Pulmonary hypertension in eosinophilic *versus* noneosinophilic COPD

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ABSTRACT

Background: The eosinophilic COPD phenotype is associated with greater airway remodelling, exacerbation risk and steroid responsiveness. However, little is known about the prevalence and characteristics of pulmonary hypertension (PH) in this patient population.

Methods: We retrospectively evaluated a cohort of COPD patients with right heart catheterisation (RHC) data at a university hospital between January 2011 and May 2019 and compared the pulmonary vascular profile and prevalence of PH between eosinophilic and noneosinophilic patients using a definition of eosinophilic COPD as at least three blood eosinophil values $\geqslant$300 cells·μL^{-1}. We used multivariable logistic regression analyses to examine the association between eosinophilic COPD and various PH categories adjusting for age, sex, body mass index, forced expiratory volume in 1 s (%), smoking status and use of supplemental oxygen.

Results: Among 106 COPD patients with RHC data and at least three blood eosinophil values, 25% met the definition of eosinophilic COPD. Fewer patients among the eosinophilic group required long-term oxygen therapy (69% *versus* 93%, p=0.001) and total lung capacity was significantly lower in the eosinophilic group (p=0.006). This group had higher mean pulmonary arterial pressure (mPAP) (median (interquartile range) 30 (27–41) mmHg *versus* 25 (22–30) mmHg, p=0.001) and pulmonary vascular resistance (PVR) (4 (2.8–5.1) Wood units *versus* 2.9 (2.1–4.1) Wood units, p=0.018). On multivariable logistic regression analyses, eosinophilic phenotype was associated with PH (adjusted (a)OR 6.5, 95% CI 1.4–30.7; p=0.018) and pre-capillary PH (aOR 3.2, 95% CI 1.1–9; p=0.027), but not severe PH (aOR 2.1, 95% CI 0.6–7.2; p=0.219).

Conclusion: Eosinophilic COPD was associated with higher mPAP and PVR and increased likelihood of PH. More studies are needed to further explore this finding.

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In COPD patients with right heart catheterisation, eosinophilic COPD confers a 7-fold increase in the likelihood of pulmonary hypertension and a 3-fold increase in the likelihood of pre-capillary pulmonary hypertension compared to noneosinophilic COPD https://bit.ly/2YJk7aB

Introduction

COPD is projected to be the third leading cause of death worldwide by 2030 [1]. The presence of pulmonary hypertension (PH) in COPD has a stronger association with mortality compared to pulmonary function test (PFT) parameters such as forced expiratory volume in 1 s (FEV$_1$ %) or gas exchange variables [2, 3]. PH has been defined as mean pulmonary artery pressure (mPAP) $\geqslant$25 mmHg, but this has been revised in chronic lung disease patients (CLD-PH) into those with mPAP 21–24 mmHg with pulmonary vascular resistance (PVR) $\geqslant$3 Wood units (WU) or mPAP $\geqslant$25 mmHg [3]. The prevalence of PH in COPD is probably underestimated, as most data were derived from patients with severe disease undergoing lung transplant evaluation. Several studies have shown that up to 90% of patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage IV have mPAP >20 mmHg and ∼5% of COPD patients have mPAP >35–40 mmHg at rest [3, 4].

Eosinophilic COPD has been increasingly recognised as a distinct phenotype. The 2019 GOLD report introduced the blood eosinophil count as a biomarker to start or de-escalate inhaled corticosteroids [5]. Thresholds for eosinophilia assessed by different studies included relative eosinophil count of 2%, and absolute eosinophil counts of 150, 300 and 340 cells·μL^{-1} [6]. Using a cut-off of 300 cells·μL^{-1}, 20% of COPD patients were reported to have an eosinophilic phenotype [7]. The role of eosinophils in the development of pulmonary arterial hypertension (PAH) has been demonstrated in few animal [8–10] and human studies [11–13]. However, to our knowledge, no previous study has evaluated PH specifically in eosinophilic COPD patients.

