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Original Article

Clinical value of $CD3^-CD56^+$ natural killer cells, IL-2, and IL-8 in acute exacerbation of chronic obstructive pulmonary disease in patients with respiratory failure

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Abstract: Objective: This study aims to investigate the the content of $CD3^-CD56^+$ Natural Killer (NK) cells in peripheral blood and the serum interleukin-2 (IL-2) and interleukin-8 (IL-8) levels in patients with acute exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD) complicated by respiratory failure (RF). Besides, their diagnostic and prognostic values for AECOPD combined with RF were also explored. Methods: This retrospective study included patients from the Affiliated Jiangning Hospital of Nanjing Medical University between December 2021 and December 2023. A total of 65 AECOPD patients with RF were selected as the RF group, 64 AECOPD patients without RF as the AE group, and 60 patients with stable COPD as the COPD group. Data on gender, age, course of disease, smoking, alcohol consumption, history of hypertension and diabetes were collected. Serum levels of IL-2 and IL-8 were detected by enzyme-linked immunosorbent assay (ELISA). Arterial oxygen partial pressure (PaO_2) and arterial carbon dioxide partial pressure ($PaCO_2$) at admission were measured by automatic blood gas analyzer. Pulmonary function was assessed using forced expiratory volume in the first second (FEV_1)/forced vital capacity (FVC) and the percentage of FEV_1 to the predicted value (FEV_1%). The percentage of NK cells ($CD3^-CD56^+$) was detected by flow cytometry. After discharge, patients were followed for one year and categorized into a favorable prognosis (FP) group or an unfavorable prognosis group (UP) based on the severity of lung function impairment. Results: Expression levels of IL-2 in the COPD group, AE group, and RF group were 2.91±0.55, 2.21±0.48 and 1.39±0.43, respectively, while the expression levels of IL-8 were 31.01±4.86, 38.02±5.16 and 44.43±6.54, respectively. The percentages of $CD3^-CD56^+$ NK cells in the three groups were 19.93±2.40%, 22.57±3.70% and 25.48±3.64%, and the levels of FEV_1/FVC were 60.81±6.00%, 51.31±4.95% and 42.67±8.77%, respectively. FEV_1% were 61.11±5.71%, 45.45±6.86% and 38.77±10.07%, and $PaCO_2$ levels were 44.08±4.91 mmHg, 53.02±10.52 mmHg and 65±6.63 mmHg, respectively. PaO_2 levels were 81.5±5.64 mmHg, 59.1±5.95 mmHg and 53.86±7.06 mmHg, respectively. The differences in the above indices were statistically significant among the three groups (all $P<0.05$). In all COPD patients, IL-2, IL-8, and $CD3^-CD56^+$ NK cells (%) were associated with lung function and arterial blood gas values. In addition, these markers demonstrated predictive value for the prognosis of AECOPD patients complicated by RF, with their combined detection showing a higher predictive value. Conclusions: The combined assessment of serum IL-2, and IL-8 levels and positive $CD3^-CD56^+$ NK cell rate in peripheral blood of AECOPD patients has diagnostic value for identifying AECOPD with RF and predicting poor prognosis. These markers can serve as predictors of outcomes.

Keywords: $CD3^-CD56^+$ NK cells, interleukin-2, interleukin-8, chronic obstructive pulmonary disease, respiratory failure

Introduction

Chronic obstructive pulmonary disease (COPD) is a prevalent and debilitating lung condition characterized by incomplete reversible airflow limitation, with high morbidity and high mortality [1, 2]. Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is a critical event in the progression of COPD and is one of the key factors affecting patient prognosis [3, 4]. AECOPD is marked by acute worsening of symptoms such as dyspnea, cough and sputum, which can eventually lead to respiratory failure (RF) [5, 6]. AECOPD complicated with RF significantly increases patient suffering and contributes to multi-organ damage, resulting in

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increased mortality [7]. The pathogenesis of AECOPD complicated with RF is complex and remains poorly understood, with a generally poor prognosis.

Recent research has mostly focused on serum indicators, which are relatively easy to measure and are favored by researchers. Studies have shown that immune-infiltrating cells, including neutrophils, macrophages, lymphocyte subsets, and dendritic cells, play a key role in airway inflammation and lung destruction in COPD [8, 9]. Natural killer (NK) cells, as part of the innate immune system, serve as the first line of defense against infections and tumors. NK cells are typically identified by the $CD3^{-}CD56^{+}$ marker, and their surface receptor recognize tumor cells, virus-infected cells, stressed cells, and various pathogens [10, 11]. Previous studies have confirmed that NK cells contribute to maintaining immunological homeostasis and play a significant role in COPD development [12-14]. Interleukin-2 (IL-2) is known for its immune-enhancing activity, which can promote the proliferation of NK [15]. A decrease in IL-2 levels results in reduced immune activity [16]. In COPD patients, viral or bacterial infections can impair body's ability to produce IL-2, leading to decreased cellular immune function during the infection stage, thereby weakening overall immune defenses and resulting in low IL-2 expression [17, 18]. Interleukin-8 (IL-8), a chemokine, promotes neutrophil recruitment to the airways and induces the release of proteases and oxygen free radicals, which damage the pulmonary capillaries and epithelial cells [19, 20]. We hypothesize that NK cells, IL-2 and IL-8 are indicative of the severity of AECOPD complicated by RF.

