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ORIGINAL RESEARCH

Identifying KL-6-Associated Immune Cell Signatures and Key Genes in Emphysematous COPD

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Background: This study aimed to evaluate the potential of Krebs von den lungen-6 (KL-6) as a biomarker for distinguishing emphysematous chronic obstructive pulmonary disease (COPD-E) from non-emphysematous COPD (COPD-NE), and to explore the underlying mechanisms associated with KL-6 expression.

Methods: We enrolled 154 patients with COPD and 170 healthy controls to assess serum KL-6 levels. Receiver operating characteristic curve was used to determine the diagnostic sensitivity and specificity. Pearson's correlation analysis was used to evaluate the correlation. Univariate and multivariate linear regression analyses were performed to explore the factors influencing KL-6 levels in COPD. Transcriptomic sequencing was performed on peripheral blood mononuclear cells from COPD patients with varying KL-6 levels to explore underlying biological mechanisms. A Mendelian randomization analysis was employed to ascertain the association between the expression quantitative trait loci of key genes and emphysema risk.

Results: Serum KL-6 levels were significantly elevated in COPD patients, particularly in COPD-E. Pearson analyses revealed that the serum KL-6 concentration was positively correlated with eosinophil count. Transcriptomic analysis revealed 237 differentially expressed genes (DEGs) between patients with high and low levels of KL-6. Gene set enrichment analysis revealed that these DEGs were associated with immune responses. No significant difference in immune cell proportions were observed between high and low KL-6 groups, but KL-6 showed a negative correlation with T cell gamma delta. By intersecting the DEGs with those from the GSE248493 dataset, we identified seven key genes and further validated their association with the risk of emphysema using Mendelian randomization, with amidohydrolase domain containing 2 (AMDHD2) potentially reducing the risk of the disease.

Conclusion: KL-6 is a promising biomarker for distinguishing COPD-E from COPD-NE and AMDHD2 may be involved in the regulation of increased KL-6 levels in COPD-E.

Keywords: chronic obstructive pulmonary disease, emphysema, Krebs von den lungen-6

Introduction

Chronic obstructive pulmonary disease (COPD) is a disease characterized by airflow limitation that is not fully reversible. As one of the leading causes of death worldwide, COPD's prevalence is on the rise,[1] and a deeper understanding of its subtypes can lead to improved treatment options.[2–4] Current classification into emphysema and chronic bronchitis phenotypes reflects divergent pathophysiological mechanisms: emphysema involves alveolar destruction and epithelial barrier disruption, while chronic bronchitis is driven by airway inflammation and remodeling.[5] It is believed that these phenotypes stem from varying sensitivities to harmful particles or gases, particularly tobacco smoke.[6] The pathophysiological pathways that lead to emphysema and small airway narrowing are distinct, yet they interconnected in intricate manners.[7] While current guidelines classify COPD by spirometric severity, growing evidence highlights the urgent need for phenotype-specific biomarkers to address two critical gaps: First, in terms of therapeutic targeting, anti-

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inflammatory therapies benefit airway inflammation-dominant COPD but show limited efficacy in emphysema-predominant COPD. Second, regarding prognostic precision, emphysema progression correlates with accelerated lung function decline,[8] yet no biomarker reliably predicts subtype trajectories.

Krebs von den Lungen-6 (KL-6) is identified as a high molecular weight, circulating mucin-like glycoprotein that is also classified as mucin 1 (MUC1). This protein is predominantly found on the surfaces of type II alveolar pneumocytes and the epithelial cells lining the bronchi. KL-6 has been studied as a biomarker for the diagnosis and prognosis of interstitial lung disease (ILD), including declines in lung function and mortality rates, as well as responses to treatment.[9–11] Beyond ILD, KL-6 has shown broad utility.[12] For instance, combining high-resolution CT imaging features with serum KL-6 levels can predict diffuse alveolar damage in acute respiratory distress syndrome (ARDS) patients. The combined detection of (1-3)-β-D-glucan and KL-6 has achieved high accuracy in diagnosing pneumocystis pneumonia. Additionally, KL-6, when combined with other markers, can enhance the accuracy of idiopathic pulmonary fibrosis (IPF) diagnosis and prognosis evaluation. Furthermore, using carcinoembryonic antigen (CEA) in combination with KL-6 as a tumor marker index is helpful for predicting the prognosis of non-small cell lung cancer (NSCLC) patients. Recent studies have also found that KL-6 levels are elevated in the serum of COPD patients, particularly during acute exacerbations. The levels of KL-6 correlate with the severity, prognosis, decline in lung function, and mortality rates associated with COPD.[13] As a marker of epithelial injury, KL-6 is released into the blood following the disruption of the alveolar-capillary barrier and becomes detectable; its accumulation can further damage the alveolar capillaries.[14,15] Unlike COPD with bronchitis as the manifestation, which is characterized by airway inflammation, emphysema-type COPD is primarily defined by the destruction of alveolar walls and damage to the alveolar-capillary membrane.[16] This suggests that KL-6 may serve as a potential biomarker to distinguish between emphysematous COPD (COPD-E) and non-emphysematous COPD (COPD-NE).

Therefore, we classified COPD patients into two subtypes according to the qualitative assessment of emphysema using chest volumetric computerized tomography (CT) scans. We investigated the levels of KL-6 and influencing factors among different subtypes, and further explored the underlying mechanisms through transcriptomic sequencing of peripheral blood.

Methods

Study Design and Subjects

This case-control study was designed to investigate the potential of KL-6 as a biomarker for COPD-E by integrating clinical phenotyping, serum biomarker analysis, and transcriptomic profiling. The study initially enrolled healthy controls and COPD patients to compare plasma KL-6 levels between the two groups and to assess the correlation of KL-6 with clinical indices. Subsequently, COPD patients were further stratified into COPD-E and COPD-NE to investigate whether KL-6 could serve as a diagnostic marker for COPD-E. Finally, transcriptomic analysis was conducted to elucidate the potential mechanisms underlying plasma KL-6 levels in COPD patients.

