BMC Pulmonary Medicine

RESEARCH

Open Access

The extracellular CIRP as a predictive marker for the endothelial dysfunction in chronic obstructive pulmonary disease combined with pulmonary hypertension

Yun Yao[1], Haibo Jiang[1], Dalin Xu[1], Bing Zhang[2], Feng Yao[3*†] and Wei Guo[1*†]

Abstract

Background Pulmonary hypertension (PH) is a serious complication of chronic obstructive pulmonary disease (COPD), distinguished by pulmonary endothelial dysfunction. The extracellular cold-inducible RNA-binding protein (eCIRP) is a damage-associated molecular pattern (DAMP) that triggers inflammation and causes vascular endothelial dysfunction in COPD-PH.

Methods The expression levels of CIRP were compared in peripheral lung tissues among 40 individuals. Moreover, A prospective analysis was conducted on serum levels of eCIRP, interleukin (IL) 1β, IL-33, endothelin-1 (ET-1), and nitric oxide (NO) in 150 COPD patients and 50 healthy control individuals at Jiangsu Taizhou Peoples Hospital. The study aimed to compare these serum levels and correlations among COPD-PH group, COPD non-PH group and the normal group.

Results We found higher CIRP levels in COPD-PH compared to COPD non-PH and the normal in lung tissue samples. A prospective analysis showed higher serum levels of eCIRP, IL-1β, IL-33, and ET 1 in COPD-PH, while a noticeable reduction in NO levels. There exists a correlation between the severity of COPD-PH and elevated levels of eCIRP, proinflammatory cytokines like IL-1β and IL-33, along with indicators of endothelial dysfunction like endothelin-1 ET-1 and NO. Moreover, the serum eCIRP level demonstrated a notable positive correlation with the levels of IL-1β, IL-33, PCT, and ET-1, while displaying a negative correlation with NO and Peripheral Oxygen Saturation (SpO_2). Moreover, the serum eCIRP level demonstrated a notable positive correlation with the levels of IL-1β, IL-33, PCT, and ET-1, while displaying a negative correlation with NO and SpO_2. Moreover, an assessment of independent risk factors for COPD-PH with ROC curve analysis, gauged the predictive value of serum eCIRP, IL-1β, IL-33, ET-1, and NO levels in diagnosing COPD-PH. Elevated eCIRP, IL-33, and ET-1 levels significantly correlated with COPD-PH, highlighting eCIRP's strong predictive value for this condition.

†Wei Guo and Feng Yao contributed equally to this work.

*Correspondence:
Feng Yao
yf301732@163.com
Wei Guo
yaoyunya2021@163.com

Full list of author information is available at the end of the article

Conclusion eCIRP levels could serve as a valuable biomarker for predicting endothelial dysfunction in COPD-PH.
Keywords COPD, PH, Inflammation, Endothelial dysfunction, eCIRP

Introduction

Chronic obstructive pulmonary disease (COPD) is a clinically common respiratory disease with high morbidity and mortality [1]. Pulmonary hypertension (PH) is an important complication of the natural progression of COPD, characterized by endothelial dysfunction, pulmonary vasoconstriction and vascular remodeling, leading to a poor prognosis [2, 3]. It was well known that disruption of endothelial homeostasis and barrier integrity, typically induced by proinflammatory cytokines, is an important factor contributing to morbidity and mortality. A study for secondary PH indicated that systemic inflammation and endothelial dysfunction play an important role in the pathogenesis of PH [4, 5]. It has found that erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein (hsCRP), procalcitonin (PCT) and Neutrophil-to-lymphocyte ratio (NLR) have been studied in acute exacerbations of COPD (AECOPD) patients as prognostic markers [6, 7]. Another study has confirmed that serum levels of interleukin (IL)-2, IL-4, IL-8, IL-10, IL-12 p70, IL-1β, IL-6, and TNF-α are elevated in patients with idiopathic and familial PH [8]. These findings not only confirm the presence of inflammation in COPD or PH but also underscore the necessity to investigate additional biomarkers that might more accurately reflect the inflammatory status in individuals with COPD combined with PH (COPD-PH).

Cold-inducible RNA-binding protein (CIRP) was found predominantly in the nucleus under physiologic conditions and acts as an RNA chaperone to coordinate stressor-related translational reprogramming [9, 10]. When cells are in stressful conditions such as hypoxia or inflammation, CIRP is released into the extracellular space [11]. Recent studies found extracellular CIRP (eCIRP) functions as a newly identified damage-associated molecular patterns (DAMP) to promote and amplify the inflammatory response [10, 12, 13]. It has been shown that CIRP expression was significantly increased in the bronchi of COPD patients [14]. Another study has also shown that CIRP was highly expressed in patients with COPD and in rats with chronic airway inflammation [15]. It has been reported that eCIRP promoted the expression of inflammatory genes in the bronchial epithelial cells to promote cold-induced exacerbation of COPD [16]. In addition, eCIRP stimulates proinflammatory cytokine release in hemorrhage and sepsis [10, 17]. These studies indicated that eCIRP could participate in the COPD process by promoting inflammation. More importantly, intravenous injection of recombinant murine CIRP induced vascular leakage and proinflammatory cytokine production in the lung tissue to cause lung injury in mice [12]. It also found that eCIRP induced mouse lung vascular endothelial cell pyroptosis by promoting the activation of NLRP3 inflammasome and caspase-1 and the release of IL-1β [12]. These results demonstrated the release of CIRP could directly activate endothelial cells and induced endothelial dysfunction to promote the occurrence and progression of inflammatory disease. However, the association between eCIRP and endothelial dysfunction in COPD-PH has not been investigated.

In summary, this study focused on the association between eCIRP and endothelial dysfunction in COPD-PH. Human lung tissue samples were collected to observe the expression of eCIRP in the normal, COPD, and COPD-PH. Following this, the serum levels of eCIRP, proinflammatory cytokines including IL-1β and IL-33, and markers of endothelial dysfunction such as endothelin-1 (ET-1) and nitric oxide (NO) were measured and analyzed in patients with the normal, COPD and COPD-PH. Furthermore, independent risk factors for COPD-PH were analyzed, and receiver operating characteristic (ROC) curve analysis were utilized to assess the predictive value of serum eCIRP, IL-1β, IL-33, ET-1 and NO in COPD-PH diagnosis.

Materials and methods

Research objects and grouping

A total of 150 COPD patients admitted to Jiangsu Taizhou Peoples Hospital from February 2023 to August 2023 were analyzed prospectively, of which 39 patients combined with PH were enrolled in the COPD-PH group by cardiac ultrasound results. Propensity score matching (PSM) was used to reduce the bias of treatment selection of four confounders, age, sex, length of hospital stays, and treatment regimen. 50 patients in the group without PH with COPD were selected as COPD non-PH group from the remaining samples using PSM. In the same period, 50 healthy individuals who underwent physical examinations in our hospital were selected as the normal group. 40 human peripheral lung tissue samples were obtained from the Department of Pathology at Jiangsu Taizhou Peoples Hospital. These samples were categorized into three groups of the normal, COPD non-PH, and COPD-PH. The research was approved by the Ethics Committee of Jiangsu Taizhou Peoples Hospital, file number KY 2023-074-01. Moreover, all the patients agreed to participate in the experiment and signed the informed consent form.