Materials and methods

General information

This retrospective study was approved by the Ethics Committee of The Affiliated Jiangning Hospital of Nanjing Medical University. COPD cases diagnosed and treated at The Affiliated Jiangning Hospital of Nanjing Medical University between December 2021 and December 2023 were included and categorized into three groups: those with AECOPD and RF (RF group, n=65 patients), those with AECOPD but without RF (AE group, n=60 patients), and those with stable COPD (COPD group, n=60 patients).

Inclusion criteria: ① A diagnosis of COPD and AECOPD based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines [21]. AECOPD complicated with RT was confirmed through blood gas analysis, pulmonary function tests, and clinical manifestations; ② Age between 18 and 79 years; ③ Complete clinical data; ④ Good compliance with relevant medical examinations during treatment period. Exclusion criteria: ① Patients with heart, liver, kidney or other organ dysfunction; ② Patients with other respiratory diseases; ③ Patients with malignant tumor or congenital immune system disease; ④ Patients with mental disorders or cognitive impairment; ⑤ Pregnant women. The experimental process is shown in **Figure 1**.

Blood value detection

Upon admission, 5 mL of peripheral venous blood was collected from all subjects, and a five-class automatic hematology analyzer (Sysmex Company, Japan, model: XN-9000) was used to conduct routine blood tests. On the next morning, 5 mL fasting venous blood was collected from each patient and stored in a non-antibacterial collection tube. After centrifugation at 3000 r/min for 10 min, the supernatant was separated, stored in EP tubes, and stored at -80°C until use. The expression levels of IL-2 (COIBO BIO, China, CB10349-Hu) and IL-8 (COIBO BIO, China, CB10376-Hu) in each group were measured using enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions. ELISA experiments were performed using a Benchmark Plus ELISA apparatus (Bio-eobrad, USA).

Arterial blood gas index detection

The arterial partial pressure of oxygen (PaO_2) and arterial partial pressure of carbon dioxide ($PaCO_2$) were detected upon admission using an automatic blood gas analyzer (Roche, USA, model: Rochecobasb123).

Pulmonary function detection

Pulmonary function was assessed using a lung function analyzer (Jaeger company, Germany, model VPAPIIIST-A). The forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) ratio (FEV_1/FVC), and the percentage of FEV_1 to the predicted value (FEV_1%) were

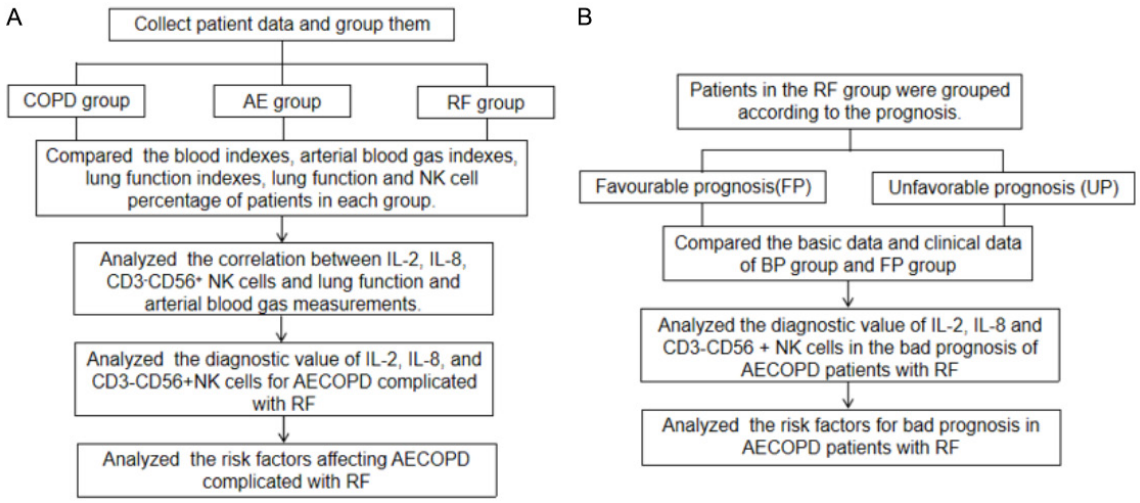

Figure 1. Flow chart of study procedures. A: Diagnostic value of IL-2, IL-8, and $CD3^-CD56^+$ NK cells for AECOPD complicated with RF; B: Diagnostic value of IL-2, IL-8 and $CD3^-CD56^+$ NK cells for unfavorable prognosis of AECOPD patients with RF. Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; AECOPD: acute exacerbation of chronic obstructive pulmonary disease; RF: respiratory failure.

measured. During the test, patients were instructed to sit upright and exhale as hard as possible for 6 s with a nose clip and a mouthpiece without interruption, coughing, or additional inhalation.

NK cell percentage detection

Whole blood anticoagulated with heparin was used for fluorescence labeling. A 100 μL whole blood sample was incubated with APC-CD45 and PerCP-Cy5-conjugated CD3 and PE-CD56 antibodies at room temperature for 30 minutes. After hemolysis using a hemolysis buffer, the cells were washed with PBS and resuspended in 500 μL of PBS for analysis by flow cytometry (FCM). The percentage of NK cells ($CD3^-CD56^+$) was determined by gating the cell population based on forward scatter (FS) and side scatter (SS) light characteristics in scatter plots. The instrument was calibrated using Flowcheck light path calibration to ensure that the half-height variation in FS and fluorescence pathways was less than 2%. Sample quality control was performed using Immuno-Trol quality control materials.

