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Inter-relationships among neutrophilic inflammation, air trapping and future exacerbation in COPD: an analysis of ECOPD study

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ABSTRACT

Background The inter-relationships among neutrophilic airway inflammation, air trapping and future exacerbation in chronic obstructive pulmonary disease (COPD) remain unclear.

Objective To evaluate the associations between sputum neutrophil proportions and future exacerbation in COPD and to determine whether these associations are modified by significant air trapping.

Methods Participants with completed data were included and followed up to the first year in the Early Chronic Obstructive Pulmonary Disease study (n=582). Sputum neutrophil proportions and high-resolution CT-related markers were measured at baseline. Sputum neutrophil proportions were dichotomised based on their median (86.2%) to low and high levels. In addition, subjects were divided into the air trapping or non-air trapping group. Outcomes of interest included COPD exacerbation (separately any, severe and frequent exacerbation, occurring in the first year of follow-up). Multivariable logistic regressions were performed to examine the risk of severe exacerbation and frequent exacerbation with either neutrophilic airway inflammation groups or air trapping groups.

Results There was no significant difference between high and low levels of sputum neutrophil proportions in the exacerbation in the preceding year. After the first year of follow-up, subjects with high sputum neutrophil proportions had increased risks of severe exacerbation (OR=1.68, 95% CI: 1.09 to 2.62, p=0.020). Subjects with high sputum neutrophil proportions and significant air trapping had increased odds of having frequent exacerbation (OR=3.29, 95% CI: 1.30 to 9.37, p=0.017) and having severe exacerbation (OR=2.72, 95% CI: 1.42 to 5.43, p=0.003) when compared with those who had low sputum neutrophil proportions and non-air trapping.

Conclusions We found that subjects with high sputum neutrophil proportions and significant air trapping are prone to future exacerbation of COPD. It may be a helpful predictor of future exacerbation.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ A previous study showed that increased neutrophil proportions and the presence of air trapping were likely at higher risk of frequent exacerbation in the preceding year. However, it is unclear whether the relationship between sputum neutrophil inflammation and future exacerbation is affected by the status of air trapping.

WHAT THIS STUDY ADDS

⇒ Chronic obstructive pulmonary disease (COPD) patients with high sputum neutrophil proportions and significant air trapping are at risk of future exacerbation in COPD, especially severe exacerbation and frequent exacerbation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This prospective cohort study provided clear evidence and showed that participants with high sputum neutrophil proportions and significant air trapping had a higher risk of severe exacerbation and frequent exacerbation than those without. Our research provides new insights into the process of COPD and may lead to the development of precision medicine strategies for future exacerbations.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a heterogeneous chronic respiratory disease that is characterised by progressive airway inflammation,[1 2] with episodes of acute exacerbations leading to significant mortality worldwide.[3–5] The infiltration of neutrophils in the wall of small airways is attributed to a remodelling process related to tissue repair and the thickening of small airways.[6] The increased resistance from small airway disease contributes to airflow limitation in COPD, causing a decline in pulmonary ventilation during expirations and parts

of air trapped in the pulmonary alveoli. Previous studies used the percentage of voxels below −856 Hounsfield unit in expiration on CT as the measure of air trapping which is a surrogate for the estimation of functional small airway disease.[7 8] Mucus plugging and air trapping were suggested as sensitive indicators of progressive lung damage.[9 10] Those with increased neutrophil proportions and significant air trapping had high percentages of frequent exacerbation in the preceding year.[11] However, it remains unclear whether sputum neutrophil proportions and air trapping are reliable predictors of future exacerbation among individuals with COPD.

Elevated neutrophil inflammation has been demonstrated in patients with COPD compared with those without,[12] but there is a mixed literature about the predictive value of neutrophil inflammation in the exacerbation of COPD.[13 14] There is limited information about the severity of exacerbation in different neutrophil proportions with current studies evaluating only the exacerbation rate.[14] There is also a lack of studies about the relationship between sputum neutrophil inflammation and future exacerbation of COPD. Most studies focus on the blood neutrophil percentage but not the sputum neutrophil inflammation.[13 15] In addition, those studies are limited to people without COPD.[15 16] Evidence about the presence of air trapping as an important factor in acute exacerbation has been established,[11 17 18] but it is unclear what the relationship between sputum neutrophil inflammation and future exacerbation is among individuals with or without significant air trapping.

Our analysis aimed to evaluate the inter-relationships among sputum neutrophilic inflammation, air trapping and future exacerbation. We hypothesised that sputum neutrophil proportions are associated with the future exacerbation of COPD, and the relationships may be modified by the presence of air trapping. COPD patients who had high sputum airway neutrophil proportions and the presence of air trapping are at risk of future exacerbation. We tested these hypotheses by applying an integrated analysis to the large-scale longitudinal cohort, the Early Chronic Obstructive Pulmonary Disease (ECOPD) cohorts. Investigating the inter-relationships among these factors would provide new insights into the process of COPD and develop precision medicine strategies for future exacerbation.

STUDY DESIGN AND METHOD

The ECOPD study is a prospective observational cohort study among individuals aged 40–80 years from the community of Guangzhou, Shaoguan, and Heyuan, Guangdong, China. Baseline data were acquired from July 2019 to August 2021. The protocol for this study design has been described previously.[19] In brief, this study focused on the population with or without COPD (indicated by the ratio of forced expiratory volume in one second (FEV_1) over forced vital capacity (FVC) less than 0.70 after bronchodilator use).[20] One of its aims was to identify the clinically relevant early COPD subtypes and the mechanism underlying them.