A total of 154 COPD subjects and 170 healthy control subjects were included. All subjects included in this study were recruited from January 1, 2024, to November 1, 2024, in the Department of Respiratory and Critical Care Medicine at the Third Affiliated Hospital of Nanjing Medical University. All COPD patients enrolled in the study fulfilled the subsequent criteria for inclusion: (1) The age range is 50 to 80 years old; (2) Meeting the diagnostic criteria for COPD as outlined in the 2024 Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines; (3) had a stable airflow limitation with forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) < 70% followed by inhaled β2-agonist administration. The criteria for exclusion were specified as follows: (1) being pregnant; (2) having a health status complicated by additional pulmonary conditions, including bronchiectasis, tuberculosis, asthma, malignant growths, active tuberculosis, interstitial lung disorders, and other inflammatory conditions that could confound the results, such as arthritis, disorders affecting connective tissue, or inflammatory bowel diseases. Furthermore, to assess serum KL-6 levels, healthy individuals were selected from the physical examination center for comparison. Exclusion criteria: (1) Ages less than 50 or greater than 80; (2) Presence of hypertension, diabetes, respiratory system diseases, autoimmune diseases. Based on prior research,[17] the mean values of KL-6 for the healthy control group and the COPD patient group were 393.5 U/mL and 589 U/mL, respectively, with standard deviations (SD) set at

393.5 and 589 for each group. For the current study, a two-sided significance level (α) of 0.05 and a power (1 - β) of 0.9 were selected. The ratio of participants in the two groups was maintained at 1:1. Utilizing the PASS 15.0 software, the total required sample size was calculated to be 280 participants, with a minimum of 140 participants per group. This sample size ensures the scientific rigor of the study design. Our sample size was thus sufficient in accordance with these parameters to ensure the scientific rigor of the study design. To investigate the levels of KL-6 in different subtypes of COPD, we categorized COPD patients as follows: (1) based on lung function (Group I: FEV_1%pred ≥ 80%; Group II: 50% ≤ FEV_1%pred < 80%; Group III: 30% ≤ FEV_1%pred < 50%; Group IV: FEV_1%pred < 30%); (2) based on CT scan (COPD-E: consistent with the clinical evaluation and radiological manifestations of emphysema; COPD-NE: no evidence of emphysema on chest CT scan). The whole blood sample was collected from participants and used for RNA sequencing. In this study, all procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki. All patients provided written informed consent, and the study was approved by the Ethics Committee of Third Affiliated Hospital of Nanjing Medical University (2024KY245-01).

Measurements of Pulmonary Function

A spirometer (Jaeger, Baglia, Germany) was utilized to document the outcomes of pulmonary function assessments with the patient in a seated position. Throughout the examination, volume and flow curves were monitored continuously. Key parameters of pulmonary function, including FVC, FEV_1, and FEV_1/FVC were assessed, with predicted values being calculated accordingly.

Measurements of KL-6

Blood samples were obtained through venous puncture and collected in coagulation tubes within a 24 h period following hospital admission. The serum was collected after centrifugation at 3000 rpm for 10 minutes, then stored in a 4°C refrigerator and tested within 48 h. The KL-6 concentration (U/mL) in the serum samples was quantified using the KL-6 Magnetic Microparticle Chemiluminescence Assay Kit (Yongheyangguang, Hunan, China), and the results were determined with a fully automated chemiluminescence immunoassay analyzer (IncreCare, Shenzhen, China).

Data Sources

We selected microarray datasets from the GEO database (http://www.ncbi.nlm.nih.gov/geo) based on the following criteria: (1) The dataset must contain whole-genome expression mRNA microarray data; (2) The dataset should include peripheral blood mononuclear cells (PBMCs) from both non-emphysematous COPD patients and emphysematous COPD patients; (3) The dataset must have more than 20 samples. Based on these criteria, we obtained the GSE248493 gene expression profile dataset, which includes PBMC samples from 14 non-emphysematous COPD patients and 8 emphysematous COPD patients.[18]

For the Mendelian randomization analysis, exposure data were sourced from the eQTLGen Consortium database, which is accessible at https://eqtlgen.org/. Outcome data were extracted from the IEU OpenGWAS project, available online at https://gwas.mrcieu.ac.uk/, with the specific GWAS ID ukb-b-7280. This dataset encompasses 6,417 cases of emphysema and 456,516 controls.

RNA Isolation and Sequencing

Participants provided whole blood samples, including four COPD patients with high KL-6 levels (> 400 U/mL) and four COPD patients with low KL-6 levels (≤ 400 U/mL), collected in PAXgene® RNA Blood tubes and stored at −80°C prior to further processing. Total RNA extraction was conducted using the PAXgene Blood RNA Kit. DNase I was utilized to digest DNA fragments present in the RNA samples, and the resulting products were purified and recovered with the aid of magnetic beads. The rRNA was subsequently removed using a commercial kit, followed by thermal cycling in a PCR instrument. Primers were added, and a complementary strand of cDNA was synthesized following the prescribed protocol. The cDNA underwent double-stranded synthesis and terminal repair, and was treated with the uracil-N-glycosylase digestion system to ensure linkage. Post-magnetic bead purification and recovery, the RNA library's quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and the ABI StepOnePlus real-time PCR system (Thermo Fisher Scientific, USA). The qualified RNA library was then subjected to denaturation with

NaOH to form single-stranded DNA, which was subsequently diluted to the appropriate concentration on the FlowCell and hybridized to the connectors present there. The hybridized molecules were amplified through bridge PCR on the cBot, and the library was sequenced on the Illumina HiSeq X-Ten platform using a paired-end 150 (PE150) strategy. Adapter sequences were removed from the reads to enhance the accuracy of the subsequent biological analysis. Bases with a mass value below 20 and those containing non-AGCT sequences at the 5' end were trimmed. Reads containing more than 10% ambiguous nucleotides were discarded, as were small fragments shorter than 25 base pairs after adapter trimming. Following these quality control measures, the data underwent statistical analysis. The sequencing reads were aligned to the GRCh38 reference genome using Hisat2 software, version 2.1.0.

Differential Gene Expression and Enrichment Analysis

We employed the Limma package in R software to identify differentially expressed genes (DEGs) among various samples, using an adjusted *P*-value threshold of less than 0.05 and a |log2 fold change| of greater than or equal to 1 as the cutoff criteria. Subsequently, we conducted Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Set Enrichment Analysis (GSEA) analyses using the clusterProfiler package in R software.

Protein-Protein Interaction (PPI) Network Analysis

The protein-protein interaction (PPI) network was established using the STRING database. DEGs were uploaded to the STRING database for analysis of protein-protein interactions, and the resulting PPI network was imported into Cytoscape software for visualization.

Immune Cell Profiling

CIBERSORTx is a computational tool that employs gene expression deconvolution to infer the proportions of different immune cell subtypes in a mixed cell population based on gene expression data. In this study, we utilized the CIBERSORTx web tool (https://cibersortx.stanford.edu) to calculate the relative abundance of 22 immune cell types within the high KL-6 group samples from our sequenced samples.

Mendelian Randomization Analysis

To ensure the robustness of the data and precision of the results, we evaluated the SNPs for quality to obtain compliant instrumental variables (IVs). SNPs were selected as exposure points if their *P*-value was below 5e-6. The default linkage disequilibrium (LD) threshold was set to a clustered distance of 10,000 kb and an r^2 value of 0.2. The *F*-statistic threshold was set above 10. This study utilized two-sample MR analysis to infer causal relationships between modifiable exposures and disease outcomes using the "TwoSampleMR" R package, aligning with our previous research.[19] In brief, we employed various methods, including inverse variance weighting (IVW), MR-Egger, weighted median, weighted mode, and simple mode, with IVW being the primary method for this study. Additionally, sensitivity analyses, such as those for heterogeneity and horizontal pleiotropy, were conducted to assess the stability of the results.

