CM between pre-treatment or post-treatment

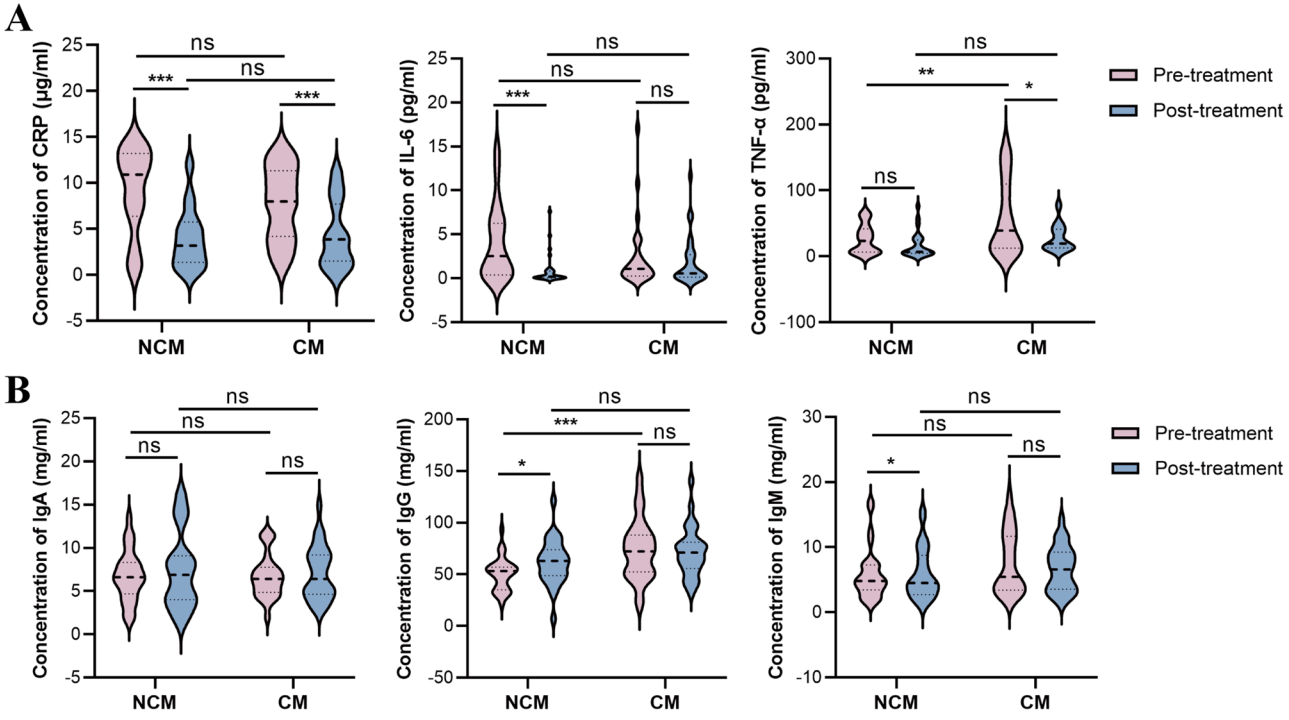

Fig. 2 Inflammatory factors and immunoglobulin levels in CAP patients after different treatments. (**A**) Changes in levels of inflammatory factors CRP, IL-6 and TNF-α. (**B**) Changes in levels of immunoglobulin IgA, IgG and IgM. CAP, community acquired pneumonia; NCM, non-Chinese medicine; CM, Chinese medicine; CRP, C-reactive protein, IL-6, interleukin 6; TNF-α, tumor necrosis factor α; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M. "*", $p<0.05$; "**", $p<0.01$; "***", $p<0.001$; "ns", no statistical significance

combination of antibiotics and PCF also significantly reduced plasma CRP levels in patients. In addition, the treatment of CAP patients with antibiotics and PCF appeared to be independent of immunoglobulin levels (Fig. 2B).

Metabolic profiling in CAP patients

The effects of different treatment modalities on patients' plasma metabolites were explored by metabolomics. The metabolic data of the samples obtained were downscaled using PCA to visualize the differences in metabolite profiles. As shown in Figs. 3A and D, pre- and post-treatment samples were clustered together in both two groups, indicating good reproducibility of the data within groups, but the differences between groups were not significant enough. Therefore, we further processed the data by PLS-DA, which enabled significant separation of the pre- and post-treatment samples (Fig. 3B and E). The PLS-DA results were also cross-validated with 200 K-folds to discern whether the PLS-DA model was overfitted. The vast majority of the samples had the R2 regression line above Q2 and the intercept of the Q2 regression line with the Y-axis was less than 0, indicating that the model had good reliability (Fig. 3C and F).