Data collection

The ECOPD cohort provided participant-reported demographic data, history of tobacco smoking, medical history, spirometric values, induced sputum data and CT scans of the chest at the baseline. The medical history included chronic respiratory symptoms, the long-term concomitant use of maintenance medications for COPD (such as long-acting muscarinic antagonists, long-acting beta-agonists and inhaled corticosteroids), previous of respiratory diseases and exacerbation in the preceding year. Participants with different smoking status were classified as never smokers, former smokers and current smokers. Never smokers were defined as those who had smoked less than 100 cigarettes in their lifetime.[21] Current smokers were those who were smoking tobacco products at the time of the survey. Former smokers were defined as those having smoked more than 100 cigarettes in the past but not having smoked tobacco products for at least 6 months. Participants were asked about their past 12-month respiratory symptoms, including the presence of chronic cough, chronic sputum and dyspnoea. The presence of chronic cough was assessed with 'Do you usually cough for three consecutive months or more per year for 2 years?'. Chronic sputum was assessed with 'Do you usually bring up phlegm for three consecutive months or more per year for 2 years?'. Dyspnoea was assessed with 'Have you had shortness of breath either when walking up a slight hill or brisk walking on the level?'. The self-reported COPD Assessment Test (CAT) Score (scores range from 0 to 40, with higher scores indicating more severe disease) was also obtained.[22] Previous respiratory diseases included physician-diagnosed asthma, tuberculosis and bronchiectasis in the past. Spirometry was performed according to 2005 American Thoracic Society/European Respiratory Society guidelines.[23 24] Spirometric values were obtained before and after bronchodilator use (20 min after the inhalation of 400 µg of albuterol). The predicted FEV_1 was derived with the use of reference values from the European Coal and Steel Community 1993, adjusted with conversion factors for the Chinese population (male 0.95 and female 0.93).[25] The Global Initiative for Chronic Obstructive Lung Disease (GOLD) staging system was used to assess the severity of lung disease. Stage I was defined as an FEV_1 of 80% or more of the predicted value. Stage II was defined as an FEV_1 between 50 and 79% of the predicted value. Stage III was defined asan FEV_1 between 30 and 49% of the predicted value. Stage IV was defined as an FEV_1 less than 30% of the predicted value.

High-resolution CT (HRCT) was performed by using a multidetector-row CT scanner (Siemens Definition AS Plus 128-slicers and United-imaging uCT 760 128-slicers). Each participant was asked to hold their breath after deep inhalation and deep expiration to achieve the best

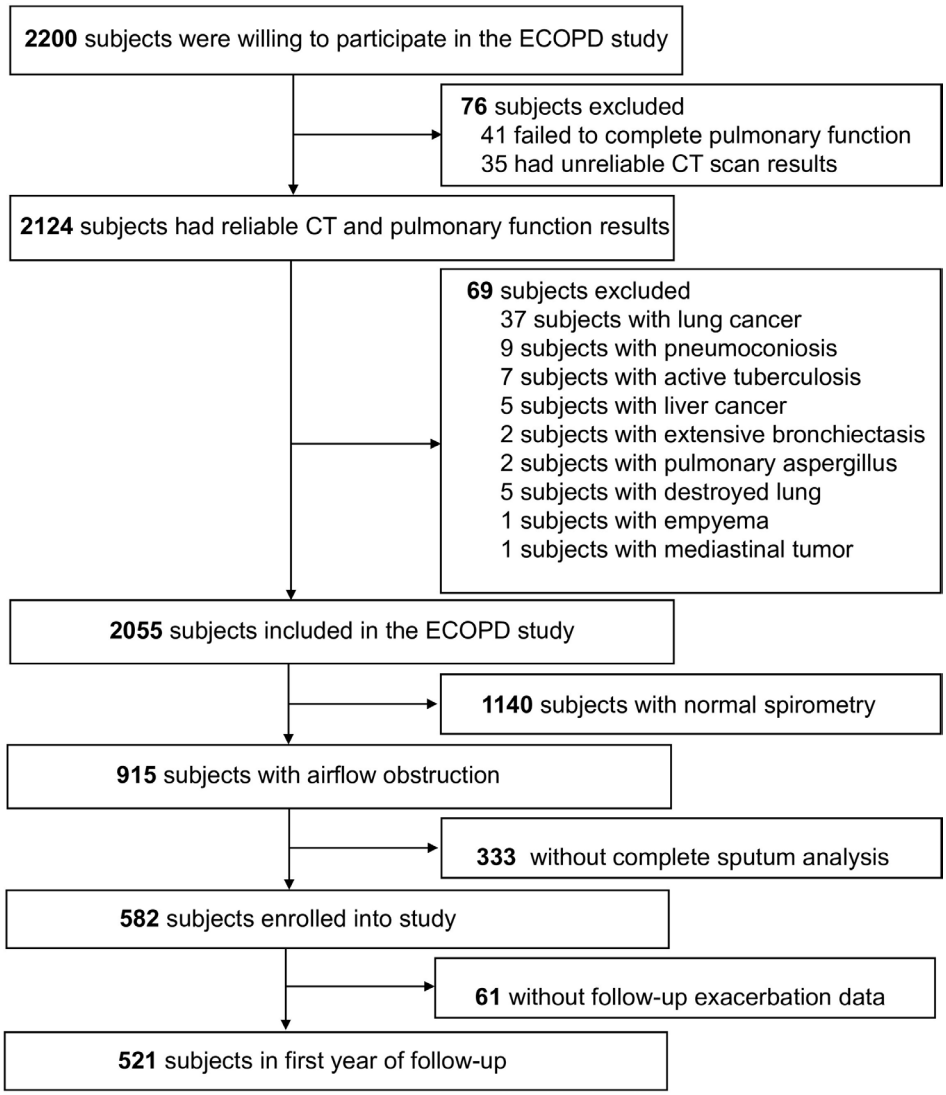

Figure 1 Participant flow diagram. Participants without complete data were excluded. Subject with normal spirometry were excluded in Early Chronic Obstructive Pulmonary Disease (ECOPD) study. A total of 582 patients with acceptable sputum proportions were included. After a year of follow-up, those with follow-up exacerbation data were finally included in our analysis (N=521).