Statistical Analysis

For the analysis, we employed the statistical software package SPSS version 21.0. GraphPad Prism version 9 was utilized to create graphical representations. The normality of the measured data was assessed using the Kolmogorov–Smirnov test. Data that adhered to a normal distribution were presented as the mean ± standard error of the mean (SEM), while data that did not follow a normal distribution were depicted as the median with interquartile ranges. Categorical variables were expressed in terms of their proportions. For dichotomous variables, Pearson's chi-squared test was applied. Continuous variables were analyzed using a *t*-test for data that were normally distributed or a Mann–Whitney *U*-test for those that were not. Correlation analyses were conducted with Pearson's correlation coefficient. The diagnostic sensitivity and specificity were assessed using the Receiver Operating Characteristic (ROC) curve, with the area under the curve (AUC) indicating the diagnostic accuracy. The effect of baseline characteristics on KL-6 in patients with COPD-E was investigated using univariate and multivariate linear regression analyses. A *P*-value of less than 0.05 was set as the threshold for statistical significance.

Results

The Overall Characteristics of the Groups

The overall characteristics of the groups were summarized in Table 1. As shown in Table 1, there were no significant differences in sex, age, body mass index (BMI), smoking history, eosinophil (EOS) counts, basophil (BASO) counts and C-reactive protein (CRP) between healthy controls and COPD patients. COPD patients had lower FEV_1/FVC, FEV_1% and lymphocyte (LYM) counts. White blood cell (WBC) counts and neutrophil (NEU) counts were higher in COPD patients when compared with the control groups.

Serum KL-6 Levels of COPD Patients and Healthy Controls

The levels of KL-6 for each group were presented in Figure 1. Serum KL-6 concentrations were found to be elevated in COPD groups relative to control groups ($P < 0.001$) (Figure 1A). To assess the diagnostic performance of KL-6 as a biomarker for COPD, we conducted a ROC analysis (Figure 1B). At a cut-off value of 224.5 U/mL, KL-6 demonstrated a sensitivity of 0.8442, a specificity of 0.6059, and an AUC of 0.783 (95% CI= 0.7335–0.8327, $P < 0.001$), indicating its optimal diagnostic value as a biomarker for COPD. Additionally, we explored the KL-6 levels across different GOLD stages of COPD patients and observed no significant differences in serum KL-6 levels among these stages. Furthermore, we examined the correlation between serum KL-6 and clinical characteristics of COPD patients and results showed that serum KL-6 level of COPD patients was positively correlated with EOS count (Figure 2).

Analysis of Risk Factors for KL-6 Elevation in COPD Patients

The impact of baseline patient information on the heightened KL-6 levels in COPD was explored. As shown in Figure 3, emphysema (β=88, 95% CI=30-147, P=0.003) and EOS (β=192, 95% CI=10-374, P=0.039) had effects on KL-6 elevation according to univariate linear regression in COPD patients. Subsequently, when emphysema and EOS were included in a multivariate linear regression model, the results indicated that only emphysema (β=82.9, 95% CI=24.7–141.1, P=0.006) significantly influenced the elevation of KL-6 levels.

Table 1 Patient Baseline Demographic and Clinical Characteristics in Control and COPD Patients.

Variables	Overall N=324	Control N=170	COPD N=154	*P*-value
Sex, n (%)				0.169
Female	55.00(16.98%)	34.00(20.00%)	21.00(13.64%)	
Male	269.00(83.02%)	136.00(80.00%)	133.00(86.36%)	
Age, Median (Q1, Q3)	73.00 (69.00, 77.00)	73.00 (68.00, 77.00)	74.00 (70.00, 77.00)	0.218
BMI, Mean ± SD	22.86 (20.28, 25.39)	23.61 (21.22, 25.97)	22.49 (19.05, 24.80)	0.076
Smoking history, n (%)				0.344
No	134.00(41.36%)	75.00(44.12%)	59.00(38.31%)	
Yes	190.00(58.64%)	95.00(55.88%)	95.00(61.69%)	
FEV1 pred%, Median (Q1, Q3)	73.95 (43.10, 95.95)	101.30 (89.00, 118.00)	45.60 (37.90, 73.10)	<0.001
FEV1/FVC, Median (Q1, Q3)	58.70 (42.07, 75.84)	78.05 (74.35, 84.14)	45.62 (39.87, 57.61)	<0.001
WBC, Median (Q1, Q3)	7.20 (5.59, 9.70)	7.09 (5.26, 9.31)	7.41 (5.99, 10.42)	0.022
NEU, Median (Q1, Q3)	5.00 (3.56, 7.69)	4.63 (3.16, 7.08)	5.39 (3.83, 8.59)	0.003
LYM, Median (Q1, Q3)	1.22 (0.81, 1.66)	1.33 (0.86, 1.67)	1.15 (0.78, 1.58)	0.042
MONO, Median (Q1, Q3)	0.54 (0.37, 0.72)	0.52 (0.34, 0.70)	0.57 (0.39, 0.81)	0.060
EOS, Median (Q1, Q3)	0.07 (0.01, 0.14)	0.06 (0.01, 0.12)	0.09 (0.01, 0.17)	0.115
BASO, Median (Q1, Q3)	0.02 (0.02, 0.04)	0.02 (0.02, 0.04)	0.02 (0.01, 0.04)	0.452
CRP, Median (Q1, Q3)	12.84 (1.70, 57.41)	11.86 (0.90, 57.41)	14.78 (2.86, 59.74)	0.428

Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV_1 pred%, Forced Expiratory Volume in 1 second as a percentage of predicted; FEV_1/FVC, forced expiratory volume in one second /forced vital capacity; WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte; MONO, monocyte; EOS, eosinophil; BASO, basophil; CRP, C-reactive protein.