Inclusion criteria and exclusion criteria

All the COPD patients fulfilled the criteria proposed by Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines and patients (to be enrolled in the COPD-PH group) meeting the diagnostic criteria in 2009 European Pulmonary Hypertension Guidelines. The diagnosis of COPD combined with PH meets the echocardiographic assessment criteria for PH diagnosis outlined in the 2015 European Society of Cardiology/European Respiratory Society (ESC/ERS) guidelines for the diagnosis and treatment of pulmonary hypertension.

Mild (36 mmHg ≤ PASP ≤ 50 mmHg).

Moderate (51 mmHg ≤ PASP ≤ 70 mmHg).

Severe (PASP > 70 mmHg).

Exclusion criteria: acute inflammatory diseases such as pulmonary and upper respiratory tract infections and other site infections. Rheumatic immune diseases, autoimmune diseases, bone metabolism related diseases and other serious systemic inflammatory diseases. Pulmonary hypertension caused by other diseases: such as interstitial lung disease, pulmonary embolism. Left heart disease, congenital heart disease, portal hypertension, sleep-disordered breathing, connective tissue disease, lung tumors, human immunodeficiency virus (HIV) infection, liver and kidney failure, hepatitis and other diseases. Long-term oral administration of drugs affects endothelial function, such as hormones, statins, ACE inhibitors, antiplatelets, or anticoagulants.

Data and laboratory test indices collection

General information of the research subjects including gender, age, body mass index (BMI), SI smoking index (SI), course of COPD, blood pressure (including systolic blood pressure and diastolic blood pressure), the presence of diabetes and coronary heart disease, serum creatinine (Scr) and blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT) levels and coagulation function data such as PLT, PT, INR, APTT, TT, Fbg and D dimer levels were collected.

Blood gas samples were analyzed using a blood gas analyzer (Wofin, GEM Premier). Various blood routine indexes were examined using the Unicel DxH800 automatic blood analyzer (Beckman Coulter, Miami, FL, USA). The blood CRP was performed with the H7600 automatic biochemical.

analyzer, and B-Type Natriuretic Peptide (BNP) was detected using Alere Triage MeterPro quantitative fluorescence immunoassay.

Color doppler echocardiography was used to measure the pulmonary artery systolic pressure (PASP). All patients underwent the assessment of pulmonary artery pressure using color Doppler echocardiography while in a peaceful supine position. Four-chamber images of their apical muscular region were captured with the sample lines maintained parallel to regurgitation. The patients' PH was calculated according to the Bernoulli simplified formula: $PASP = 4V^2$ (maximum tricuspid regurgitation rate) + 5 mmHg [18]. Based on the echocardiography results, the study subjects in the COPD-PH group were divided into mild, moderate, and severe COPD-PH groups according to the severity of pulmonary hypertension.

5 mL of fasting venous blood taken in the next morning after admission was centrifuged at 3000 r/min and stored at -80 °C for later detection. When measuring indicators, remove the cryopreservation tube and place it at room temperature. Allow for a gradual thawing process, ensuring that the specimen thaws evenly and thoroughly. The levels of eCIRP, ET-1, NO, IL-3 and IL-1β were determined by ELISA.CIRP, ET-1, IL-3, and IL-1β ELISA kits were acquired from China Jiangsu Meimian Industrial Co, Ltd, while NO ELISA kits were sourced from China Hebei Yancheng Biotechnology Co, Ltd. All procedures were meticulously conducted in strict adherence to the kit instructions.

Processing of human lung tissue

Lung tissue samples were obtained from 40 patients during lobectomy through resection of the adjacent noncancerous lung tissues. Partial samples were fixed in 10% formalin solution at room temperature for 12 h and cut into 5 μm paraffin-embedded sections, which processed for immunohistochemical analysis.

The tissue samples were fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.3% Triton X-100 for another 30 min, and then blocked with goat serum for 1 h at room temperature. Subsequently, the tissue slides were incubated with rabbit anti-CIRP antibody (dilution 1:200) at 4 °C overnight. The tissue slides were then exposed to biotinylated secondary antibody (goat anti-rabbit antibody, dilution 1:200) for 30 min and streptavidin-peroxidase complex (dilution 1:100) for another 45 min. The tissue slides were observed and imaged under an ultrahigh resolution laser confocal microscope (Leica, Germany).

Statistical analysis

In the study, SPSS 27.0 software was adopted for statistical analysis of experiment data. Metric data conforming to a normal distribution are expressed as mean ± SD, but are presented as mean ± SEM in the graphs. The independent sample t-test is used for comparing two groups, one-way analysis of variance (one-way ANOVA) is utilized for comparing three groups, and further pairwise comparisons are conducted using the Bonferroni test. Non-normally distributed metric data are represented as median (interquartile range), with the Mann-Whitney U test employed for comparisons between two groups, the

Kruskal-Wallis H test for three-group comparisons, and the Nemenyi test for subsequent pairwise comparisons. The count data cases, presented as the number [n (%)], involve between-group comparisons conducted using the chi-square test. Correlation analysis was conducted using Spearman's rank correlation test. Logistic multivariate analysis was performed for risk factors of PH.

Results

CIRP expression is upregulated in lung tissues from COPD-PH patients

The results of immunohistochemical experiments are presented in Fig. 1. The brown areas (positive areas stained by the CIRP antibody) indicated CIRP expression. The results showed that CIRP was highly expressed in the lung tissues from COPD-PH (Fig. 1a). AOD (average optical density) of the positive area for the lung tissues from the COPD-PH group was higher than normal and COPD non-PH group (Fig. 1b). Therefore, CIRP expression is upregulated in lung tissues from COPD-PH patients.

Patient characteristics and clinical data

The general data of subjects in the three groups are shown in Table 1. The sex, age, blood pressure, the presence of diabetes, the presence of coronary heart disease, liver function, and renal function of the subjects in the 3 groups were not significantly different. The average COPD course and coagulation function in the COPD non-PH group and the COPD-PH group were not significantly different. The BMI of the COPD-PH group was significantly lower than that of the other two groups, and the SI of the COPD-PH group was significantly higher than that of the other two groups. Analysis of Blood oxygen saturation and related blood gas index detection showed that SpO_2 was statistically significantly lower in COPD-PH than in COPD non-PH. $PaCO_2$ was statistically significantly higher in COPD-PH than in COPD non-PH. PaO_2 in the COPD-PH group was lower than in the COPD non-PH group, but there was no significant significance. BNP were significant higher in the COPD-PH group compared with the COPD non-PH group.

Comparison among the three groups in serum eCIRP, IL-1β, IL-33, ET-1 and NO levels

ELISA assays revealed a significant increase in serum eCIRP levels in the COPD-PH group compared to both the normal and COPD non-PH groups. (Fig. 2a). Moreover, serum IL-1β, IL-33 and ET-1 levels were significantly increased in the COPD non-PH and COPD-PH group compared with the normal group, and serum IL-33 and ET-1 levels were significantly increased in the COPD-PH group compared with the COPD non-PH group (Fig. 2b, c and d). In addition, serum NO level was significantly decreased in the COPD non-PH and COPD-PH group compared with the normal group (Fig. 2e).