condition before the scan. HRCT analysis was performed by using the Chest Imaging Platform (https://www.chestimagingplatform.org) on the semiautomated 3D Slicer V.4.11 software (https://www.slicer.org).[19 26] We recorded the CT marker, $\%LAA_{-856}$, defined as the percentage of low-attenuation areas below −856 Hounsfield units on full-expiration CT.[7] Significant air trapping was defined according to $\%LAA_{-856}$ exceeding 20%.[8]

Sputum was induced in a subset of ECOPD participants, which was based on those who produced a sufficient sputum sample and were capable of sputum induction. Prior to the induced sputum examination, participants were asked to rinse their mouths three times. The participants were asked to inhale nebulised saline (3%) in three sequential 7 min inhalation periods using an ultrasonic nebulizer with an output of 1.5 mL/min. The process of induced sputum examination was described in detail in a previous study.[27] Counts and proportions of each type of cell were recorded. The proportions of neutrophils were analysed in this study. We dichotomised participants by the median (86.2%) of the sputum neutrophil proportions (low levels vs high levels). More details about HRCT analysis and sputum induction are shown in the protocol of the ECOPD cohort.[19]

Baseline exacerbation data were obtained through participant self-report at the time of enrolment. Prospective exacerbation data were captured after the first year of follow-up. The longitudinal follow-up data were collected in August 2022. Acute exacerbation for COPD (AECOPD) was defined according to symptom worsening (eg, cough, sputum production, sputum purulence, wheezing or dyspnoea) for at least 48 hours, after the presence of cardiac insufficiency, pulmonary embolism, pneumothorax, pleural effusion or cardiac arrhythmia had been ruled out.[28] Severe exacerbation was defined as hospitalisation or emergency visits. Patients who

Table 1 Clinical characteristics of subjects in the low or high levels of sputum neutrophil proportions

Characteristics	Low (<86.2%)	High (≥86.2%)	P value
N	287	295	
Age (years)	63.8±6.6	65.7±8.0	**0.001**
Sex (male, %)	269 (93.7)	280 (94.9)	0.593
BMI	22.7±3.3	21.8±4.9	**0.001**
Smoking status (%)			0.138
Never smoked	27 (9.4)	31 (10.5)	
Former smoking	73 (25.4)	95 (32.2)	
Current smoking	187 (65.2)	169 (57.3)	
Smoking index (pack-year)	41.1±33.6	37.6±37.0	0.083
GOLD stage (%)**			**0.001**
Stage I/II/III/IV	43.2/48.4/6.3/2.1	36.3/46.1/16.6/1.0	
Sputum neutrophil proportion (%)‡‡	77.8 (69.9–81.8)	92.0 (89.1–95.0)	**<0.001**
Respiratory symptoms			
Chronic cough	118 (41.1)	146 (49.5)	**0.042**
Chronic sputum	154 (53.7)	174 (59.0)	0.210
Dyspnoea	122 (42.5)	147 (49.8)	0.081
CAT scores≥10	37 (12.9)	72 (24.4)	**<0.001**
Exacerbation in the preceding year (%)	40 (13.9)	52 (17.6)	0.256
Previous diagnosis of respiratory disease	24 (8.4)	15 (5.1)	0.136
Asthma	10 (3.5)	6 (2.0)	0.320
Tuberculosis	12 (4.2)	7 (2.4)	0.250
Bronchiectasis	2 (0.7)	2 (0.7)	1.000
Inhaled medications	19 (6.6)	22 (7.5)	0.747
LAMA§	4 (1.4)	8 (2.7)	0.383
LABA§	17 (5.9)	13 (4.4)	0.456
ICS§	13 (4.5)	12 (4.1)	0.840
pre-bronchodilator FEV_1% predicted	70.8±19.0	66.3±21.1	**0.010**
pre-bronchodilator FVC% predicted	101.4±18.0	90.8±20.0	**<0.001**
pre-bronchodilator FEV_1/FVC	57.3±8.9	54.5±11.3	**<0.001**
Air trapping (%)	146 (50.9)	205 (69.5)	**<0.001**
Expiratory $\%LAA_{-856}$ (%)‡‡	20.3 (9.5–36.3)	32.3 (17.0–51.9)	**<0.001**

Data are presented by means±SD or number (percentages). T-test or χ^2 test is applied to compare the difference between two groups. Bold indicates p<0.05.
‡‡Data are presented by median (IQR). Mann-Whitney U test is applied.
§Used alone or in combination.
**The Global Initiative for Chronic Obstructive Lung Disease (GOLD) staging systerm is used to assess the severity of lung disease. Stages range from I to IV. Stage I is defined as an FEV_1 of 80% or more of the predicted value. Stage II is defined as an FEV_1 between 50 and 79% of the predicted value. Stage III is defined as an FEV_1 between 30 and 49% of the predicted value. Stage IV is defined as an FEV1 less than 30% of the predicted value.
BMI, body mass index; CAT, COPD Assessment Test; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; ICS, inhaled corticosteroids; LABA, long-acting beta-agonists; LAMA, long-acting muscarinic antagonists.

experienced two or more episodes of acute exacerbations in a year of follow-up were denoted as having frequent exacerbations.