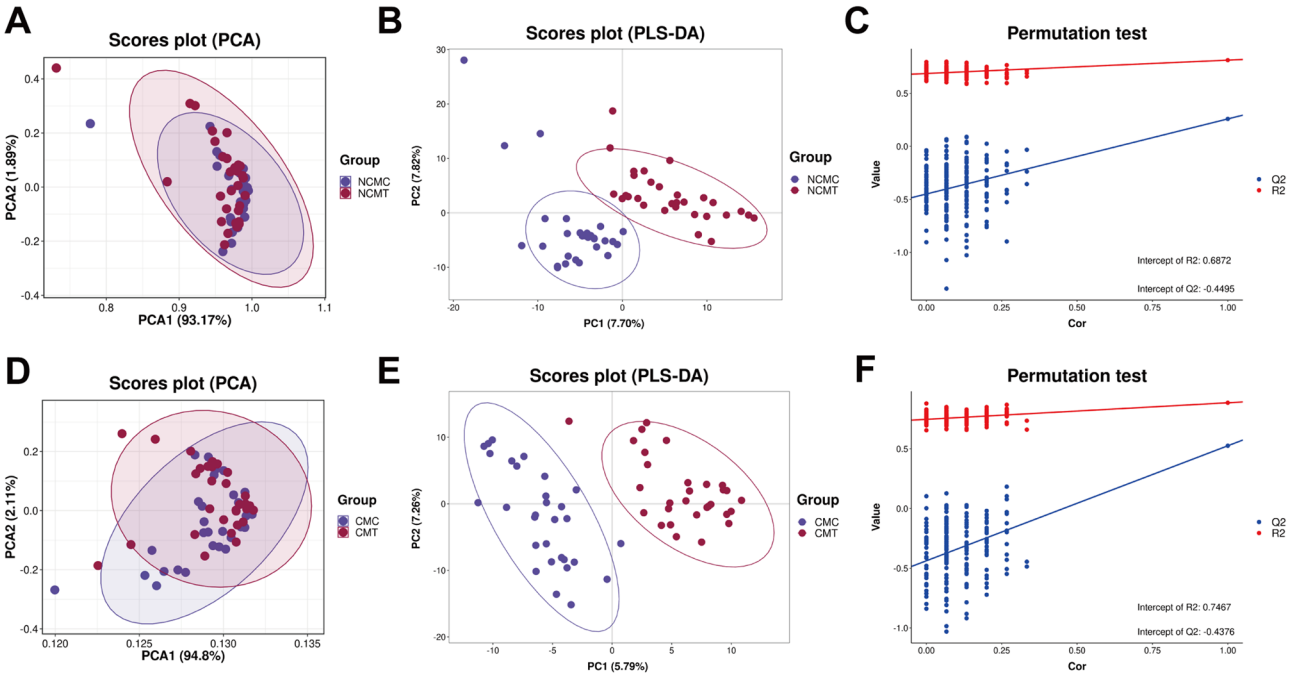

Fig. 3 Metabolic profiles of plasma samples from CAP patients between pre- and post-treatment in NCM and CM groups. (**A**) Scores plot of PCA from NCMT and NCMC. Scores plot (**B**) and permutation test (**C**) of PLS-DA from NCMT and NCMC. (**D**) Scores plot of PCA from CMT and CMC. Scores plot (**E**) and permutation test (**F**) of PLS-DA from CMT and CMC. NCMT, non-Chinese medicine treatment; NCMC, non-Chinese medicine control; CMT, Chinese medicine treatment; CMC, Chinese medicine control

Differential metabolites in CAP patients before and after treatment

Differential metabolites were identified based on VIP scores, *p*-values, and fold changes. A total of 88 differential metabolites were identified in CAP patients before and after antibiotic treatment, with 38 upregulated and 50 downregulated (Fig. 4A). In CAP patients treated with a combination of antibiotics and PCF, 92 differential metabolites were identified, with 57 upregulated and 35 downregulated (Fig. 4B). Among these, 21 differential metabolites were common to both groups (Fig. 4C). Interestingly, only five metabolites exhibited consistent trends in both groups (Table 3), suggesting that these metabolites may be predominantly influenced by the antibiotic treatment.