Methods

We retrospectively evaluated a cohort of COPD patients who underwent right heart catheterisation (RHC) for evaluation of PH at the University of Florida between January 2011 and May 2019. We compared the pulmonary haemodynamic profile and prevalence of PH between patients with eosinophilic and noneosinophilic COPD. Study was approved by the University of Florida institutional review board (IRB 201901525).

Study subjects and clinical variables

We initially identified 119 patients previously diagnosed with COPD who also had available RHC data. The indication for RHC was either lung pre-transplant work-up or suspected PH. Information was collected about baseline characteristics, comorbidities, PFTs, echocardiogram, RHC, 6-min walk distance (6MWD), imaging and laboratory data. A board-certified pulmonary attending (HA) and a pulmonary fellow (BNA) reviewed the patients' chest computed tomography (CT) scans and PFTs and excluded patients who were mislabelled as COPD or had underlying interstitial lung disease (ILD). COPD was defined per the American Thoracic Society (ATS)/European Respiratory Society guidelines [14]. One patient did not have spirometry available, but had significant emphysema on chest CT and was included in the analysis [15]. Nine patients (previously labelled to have COPD, but did not have any spirometric or radiological evidence of COPD, or had underlying ILD), were excluded, bringing the final number to 111 patients: 92 who underwent RHC for pre-transplant work-up and 19 for suspected PH. Vital signs were obtained on the day of RHC. Laboratory values closest to the RHC date were reported.

We classified the patients into eosinophilic *versus* noneosinophilic COPD. Eosinophilia was defined as having at least three absolute blood eosinophil counts $\geqslant$300 cells·μL^{-1} [5, 6, 16, 17]. We only included patients who had at least three separate complete blood count (CBC) results available (106 patients).

COPD severity parameters and PFT

Post-bronchodilator spirometry, plethysmography and diffusing capacity of the lung for carbon monoxide (D_{LCO}) data were collected for each patient (Zan 500 Body; nSpire Health, Louisville, CO, USA). These were performed according to ATS guidelines [18] using predicted values according to the third National Health and Nutrition Examination Survey [19]. PFT values were not available in one (0.9%) patient. Patients were classified into four classes based on airflow limitation according to the GOLD 2020 report [5]. In addition, we compared supplemental oxygen for each patient, smoking history, α_1-antitrypsin deficiency, 6MWD, BODE (body mass index, airflow obstruction, dyspnoea and exercise) COPD severity index, New York Heart Association functional classification and number of COPD exacerbations requiring hospitalisation in the previous year.

Echocardiography

Transthoracic echocardiography was performed using a Philips EPIQ 7 system (Philips Healthcare, Andover, MA, USA). We used echocardiographic values measured as described in the American Society of Echocardiography guidelines [20] as reported by board-certified cardiologists. The echocardiogram closest

in time to the RHC was selected. The median time difference between obtaining the echocardiogram and RHC was 3 days (interquartile range (IQR) 1–46 days).

Right heart catheterisation

RHC was performed by a board-certified cardiologist or pulmonologist as part of the lung transplantation evaluation (82.9%) and/or if they had clinical suspicion for PH (17.1%). The majority of RHCs (90%) were performed as outpatient cases with no statistically significant difference between the two groups, and only one patient in each group was being treated for COPD exacerbation when the RHC was performed. End-expiration values were recorded. Cardiac output was measured using either the thermodilution (74.8%) or indirect Fick method (25.2%) [21]. PVR was calculated as (mPAP − pulmonary capillary wedge pressure (PCWP))/cardiac output expressed in Wood units. Diastolic pulmonary gradient was calculated as the difference between the diastolic pulmonary artery pressure and PCWP [22]. We defined PH as mPAP ⩾25 mmHg [2] and reported the prevalence of PH as per the 2018 World Symposium on Pulmonary Hypertension (WSPH) consensus definition of CLD-PH (mPAP 21–24 mmHg with PVR ⩾3 WU, or mPAP ⩾25 mmHg) [3]. Pre-capillary PH was defined as mPAP ⩾25 mmHg, PVR ⩾3 WU and PCWP ⩽15 mmHg, and severe PH as mPAP ⩾35 mmHg or 25–34 mmHg with cardiac index) <2 L·min^{-1}·m^{-2} [3].