Treatment and follow-up

All patients received electrocardiographic monitoring upon admission, with mechanical ventilation provided as needed. Treatments included oxygen inhalation, expectoration assistance, electrolyte disturbance correction, and nutritional support. After discharge, patients were monitored for one year, and categorized into a favorable prognosis (FP) group and an unfavorable prognosis group (UP) based on the severity of lung function impairment. In the FP group, clinical symptoms such as cough and dyspnea improved, while in the UP group, lung function remained impaired without improvement.

Statistical analysis

Data analysis was conducted using SPSS 26.0. Measured data were presented as mean ± standard deviation and compared using t test or analysis of variance (ANOVA) with post-hoc LSD-t test. Counted data were presented as percentage and compared using χ^2 test. Pearson correlation analysis was applied to assess the relationship between two variables. The predictive value of variables was tested using a receiver operating characteristic (ROC) curve. A *P*-value of <0.05 was considered significant.

Results

Comparison of general data among patients in the RF, AE, and COPD groups

There was no significant difference in gender composition, age, disease duration, smoking history, alcohol consumption, hypertension, or diabetes history among the RF group, AE group, and COPD group (all $P>0.05$) (**Table 1**).

Table 1. Comparison of general data among groups

		COPD (n=60)	AE (n=64)	RF (n=65)	X^2/t	P
Age (years)		57.12±14.26	56.42±14.77	56.94±14.27	0.039	0.962
Gender	Male	41.67%	42.19%	40.00%	0.069	0.966
	Female	58.33%	57.81%	60.00%		
Disease duration (months)		12.12±4.55	11.98±4.75	12.05±4.68	0.012	0.988
Smoking	Yes	46.67%	42.19%	36.92%	1.224	0.542
	No	53.33%	57.81%	63.08%		
Alcohol consumption	Yes	33.33%	35.94%	23.08%	2.797	0.247
	No	66.67%	64.06%	76.92%		
Hypertension	Yes	40.00%	35.94%	40.00%	0.295	0.863
	No	60.00%	64.06%	60.00%		
Diabetes	Yes	23.33%	23.44%	40.00%	5.705	0.058
	No	76.67%	76.56%	60.00%		

Notes: COPD refers to the group of patients with stable chronic obstructive pulmonary disease; AE refers to the group of patients with acute exacerbation of chronic obstructive pulmonary disease; RF refers to the group of patients with acute exacerbation of chronic obstructive pulmonary disease complicated by respiratory failure.

Table 2. Comparison of clinical data among patients in each of the RF, AE, and COPD groups

Index	Group		X^2/t	P
IL-2 (μg/L)	COPD	2.91±0.55	152.695	<0.001
	AE	2.21±0.48*		
	RF	1.39±0.43*,#		
IL-8 (μg/L)	COPD	31.01±4.86	89.93	<0.001
	AE	38.02±5.16*		
	RF	44.43±6.54*,#		
$CD3^-CD56^+$ NK cells (%)	COPD	19.93±2.40	43.673	<0.001
	AE	22.57±3.70*		
	RF	25.48±3.64*,#		
FEV_1/FVC (%)	COPD	60.81±6.00	111.380	<0.001
	AE	51.31±4.95*		
	RF	42.67±8.77*,#		
FEV_1% (%)	COPD	61.11±5.71	132.891	<0.001
	AE	45.45±6.86*		
	RF	38.77±10.07*,#		
$PaCO_2$ (mmHg)	COPD	44.08±4.91	114.619	<0.001
	AE	53.02±10.52*		
	RF	65±6.63*,#		
PaO_2 (mmHg)	COPD	81.5±5.64	338.584	<0.001
	AE	59.1±5.95*		
	RF	53.86±7.06*,#		

Notes: *P<0.05 compared to COPD group; #P<0.05 compared to AE group. IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; FEV_1/FVC: the forced expiratory volume in the first second/forced vital capacity; FEV_1%: the percentage of forced expiratory volume in the first second to the predicted value; $PaCO_2$: arterial carbon dioxide partial pressure; PaO_2: the levels of arterial oxygen partial pressure. COPD refers to the group of patients with stable chronic obstructive pulmonary disease; AE refers to the group of patients with acute exacerbation of chronic obstructive pulmonary disease; RF refers to the group of patients with acute exacerbation of chronic obstructive pulmonary disease complicated by respiratory failure.

Comparison of clinical data among RF, AE and COPD groups

Analysis of medical records from the three groups revealed significant differences in serum IL-2 and IL-8 levels, peripheral blood $CD3^-CD56^+$ NK cell expression, FEV_1/FVC, FEV_1%, $PaCO_2$, and PaO_2 among the three groups (all P<0.001). Compared to the COPD group, the RF and AE groups showed higher levels of IL-8, $CD3^-CD56^+$ NK cells, and $PaCO_2$, but lower levels of IL-2, FEV_1/FVC, FEV_1%, and PaO_2. Additionally, significant differences were observed between the RF and AE groups for these values (**Table 2** and **Figure 2**).