Patient and public involvement

No patients or members of the public were involved in the design, conducted research questions or reported the outcome measures directly. No patients were asked to advise on interpretation or writing up of results.

Statistical analyses

Two-sample Student's t-test, Mann-Whitney U tests or χ^2 test was performed to identify any differences between the groups with low levels and high levels of sputum

Table 2 The association between future exacerbation and neutrophil proportions levels in the air trapping group, non-air trapping group and total group

Variables	N	Low (<86.2%)	High (≥86.2%)	OR (95% CI)		
				All	Air trapping	Non-air trapping
Exacerbation≥1/year (%)	521	69 (27.4)	96 (35.7)	1.41 (0.95 to 2.09)	**1.69 (1.01 to 2.84)**	0.97 (0.47 to 1.96)
Severe exacerbation (%)	521	47 (18.7)	79 (29.4)	**1.68 (1.09 to 2.62)**	**1.79 (1.03 to 3.15)**	1.45 (0.65 to 3.19)
Exacerbation≥2/year (%)	521	24 (9.5)	39 (14.5)	1.28 (0.67 to 251)	1.04 (0.28 to 2.84)	1.95 (0.47 to 8.51)

The OR for clinical outcomes is obtained by using the logical regression model. All models are adjusted for age, sex, body mass index, smoking status, smoking index, prior exacerbation, forced expiratory volume in one second % predicted and inhaled medication.
Bold indicates p<0.05.

neutrophil proportions. The logistic regression model was applied to evaluate the relationships between the levels of sputum neutrophil proportions and severe exacerbation as well as frequent exacerbation. Furthermore, patients were stratified as with or without the presence of air trapping (air trapping group vs non-air trapping group), and the effects of sputum neutrophil proportions on future exacerbation were assessed in each group. Four groups were analysed (low level of sputum neutrophil proportion/non-air trapping (LN/NA), high level of sputum neutrophil proportion/non-air trapping (HN/NA), low level of sputum neutrophil proportion/air trapping (LN/AT) and high level of sputum neutrophil proportion/air trapping (HN/AT)). Continuous variables were analysed by one-way analysis of variance with the Bonferroni method for multiple comparisons. Categorical variables were analysed by χ^2 analysis with the Bonferroni method for multiple comparisons. A logistic regression model for future exacerbation was applied with either the levels of sputum neutrophil proportions or air trapping as the independent variables. Covariates in the afore-mentioned models were selected on the basis of clinical relevance. Covariates included age, sex, body mass index (BMI), smoking status, smoking index, exacerbation in the preceding year, FEV_1% predicted and inhaled medication. All statistical analyses were conducted in R software (V.4.1.1). The cut point for statistical significance was denoted as a p value less than 0.05. All reported P values are two-sided.

RESULTS

Participant characteristics

Of the 2055 participants recruited to ECOPD, 915 had COPD. Among them, 582 produced an induced sputum sample at baseline. A total of 521 (89.5%) subjects finished the first year of follow-up (figure 1). Table 1 shows the demographic data, sputum neutrophil proportions, percentages of air trapping and exacerbation information in COPD subjects enrolled in this analysis. Compared with participants with low levels of sputum neutrophil proportions, those with high levels were older (63.8±6.6 years old vs 65.7±8.0 years old, p=0.001), had a lower BMI (22.7±3.3 kg/m^2 vs 21.8±4.9 kg/m^2, p=0.001) and were more likely to report chronic respiratory symptoms (chronic cough, 41.1% vs 49.5%, p=0.042; CAT scores≥10, 12.9% vs 24.4%, p<0.001). Those with high levels of sputum neutrophil proportions had lower pre-bronchodilator FEV_1% predicted (70.8±19.0% vs 66.3±21.1%, p=0.010), lower pre-bronchodilator FEV_1/FVC (57.3±8.9% vs 54.5±11.3%, p<0.001) and higher percentages of air trapping (50.9% vs 69.5%, p<0.001).

To understand whether sputum neutrophil proportions are major contributors to future exacerbation of COPD, we assessed the relationships between sputum neutrophil proportions and future exacerbation of COPD in the non-air trapping group, air trapping group (table 2). Participants who had high levels of sputum neutrophil proportions had increased risks of severe exacerbation (OR=1.68, 95% CI: 1.09 to 2.62, p=0.020) compared those with the low levels of sputum neutrophil proportions. In the air trapping group, participants who had the high levels of sputum neutrophil proportions had increased risks of acute exacerbation (OR=1.69, 95% CI: 1.01 to 2.84, p=0.046), severe exacerbation (OR=1.79, 95% CI: 1.03 to 3.15, p=0.042) compared those with the low levels. In the non-air trapping group, no significant effects of sputum neutrophil proportions were found on severe exacerbation or frequent exacerbation in a year of follow-up. The impacts of other relative covariables on future AECOPD are presented in online supplemental table S1.