KEGG pathway enrichment analysis of differential metabolites

Subsequently, we explored the metabolic pathways primarily involved with the identified differential metabolites. In the NCM group, these metabolites were predominantly enriched in pathways such as glycerophospholipid metabolism, arachidonic acid metabolism, alpha-linolenic acid metabolism, pathogenic Escherichia coli infection, and necroptotic apoptosis (Fig. 5A). In the CM group, after excluding the five common differential metabolites, the remaining metabolites were chiefly enriched in glycerophospholipid metabolism, phosphatidylinositol signaling system, inositol phosphate metabolism, the biosynthesis of glycosylphosphatidylinositol (GPI) anchors, the biosynthesis of lipoarabinomannan (LAM), and autophagy pathways (Fig. 5B). The glycerophospholipid metabolism pathway was significantly enriched in both CM and NCM groups, with the CM group showing a notably higher enrichment of differential metabolites in this pathway compared to the NCM group (Fig. 5C).

Effect of PCF on glycerophospholipid metabolic pathway in CAP patients

As depicted in Fig. 6, a total of 26 differential metabolites were involved in the regulation of the glycerophospholipid metabolism pathway in CAP patients treated with PCF. Among these, the levels of 23 metabolites increased after treatment, while the levels of 3 metabolites decreased.

The 26 differential metabolites identified were ultimately annotated to five specific metabolites: phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and 1-acyl-sn-glycero-3-phosphoethanolamine (LysoPE). Following treatment with PCF, the metabolic levels of PI, PG, and LysoPE were upregulated in CAP patients. In contrast, the levels of PC and PE were downregulated (Fig. 7).