Sensitivity analysis

We performed a subgroup analysis comparing eosinophilic and noneosinophilic COPD using only the pre-transplant cohort (92 patients). Additionally, we ran the analysis using an alternative definition of eosinophilic COPD as a single blood eosinophil count ⩾340 cells·µL^{-1} [23] (111 patients). Furthermore, using a definition of PH as mPAP ⩾25 mmHg, we determined the prevalence of eosinophilia in those with and without PH.

Statistical analysis

We summarised the data as percentages for categorical variables, mean±SD for normally distributed continuous variables and median (IQR) for non-normally distributed continuous variables. Shapiro–Wilk testing and visual inspection of variables' histograms were used to assess distribution normality. An independent-sample t-test was used to compare variables with normal distribution and the Mann–Whitney U-test was used for variables with non-normal distribution. We constructed a clustered bar chart to demonstrate the prevalence rates of PH and its subgroups in eosinophilic versus noneosinophilic COPD. We used multivariable logistic regression analyses to examine the association between eosinophilic COPD and various PH categories. In multivariable models, we adjusted for age, sex, body mass index (BMI) [24], FEV$_1$%, smoking status (active versus former) and need for supplementary oxygen during RHC, which were chosen on an a priori basis. We then presented the adjusted odds ratio (95% confidence interval) of having various PH categories in eosinophilic versus noneosinophilic COPD. Statistical analyses were performed using IBM SPSS Statistics for Windows (version 23.0; released 2015, IBM Corp, Armonk, NY, USA).

Results

111 patients with confirmed diagnosis of COPD who also had RHC were identified, of whom 106 had at least three CBC values and were included in the primary analysis. 26 (24.5%) patients met the definition of eosinophilic COPD. Both groups were generally middle-aged Caucasians and almost equally distributed between males and females (table 1). Eosinophilic patients had higher BMI (p=0.006), more history of rheumatological diseases (p=0.045) and were marginally more likely to have a history of marijuana smoking and diabetes mellitus (p=0.052 and 0.057, respectively). There were no significant differences in other comorbidities (table 1).

There were no significant differences in FEV$_1$ (median (IQR) 24% (19–48%) versus 21% (17–26%), p=0.101) or GOLD airflow limitation severity. Fewer patients among the eosinophilic group required long-term supplemental oxygen therapy (69% versus 93%, p=0.001). Furthermore, total lung capacity (TLC) was significantly lower in the eosinophilic COPD group (p=0.006) (table 2). There were no statistically significant differences between the two groups in the use of inhaled corticosteroids (77% versus 85%, p=0.341), chronic oral steroid therapy (23% versus 17.5%, p=0.528) or chronic azithromycin therapy (7.7% versus 10%, p=0.727). Roflumilast use was more common in the eosinophilic COPD group, but did not reach statistical significance (23% versus 10%, p=0.087). There was no statistically significant difference in any other COPD-related measured values (table 2).

The reported echocardiographic parameters are summarised in table 3. Eosinophilic COPD patients had more left atrial dilation (27% versus 10%, p=0.039) and marginally more left ventricular hypertrophy (did not reach statistical significance, p=0.059). There was no statistically significant difference in the other