Correlation of IL-2, IL-8 and $CD3^-CD56^+$ NK cells with pulmonary function and arterial blood gas indices

Pearson correlation analysis demonstrated that in all in COPD patients, serum IL-2 levels were positively correlated with FEV_1/FVC (R=0.639,

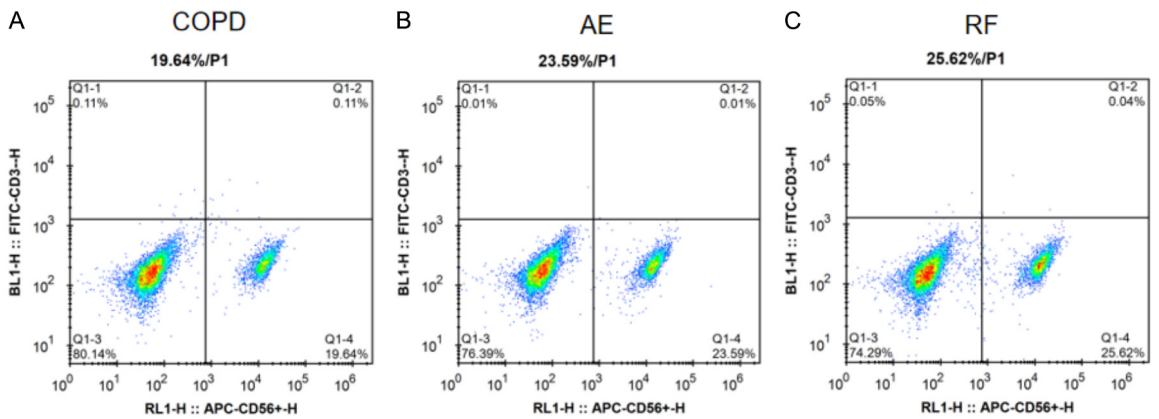

Figure 2. Contents of $CD3^-CD56^+$ NK cells in peripheral blood of each groups. A: COPD group; B: AE group; C: RF group. Notes: COPD refers to the group of patients with stable chronic obstructive pulmonary disease; AE refers to the group of patients with acute exacerbation of chronic obstructive pulmonary disease; RF refers to the group of patients with acute exacerbation of chronic obstructive pulmonary disease complicated by respiratory failure. COPD: chronic obstructive pulmonary disease; AE: acute exacerbation; RF: respiratory failure.

Table 3. Correlation of IL-2, IL-8, and $CD3^-CD56^+$ NK cells with pulmonary function and arterial blood gas measures

Value	FEV_1/FVC (%)		FEV_1%		$PaCO_2$ (mmHg)		PaO_2 (mmHg)	
	R value	*P* value	R value	*P* value	R value	*P* value	R value	*P* value
IL-2	0.433	<0.001	0.362	<0.001	-0.266	0.002	0.375	<0.001
IL-8	-0.436	<0.001	-0.344	<0.001	0.312	<0.001	-0.381	<0.001
$CD3^-CD56^+$ NK cells (%)	-0.407	<0.001	-0.032	0.719	0.109	0.221	-0.273	0.002

Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; FEV_1/FVC: the forced expiratory volume in the first second/forced vital capacity; FEV_1%: the percentage of forced expiratory volume in the first second to the predicted value; $PaCO_2$: arterial carbon dioxide partial pressure; PaO_2: the levels of arterial oxygen partial pressure.

P<0.001), FEV_1% (R=0.619, P<0.001) and PaO_2 (R=0.669, P<0.001), and negatively correlated with $PaCO_2$ (R=-0.524, P<0.001). Furthermore, IL-8 level was positively correlated with $PaCO_2$ levels (R=0.312, P<0.001), but negatively correlated with FEV_1/FVC (R=-0.436, P<0.001), FEV_1% (R=-0.344, P<0.001) and PaO_2 (R=-0.381, P<0.001). $CD3^-CD56^+$ NK cell proportion in peripheral blood was negatively correlated with FEV_1/FVC (R=-0.407, P<0.001), and PaO_2 (R=-0.273, P=0.002), see **Table 3**.

Diagnostic value of IL-2, IL-8, and $CD3^-CD56^+$ NK cells in AECOPD complicated by RF

The diagnostic value of IL-2, IL-8, and $CD3^-CD56^+$ NK cells in predicting AECOPD complicated by RF was examined using binary logistic regression analysis. The dependent variable was the presence of RF (0= concurrent RF, 1= not concurrent RF), and the independent variables were IL-2, IL-8, and $CD3^-CD56^+$ NK cell levels. The predictive value of the variables was assessed using ROC curve analysis. The results showed that the area under the curve (AUC) for serum IL-2 in predicting RF in AECOPD patients was 0.903 (95% CI 0.850-0.956), for serum IL-8 it was 0.780 (95% CI 0.701-0.858), and for $CD3^-CD56^+$ NK cell expression rate in peripheral blood it was 0.714 (95% CI 0.626-0.802).

A combined detection equation for diagnosing AECOPD patients with RF was established using binary logistic regression analysis: Logit(P) = -3.622 × IL-2 + 0.148 × IL-8 + 0.164 × $CD3^-CD56^+$ NK cells - 3.272. The ROC curve for this combined prediction model showed an AUC of 0.927 (95% CI 0.882-0.971), indicating that the combined model outperformed the individual factors (**Table 4** and **Figure 3**).

Risk factors for AECOPD complicated by RF identified by logistic regression analysis

Cut-off values from ROC curve for serum IL-2, IL-8 levels and $CD3^-CD56^+$ NK cells were used to define high and low expression levels. Serum

Table 4. Diagnostic value of IL-2, IL-8, and $CD3^-CD56^+$ NK cells for AECOPD complicated with RF

Value	AUC	95% CI	Cutoff value	Sensitivity	Specificity	Youden Index
IL-2	0.903	0.850-0.956	1.75 μg/L	80.0%	89.10%	0.501
IL-8	0.78	0.701-0.858	31.95 μg/L	98.50%	52.50%	0.860
$CD3^-CD56^+$ NK cell	0.714	0.626-0.802	24%	67.70%	65.60%	0.333
Combined diagnosis	0.927	0.882-0.971	-	84.60%	93.70%	0.783

Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; AECOPD: acute exacerbation of chronic obstructive pulmonary disease; RF: respiratory failure.