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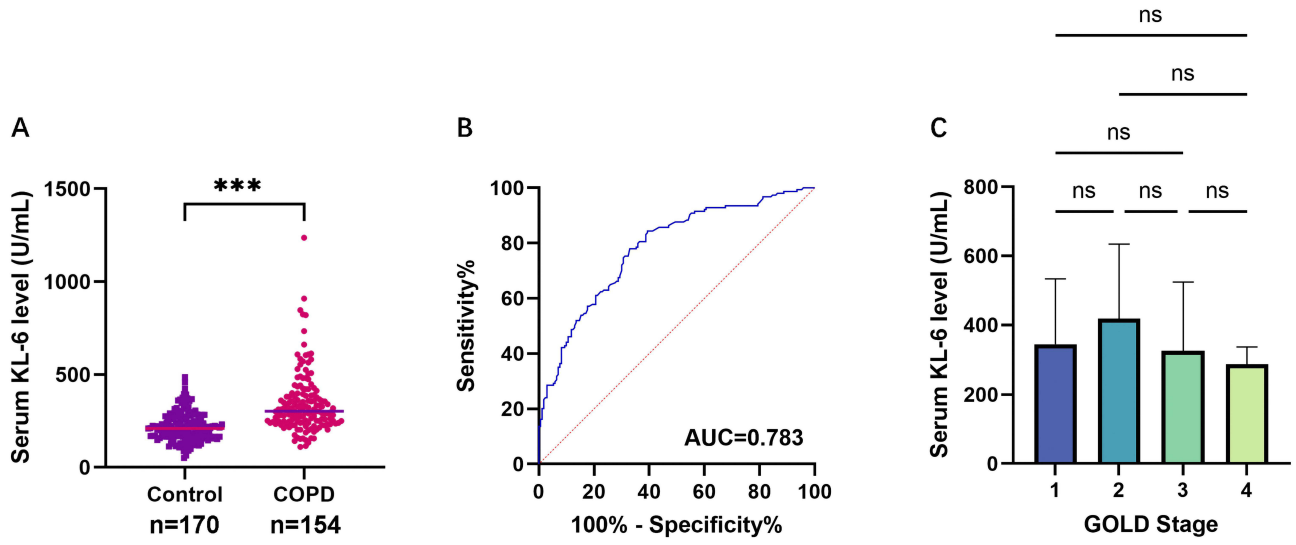

Figure 1 KL-6 expression and diagnostic value in COPD. (**A**) Serum levels of KL-6 between COPD patients and healthy control. (**B**) Performances of KL-6 in the diagnosis of COPD. (**C**) Serum levels of KL-6 across different GOLD stages in COPD patients. ****P* <0.001, ns: no significant.

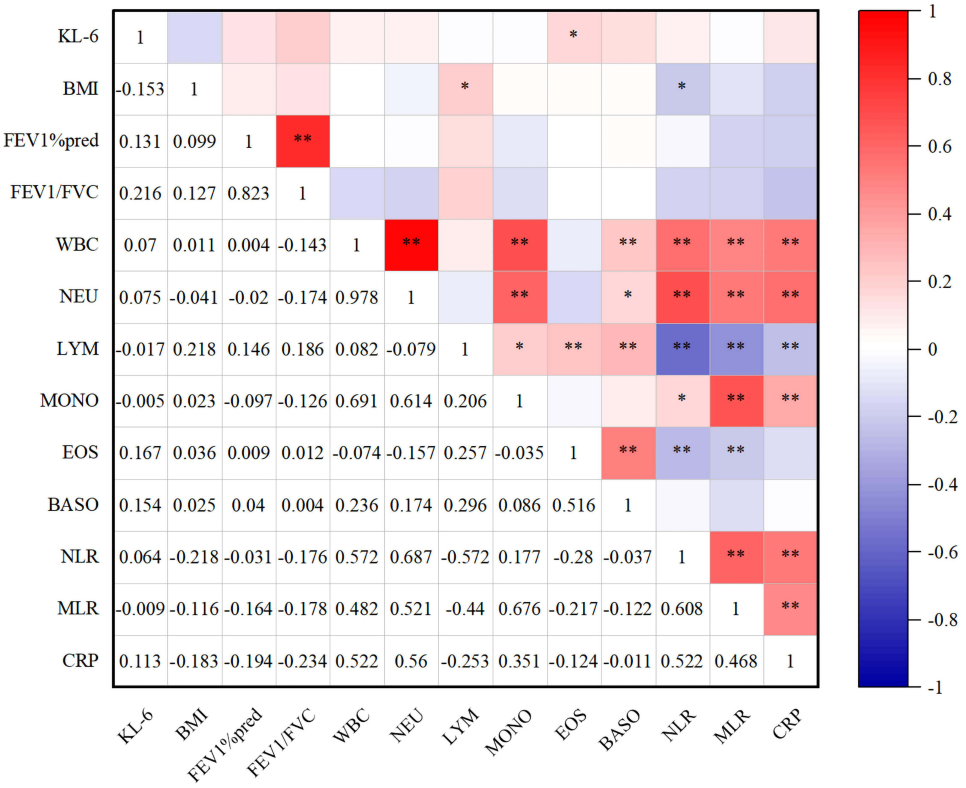

Figure 2 Associations between serum KL-6 level and different clinical characteristics in COPD patients. **P* <0.05, ***P* <0.01.

Serum KL-6 Level Distinguishing COPD-E From COPD-NE in COPD Patients

The overall characteristics of the groups were summarized in Table 2. Serum KL-6 concentrations were found to be elevated in COPD-E groups relative to COPD-NE groups ($P < 0.001$) (Figure 4A). ROC analysis was used to assess the value of KL-6 as COPD-E biomarker (Figure 4B). When the cut-off value was 301.5 U/mL, the sensitivity of KL-6 as a biomarker for COPD-E was 0.6068, the specificity was 0.7949, and the AUC was 0.714 (95% CI=0.6226–0.8046, $P < 0.001$), which has the best diagnostic value as a biomarker for COPD-E.