The serum levels of eCIRP, IL-1β, IL-33, CRP, PCT, ET-1, NO and SpO2 correlated with the severity of COPD-PH

A comparison was conducted among patients in the COPD-PH group who exhibited varying degrees of PH. There were significant differences observed in the levels of eCIRP, IL-1β, IL-33, CRP, ET-1, NO, and SpO_2 between the mild COPD-PH group and the severe COPD-PH group (Fig. 3). However, significant differences were observed only in the serum eCIRP levels among the mild, moderate, and severe COPD-PH groups (Fig. 3a). With an escalation in the severity of pulmonary hypertension, a noteworthy increase in eCIRP levels is observed, highlighting a strong correlation between eCIRP levels and the severity of PH.

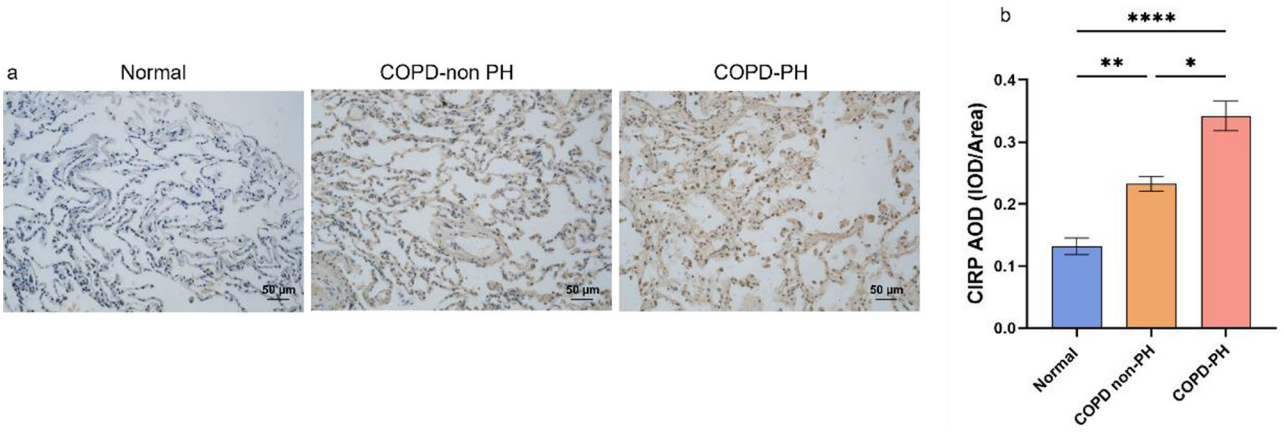

Fig. 1 CIRP expression is upregulated in lung tissues from COPD-PH patients. The lung tissues were stained with immunohistochemistry. (**a**) Representative images of CIRP immunostaining of lung specimens. (**b**) CIRP expression level in lung tissues with AOD. Image quantification was performed using Image J. Scale bar = 50 μm. AOD = IOD (integrated optical density)/area. $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{****}P < 0.0001$

Table 1 Analysis of the clinical data among different groups of subjects

Factor	Normal (*N* = 50)	COPD non-PH (*N* = 50)	COPD-PH (*N* = 39)	X^2/F/H	*P*
Gender, n (%)				3.089	2.869
Male	18 (72)	22 (88)	34 (87.2)		
Female	7 (28)	3 (12)	5 (12.8)		
Age (years), n (%)				2.167	0.338
≤ 60	5 (20)	4 (16)	3 (7.7)		
> 60	20 (80)	21 (84)	36 (92.3)		
BMI (kg/m^2)	23.79 ± 2.89	23.53 ± 2.80	21.06 ± 3.51	7.498	0.001*
SI (pack-years)	0 (0,0)	200 (0, 700)	400 (0, 600)	10.128	0.006*
Course of disease (years)	&	11.08 ± 10.996	13.21 ± 9.117	0.704	0.405
Blood pressure (mmhg)					
Systolic blood pressure	130.84 ± 10.131	133.04 ± 15.358	138.08 ± 15.964	2.138	0.124
Diastolic blood pressure	79.76 ± 6.863	84.56 ± 11.248	80.5 ± 11.450	1.633	0.201
Diabetes, n (%)				0.375	0.829
Yes	2 (8)	3 (12)	5 (12.8)		
NO	23 (92)	22 (88)	34 (87.2)		
Coronary heart disease, n (%)				1.272	0.529
Yes	1 (4)	0 (0)	2 (5.1)		
NO	24 (96.0)	25 (100)	37 (94.9)		
Renal function					
SCr (umol/L)	73.25 ± 14.205	76.88 ± 21.807	74.07 ± 30.410	0.357	0.701
BUN (mmol/L)	5.84 ± 3.151	6.03 ± 1.771	6.35 ± 3.176	0.557	0.757
Liver function					
AST (U/L)	22.00 ± 3.122	21.04 ± 10.122	24.23 ± 12.621	3.495	0.174
ALT (U/L)	18.56 ± 3.441	23.44 ± 18.945	19.00 ± 15.735	4.102	0.129
Coagulationfunction					
PLT (10^9/L)	182.80 ± 44.500	167.90 ± 69.50	161.87 ± 71.514	4.484	0.106
PT (s)	a	12.78 ± 1.165	13.91 ± 2.629	2.636	0.104
INR	a	1.05 ± 0.086	1.13 ± 0.187	2.733	0.098
APTT (s)	a	30.83 ± 6.601	34.77 ± 6.913	2.029	0.154
TT (s)	a	18.06 ± 0.650	18.09 ± 1.008	0.384	0.535
Fbg (g/L)	a	4.63 ± 6.001	4.07 ± 1.617	2.009	0.156
D dimer (mg/L)	a	1.15 ± 1.241	1.10 ± 0.660	2.250	0.134
SpO_2 (%)	a	96.00 (92.00, 98.00)	91.00 (74.00, 94.00)	-3.520	< 0.001*
PaO_2 (mmhg)	a	86.15 ± 4.348	84.63 ± 4.195	-0.235	0.815
$PaCO_2$ (mmhg)	a	46.80 (43.95, 48.60)	52.10 (44.50, 59.80)	-2.275	0.023*
BNP (ng/L)	a	27.70 (18.45, 52.32)	237.5 (61.65, 509.60)	-2.497	0.013*

Data are presented as mean ± SD, median (interquartile range), or NO (%). [a]Patient with missing data who did not have this test. *$P < 0.05$ was considered to indicate a statistically significant difference

Correlation analysis of serum eCIRP, IL-1β, IL-33, CRP, PCT, ET-1, NO, SpO2, PaO2 and PaCO2 levels

Through Spearman correlation analysis, serum eCIRP levels is positively correlated with inflammatory markers IL-1β, IL-33, CRP and PCT (Fig. 4a, b, c and d). Additionally, serum eCIRP levels shows a significant positive correlation with endothelial function indicator ET-1 and a significant negative correlation with endothelial function marker NO (Fig. 4e and f). At the same time, there is a significant negative correlation between serum eCIRP levels and SpO_2 (Fig. 4g). This further confirms the close association between serum eCIRP levels and the inflammatory response and endothelial dysfunction in COPD-PH patients, potentially contributing to the development of PH and systemic hypoxia.