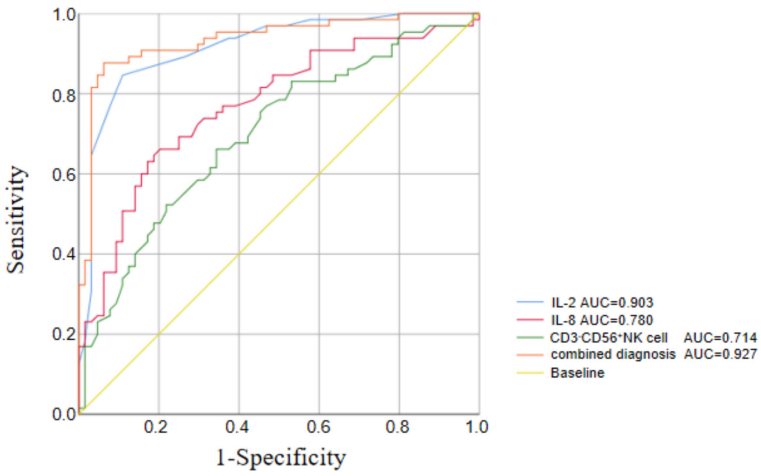

Figure 3. ROC curve for each index in diagnosing RF in AECOPD patients. Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; AECOPD: acute exacerbation of chronic obstructive pulmonary disease; RF: respiratory failure.

Table 5. Logistic regression analysis of risk factors for RF in AECOPD patients

Variable	β	SE	Wald X^2	OR	95% CI	P
IL-2	-0.339	0.049	-0.414	-6.898	-0.436 - -0.241	<0.001
$PaCO_2$	0.020	0.003	0.424	7.866	0.015 - -0.025	<0.001
FEV_1/FVC	-0.012	0.004	-0.195	-3.311	-0.019 - -0.005	0.001
PaO_2	-0.012	0.004	-0.163	-2.8299	-0.020 - -0.004	0.005

Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; FEV_1/FVC: the forced expiratory volume in the first second/forced vital capacity; PaO_2: the levels of arterial oxygen partial pressure; AECOPD: acute exacerbation of chronic obstructive pulmonary disease; RF: respiratory failure.

IL-2≥1.75 μg/L was considered as high expression, while <1.75 μg/L was considered as low. Serum IL-8≥31.95 μg/L was defined as high expression, and <331.95 μg/L as low. A $CD3^-CD56^+$ NK cell expression rate in peripheral blood ≥24% was considered high expression, and <24% as low. The median values of FEV_1/FVC, $FEV_1\%$, PaO_2, and $PaCO_2$ were 48.8%, 43.8%, 56.7 mmHg, and 59.4 mmHg, respectively, which were used as the cut-off values for high and low groups.

Taking patients who had AECOPD, and using complication by RF as the dependent variable (complicated by RF=0, not complicated by RF=1), significant variables from the univariate analysis were included in the multivariate logistic regression analysis, including FEV_1/FVC (low level =1, high level =0), $FEV_1\%$ (low level =1, high level =0), PaO_2 (low level =1, high level =0), $PaCO_2$ (low level =1, high level =0), IL-2 (high expression =1, low expression =0), IL-8 (high expression =1, low expression =0), the $CD3^-CD56^+$ NK cell proportion (high rate =1, low rate =0). Results showed that IL-2, FEV_1/FVC, $PaCO_2$, and PaO_2 were independent risk factors for AECOPD complicated by RF (all P<0.05), as shown in **Table 5**.

Prognosis comparison between FP and UP subgroups in patients who have AECOPD complicated by RF

After one-year follow-up, 36 patients with favorable prognosis were included in the FP group, and 29 patients with unfavorable prognosis were included in the UP group. There were no notable disparities in the gender composition, age, course of disease, smoking history, alcohol consumption, hypertension and diabetes history between the UP group and the FP group (all P>0.05). However, significant differences were observed between the FP and UP groups for serum levels of IL-2 and IL-8, the expression rate of $CD3^-CD56^+$ NK cells in peripheral blood, FEV_1/FVC, $FEV_1\%$, PaO_2 and

Table 6. Comparison of baseline data between the UP group and the FP group in AECOPD patients with RF

Value	FP (n=36)	UP (n=29)	X^2/t	P
Age (years)	55.58±14.96	58.62±13.44	0.281	0.398
Gender			0.093	0.803
Male	41.67%	72.41%		
Female	30.56%	62.07%		
Disease duration (months)	11.42±4.05	12.83±5.32	1.711	0.229
Smoking			0.780	0.444
Yes	41.67%	72.41%		
No	25.00%	68.97%		
Drinking			1.004	0.384
Yes	27.78%	89.66%		
No	13.89%	82.76%		
Hypertension			0.093	0.803
Yes	41.67%	72.41%		
No	30.56%	62.07%		
Diabetes			0.093	0.803
Yes	41.67%	72.41%		
No	30.56%	62.07%		
IL-2 (μg/L)	1.62±0.39	1.1±0.29	0.651	<0.001
IL-8 (μg/L)	41.51±5.37	48.05±6.1	0.261	<0.001
$CD3^-CD56^+$ NK cells (%)	24.14±3.39	27.14±3.27	1.658	0.001
FEV_1/FVC (%)	47.21±8.63	37.03±4.81	8.004	<0.001
FEV_1% (%)	42.66±6.53	33.94±11.62	14.769	0.001
$PaCO_2$ (mmHg)	68.17±5.65	61.05±5.61	0.048	<0.001
PaO_2 (mmHg)	57.89±4.11	48.86±6.78	3.406	<0.001

Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; FEV_1/FVC: the forced expiratory volume in the first second/forced vital capacity; FEV_1%: the percentage of forced expiratory volume in the first second to the predicted value; $PaCO_2$: arterial carbon dioxide partial pressure; PaO_2: the levels of arterial oxygen partial pressure; AECOPD: acute exacerbation of chronic obstructive pulmonary disease; RF: respiratory failure; UP: unfavorable prognosis; FP: favorable prognosis.

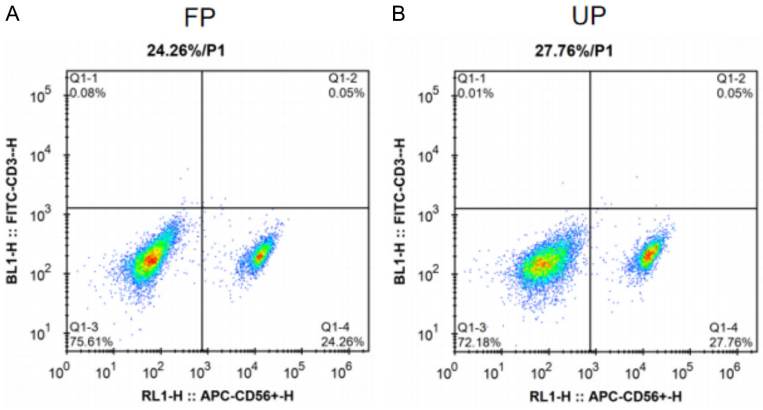

Figure 4. Contents of $CD3^-CD56^+$ NK cells in peripheral blood of each group. A: FP group; B: UP group. Notes: FP: favorable prognosis; UP: unfavorable prognosis.

$PaCO_2$ levels (all P<0.001). Compared to the FP group, the UP group had significantly higher serum IL-8 level, peripheral blood $CD3^-CD56^+$ NK cell rate, and PaCO2, and significantly lower FEV_1/FVC, FEV_1% and PaO_2 levels (all P<0.001), as shown in **Table 6** and **Figure 4**.

Diagnostic value of IL-2, IL-8, and $CD3^-CD56^+$ NK cells in prognosis of AECOPD patients with RF

To assess the prognosis of AECOPD patients with RF (FP=0, UP=1), we evaluated IL-2, IL-8, and $CD3^-CD56^+$ NK cells as predictive variables using binary logistic regression analysis. The AUC for serum IL-2 in predicting poor prognosis in

Table 7. Prognostic value of IL-2, IL-8, and $CD3^-CD56^+$ NK cells for AECOPD complicated by RF

Measure	AUC	95% CI	Cutoff value	Sensitivity	Specificity	Youden Index
IL-2	0.862	0.770-0.954	1.45 μg/L	77.80%	89.70%	0.675
IL-8	0.802	0.692-0.912	55.2 μg/L	97.20%	60.30%	0.869
$CD3^-CD56^+$ NK cells	0.752	0.631-0.874	27.55%	91.70%	51.70%	0.434
Combined diagnosis	0.928	0.867-0.989	-	86.10%	89.70%	0.758

Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells.

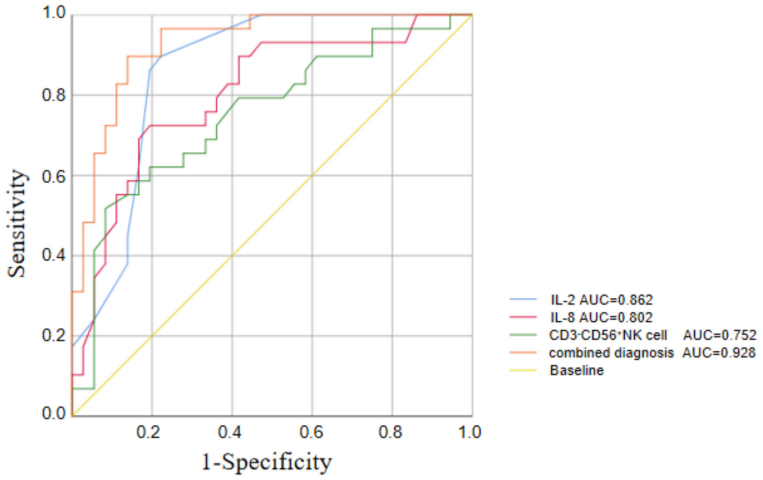

Figure 5. ROC curve for each index in diagnosing poor prognosis in AECOPD patients with RF. Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; AECOPD: acute exacerbation of chronic obstructive pulmonary disease; RF: respiratory failure.