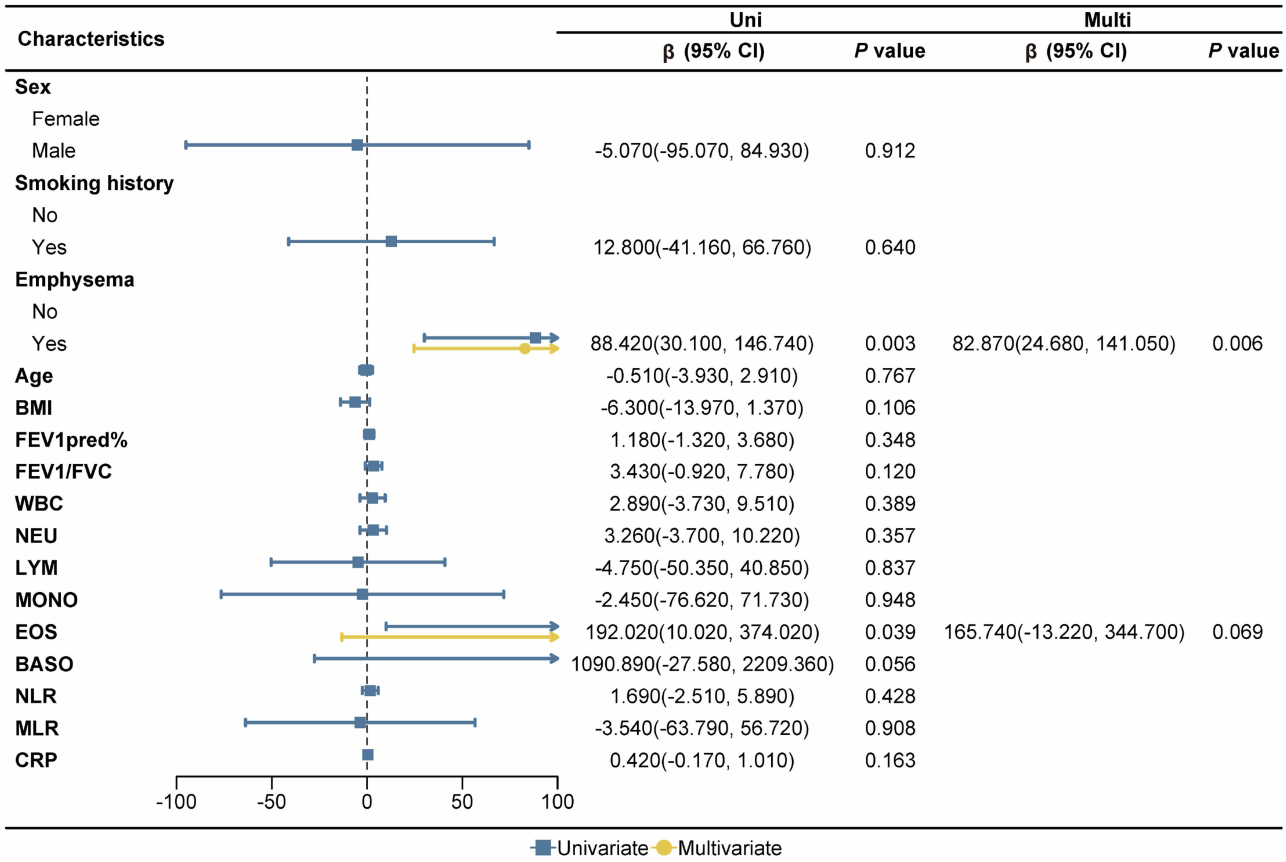

Figure 3 Risk factors for KL-6 elevation in COPD patients.

Transcriptome Sequencing Based on KL-6 Levels

To investigate the potential mechanisms affecting serum KL-6 levels in COPD patients, we conducted transcriptomic sequencing on PBMCs from COPD patients with elevated KL-6 levels and those with reduced KL-6 levels. The analysis

Table 2 Patient Baseline Demographic and Clinical Characteristics in COPD Subgroups

Variables	Overall N=154	COPD-NE N=38	COPD-E N=116	*P*-value
Sex, n (%)				0.098
Male	140.00(90.91%)	32.00(84.21%)	108.00(93.10%)	
Female	14.00(9.09%)	6.00(15.79%)	8.00(6.90%)	
Age, Median (Q1, Q3)	74.00 (70.00, 77.00)	73.00 (71.00, 78.00)	74.00 (69.50, 77.00)	0.990
BMI, Median (Q1, Q3)	22.49 (19.05, 24.80)	23.39 (19.63, 25.50)	21.77 (18.82, 24.22)	0.053
Smoking history, n (%)				0.084
No	55.00(35.71%)	18.00(47.37%)	37.00(31.90%)	
Yes	99.00(64.29%)	20.00(52.63%)	79.00(68.10%)	
FEV1 pred%, Median (Q1, Q3)	45.60 (37.90, 73.10)	78.30 (45.00, 90.00)	44.10 (35.10, 62.30)	0.009
FEV1/FVC, Mean ± SD	46.99 ± 12.24	55.82 ± 10.42	44.68 ± 11.71	0.007
GOLD Stage, n (%)				0.107
1	10.00(18.87%)	5.00(45.45%)	5.00(11.90%)	
2	11.00(20.75%)	2.00(18.18%)	9.00(21.43%)	
3	28.00(52.83%)	4.00(36.36%)	24.00(57.14%)	
4	4.00(7.55%)	0.00(0.00%)	4.00(9.52%)	

(*Continued*)

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Table 2 (Continued).

Variables	Overall N=154	COPD-NE N=38	COPD-E N=116	*P*-value
WBC, Median (Q1, Q3)	7.41 (5.99, 10.42)	7.45 (6.11, 9.31)	7.37 (5.74, 10.49)	0.661
NEU, Median (Q1, Q3)	5.39 (3.83, 8.59)	5.43 (3.67, 7.51)	5.38 (3.86, 8.91)	0.567
LYM, Median (Q1, Q3)	1.15 (0.78, 1.58)	1.31 (0.82, 1.73)	1.10 (0.76, 1.50)	0.308
MONO, Median (Q1, Q3)	0.57 (0.39, 0.81)	0.53 (0.36, 0.71)	0.59 (0.40, 0.82)	0.376
EOS, Median (Q1, Q3)	0.09 (0.01, 0.17)	0.07 (0.00, 0.14)	0.10 (0.02, 0.18)	0.150
BASO, Median (Q1, Q3)	0.02 (0.01, 0.04)	0.03 (0.01, 0.04)	0.02 (0.01, 0.04)	0.884
CRP, Median (Q1, Q3)	14.78 (2.86, 59.74)	17.51 (1.28, 45.12)	14.25 (3.10, 63.10)	0.525

Abbreviations: COPD, chronic obstructive pulmonary disease; COPD-NE, non-emphysematous COPD; COPD-E, emphysematous COPD; BMI, body mass index; FEV_1 pred%, Forced Expiratory Volume in 1 second as a percentage of predicted; FEV_1/FVC, forced expiratory volume in one second /forced vital capacity; GOLD stage, global initiative for chronic obstructive lung disease stage; WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte, MONO, monocyte; EOS, eosinophil; BASO, basophil; CRP, C-reactive protein.

demonstrated that, compared to the low KL-6 group, the high KL-6 group had 159 genes upregulated and 78 genes downregulated (Figure 5A). To explore the protein interactions among the 137 DEGs, we constructed a PPI network, with the top 20 genes highlighted (Figure 5B). Furthermore, we performed GO and KEGG enrichment analyses on these DEGs. Figure 5C illustrated the pathways enriched by DEGs, while Figure 5D and Supplementary Figure 1A and B displayed the biological processes (BP), cellular components (CC), and molecular functions (MF) enriched by DEGs. Further investigation into the expression trends of DEGs and their relationship with pathways, BP, CC, and MF was conducted using GSEA, and the top 5 results were presented (Figure 5E and F and Supplementary Figure 1C and D).

Correlation Between KL-6 and Immune Cell Signatures

We further investigated the proportions of immune cells in the high KL-6 and low KL-6 groups. However, the results indicated that there were no significant differences in the proportions of immune cells between the low KL-6 group and the high KL-6 group (Figure 6A). Correlation analysis revealed that KL-6 showed a negative correlation with T cell gamma delta and dendritic cells (DCs) activated (Figure 6B).