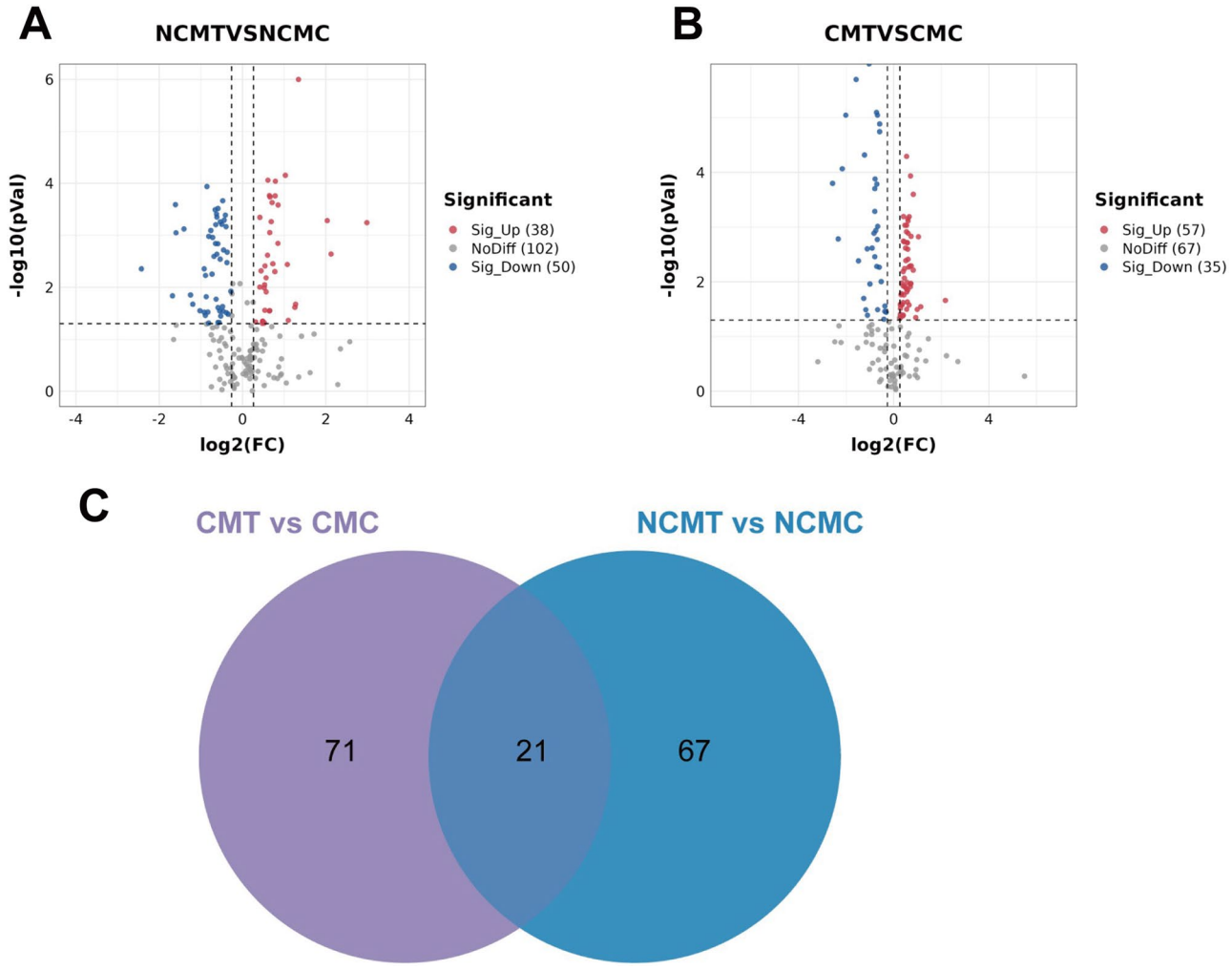

Fig. 4 Differential metabolites between pre- and post-treatment in NCM and CM groups. NCMT, non-Chinese medicine treatment; NCMC, non-Chinese medicine control; CMT, Chinese medicine treatment; CMC, Chinese medicine control

Discussion

In recent years, as the incidence of pneumonia has surged globally, this disease has become a severe public health concern, not only endangering the lives of patients but also significantly impacting global socio-economic development [27]. Against this backdrop, TCM, as an integral part of Chinese medical practices, has played a crucial role in treating various types of pneumonia and has gradually gained recognition and application internationally [28]. Particularly in the face of the COVID-19 challenge, our institution has developed a novel TCM formula named "Pneumonia Compound Formulation" (PCF), based on classical prescriptions combined with clinical practices. Preliminary observations indicate that, in addition to demonstrating potential in treating COVID-19, PCF has shown promising therapeutic effects on CAP caused by other pathogens. However, the clinical efficacy and specific mechanisms of PCF in treating CAP still require further evaluation and study.

This study recruited 100 CAP patients who met the inclusion criteria and treated them with either antibiotics alone or a combination of antibiotics and PCF. Both treatment modalities effectively improved neutrophil abnormalities and inflammation markers (CRP). Studies have shown that serum immunoglobulin levels are inversely correlated with the severity of CAP in patients, and adjusting the levels of IgM, IgA, and IgG can expedite the resolution of dysregulated inflammatory responses in CAP patients [29, 30]. However, our study found no significant changes in immunoglobulin levels in CAP patients before and after treatment, suggesting that neither PCF nor antibiotics function by modulating these levels. Encouragingly, significant differences were observed in the main outcome indicators (TEI) between the two treatment modalities, indicating real benefits of PCF in treating CAP patients. PCF may have the potential to reduce the use of antibiotics.

Table 3 Differential metabolites common to NCM and CM groups

Metabolites name	NCMT vs. NCMC	CMT vs. CMC
PC 38:4; PC(18:0/20:4)	Up	Up
LysoPE 20:1	Up	Up
Triethylene glycol monobutyl ether	Down	Up
Ambroxol	Down	Down
Ile-Phe	Up	Up
Diglyme	Down	Up
Monoisobutyl phthalate	Up	Down
Tetraethylene glycol	Up	Down
5,9:6,9-Diepoxy-3-megastigmene	Down	Up
Tetraethylene glycol monomethyl ether	Up	Down
SM 36:2; SM(d14:0/22:2)	Down	Up
Deoxyvasicinone	Up	Down
Octamethylpyrophosphoramide	Up	Down
Sempervirine	Up	Down
(+/-)-trans- and cis-4,8-Dimethyl-3,7-nonadien-2-ol	Down	Up
Carbanilide	Down	Up
Metyrapol	Down	Up
Timolol	Up	Down
1-(3-(Trifluoromethyl)phenyl)piperazine	Up	Down
Benzidine	Up	Up
Huperzine B	Down	Up