AECOPD patients with RF was 0.862 (95% CI: 0.770-0.954), for serum IL-8, it was 0.802 (95% CI: 0.692-0.912), and for $CD3^-CD56^+$ NK cell rate in peripheral blood, it was 0.752 (95% CI: 0.631-0.874). A combined detection equation for predicting poor prognosis in AECOPD patients with RF was established as follows: Logit(P) = -4.089 × IL-2 + 0.179 × IL-8 + 0.309 × $CD3^-CD56^+$ NK cells - 10.693. The ROC curve for this combined model showed an AUC of 0.928 (95% CI: 0.867-0.989), which was superior to the individual markers alone (**Table 7** and **Figure 5**).

Risk factors affecting the prognosis of AECOPD patients with RF

Based on the ROC curve cut-off values, serum IL-2 level ≥1.45 μg/L was defined as high expression, and <1.45 μg/L as low. Serum IL-8 level ≥55.2 μg/L was considered high expression, and <55.2 μg/L as low. For percentage of $CD3^-CD56^+$ NK cells in peripheral blood, ≥27.55% was high expression, and <27.55% was low. The median values of FEV_1/FVC, FEV_1%, PaO_2 and $PaCO_2$ were 42.3%, 40.4%, and 65.4 mmHg and 55.1 mmHg, respectively, serving as cut-off values to categorize high and low levels. By multivariate logistic regression analysis, the statistically significant variables identified from univariate analysis were included, using patients with AECOPD, complicated by RF as the dependent variable (UP=1, FP=0). These variables included FEV_1/FVC (low =1, high =0), FEV_1% (low =1, high =0), PaO_2 (low =1, high =0), $PaCO_2$ (low =1, high =0), IL-2 (high =1, low =0), IL-8 (high =1, low =0), and $CD3^-CD56^+$ NK cells (high =1, low =0). The results indicated that IL-2, $CD3^-CD56^+$ NK cells, FEV_1/FVC, FEV_1%, $PaCO_2$ and PaO_2 were independent risk factors for poor prognosis in AECOPD patients with RF (all P<0.05), as shown in **Table 8**.

Discussion

Respiratory failure (RF) is a common complication of AECOPD. Clinical studies suggest that the progression of AECOPD is primarily driven by infectious injury, which destroys the body's immune anti-inflammatory mechanisms. This leads to an over-activation of the inflammatory response, causing cell infiltration, damage to airway and lung tissue function, and severe ischemia and hypoxia in the lungs, ultimately resulting in RF [22-24]. Exploring the diagnostic and prognostic value of hematologic indices can aid in early disease prediction and the identification of a poor prognosis, allowing for timely formulation of individualized treatment plans.

Table 8. Logistic regression analysis of risk factors for poor prognosis in AECOPD patients complicated by RF

Variable	β	SE	Wald X^2	OR	95% CI	P
IL-2	-0.269	0.103	-0.231	-2.599	-0.476-0.062	0.012
$CD3^-CD56^+$ NK cell	0.026	0.011	0.192	2.349	0.004-0.049	0.022
FEV_1/FVC	-0.014	0.005	-0.237	-2.712	-0.024 - -0.004	0.009
FEV_1%	-0.011	0.004	-0.217	-2.&50	-0.019 - -0.003	0.008
$PaCO_2$	-0.014	0.007	-0.181	-2.086	-0.027 - -0.001	0.041
PaO_2	-0.016	0.007	-0.230	-2.497	-0.029 - -0.003	0.015

Notes: IL-2: interleukin-2; IL-8: interleukin-8; NK cells: natural killer cells; FEV_1/FVC: the forced expiratory volume in the first second/forced vital capacity; FEV_1%: the percentage of forced expiratory volume in the first second to the predicted value; $PaCO_2$: arterial carbon dioxide partial pressure; PaO_2: the levels of arterial oxygen partial pressure; AECOPD: acute exacerbation of chronic obstructive pulmonary disease; RF: respiratory failure.

NK cells are lymphocytes that function as part of the innate immune system and are now classified as part of the innate lymphoid cell family 1 (ILC1). NK cells play a crucial role in both innate and adaptive immune responses. Studies have confirmed alterations in NK cell function in COPD [25]. Interleukins are cytokines produced by a variety of cells that regulate immune responses by interacting with and coordinating cellular growth factors [26]. IL-2 is a growth factor for T cell subsets that promotes the proliferation of T and B cells. It also induces lymphocytes to secrete antibodies, activates killer cells, and enhances the functional activity of NK cells [27, 28]. Previous studies have shown that IL-2 expression in AECOPD patients was lower than that in healthy individuals [29]. In addition, IL-8 activates neutrophils, inhibits extracellular matrix degradation, promotes fibroblast proliferation, and alters the structure and function of alveolar tissues. It can also activate the coagulation mechanism, promote thrombosis, which hinders gas exchange and perpetuates inflammatory damage to lung tissue [30, 31].