The heatmap illustrates the correlations among eCIRP, IL-1β, IL-33, CRP, PCT, ET-1, NO, SpO_2, PaO_2, $PaCO_2$, and PASP (Fig. 5). This study reveals a significant positive correlation with serum ET-1 levels and between serum IL-1β and IL-33 levels. Moreover, SpO_2 shows a significant negative correlation with the endothelial function indicator ET-1 and a positive correlation with NO. These

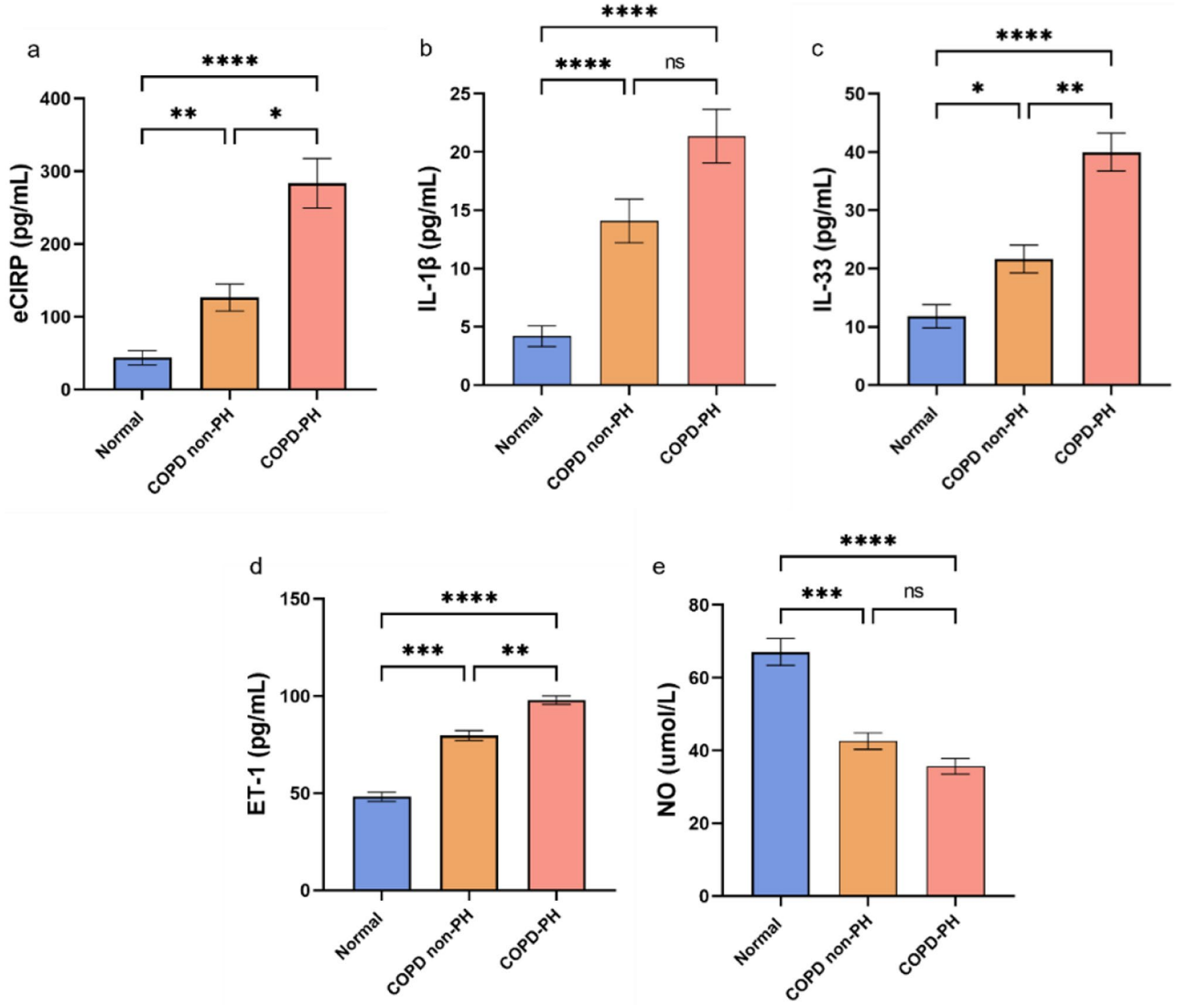

Fig. 2 Comparison among the three groups in serum eCIRP, IL-1β, IL-33, ET-1 and NO levels. Bar graphs presented serum eCIRP, IL-1β, IL-33, ET-1, NO levels. (**a**) eCIRP levels among the three groups. (**b**) IL-1β levels among the three groups. (**c**) IL-33 levels among the three groups. (**d**) ET-1 levels among the three groups. (**e**) NO levels among the three groups. Data are presented as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ or $^{****}P < 0.0001$

findings suggest a potential interplay between pulmonary vascular inflammation and endothelial dysfunction, collectively contributing to the occurrence and progression of COPD-PH.

Predictive value of eCIRP, biomarkers of endothelial dysfunction and inflammation correlated on COPD-PH

To investigate whether eCIRP, biomarkers of endothelial dysfunction and inflammation was related to COPD-PH, univariate analysis were conducted. The crude odds ratios for the influencing factors of COPD-PH occurrence are provided in Table 2, along with the adjusted OR adjusted for age, gender, BMI, and SI (Fig. 6). Higher expression of eCIRP, IL-1β, IL-33 and ET-1 and lower expression of NO and SpO_2 was significantly related to the occurrence of COPD-PH on univariate analysis. After adjusting for age, gender, BMI, and systemic inflammation (SI), multivariate logistic regression validated eCIRP, IL-33, ET-1, and SpO_2 as significant independent factors associated with an increased risk of COPD-PH.

ROC analysis of eCIRP, IL-1β, IL-33, ET-1 and NO to COPD-PH

In terms of COPD-PH diagnosis, eCIRP sensitivity is 94.87%, specificity is 47.83%, area under the curve (AUC) is 0.749, and cut-off value is 69.78. IL-1β sensitivity 69.23%, specificity 65.22%, area under the curve (AUC) 0.660, cut-off value 13.31. IL-33 sensitivity was 76.92%, specificity was 82.61%, area under the curve (AUC) was 0.802, and the critical value was 22.48. The sensitivity of ET-1 is 89.74%, the specificity is 65.22%, the area under the curve (AUC) is 0.837, and the critical value is 79.93. NO sensitivity is 66.67%, specificity is 73.91%, area under