Metabolomics has shown considerable potential in evaluating the efficacy of traditional Chinese medicine. By employing metabolomic techniques, researchers can delve into the specific impacts of Chinese medicine on human metabolic pathways, thereby uncovering their mechanisms of action [31, 32]. Our study identified 88 and 92 differentially expressed metabolites by comparing the metabolic changes in CAP patients before and after treatment with antibiotics alone and in combination with PCF, respectively. Among these, five metabolites exhibited similar trends under both treatment conditions, likely influenced primarily by the antibiotics. The remaining 87 metabolites appear to be primarily related to the therapeutic effects of PCF, focusing mainly on glycerophospholipid metabolism. This finding suggests that PCF may promote the recovery of CAP patients by regulating this metabolic pathway, providing significant insights into its potential mechanisms.

Glycerophospholipids are essential phospholipid molecules widely present in the cell membranes of organisms, crucial for maintaining cell structure and function [33]. Studies have indicated significant alterations in glycerophospholipid metabolism in pneumonia patients [34, 35]. Clinical studies have confirmed that plasma phospholipid concentrations in patients with CAP are significantly reduced and return to normal as clinical conditions improve, suggesting that plasma phospholipids are potential effective biomarkers for CAP [36]. In our study, PCF treatment significantly increased the levels of PI, PG, and LysoPE, while reducing levels of PC and PE. Furthermore, a study found significant reductions in PI and LysoPE in COVID-19 patients compared to healthy controls, while PC and PE were significantly increased, consistent with our findings [37]. PI, involved in the cell autophagy pathway, enhances pathogen clearance, regulates inflammatory responses, and optimizes lung biophysical activity to help defend against lung infections [38, 39]. LysoPE has anti-inflammatory effects, while PC and PE are involved in generating certain inflammatory mediators [40]. As a component of lung surfactant, PG has been proven to inhibit the activation of toll-like receptors (TLRs) and their induced inflammatory responses in the lung's innate immune system by microbial components [41, 42]. Among the three inflammatory mediators we measured, no significant differences were observed in CRP and IL-6 levels between the two groups before and after treatment. However, TNF-α levels showed a significant difference before treatment, which became non-significant after treatment, suggesting that PCF may inhibit the accumulation of TNF-α. Interestingly, a previous study reported that PE can activate the TNF-α pathway [43]. Our findings demonstrate that PCF reduces both PE and TNF-α levels in patients, suggesting that PCF may inhibit TNF-α accumulation through the regulation of PE. In summary, we hypothesize that PCF may inhibit the release of pro-inflammatory mediators, particularly TNF-α, by modulating the levels of these key glycerophospholipids, thereby protecting lung tissue from damage.

However, this study has several limitations. First, due to the small sample size and being limited to a single center, the generalizability of our results may be restricted. Therefore, future studies with larger samples and multicenter collaboration are needed to further validate the effects of PCF adjunct treatment for CAP. Second, given that both groups of patients received antibiotics, the potential influence of antibiotics on glycerophospholipid-related metabolites cannot be entirely ruled out at this stage. Therefore, future studies could involve constructing animal models and adding a group where PCF is used alone to treat CAP to clearly define the independent effects of PCF. Additionally, this study only collected TCM symptom scores before treatment and after one treatment course, without assessing their trajectory over time to more comprehensively capture the improvement process. Lastly, although changes in the levels of several glycerophospholipid-related metabolites were observed following treatment, the specific mechanisms underlying these changes remain unclear. More in-depth studies are required to explore their molecular mechanisms.