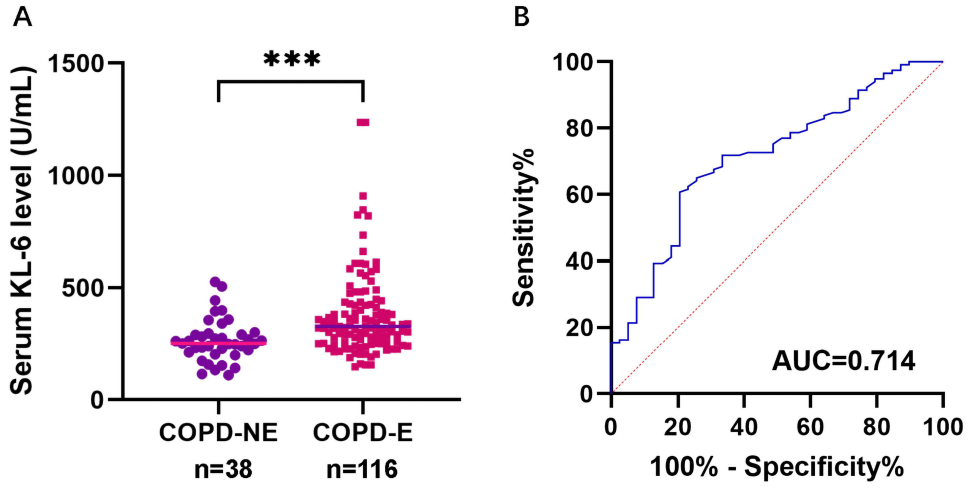

Figure 4 Serum KL-6 level distinguishing COPD-E from COPD-NE in COPD patients. (**A**) Serum levels of KL-6 between COPD-NE patients and COPD-E patients. (**B**) Performances of KL-6 in the diagnosis of COPD-E. ***P <0.001.

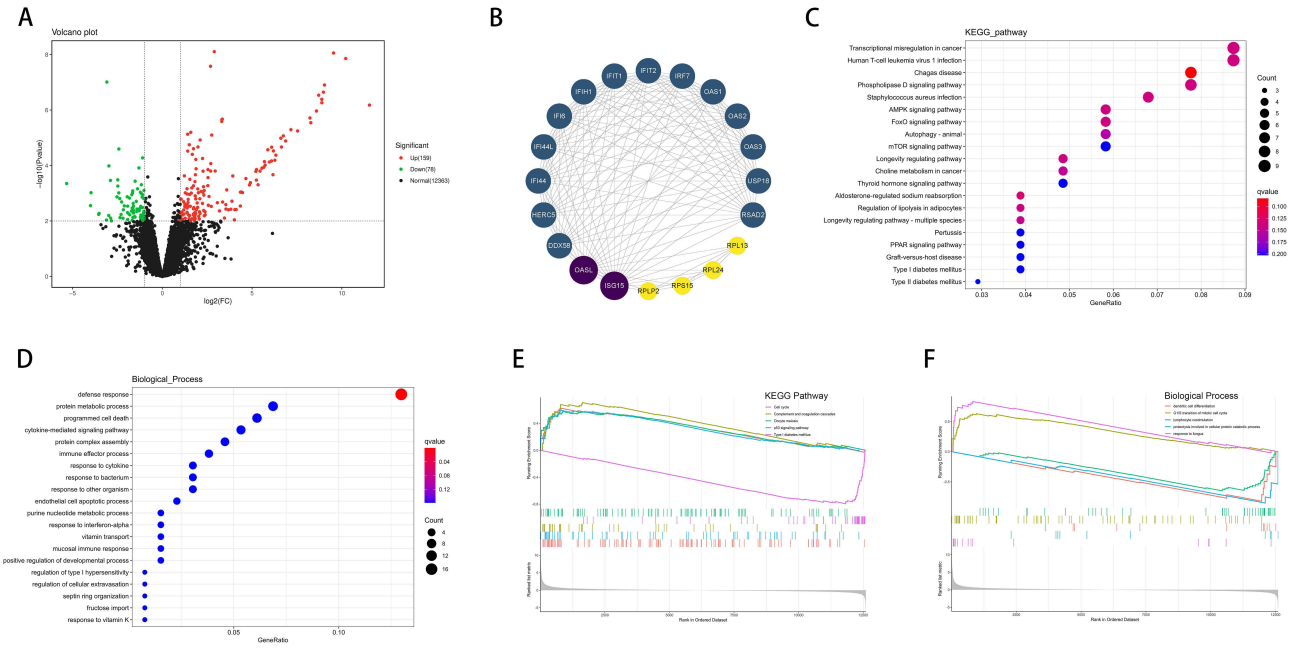

Figure 5 Identification and enrichment analysis of DEGs in COPD patients with high and low levels of KL-6. (**A**) Volcano plot displaying DEGs. (**B**) PPI network of the top 20 DEGs. (**C**) Dotplot analysis of KEGG pathway enrichment for DEGs. (**D**) GO classification analysis of DEGs associated with biological processes. (**E**) GSEA analysis of KEGG pathways. (**F**) GSEA analysis of biological processes.