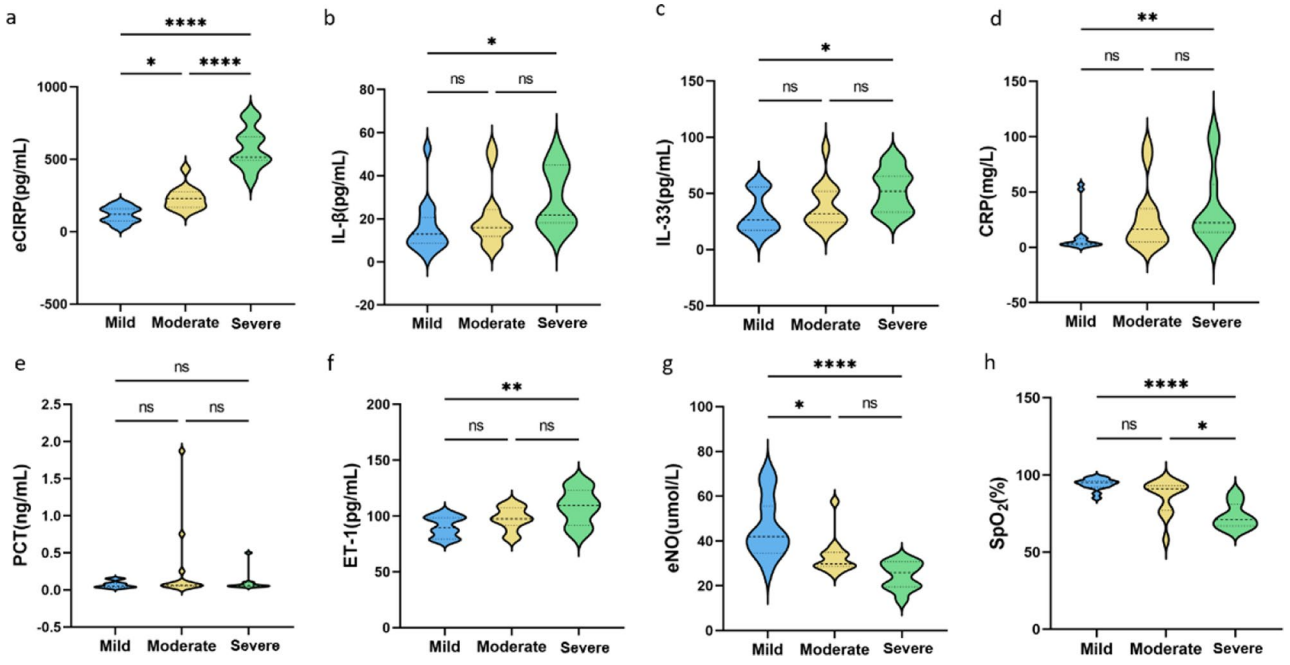

Fig. 3 The serum levels of eCIRP, IL-1β, IL-33, CRP, PCT, ET-1, NO, SpO_2 and PaO_2 correlated with the severity of COPD-PH. *$P < 0.05$, **$P < 0.01$ or ****$P < 0.0001$

the curve (AUC) is 0.701, critical value is 35.91. See more details in Fig. 7.

Discussion

COPD is a respiratory disorder characterized by impaired airflow and compromised lung tissue integrity [4]. Airway obstruction and damage to the pulmonary parenchyma and vasculature promote hyperplasia and fibrosis in the lung vascular endothelium [19, 20]. This exacerbates pulmonary artery remodeling, ultimately culminating in pulmonary hypertension and right heart failure [21]. The coexistence of COPD and PH represents an irreversible pathological alteration that exerts a significant influence on the unfavorable prognosis of patients [22]. Our study reveals elevated expression of eCIRP in the lung tissue and serum of individuals with COPD-PH. Further research identified a correlation between the levels of eCIRP and the severity of COPD-PH. There was a significant correlation between eCIRP and inflammatory factors including IL-1β and IL-33, and indicators of endothelial dysfunction, as evidenced by increased ET-1 levels and reduced NO. Thus, the eCIRP level can serve as a biomarker for predicting endothelial dysfunction in COPD-PH.

It has been reported that CIRP was highly expressed in patients with COPD and in rats with chronic airway inflammation [23]. In this study, we further investigated the levels of eCIRP in lung tissue and serum, which were found to be significantly elevated in the COPD-PH group compared to both the COPD non-PH group and the normal group. Additionally, the serum eCIRP level demonstrated an increase in accordance with the severity of the disease. This implies a close association between eCIRP and the progression as well as the prognosis of PH. Prior investigations have indicated that prolonged chronic hypoxia has a direct impact on endothelial cells and epithelial cells, leading to endothelial and epithelial dysfunction, as well as on pulmonary artery smooth muscle cells. Alternatively, it can induce pulmonary vascular remodeling through its influence on inflammatory mechanisms, constituting a crucial factor in the elevation of pulmonary vascular resistance (PVR) [24–27]. This indicates that the progression of COPD-PH may be linked to endothelial dysfunction and inflammatory responses induced by chronic hypoxia. Moreover, our findings further revealed a significant increase in IL-1β, IL-33, and ET-1 levels in the COPD-PH group, with their expression intensifying proportionally as the severity of PH advanced. The levels of NO, SpO_2, and PaO_2 in the COPD-PH group exhibited a significant decrease, with their expression diminishing as the severity of PH increased. These findings suggest a potential association between the development of COPD-PH and hypoxemia, along with endothelial dysfunction. It is further suggested that the inflammatory response triggered by chronic hypoxia may be linked to the endothelial dysfunction observed in COPD-PH.

In the present study, we further investigated the expression of serum BNP in each group, revealing a significant increase in the COPD-PH group. This finding is likely attributed to elevated mean pulmonary arterial pressures, subsequently leading to heightened afterload of the