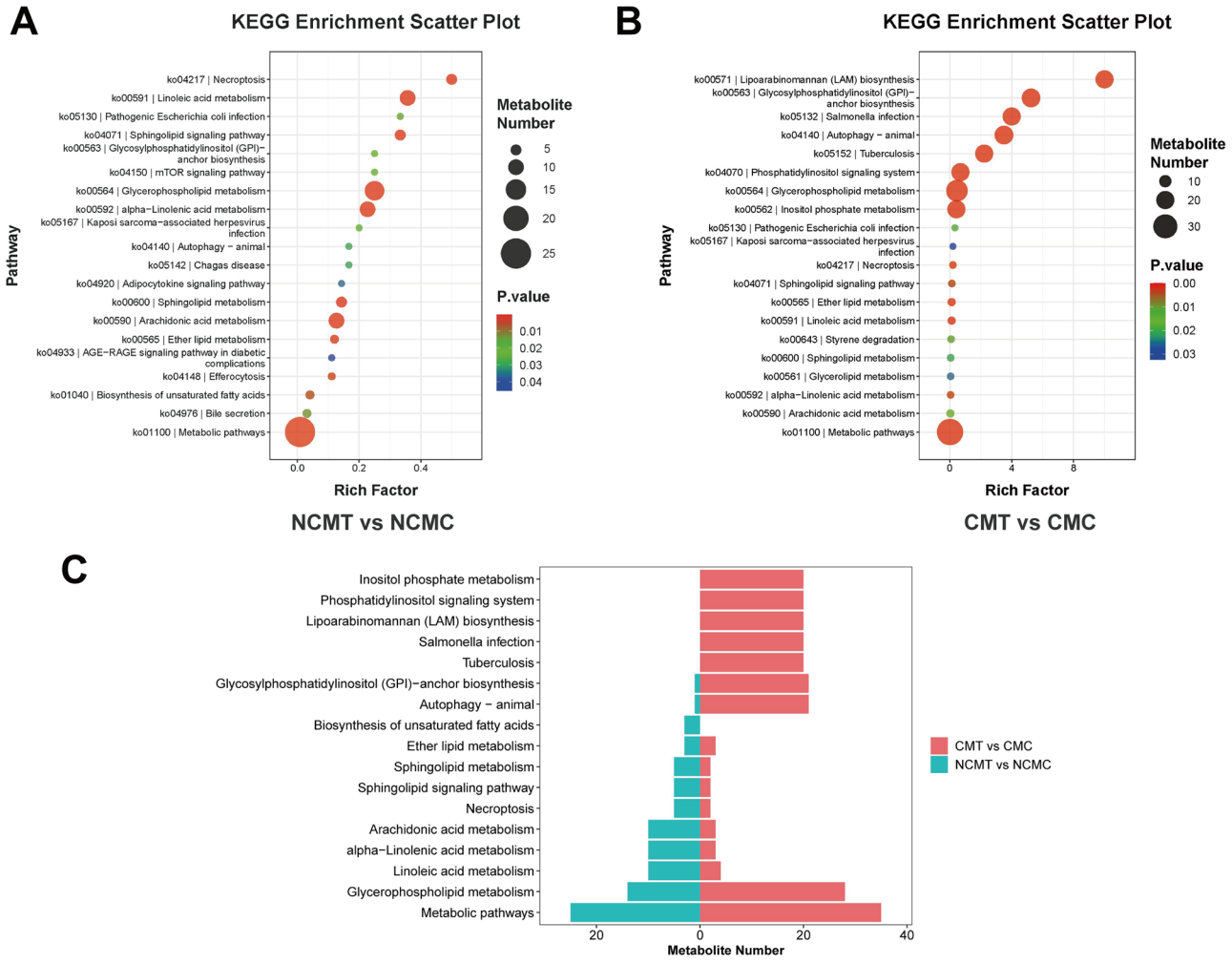

Fig. 5 KEGG-enriched pathways of differential metabolites between pre- and post-treatment in NCM and CM groups. (**A**) KEGG enrichment bubble diagram in NCM group. (**B**) KEGG enrichment bubble diagram in CM group. (**C**) Comparison of KEGG-enriched pathways between NCM and CM groups. NCMT, non-Chinese medicine treatment; NCMC, non-Chinese medicine control; CMT, Chinese medicine treatment; CMC, Chinese medicine control

Conclusion

In this clinical controlled trial, our self-formulated Chinese herbal compound mixture (pneumonia compound formula, PCF) demonstrated a beneficial adjunctive therapeutic effect on patients with CAP, significantly enhancing the improvement of clinical symptoms by antibiotics. PCF may have the potential to reduce the use of antibiotics. Furthermore, based on metabolomics studies, PCF appears to exert its therapeutic effects primarily by regulating plasma glycerophospholipid levels. Specifically, PCF increases the levels of PI, PG, and LysoPE, while decreasing PC and PE. This modulation inhibits the production of inflammatory mediators such as TNF-α, thereby protecting lung tissue. Combined with clinical treatment outcomes, these metabolites may serve as important biomarkers and potential therapeutic targets for CAP.