TABLE 1 Baseline demographics and clinical characteristics of eosinophilic COPD as compared to noneosinophilic COPD[#]			
	Eosinophilic COPD	**Noneosinophilic COPD**	**p-value**
Patients n	26	80	
Demographics			
Age years	64±7.4	61.3±7.7	0.124
Female	12 (46.2)	45 (56.3)	0.370
Race			
Caucasian	24 (92.3)	75/79 (94.9)	0.616
African-American	2 (7.7)	4/79 (5.1)	
Clinical characteristics			
Body mass index $kg·m^{-2}$	27±4.3	24.3±4.3	0.006
Heart rate $beats·min^{-1}$[¶]	81.3±15.5	80.8±12.3	0.868
Oxygen saturation %[¶]	96.3±2.3	97.3±2.7	0.093
Mean systemic blood pressure mmHg[¶]	96.5±14.4	99±11.7	0.377
Marijuana use	7 (26.9)	9 (11.3)	0.052
Asthma	1 (3.8)	3 (3.8)	0.682
Atopic dermatitis	3 (11.5)	3 (3.8)	0.156
Systemic hypertension	14 (53.8)	44 (55)	0.918
Diabetes mellitus	10 (38.5)	16 (20)	0.057
Congestive heart failure	2 (7.7)	7 (8.8)	0.867
Obstructive sleep apnoea	4 (15.4)	9 (11.3)	0.577
Rheumatological disease[+]	3 (11.5)	1 (1.3)	0.045
Laboratory findings[¶]			
WBC $×10^9$ $cells·L^{-1}$	8.5±2.7	8.7±3.6	0.820
P_{aO_2} mmHg[¶]	83 (77–89)	80 (69–91)	0.541
Eosinophils $cells·µL^{-1}$	333.5±189.6	164.6±70	<0.001
Brain natriuretic peptide $pg·mL^{-1}$	75.1 (16–184)	37.1 (20–122)	0.881

Data are presented as n, mean±SD, n (%), n/N (%) or median (interquartile range), unless otherwise stated. WBC: white blood cells; P_{aO_2}: arterial oxygen tension. [#]: eosinophilic COPD was defined as having at least three separate absolute blood eosinophil counts ⩾300 $cells·µL^{-1}$. Five patients did not have at least three eosinophil count values and were not classified based on this definition; [¶]: vital signs were reported on the day of right heart catheterisation (RHC). Lab measures closest to the RHC date were reported. P_{aO_2} on the RHC day was only available in 26% of the patients; [+]: of the three patients in the eosinophilic group with rheumatological disorders, one had rheumatoid arthritis with relapsing polychondritis (mean pulmonary arterial pressure (mPAP) 40 mmHg), one had systemic lupus erythematosus (SLE) (mPAP 32 mmHg) and one had scleroderma (mPAP 55 mmHg), and the one patient from the noneosinophilic group had SLE (mPAP 21 mmHg). All of these four patients underwent RHC for pulmonary hypertension evaluation and were not pre-transplant patients.

echocardiographic parameters. On RHC, the eosinophilic patients had higher systolic and diastolic pulmonary artery pressures (p=0.004 and 0.046, respectively), higher mPAP (median (IQR) 30 (27–41) mmHg *versus* 25 (22–30) mmHg, p=0.001) and higher PVR (4 (2.8–5.1) WU *versus* 2.9 (2.1–4.1) WU, p=0.018). There was no statistically significant difference in PCWP, cardiac output and cardiac index (table 3).

On univariable analysis, eosinophilic patients had more PH (OR 8, 95% CI 1.8–36.2; p=0.002), CLD-PH (2018 WSPH definition) (6.1, 1.3–27.8; p=0.01), pre-capillary PH (3.3, 1.3–8.3; p=0.01) and marginally more severe PH (2.5, 0.96–6.5; p=0.057) (figure 1). On multivariable logistic regression adjusting for potential confounders, this phenotype was associated with PH (adjusted (a)OR 6.9, 95% CI 1.5–32.4; p=0.015) and pre-capillary PH (aOR 3.3, 95% CI 1.2–9.1; p=0.023), but not with severe PH (aOR 1.7, 95% CI 0.5–5.3; p=0.365) (table 4).