Data on the four-group analysis are shown in table 3. Of note, there were 141 participants in the LN/NA group (n=141, 24.2%), almost the same number as those who were in the LN/AT group (n=146, 25.1%), 90 (15.5%) participants in the HN/NA group and 205 (35.2%) participants in the HN/AT group. Those in the HN/AT group had older ages, lower BMI, lower pre-bronchodilator FEV_1% predicted and lower pre-bronchodilator FVC% predicted when compared with the other groups. Those in the HN/AT group had a higher percentage of chronic respiratory symptoms (chronic cough, 54.6% vs 34.8%, p<0.001; chronic sputum, 63.4% vs 48.9%, p=0.023; dyspnoea, 42.8% vs 30.5%, p<0.001; CAT scores≥10, 29.3% vs 6.4%, p<0.001) and higher percentages of prior exacerbation (18.5% vs 5.0%, p<0.001) than those in LN/NA group. After the first year of follow-up, the

Table 3 Clinical characteristics of four groups

Characteristics	LN/NA	HN/NA	LN/AT	HN/AT	P value
N	141	90	146	205	
Age (years)	63.0±6.7	62.1±7.5	64.5±6.5	67.3±6.3*†‡	**<0.001**
Sex (male %)	128 (90.8)	81 (96.6)‡	141 (96.6)‡	199 (97.1)‡	**0.013**
BMI	23.6±3.1	23.4±3.1	21.8±3.2†‡	21.0±3.1†‡	**<0.001**
Smoking status (%)					**<0.001**
Never smoked	18 (12.8)	17 (18.9)	9 (6.2)†	14 (6.8)†	
Former smoking	25 (17.7)	20 (22.2)	48 (32.9)‡	75 (36.6)‡	
Current smoking	98 (69.5)	53 (58.9)	89 (61.0)	116 (61.2)	
Smoking index (pack-year)	41.4±34.7	33.8±30.8	40.9±32.6	39.9±30.9	0.308
GOLD stage (%)**					**<0.001**
Stage I/II/III/IV	61.0/39.0/0/0	63.4/33.3/3.3/0	26.0/57.5/12.3/4.1	24.4/51.7/11.5/1.5	
Respiratory symptoms					
Chronic cough (n, %)	49 (34.8)	34 (37.8)	69 (47.3)	112 (54.6)‡	**<0.001**
Chronic sputum	69 (48.9)	44 (48.9)	85 (58.2)	130 (63.4)†‡	**0.023**
Dyspnoea	43 (30.5)	32 (35.6)	79 (54.1)†‡	115 (42.8)†‡	**<0.001**
CAT scores≥10	9 (6.4)	12 (13.3)	28 (19.2)‡	60 (29.3)†‡	**<0.001**
Exacerbation in the preceding year (%)	7 (5.0)	14 (15.6)	33 (22.6)‡	38 (18.5)‡	**<0.001**
Previous respiratory disease	7 (5.0)	7 (7.8)	17 (11.6)	8 (3.9)*	0.028
Asthma	5 (3.5)	3 (3.3)	5 (3.4)	3 (18.8)	0.579
Tuberculosis	2 (1.4)	3 (3.3)	10 (6.8)†‡	4 (2.0)*	**0.035**
Bronchiectasis	0 (0.0)	1 (1.1)	2 (1.4)	1 (0.5)	0.507
Inhaled medications	5 (3.5)	5 (5.6)	14 (9.6)	17 (8.3)	0.181
LAMA§	2 (1.4)	2 (2.2)	2 (1.4)	6 (2.9)	0.704
LABA§	4 (2.8)	2 (2.2)	13 (8.9)	11 (5.4)	0.062
ICS§	3 (2.1)	2 (2.2)	10 (6.8)	10 (4.9)	0.169
pre-bronchodilator FEV_1% predicted	78.6±14.3	80.1±17.5	63.3±20.0†‡	60.6±19.8*†‡	**<0.001**
pre-bronchodilator FVC% predicted	103.3±17.3	101.2±18.1	99.5±18.5	86.2±19.0*†‡	**<0.001**
pre-bronchodilator FEV_1/FVC	54.8±8.7	58.5±8.4‡	59.8±8.4‡	52.778±11.9*†	**<0.001**
1-year follow-up	N=119	N=78	N=133	N=191	
Exacerbation≥1/year (%)	32 (26.9)	22 (28.2)	37 (27.8)	74 (38.7)	0.071
Severe exacerbation (%)	19 (16.0)	18 (23.1)	28 (21.1)	61 (31.9)‡	**0.010**
Exacerbation≥2/year (%)	7 (5.9)	10 (12.8)	17 (12.8)	29 (15.2)	0.105

Data are presented by means±SD or number (percentages). Continuous variables were analysed by one-way analysis of variance with the Bonferroni method for multiple comparisons. Categorical variables were analysed by chi-square analysis with the Bonferroni method for multiple comparisons.

Bold indicates p<0.05.
*Compared with LN/AT group. P value less than 0.05.
†Compared with HN/NA group. P value less than 0.05.
‡Compared with LN/NA group. P value less than 0.05.
§Used alone or in combination.
**The Global Initiative for Chronic Obstructive Lung Disease (GOLD) staging system is used to assess the severity of lung disease. Stages range from I to IV. Stage I is defined as an FEV_1 of 80% or more of the predicted value. Stage II is defined as an FEV_1 between 50 and 79% of the predicted value. Stage III is defined as an FEV_1 between 30 and 49% of the predicted value. Stage IV is defined as an FEV_1 less than 30% of the predicted value.

BMI, body mass index; CAT, COPD Assessment Test; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; HN/AT, high levels of sputum neutrophil proportion/air trapping; HN/NA, high levels of sputum neutrophil proportion/non-air trapping; ICS, inhaled corticosteroids; LABA, long-acting beta-agonists; LAMA, long-acting muscarinic antagonists; LN/AT, low levels of sputum neutrophil proportion/air trapping; LN/NA, low levels of sputum neutrophil proportion/non-air trapping.