$PaCO_2$ and PaO_2 are regulated by the respiratory system and are important indicators of respiratory function. When the body produces excess carbon dioxide or fails to expel it adequately, the $PaCO_2$ levels increase and PaO_2 levels decrease [32, 33]. FEV_1/FVC and FEV_1% are commonly used to assess the degree of pulmonary airflow limitation. The findings in this study revealed that the serum levels of IL-8, peripheral blood $CD3^-CD56^+$ NK cell rate, and $PaCO_2$ levels were higher in the RF and AE groups compared to the COPD group. Conversely, the levels of IL-2, FEV_1/FVC, FEV_1%, and PaO_2 were lower in the RF and AE groups. In addition, these values in the RF group were also significantly different from those in the AE group, indicating that AECOPD patients with RF had more severe inflammation, impaired lung ventilation, and possible lung injury. Pearson correlation analysis showed that in all COPD patients, the serum level of IL-2 was positively correlated with FEV_1/FVC, FEV_1% and PaO_2 levels, and negatively correlated with $PaCO_2$ levels. Zhang Y et al. [29] pointed out that increased serum soluble IL-2 receptor level was correlated with the severity of AECOPD. Combined with the results of this study, it is suggested that decreased IL-2 level may contribute to the exacerbation of lung function and respiratory impairment. In addition, Nam JH et al. [15] highlighted that IL-2 has immune-enhancing properties, promoting the proliferation of NK cells. When IL-2 levels are reduced, the body's immune activity decreases. In this study, we observed that lower IL-2 levels were associated with a decreased expression rate of $CD3^-CD56^+$ NK cells, further supporting the idea that reduced IL-2 levels impair immunity in AECOPD patients, aggravating lung function decline. The study also found that serum IL-8 levels were negatively correlated with PaO_2, FEV_1%, and FEV_1/FVC, while being positively correlated with $PaCO_2$. Similarly, the expression rate of $CD3^-CD56^+$ NK cells was negatively correlated with FEV_1/FVC, and PaO_2. Zhang et al. [34-36] showed that IL-8 levels increased significantly during the AECOPD stage, confirming that serum IL-8 was more sensitive than IL-6 and TNF-α in the Chinese population. These findings, in addition to those of this study, sugges that elevated IL-8 levels may contribute to deterioration of lung and respiratory function.

Based on whether complicated with RF, the ROC curves for IL-2, IL-8, $CD3^-CD56^+$ NK cells, and their combined detection were analyzed. Results showed that the sensitivity of serum IL-2, IL-8, peripheral blood $CD3^-CD56^+$ NK cell positivity, and the combined detection for diagnosing RF in AECOPD patients was 80.0%, 98.50%, 67.70% and 84.60%, respectively, with AUCs of 0.903, 0.78, 0.714 and 0.927, respectively. This indicates that IL-2, IL-8, $CD3^-CD56^+$ NK cells and their combination have high diagnostic value for predicting RF in AECOPD patients. The combined detection of these markers offers superior predictive value. In addition, multivariate analysis identified IL-2, IL-8, $CD3^-CD56^+$ NK cells, FEV_1/FVC, and PaO_2 as independent risk factors for AECOPD patients complicated by RF. This suggests that simultaneous detection of serum IL-2, IL-8, and peripheral blood $CD3^-CD56^+$ NK cells, along with lung function and blood gas values, can improve the prediction of RF risk in AECOPD patients.

Despite advancements in medical technology for the diagnosis and treatment of AECOPD complicated by RF, some patients still experience poor outcome [34-36]. In this study, 29 patients (44.61%) in the RF group had a poor prognosis. The UP group showed higher levels of serum IL-8, peripheral blood $CD3^-CD56^+$ NK cell positivity, and $PaCO_2$ level, while IL-2, FEV_1/FVC, FEV_1%, and PaO_2 level were significantly lower compared to the FP group. Additionally, ROC analysis demonstrated that IL-2, IL-8, $CD3^-CD56^+$ NK cells, and their combined detection had a higher diagnostic value for predicting poor prognosis in AECOPD patients with RF, with the combined detection offering the highest diagnostic value. Multivariate analysis further confirmed that IL-2, $CD3^-CD56^+$ NK cells, FEV_1/FVC, $PaCO_2$, and PaO_2 were independent risk factors for poor prognosis. This suggests that detecting serum IL-2, IL-8, and peripheral blood $CD3^-CD56^+$ NK cells, along with lung function and blood gas values, can enhance the prediction of poor outcome in AECOPD patients with RF.

Above all, serum IL-2, IL-8, and peripheral blood $CD3^-CD56^+$ NK cell levels are significantly different in AECOPD patients with RF compared to those without RF and are independent risk factors for concurrent RF. These three markers have strong predictive value for AECOPD complicated by RF, and their combined detection is more efficient. Furthermore, they can also serve as prognostic indicators, aiding in the formulation of clinical treatment plans for AECOPD patients with RF.

NK cells have been associated with an increased risk of COPD. IL-2 promotes the proliferation of NK cells, and IL-8 induces neutrophils migration to the airway, resulting in increased inflammation. The innovation of this study lies in selecting patients with AECOPD combined by RF as research objects and collecting data on their NK cells, IL-2 and IL-8 levels and prognosis. It was found for the first time that the combined detection of serum IL-2 and IL-8 levels and peripheral blood $CD3^-CD56^+$ NK cell positive rate in patients with AECOPD had certain diagnostic value for identifying AECOPD complicated by RF and predicting poor prognosis. These markers can serve as predictors for both RF and prognosis in AECOPD patients, making this study both innovative and clinically significant.

However, the limitations of this study include a relatively small blood sample size and a concentrated sample population, which did not include patients from various stages of the disease. Additionally, dynamic analysis of the relationship between serum IL-2, IL-8, and peripheral blood $CD3^-CD56^+$ NK cell expression and AECOPD progression was not conducted. Future research will aim to increase the sample size and further investigate the relationship between these biomarkers and AECOPD. Additionally, exploring the underlying mechanisms will provide more robust evidence to support the use of IL-2, IL-8, and NK cell positivity as clinical predictive biomarkers.

Disclosure of conflict of interest

None.

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