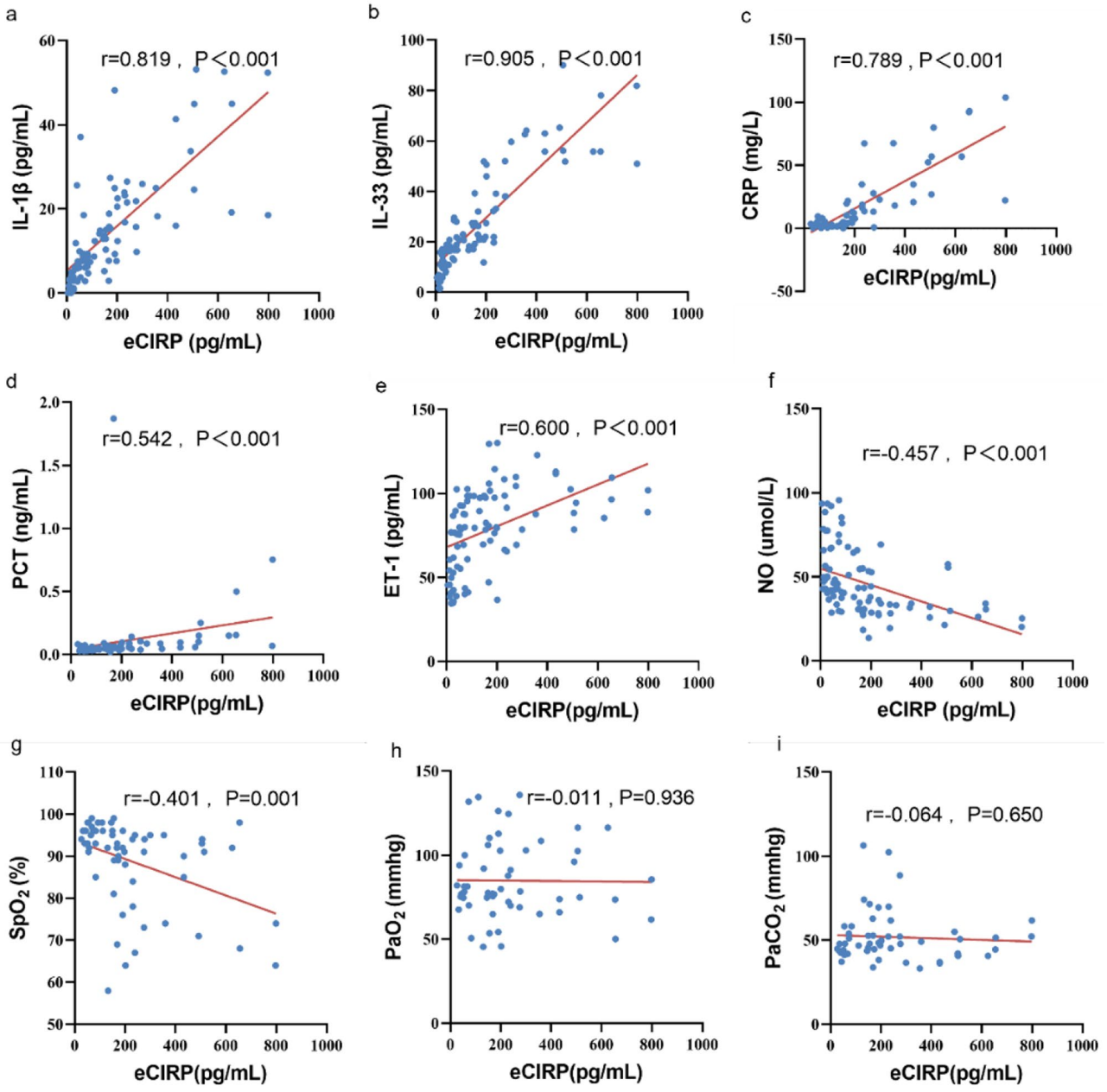

Fig. 4 Correlation analysis of serum eCIRP, IL-1β, IL-33, CRP, PCT, ET-1, NO, SpO2, PaO2 and PaCO2 levels. Spearman correlation analysis was used. r, Pearson coefficient. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 or *****P* < 0.0001

right ventricle (RV) and causing right heart hypertrophy. This adaptive hypertrophy helps the heart cope with high PVR. However, over time, this beneficial adaptive cardiac hypertrophy can transition into maladaptive RV dilation and eventually result in heart failure [28].

Previous studies have shown the existence of eCIRP in different inflammatory conditions, indicating its potential as a pro-inflammatory factor [17]. However, further investigation is needed to explore the correlation between these factors. The correlation analysis performed in this study revealed a significant positive association between serum eCIRP and inflammatory factors, including CRP, PCT, IL-1β, and IL-33. Moreover, there was a significant correlation between serum eCIRP and endothelial functional response indicators, specifically ET-1 and NO. In addition, this study showed that serum IL-1β and IL-33 were significantly positively correlated with ET-1, and SpO_2 was significantly negatively correlated with serum ET-1 and positively correlated with NO. These findings support the notion that chronic hypoxia triggers an inflammatory response, exacerbating endothelial. Consequently, this endothelial dysfunction may be responsible

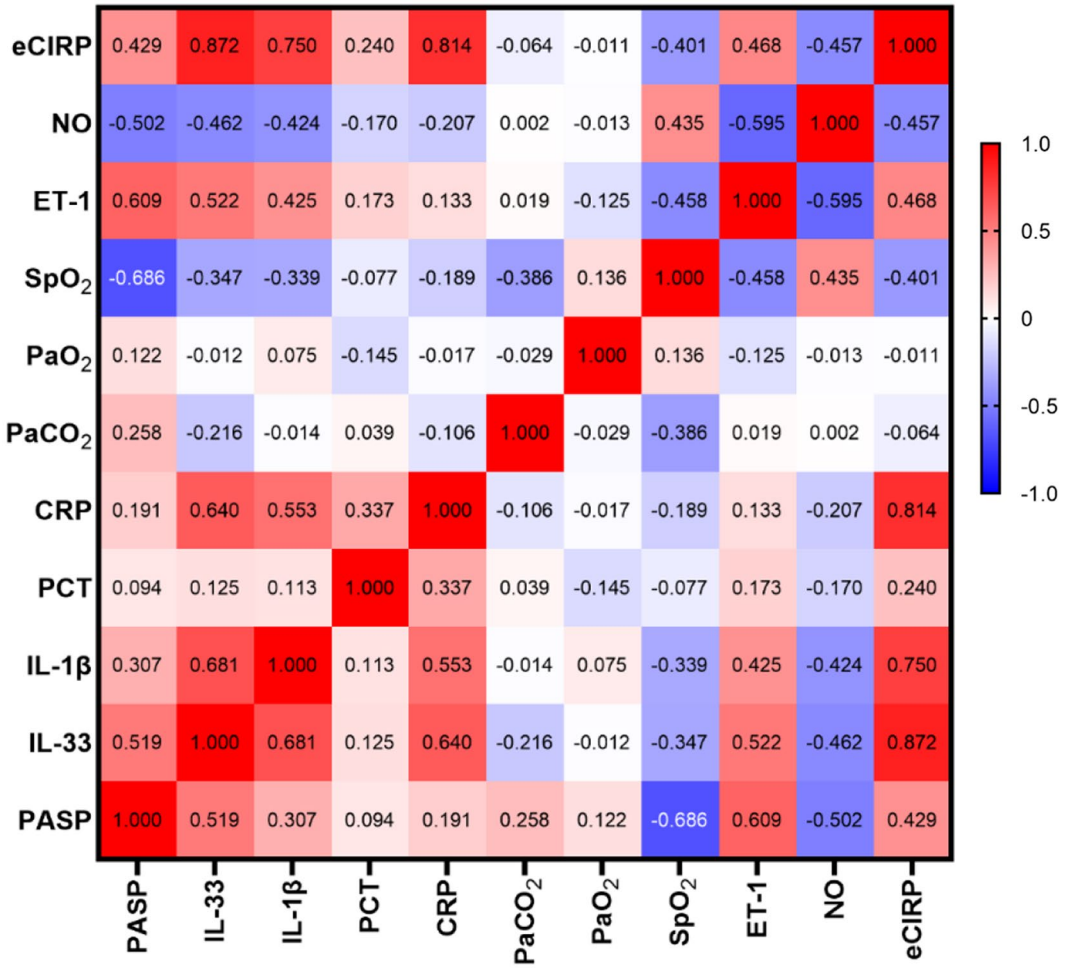

Fig. 5 The heatmap present correlations of eCIRP, IL-1β, IL-33, CRP, PCT, ET-1, NO, SpO_2, PaO_2, $PaCO_2$ and PASP Numbers in heatmap represent Pearson's correlation coefficient. Red numbers represent positively correlated. Blue numbers represent negatively correlated

Table 2 The risk factors of COPD-PH

Variables	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
eCIRP	1.008 (1.002–1.013)	0.007*	1.007 (1.001–1.012)	0.014*
IL-1β	1.056 (1.001–1.114)	0.044*	1.050 (0.997–1.106)	0.063
IL-33	1.084 (1.029–1.141)	0.002*	1.067 (1.015–1.121)	0.010*
CRP	1.024 (0.996–1.053)	0.093	1.013 (0.982–1.044)	0.418
PCT*100	1.177 (0.973–1.424)	0.093	1.120 (0.912–1.375)	0.281
ET-1	1.125 (1.059–1.196)	< 0.001*	1.144 (1.056–1.239)	0.001*
NO	0.958 (0.918–0.999)	0.047*	0.954 (0.908–1.002)	0.058
SpO_2	0.839 (0.738–0.954)	0.007*	0.829 (0.732–0.939)	0.003*
$PaCO_2$	1.062 (0.998–1.131)	0.058	1.087 (0.999–1.182)	0.053
BNP	1.026 (0.990,1.063)	0.164	1.069 (0.959–1.191)	0.228