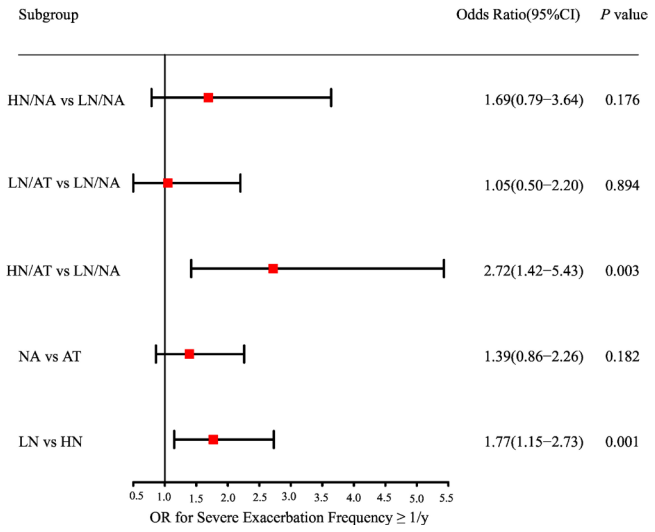

Figure 2 OR for severe exacerbation frequency≥1 in the first year of follow-up. Adjusted by age, sex, body mass index, smoking status, smoking index, prior exacerbation, forced expiratory volume in one second % predicted and inhaled medication. HN/AT, high levels of sputum neutrophil proportion/air trapping; HN/NA, high levels of sputum neutrophil proportion/non-air trapping; LN/AT, low levels of sputum neutrophil proportion/air trapping; LN/NA, low levels of sputum neutrophil proportion/non-air trapping.

proportions of severe exacerbation were higher in the HN/AT group compared with LN/NA group (16.0% vs 31.9%, p=0.010). No significant differences in the proportions of frequency exacerbation were found among those four groups (p=0.105).

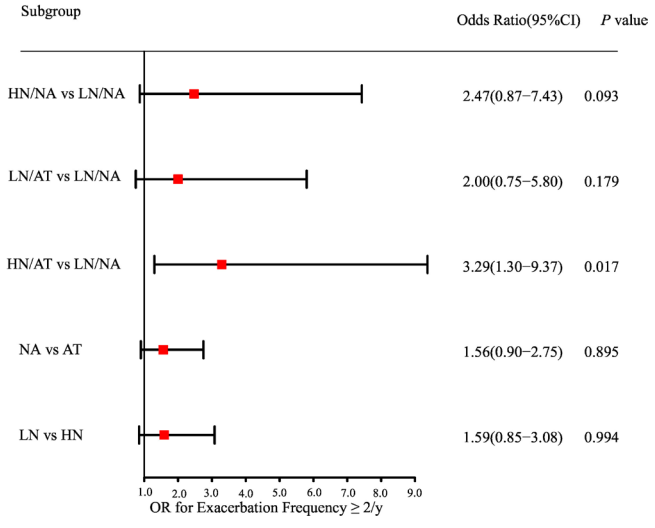

Figure 3 OR for exacerbation frequency≥2 in the first year of follow-up. Adjusted by age, sex, body mass index, smoking status, smoking index, prior exacerbation, forced expiratory volume in one second % predicted and inhaled medication. HN/AT, high levels of sputum neutrophil proportion/air trapping; HN/NA, high levels of sputum neutrophil proportion/non-air trapping; LN/AT, low levels of sputum neutrophil proportion/air trapping; LN/NA, low levels of sputum neutrophil proportion/non-air trapping.

Figures 2 and 3 represent the OR for frequent exacerbation and severe exacerbation in a year of follow-up. The HN/AT group had an increased OR for severe exacerbation in multivariable logistic regression (OR=2.72, 95% CI: 1.42 to 5.43, p=0.003) compared with the LN/NA group. Similarly, the HN/AT group had increased risk of having frequent exacerbation compared with the LN/NA group (OR=3.29, 95% CI: 1.30 to 9.37, p=0.017). No significant differences in severe exacerbation and frequent exacerbation were found when comparing the HN/NA group or LN/AT groups with the LN/NA group.

DISCUSSION

Our analysis found that COPD patients with airway neutrophilic inflammation had increased risks of future exacerbation, especially in those with significant air trapping. In patients with air trapping, those who had high sputum neutrophil proportions had an increased risk of severe exacerbation in a year of follow-up. In the non-air trapping group, no significant associations were noted. Those with high sputum neutrophil proportions and the presence of air trapping had an increased risk of severe exacerbation and frequent exacerbation compared with those without. In the context of increasing efforts to predict of future exacerbation in COPD, this study highlights sputum neutrophils and air trapping as simple, reliable markers that may add value to the prevention of future exacerbation.

There are no definite cut-offs for neutrophil inflammation in COPD patients. For the skewed proportions of sputum neutrophils, the linear relationship between sputum neutrophil proportions and future AECOPD is suggested to be not suitable. We divided the sputum neutrophil proportions by its median (86.2%) into high and low levels before our analysis. Notably, sputum neutrophil cut-offs are higher than the cut-offs (86.2% vs 61.0%) in previous studies.[29 30] The 61.0% cut-off of sputum neutrophil proportion was derived from the asthma population but not the COPD population in previous studies. Neutrophils are prominent in COPD, but less prominent in asthma.[31] The 61.1% cut-off may be lower in the COPD population.