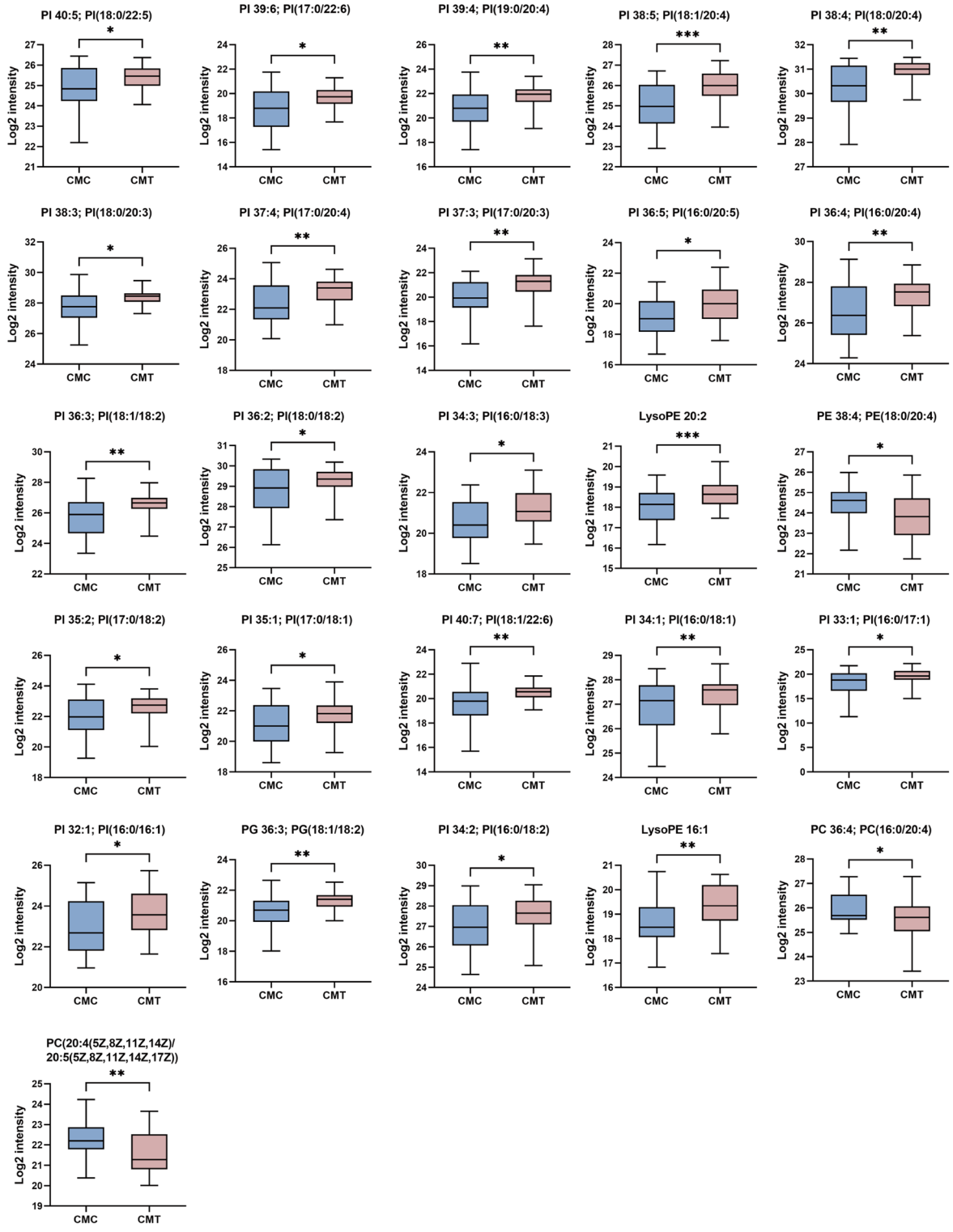

Fig. 6 Changes in the relative levels of plasma metabolites involved in the glycerophospholipid metabolic pathway in CAP patients treated with PCF. CMT, Chinese medicine treatment; CMC, Chinese medicine control. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