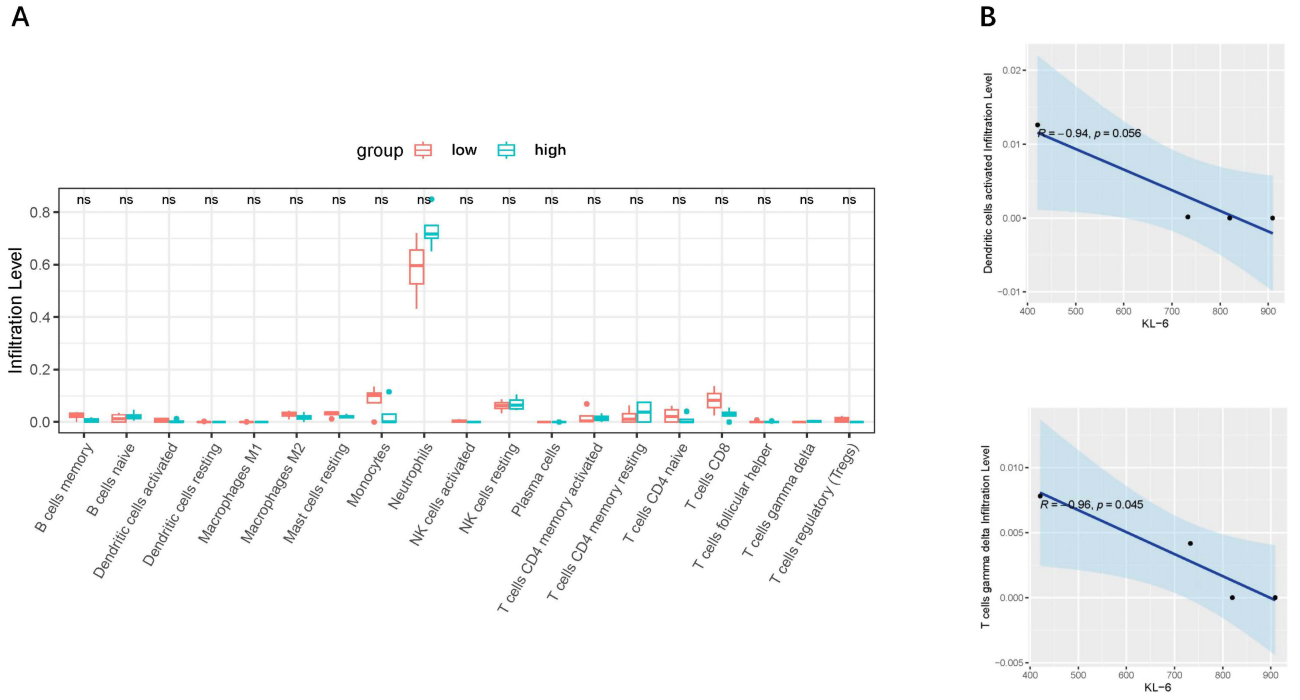

Figure 6 The landscape of immune cells between COPD patients with high and low levels of KL-6. (**A**) violin graphs show the distribution of immune cells between high and low KL-6 patients. (**B**) Association of KL-6 with DCs activated and T cell gamma delta level in COPD-E. ns: no significant.

Identification of Key Genes Associated with KL-6 Expression in Emphysema Pathogenesis

We obtained the GSE248493 dataset from the GEO database, which comprises transcriptome sequencing data from PBMCs of 14 patients with COPD-NE and 8 patients with COPD-E. By intersecting the differentially expressed genes

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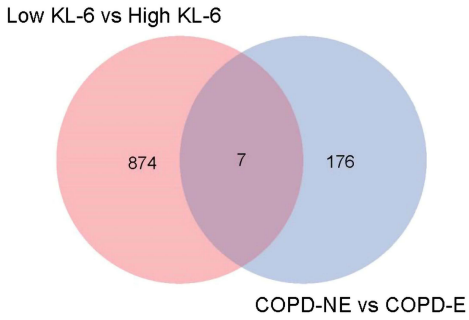
A



B

exposure	outcome	method	nsnp	OR (95% CI)	pval
AMDHD2	emphysema	MR Egger	13	0.9956 (0.9910 to 1.0003)	6.72e-02
AMDHD2	emphysema	Weighted median	13	0.9985 (0.9954 to 1.0015)	3.27e-01
AMDHD2	emphysema	Inverse variance weighted	13	0.9969 (0.9948 to 0.9990)	3.61e-03
AMDHD2	emphysema	Simple mode	13	0.9981 (0.9925 to 1.0038)	5.17e-01
AMDHD2	emphysema	Weighted mode	13	0.9986 (0.9941 to 1.0031)	5.44e-01

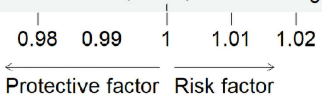


Figure 7 Identification of key genes associated with KL-6 expression in emphysema pathogenesis. (**A**) Intersection of differential genes between two groups. (**B**) Forest plot of AMDHD2 in relation to emphysema risk.

from this dataset with those identified in our sequencing analysis, we identified seven genes—protein kinase, membrane associated tyrosine/threonine 1 (PKMYT1), amidohydrolase domain containing 2 (AMDHD2), ADAM metallopeptidase with thrombospondin type 1 motif 10 (ADAMTS10), high mobility group box 3 (HMGB3), fragile histidine triad (FHIT), TATA-box binding protein associated factor 6 like (TAF6L), and solute carrier family 26 member 8 (SLC26A8)—that may play a significant role in the pathogenesis of emphysema involving KL-6 (Figure 7A). Following a stringent selection process for instrumental variables, we ultimately identified five genetic instrumental variables (AMDHD2, ADAMTS10, FHIT, TAF6L, and SLC26A8). We then performed an MR analysis with these five gene expression quantitative trait loci (eQTLs) as exposures and emphysema as the outcome (Supplementary Table 1). The findings revealed that genetic susceptibility associated with the AMDHD2 cis-eQTL decreased the risk of emphysema (OR=0.9969, 95% CI=0.9948–0.9990, P=0.004, heterogeneity=0.562, pleiotropy=0.520, Figure 7B).

Discussion

Our study provided important insights into the role of KL-6 as a biomarker for COPD, particularly in distinguishing between COPD-E and COPD-NE subtypes. Compared to healthy controls, COPD patients exhibited significantly higher serum KL-6 levels, with multivariate regression analysis showing that emphysema was a risk factor for elevated serum KL-6 levels in COPD. Subsequent subgroup analysis indicated that KL-6 can serve as a biomarker to differentiate COPD-NE from COPD-E.

KL-6, a mucinous high molecular weight glycoprotein expressed on epithelial and alveolar type II epithelial cells, is part of the MUC1 protein family.[20–22] Elevated KL-6 in the serum can be detected when the alveolar-capillary basement membrane is compromised.[23,24] Additionally, a study showed that serum KL-6 levels raised in patients with acute respiratory distress syndrome due to systemic inflammation when the blood-air barrier is damaged.[25] Oxidative stress can also upregulate KL-6/MUC1 expression, which subsequently induces the expression of various antioxidant enzymes, mitigating the apoptotic effects of oxidative stress.[25] Research has demonstrated that KL-6 is crucial in the progression of several pulmonary diseases,

such as idiopathic pulmonary fibrosis, interstitial lung disease, and acute lung injury.[23,26–30] Furthermore, compared to healthy participants, COPD patients have elevated KL-6 levels,[17,31,32] a finding that our study also confirms. Notably, KL-6 is primarily expressed on alveolar type II cells and is released in response to alveolar epithelial injury. However, in COPD, the primary pathology involves airway remodeling and alveolar structural destruction, which may not uniformly induce significant alveolar type II cell injury across disease severities. This could explain why KL-6 levels remain stable across COPD severity stages (GOLD I–IV) observed in our study, yet exhibit marked differences between emphysema-dominant (COPD-E) and airway-centric (COPD-NE) subtypes. Wang et al[13] further found that elevated KL-6 can predict the severity and prognosis of COPD, with patients having higher KL-6 levels experiencing more frequent future exacerbations and longer hospital stays. However, no studies have analyzed KL-6 levels in COPD subtypes; in this study, we found that emphysema is a factor affecting the elevation of serum KL-6 levels in COPD patients. By classifying patients into COPD-NE and COPD-E groups based on chest CT, and analyzing KL-6 levels between different subgroups, we discovered that KL-6 can serve as a biomarker to differentiate COPD-NE from COPD-E.