Previous studies have demonstrated that neutrophilic airway inflammation is one of the main contributions against bacterial infection and other microorganisms.[1 4 32 33] We must realise the double-edged sword of neutrophilic inflammation in disease progression.[34] Neutrophils are suggested to play an important role in innate immunity to prevent people from becoming infected. However, in COPD, elevated neutrophil inflammation can release many molecules that are indices of oxidative stress, directly alter airway pathophysiology and lead to disease progression. These factors may worsen the health status of COPD patients.[35]

Given that the increased neutrophilic inflammation had relatively higher, although not significant,

percentages of previous exacerbation in the preceding year, which was predicted to be significantly associated with a high risk of subsequent exacerbation,[36] our analysis suggests that high neutrophil inflammation may be a biomarker of future exacerbation. Additionally, our study found that neutrophilic inflammation was associated with higher percentages of chronic respiratory symptoms, higher percentages of air trapping and lower lung function. Those clinical characteristics were also great predictors for future exacerbation.[18 37] Those with neutrophilic inflammation were prone to be an independent predictor of emergency department or intensive care unit admission.[38 39] Neutrophilic inflammation is positively associated with bacterial load,[12] especially that of Proteobacteria. Proteobacteria dominant during clinically stable periods is associated with more severe exacerbation in the 4-year median follow-up, although not the risk of acute exacerbation.[40 41] In addition, neutrophils can be triggered to release oxidative stress, which is increased further in severe and very severe exacerbations of COPD.[42] Therefore, high neutrophil inflammation predisposes subjects are susceptible to severe exacerbation because of high bacterial load and high levels of oxidative stress.

The relationship between neutrophil proportion and future AECOPD differed between those with or without air trapping. While our study did not present a significant relationship between air trapping and the future exacerbation of COPD, we found that increased sputum neutrophil proportions increased the risk of severe exacerbation only in the presence of air trapping. High airway neutrophil inflammation may lead to a remodelling process related to tissue repair, loss and thickening of small air way which causes parts of the air to be trapped in the pulmonary alveoli.[6] The higher the percentages of air trapping patients have, the more severe the damage to lung structure and poorer the lung function they have. Patients with high airway neutrophil inflammation and significant air trapping were suggested to be more prone to the exacerbation of clinical respiratory symptoms and the decline of lung function, which may trigger the exacerbation of AECOPD.[43 44]

The subcluster of high sputum neutrophil proportions and significant air trapping had significantly increased odds of severe exacerbation and frequent exacerbation compared with those without air trapping and low sputum neutrophil proportions. The same cluster was found in previous studies. Day *et al* demonstrated that the subcluster of frequent exacerbators with excessive neutrophilic inflammation has significantly higher percentages of air trapping than those without excessive neutrophilic inflammation.[11] Agache *et al*. applied unsupervised clustering of biomarkers to describe asthma endotypes and found a special cluster type. Cluster patients had significant air trapping and higher oxidative stress levels, which were significantly associated with neutrophilic inflammation and infection activation.[45] A cluster analysis found that this special cluster had lower lung function and greater urgent care visit history in asthma patients.[46]

Several mechanisms may explain the effect of this cluster. Increased airway inflammation and air trapping may cause dynamic lung hyperinflation as well as changes in lower airway bacterial colonisation, which may result in exacerbation being more easily triggered in those subjects.[46] In addition, frequent exacerbations may cause air trapping through increased neutrophil proportions and neutrophil-related cytokines, causing mucus production, airway thickening and occlusion, which may cause a vicious cycle of exacerbation.[47 48]

The strengths of this study merit emphasis. First, our study demonstrated the effects of sputum neutrophil inflammation on further exacerbation of COPD, and those effects were heightened in the presence of significant air trapping. Our study provides new insights into the inter-relationships among neutrophilic inflammation, air trapping and future exacerbation of COPD. Second, our analysis was based on data from a larger community-based cohort of COPD patients whose clinical characteristics were described, allowing for adequate assessment of the inter-relationships among sputum neutrophil proportion, air trapping and further exacerbation of COPD. Finally, the analysis of our study was based on a prospective cohort design, which could infer causation among different variables.

Several limitations should be noted in this study. First, the ECOPD study captured patient-reported outcomes to evaluate the incidence of acute exacerbation. These events were collected at baseline and each visit after enrolment, potentially introducing differential recall bias that may contribute to the differential effect of sputum neutrophil inflammation on prior versus 1-year acute exacerbation assessments. Second, sufficient data on sputum induction were obtained from 582 of the 915 COPD patients (63.6%), which was lower than those in the entire population of the ECOPD study (80.6%).[27] This might introduce selection bias in our analysis. Although technicians were well trained for sputum induction and processing, some factors still prevented sputum induction in all eligible patients. In addition, for safety reasons, those with poor health status that might be life-threatening were excluded from the sputum induction process. Last, patients in our study, who were derived from the community, were mainly in the mild–moderate status (86.9%). This might introduce selection bias in our analysis because of different clinical characteristics between mild–moderate and severe–very severe patients. Further cohorts that are hospital-based are needed to estimate the association between sputum neutrophil proportions, air trapping and future exacerbation in patients with GOLD stage III or IV.

Interpretation

This analysis of the ECOPD study aimed to demonstrate the association between neutrophilic airway inflammation and future exacerbations in COPD patients and to evaluate the modified effect of air trapping. High sputum neutrophil proportions are associated with greater odds of severe exacerbation of COPD. In addition, people who had air trapping and high sputum neutrophil proportions had an increased risk of severe and frequent exacerbation. The results describe potential factors for future exacerbation in those with COPD. Those with high sputum neutrophil proportions and significant air trapping may be reliable predictors of future exacerbation. Our findings encourage the use of sputum neutrophils and CT-based air trapping in clinical practice for precise treatments in clinical intervention.