Crude OR=odds ratio. Adjusted OR=crude OR adjusted for age, gender, BMI and SI

*$P < 0.05$, indicates statistical significance

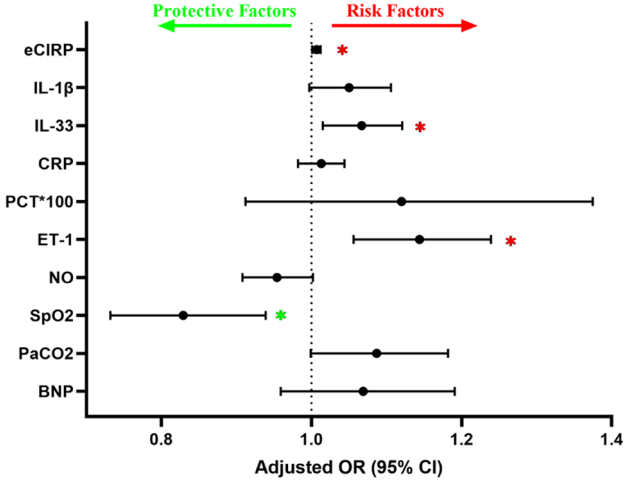

Fig. 6 Forest plots of the univariate regression analysis adjusted for age, gender, BMI and SI. *$P < 0.05$, indicates statistical significance

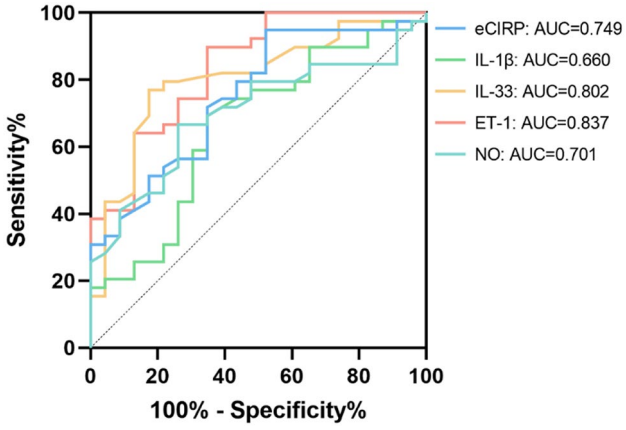

Fig. 7 ROC analysis of eCIRP, IL-1β, IL-33, ET-1 and NO to COPD-PH

for the development of PH and further exacerbation of hypoxia, creating a detrimental cycle. Hence, the involvement of eCIRP in pulmonary vascular inflammatory response and vascular endothelial dysfunction is evident. Therapeutic strategies that reduce eCIRP expression or block its transport to the cytosol may potentially help manage COPD combined with PH. While such strategies could contribute to the reduction of airway inflammation, overall inflammatory response, and endothelial dysfunction [29], it remains uncertain if these effects directly influence COPD-PH progression.

Consequently, a univariate logistic regression analysis was conducted on patients with COPD-PH. We recognized increased levels of serum eCIRP, IL-1β, IL-33, and ET-1, along with reduced NO, as risk factors associated with the development of COPD-PH. The study findings did not demonstrate a statistically significant association between the occurrence of COPD-PH and serum levels of CRP, PCT, IL-1β, NO, $PaCO_2$, and BNP. This lack of significance may be potentially influenced by various factors, including the sample size and the specific characteristics of the study population. After adjusting for age, gender, BMI, and SI, the multivariate logistic regression confirmed that eCIRP, IL-33, ET-1, and SpO_2 were significant independent factors associated with an increased risk of COPD-PH. Further binary logistic regression analysis and ROC curve analyses revealed that eCIRP, IL-33, ET-1, and NO have significant predictive value for COPD-PH. This reinforces the notion that inflammatory factors like eCIRP, IL-1β, IL-33, along with markers of endothelial dysfunction such as elevated ET-1 and decreased NO, play a pivotal role in the onset and progression of COPD-PH.

Overall, serum eCIRP levels has a predictive value in COPD combined with PH, though their precise role remains to be clarified. Elevated eCIRP levels may be associated with pulmonary vascular endothelial dysfunction, potentially linked to the body's inflammatory response. However, whether this relationship directly contributes to the initiation and progression of pulmonary hypertension or merely reflects a downstream effect of COPD-PH requires further investigation. Thus, a therapeutic approach targeting eCIRP might offer some benefit in modulating inflammation and endothelial function but requires additional validation before establishing its role in COPD-PH treatment. At the same time, it is important to note the limitations regarding the diagnosis of PH in our study. We acknowledge that while echocardiography is a widely used and non-invasive method, it is not considered the gold standard for diagnosing PH. Due to the study's constraints, right heart catheterization (RHC) was not feasible for all patients. There remains some uncertainty in the etiology. Additionally, one limitation of this study is the absence of stratification based on COPD severity, as we did not include baseline FEV_1 and FEV_1/FVC ratio values for each cohort. While our focus was specifically on endothelial dysfunction in the COPD-PH group, stratifying patients by COPD severity might have provided further insights into the variability of endothelial function across different stages of disease progression. Future studies should aim to incorporate COPD severity stratification to better assess the impact of endothelial dysfunction across COPD severity levels and its potential interaction with PH. On top of that, this study is a single-center, small observational investigation characterized by a limited sample size and a relatively short duration. The absence of dynamic monitoring for the research indicators may have certain implications on the robustness of the experimental outcomes. Hence, it is crucial to conduct extensive multi-center, prospective studies in order to establish a more robust foundation for clinical practices. By incorporating a larger sample size and involving multiple centers, these studies will offer more reliable and comprehensive evidence for informing clinical decisions.

Acknowledgements
Not applicable.

Author contributions
Yun Yao take responsibility for the integrity of the work as a whole, including all data and content of the manuscript; Wei Guo and Feng Yao was responsible for study design conception, design of analyses, and revising the manuscript. Haibo Jiang was responsible for the data management, and statistical analysis, and drafted the manuscript. All authors read and approved the final manuscript.

Funding
This study was supported by grants from the National Natural Science Foundation of China (No. 82304505).

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

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Jiangsu Taizhou Peoples Hospital (KY 2023-074-01). Written informed consent was obtained from all subjects and/or their legal guardian(s).

Consent for publication

Written informed consent was obtained from all subjects and/or their legal guardian(s). The patients participating in the study all agree to publish the research results.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Respiratory and Critical Care Medicine, Anhui Provincial Lujiang County People's Hospital, Hefei, Anhui, P.R. China
[2]Department of Internal Medicine, Taizhou People's Hospital, Taizhou, Jiangsu, P.R. China
[3]Department of Clinical Pharmacology, The Second Affiliated Hospital of Anhui Medical University, Hefei, Anhui, P.R. China

Received: 3 September 2024 / Accepted: 25 November 2024
Published online: 18 December 2024