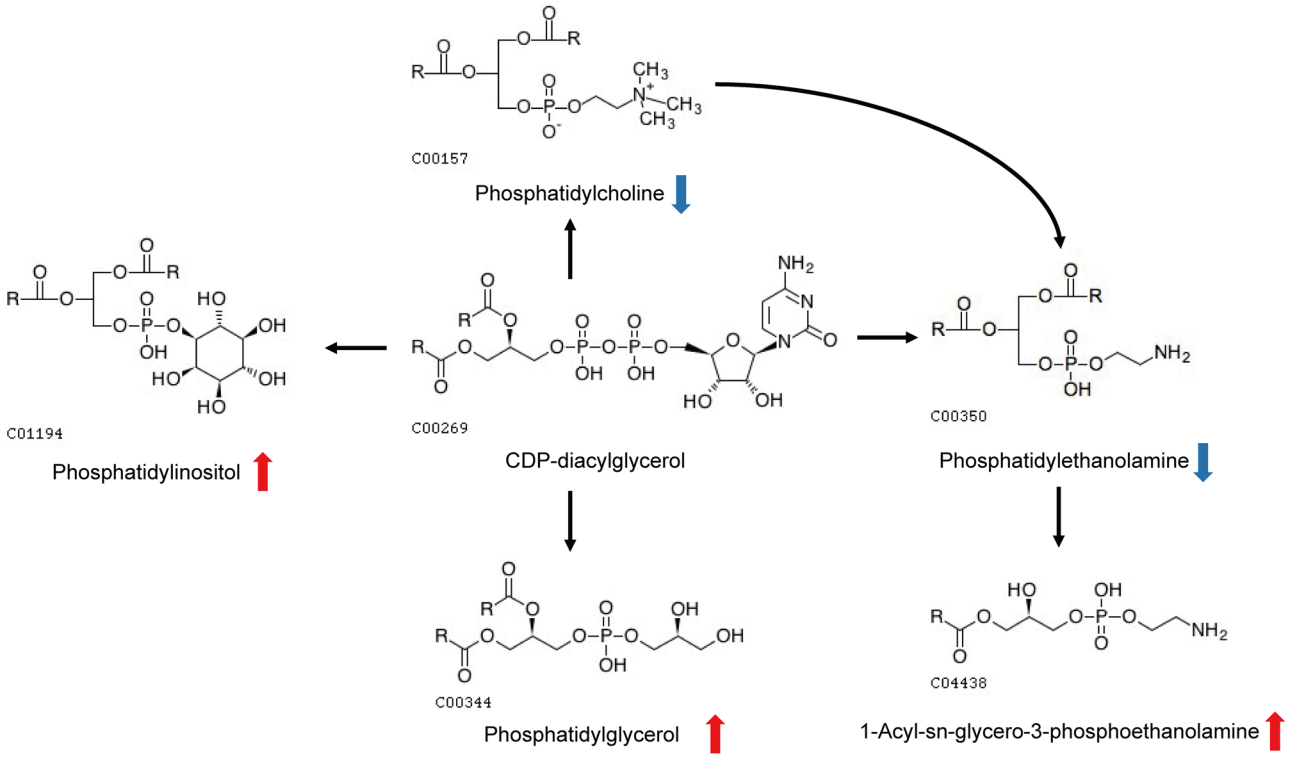

Fig. 7 Glycerophospholipid metabolic pathway in CAP patients treated with PCF. Red arrows represent ups, blue arrows represent downs

Abbreviations

CAP	Community-acquired pneumonia
CM	Chinese medicine (combined antibiotics and PCF treatment)
GPI	Glycosylphosphatidylinositol
LAM	Lipoarabinomannan
LysoPE	1-acyl-sn-glycero-3-phosphoethanolamine
NCM	Non-Chinese medicine (antibiotics-only treatment)
PC	Phosphatidylcholine
PCA	Principal component analysis
PCF	Pneumonia Compound Formula
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PLS-DA	Partial least squares discriminant analysis
TCM	Traditional Chinese medicine
TEI	Treatment effectiveness index
VIP	Variable importance in projection

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
Z. Y. participated in the conception and design of the work as well as in the writing of the manuscript. W. Y. and W. JQ. were responsible for subject recruitment. W. LQ. and Z. MX. performed the metabolomic testing of the samples. Z. FX. and W. WM. were responsible for data analysis. H. FH. was responsible for the revision of the manuscript. All authors read and approved the final manuscript.

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

Declarations

Ethics approval and consent to participate
This study protocol was approved by the Ethics Committee of Tongde Hospital of Zhejiang Province (Approval No. 2023-045(K)).

Consent for publication
Not applicable.

Consort statement
Our study adheres to Consort guidelines.

Competing interests
The authors declare no competing interests.

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