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Patient consent for publication Consent obtained directly from patient(s)

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Table S1. The association of covariates in logistical regression model with future exacerbation

Variables	Exacerbation ≥1/y (%)	P vale	Severe exacerbation (%)	P value	Exacerbation≥2/y (%)	P value
All patients						
Male (vs. female)	0.51 (0.14-1.79)	0.304	0.45 (0.1-1.88)	0.288	0.49 (0.02-6.16)	0.592
Age (per years)	0.99 (0.96-1.02)	0.533	0.99 (0.96-1.03)	0.728	0.99 (0.95-1.04)	0.757
BMI (per kg/m^2)	0.99 (0.93-1.06)	0.83	0.98 (0.91-1.05)	0.491	0.98 (0.88-1.09)	0.715
Exacerbation in the preceding year (Yes vs. No)	**3.02 (1.82-5.05)**	**<0.001**	**3.22 (1.91-5.43)**	**<0.001**	**3.61 (1.8-7.12)**	**<0.001**
Smoking status (%)						
Never smoked	1		1		1	
Former smoking	1.36 (0.46-4.51)	0.595	1.91 (0.56-8.06)	0.332	3.36 (0.49-70.06)	0.297
Current smoking	1.24 (0.45-3.89)	0.694	1.45 (0.46-5.84)	0.557	2.59 (0.41-52.26)	0.402
Smoking index (per pack-yr)	1.00 (0.99-1.01)	0.843	1.00 (0.99-1.01)	0.68	1.00 (0.98-1.01)	0.439
FEV_1% pred (per percentage)	0.99 (0.98-1.00)	0.098	0.99 (0.98-1.00)	0.065	0.99 (0.97-1.00)	0.119
High neutrophil proportion level (vs. low neutrophil proportion level)	1.41 (0.95-2.09)	0.090	**1.68 (1.09-2.62)**	**0.020**	1.28 (0.67-2.51)	0.460
Inhaled medications (Yes vs No)	1.43 (0.69-2.92)	0.329	1.66 (0.77-3.44)	0.182	1.59 (0.54-4.07)	0.367
Patients with Air trapping						
Male (vs. female)	0.4 (0.04-3.19)	0.402	1.11 (0.09-12.83)	0.932	-	0.989
Age (per years)	0.99 (0.95-1.03)	0.468	1.00 (0.95-1.04)	0.857	1.01 (1.07-1.03)	0.794
BMI (per kg/m^2)	0.96 (0.88-1.04)	0.327	0.96 (0.88-1.05)	0.413	1.03 (1.16-1.04)	0.647
Exacerbation in the preceding year (Yes vs. No)	**2.23 (1.24-4.02)**	**0.007**	**2.72 (1.49-4.97)**	**0.001**	**3.64 (7.8-4.02)**	**0.001**
Smoking status (%)						
Never smoked	1		1		1	
Former smoking	3.58 (0.71-28.36)	0.159	2.64 (0.51-21.56)	0.293	-	0.989
Current smoking	2.18 (0.46-16.75)	0.375	1.49 (0.3-11.81)	0.656	-	0.989
Smoking index (per pack-yr)	0.99 (0.98-1.00)	0.125	0.99 (0.98-1.00)	0.221	0.99 (1.01-1)	0.351

FEV_1% pred (per percentage)	0.99 (0.97-1.00)	0.069	0.99 (0.97-1.00)	0.070	0.98 (1-1)	0.079
High neutrophil proportion level (vs. low neutrophil proportion level)	**1.69 (1.01-2.84)**	**0.046**	**1.79 (1.03-3.15)**	**0.042**	1.04 (2.28-2.84)	0.912
Inhaled medications (Yes vs No)	1.97 (0.86-4.54)	0.108	1.96 (0.83-4.55)	0.119	1.48 (4.23-4.54)	0.493
Non-Air trapping group						
Male (vs. female)	0.60 (0.11-3.00)	0.540	0.27 (0.03-1.72)	0.194	1.5 (0.04-47.83)	0.194
Age (per years)	0.99 (0.94-1.05)	0.792	0.99 (0.93-1.05)	0.66	0.94 (0.85-1.04)	0.66
BMI (per kg/m^2)	1.04 (0.92-1.17)	0.508	0.98 (0.86-1.12)	0.756	0.86 (0.65-1.08)	0.756
Exacerbation in the preceding year (Yes vs. No)	**9.01 (2.78-35.48)**	**0.001**	**5.66 (1.77-18.5)**	**0.003**	**2.23 (0.23-13.02)**	**0.003**
Smoking status (%)						
Never smoked	**1**		**1**		**1**	
Former smoking	0.37 (0.06-2.27)	0.265	0.79 (0.1-8.57)	0.832	0.66 (0.03-27.21)	0.832
Current smoking	0.76 (0.18-3.79)	0.719	1.31 (0.23-11.64)	0.782	0.51 (0.04-15.76)	0.782
Smoking index (per pack-yr)	1.01 (1.00-1.02)	0.252	1.01 (0.99-1.02)	0.446	1.01 (0.98-1.03)	0.446
FEV_1% pred (per percentage)	1.00 (0.98-1.03)	0.794	1 (0.97-1.02)	0.723	1.01 (0.97-1.06)	0.723
High neutrophil proportion level (vs. low neutrophil proportion level)	0.97 (0.47-1.96)	0.937	1.45 (0.65-3.19)	0.359	1.95 (0.47-8.51)	0.359
Inhaled medications (Yes vs No)	0.61 (0.07-3.10)	0.590	0.98 (0.12-5.12)	0.981	2.3 (0.08-22.33)	0.981

The effect values for clinical outcomes are obtained by using the linear regression model. All models are adjusted for age, sex, body mass index, smoking status, smoking index, prior exacerbation, FEV_1% pred, inhaled medication, and levels of sputum neutrophil proportion. BMI=body mass index; FEV_1=Forced Expiratory Volume in one second.