References

1. Chen Y, Lu L, Li X, Liu B, Zhang Y, Zheng Y, et al. Association between chronic obstructive pulmonary disease and 28-day mortality in patients with sepsis: a retrospective study based on the MIMIC-III database. BMC Pulm Med. 2023;23(1):435. https://doi.org/10.1186/s12890-023-02729-5.
2. Liao YX, Wang XH, Bai Y, Lin F, Li MX, Mi WJ, et al. Relationship between endogenous hydrogen sulfide and pulmonary vascular indexes on high-resolution computed Tomography in patients with Chronic Obstructive Pulmonary Disease. Int J Chronic Obstruct Pulm Dis. 2021;16:2279–89. https://doi.org/10.2147/COPD.S314349.
3. Chai T, Qiu C, Xian Z, Lu Y, Zeng Y, Li J. A narrative review of research advances in hypoxic pulmonary hypertension. Ann Transl Med. 2022;10(4):230. https://doi.org/10.21037/atm-22-259.
4. Yang D, Wang L, Jiang P, Kang R, Xie Y. Correlation between hs-CRP, IL-6, IL-10, ET-1, and Chronic Obstructive Pulmonary Disease Combined with Pulmonary Hypertension. J Healthc Eng. 2022;3247807. https://doi.org/10.1155/2022/3247807.
5. Chen IC, Liu YC, Wu YH, Lo SH, Wang SC, Li CY, et al. Proteasome inhibitors decrease the viability of pulmonary arterial smooth muscle cells by restoring Mitofusin-2 expression under hypoxic conditions. Biomedicines. 2022;10(4):873. https://doi.org/10.3390/biomedicines10040873.
6. Singh B, Kampani G, Lall B, Singh M. Study of inflammatory markers in Chronic Obstructive Pulmonary Disease. J Assoc Physicians India. 2022;70(12):11–2. https://doi.org/10.5005/japi-11001-0165.
7. Lenártová P, Kopčeková J, Gažarová M, Mrázová J, Wyka J. Biochemical parameters as monitoring markers of the inflammatory reaction by patients with chronic obstructive pulmonary disease (COPD). Rocz Panstw Zakl Hig. 2017;68(2):185–90. PMID:28646836.
8. Soon E, Holmes AM, Treacy CM, Doughty NJ, Southgate L, Machado RD, et al. Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. Circulation. 2010;122(9):920–7. https://doi.org/10.1161/CIRCULATIONAHA.109.933762.
9. Zhong P, Huang H. Recent progress in the research of cold-inducible RNA-binding protein. Future Sci OA. 2018;4(5):FSO246. https://doi.org/10.4155/fsoa-2017-0077.
10. Aziz M, Brenner M, Wang P, Extracellular. CIRP (eCIRP) and inflammation. J Leukoc Biol. 2019;106(1):133–46. https://doi.org/10.1002/JLB.3MIR1118-443R.
11. Shimizu J, Murao A, Nofi C, Wang P, Aziz M. Extracellular CIRP promotes GPX4-Mediated ferroptosis in Sepsis. Front Immunol. 2022;13:903859. https://doi.org/10.3389/fimmu.2022.903859.
12. Yang WL, Sharma A, Wang Z, Li Z, Fan J, Wang P. Cold-inducible RNA-binding protein causes endothelial dysfunction via activation of Nlrp3 inflammasome. Sci Rep. 2016;6:26571. https://doi.org/10.1038/srep26571.
13. Han J, Zhang Y, Ge P, Dakal TC, Wen H, Tang S, et al. Exosome-derived CIRP: an amplifier of inflammatory diseases. Front Immunol. 2023;14:1066721. https://doi.org/10.3389/fimmu.2023.1066721.
14. Ran D, Chen L, Xie W, Xu Q, Han Z, Huang H, et al. Cold-inducible RNA binding protein regulates mucin expression induced by cold temperatures in human airway epithelial cells. Arch Biochem Biophys. 2016;603:81–90. https://doi.org/10.1016/j.abb.2016.05.009.
15. Juan Y, Haiqiao W, Xie W, Huaping H, Zhong H, Xiangdong Z, et al. Cold-inducible RNA-binding protein mediates airway inflammation and mucus hypersecretion through a post-transcriptional regulatory mechanism under cold stress. Int J Biochem Cell Biol. 2016;78:335–48. https://doi.org/10.1016/j.biocel.2016.07.029.
16. Chen L, Ran D, Xie W, Xu Q, Zhou X. Cold-inducible RNA-binding protein mediates cold air inducible airway mucin production through TLR4/NF-κB signaling pathway. Int Immunopharmacol. 2016;39:48–56. https://doi.org/10.1016/j.intimp.2016.07.007.
17. Zhong P, Zhou M, Zhang J, Peng J, Zeng G, Huang H. The role of Cold-Inducible RNA-binding protein in respiratory diseases. J Cell Mol Med. 2022;26(4):957–65. https://doi.org/10.1111/jcmm.17142.
18. Fisher MR, Criner GJ, Fishman AP, Hassoun PM, Minai OA, Scharf SM. Estimating pulmonary artery pressures by echocardiography in patients with emphysema. Eur Respir J. 2007;30(5):914–21. https://doi.org/10.1183/09031936.00033007.
19. Ando K, Kuraishi H, Nagaoka T, Tsutsumi T, Hoshika Y, Kimura T, et al. Potential role of CT Metrics in Chronic Obstructive Pulmonary Disease with Pulmonary Hypertension. Lung. 2015;193(6):911–8. https://doi.org/10.1007/s00408-015-9813-8.
20. Tanabe N, Taniguchi H, Tsujino I, Sakamaki F, Emoto N, Kimura H, et al. Multi-institutional retrospective cohort study of patients with severe pulmonary hypertension associated with respiratory diseases. Respirology. 2015;20(5):805–12. https://doi.org/10.1111/resp.12530.
21. Eichstaedt CA, Pagani L, Antao T, Inchley CE, Cardona A, Mörseburg A, et al. Evidence of early-stage selection on EPAS1 and GPR126 genes in Andean High Altitude populations. Sci Rep. 2017;7(1):13042. https://doi.org/10.1038/s41598-017-13382-4.
22. Yano S, Kobayashi K, Kato K, Ikeda T. The stady of pulmonary hypertension and plasma BNP values in respiratory diseases. Nihon Kokyuki Gakkai Zasshi. 2006;44(2):99–103. PMID:17228802.
23. Zhuang R, Wu J, Lin F, Han L, Liang X, Meng Q, et al. Fasudil preserves lung endothelial function and reduces pulmonary vascular remodeling in a rat model of end–stage pulmonary hypertension with left heart disease. Int J Mol Med. 2018;42(3):1341–52. https://doi.org/10.3892/ijmm.2018.3728.
24. Ferrer E, Peinado VI, Díez M, Carrasco JL, Musri MM, Martínez A, et al. Effects of cigarette smoke on endothelial function of pulmonary arteries in the guinea pig. Respir Res. 2009;10(1):76. https://doi.org/10.1186/1465-9921-10-76.
25. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. J Dent Res. 2012;91(2):142–9. https://doi.org/10.1177/0022034511421200.
26. Wright JL, Churg A. Smoking cessation decreases the number of metaplastic secretory cells in the small airways of the Guinea pig. Inhal Toxicol. 2002;14(11):1153–9. https://doi.org/10.1080/08958370290084836.
27. Gredic M, Blanco I, Kovacs G, Helyes Z, Ferdinandy P, Olschewski H, et al. Pulmonary hypertension in chronic obstructive pulmonary disease. Br J Pharmacol. 2021;178(1):132–51. https://doi.org/10.1111/bph.14979.
28. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53(1):1801913. https://doi.org/10.1183/13993003.01913-2018.
29. Borjas T, Jacob A, Kobritz M, Ma G, Tan C, Patel V, et al. An engineered miRNA PS-OMe miR130 inhibits acute lung injury by targeting eCIRP in sepsis. Mol Med. 2023;29(1):21. https://doi.org/10.1186/s10020-023-00607-8.

Publisher's note