KL-6 is an inflammatory factor released during the inflammatory storm caused by alveolar injury. Therefore, as a marker of epithelial and alveolar cell injury, KL-6 is closely associated with the pathogenesis of COPD through various pathways. In COPD patients, a potential mechanism for elevated KL-6 levels is chronic exposure to cigarette smoke, which causes the damage of alveolar epithelial cells and alveolar basement membrane, consequently leading to increased serum KL-6 levels. While the mechanisms influencing serum KL-6 levels in COPD patients remain unclear, some reports have detailed the pathogenic mechanisms of KL-6 in other lung diseases. Previous studies have shown that KL-6 increases the motility and volume of pulmonary fibroblasts and inhibits their apoptosis.[33] A study suggests that serum KL-6 levels are a biomarker for structural lung damage and are involved in airway remodeling in severe asthma.[34] Furthermore, KL-6 can be detected in mice expressing human MUC1 (hMUC1-exp), and it can reflect the severity of bleomycin-induced pulmonary fibrosis.[35]

In our understanding, chronic inflammation plays a pivotal role in the pathogenesis of COPD.[36] Upon exposure to smoke or certain pathogens, the initiation of pathogen-associated molecular patterns and the subsequent enhancement of airway inflammation trigger an inflammatory response. This leads to the recruitment and accumulation of immune cells like macrophages, neutrophils, and eosinophils, potentially involving KL-6. Previous research has found that COPD patients with elevated blood eosinophils have more severe emphysema.[37–39] Our research indicates a correlation between serum KL-6 levels and eosinophil counts in COPD patients, hinting at a possible association between KL-6 and type 2 inflammatory pathways within the disease. This relationship further suggests that KL-6 can serve as a reliable biomarker for COPD-E. Therefore, in the subsequent transcriptomic sequencing and enrichment analysis of high KL-6 and low KL-6 PBMCs, we focused on the signal transduction of immune cells and related pathways. GO analysis identified key immune processes including Defense response, Cytokine-mediated signaling pathway, Immune effector process, Response to cytokine, Response to bacterium, Mucosal immune response, and Regulation of cellular extravasation, indicating KL-6's involvement in epithelial barrier maintenance and neutrophil recruitment—both hallmark features of COPD progression. KEGG pathway analysis revealed metabolic reprogramming through AMPK, FoxO, and PPAR signaling, potentially linking KL-6 to oxidative stress regulation and cellular senescence—critical mechanisms in emphysema development. GSEA highlighted fundamental immune regulation through dendritic cell differentiation and lymphocyte costimulation, suggesting proliferative exhaustion and T-cell dysfunction in KL-6-high COPD. Cell cycle-related pathways and p53 signaling indicate KL-6's potential role in apoptosis regulation of structural cells. We then conducted further immune infiltration analysis to explore the differences in immune cell proportions between the high KL-6 group and the low KL-6 group. It was unexpected that there were no significant differences in immune cell proportions between the high KL-6 group and the low KL-6 group. Although overall immune cell proportions did not show significant differences between KL-6 groups, deconvolution analysis revealed two critical insights: KL-6 levels were negatively correlated with γδ T cell, a population implicated in mucosal immunity and bacterial response. KL-6-high PBMCs exhibited transcriptional signatures of impaired dendritic cell differentiation, potentially linking KL-6 to antigen-presentation defects via downregulation of the PPAR γ /FoxO1 axis. The integration of immune response pathways from GO analysis with metabolic signaling pathways from KEGG suggests a potential KL-6-mediated interaction between epithelial injury responses and metabolic reprogramming in immune cells.

This study only performed transcriptome sequencing on PBMCs from COPD patients. To further explore the role of KL-6 in COPD-E, we obtained the PBMC sequencing results from Takuro et al[16] for COPD-NE and COPD-E. By intersecting their DEGs with ours, we identified 7 key genes potentially implicated in the elevated KL-6 levels characteristic of COPD-E. A subsequent Mendelian randomization analysis of the eQTLs for these seven genes in relation to emphysema risk indicated that AMDHD2 might mitigate the risk of emphysema. AMDHD2 is involved in the hexosamine biosynthetic pathway,[40] which generates the essential metabolite UDP-GlcNAc and is crucial for metabolism, health, and aging. The deficiency of AMDHD2 markedly elevates UDP-GlcNAc levels, which serves as a direct glycosyl donor for protein N-glycosylation and O-GlcNAc glycosylation.[41] Elevated plasma fibroblast growth factor 23 (FGF23) levels in COPD patients have been shown to stimulate human bronchial epithelial cells via the hexosamine biosynthetic pathway, resulting in the production of O-GlcNAc-modified proteins. This leads to the downstream activation of the nuclear factors of activated T cells (NFAT) signaling pathway and the secretion of interleukin 6 (IL-6).[42] AMDHD2 may inhibit airway inflammation in COPD by suppressing the donor of O-GlcNAc, UDP-GlcNAc. This discovery is intriguing as it points to a potential therapeutic target for emphysema. However, the underlying mechanisms necessitate further investigation through additional cellular and animal studies.

While this research has uncovered significant findings, it is not without its constraints. Firstly, the study's confinement to a single center restricts the diversity of the sample pool. Additionally, the research lacks longitudinal data on KL-6 levels in COPD patients. Tracking these levels over time could offer insights into disease activity and serve as an early indicator of exacerbations in COPD. Furthermore, the study only performed transcriptome sequencing on PBMCs from COPD patients, which may not fully represent the complex pathological processes in the lungs. Future studies should consider incorporating data from lung tissue samples and other relevant sources to provide a more comprehensive understanding. Nevertheless, it is improbable that a single biomarker for COPD will cover all diagnostic and prognostic needs; instead, the combined use of various biomarkers may enhance the precision and efficacy of disease prognosis. Despite these limitations, our findings advance COPD precision medicine by proposing KL-6 as a cost-effective tool for COPD-E screening in resource-limited settings and identifying AMDHD2 as a druggable target for metabolic modulation in COPD-E.

Conclusion

In conclusion, our study highlights the importance of KL-6 as a biomarker in COPD, particularly in the context of emphysematous changes. The findings from this study set the stage for future investigations into the intricate pathophysiological mechanisms involving KL-6 in COPD and the development of targeted therapeutic approaches. Future directions should focus on validating KL-6 thresholds for COPD subtyping in multi-ethnic cohorts, exploring the mechanisms underlying KL-6/AMDHD2 crosstalk in alveolar repair, and testing KL-6 as a stratification marker for anti-inflammatory therapies.

Data Sharing Statement

The datasets generated for this study are available upon request to the corresponding author.

Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of Third Affiliated Hospital of Nanjing Medical University, Jiangsu Province (Ethics Approval Number: 2024KY245-01). Each participant signed informed consent before participating in this study.

Consent for Publication

All authors have read and agreed to the published version of the manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest.

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