Using the same definition of eosinophilic COPD, but including pre-transplant patients only, the eosinophilic group had higher mPAP (p=0.008) but not PVR (p=0.108). Additionally, they had more PH (89% *versus* 56%, p=0.011) and CLD-PH (89% *versus* 62%, p=0.030), but not pre-capillary or severe PH (p=0.108 and 0.224, respectively) (supplementary table S1). Using a single eosinophil count of ⩾340 $cells·µL^{-1}$ to define eosinophilic COPD, eosinophilic patients had higher mPAP (p=0.013) and PVR (p=0.047), more PH (p=0.016), CLD-PH (p=0.019) and pre-capillary PH (p=0.036), but not severe PH (p=0.164) (supplementary table S2). Comparing patients with and without PH, COPD-PH patients had higher blood eosinophil counts measured closest in time to the RHC (p=0.046), had marginally higher

TABLE 2 COPD parameters and pulmonary function test data of eosinophilic COPD as compared to noneosinophilic COPD[#]

	Eosinophilic COPD	Noneosinophilic COPD	p-value
Patients	26	80	
Long-term supplemental oxygen use	18 (69.2)	71/76 (93.4)	0.001
Supplemental oxygen during RHC	21 (81)	66/79 (83.5)	0.754
Active smokers	0	2 (2.5)	0.416
Smoking pack-years	36.8 (25–61.1)	40 (30–60)	0.390
α_1-antitrypsin deficiency	4/18 (22.2)	6/60 (10)	0.174
6-min walk distance m[¶]	226±102.9	240.6±107.9	0.554
BODE index[¶]	6 (5–8)	7 (6–8)	0.104
NYHA class[¶]	3	3	0.074
COPD exacerbations in past year[¶]	1 (0–2)	1 (0–2)	0.784
Inhaled corticosteroids use	20 (77)	68 (85)	0.341
Chronic azithromycin use	2 (7.7)	8 (10)	0.727
Roflumilast use	6 (23)	8 (10)	0.087
Chronic oral steroids use[+]	6 (23)	14 (17.5)	0.528
Pulmonary function tests[¶]			
$\quad$ FEV$_1$ % pred	24 (19–48)	21 (16.9–26.3)	0.101
$\quad$ FVC % pred	56±16.9	53.2±17	0.454
$\quad$ FEV$_1$/FVC %	34 (28.1–60.5)	32 (26–39)	0.121
$\quad$ Positive bronchodilator response n/N (%)	1/23 (4.3)	6/65 (9.2)	0.457
$\quad$ TLC % pred	96.5±25.5	117.9±27.2	0.006
$\quad$ $D_{LCO_{Hb}}$ % pred	29.5 (20.3–46.3)	24.7 (18.5–34)	0.226
GOLD airflow limitation severity n/N (%)			
$\quad$ GOLD 1 (FEV$_1$ $\geqslant$80% pred)	0	3/79 (3.8)	0.422
$\quad$ GOLD 2 (50%$\leqslant$FEV$_1$<80% pred)	6 (23.1)	4/79 (5.1)	0.007
$\quad$ GOLD 3 (30%$\leqslant$FEV$_1$<50% pred)	3 (11.5)	9/79 (11.4)	0.984
$\quad$ GOLD 4 (FEV$_1$ <30% pred)	17 (65.4)	63/79 (79.7)	0.136

Data are presented as n, n (%), n/N (%), median (interquartile range) or mean±SD, unless otherwise stated. RHC: right heart catheterisation; BODE: body mass index, airflow obstruction, dyspnoea and exercise COPD severity index; NYHA: New York Heart Association functional class; FEV$_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; $D_{LCO_{Hb}}$: diffusing capacity of the lung for carbon monoxide corrected for haemoglobin; GOLD: Global Initiative for Chronic Obstructive Lung Disease. [#]: eosinophilic COPD was defined as having at least three separate absolute blood eosinophil counts $\geqslant$300 cells·µL^{-1}. Five patients did not have at least three eosinophil count values and were not classified based on this definition; [¶]: information regarding 6-min walk distance, BODE index, NYHA and COPD exacerbations was available in 97.1%, 92.4%, 94.3% and 62.2% of the patients, respectively. Data regarding FEV$_1$%, FVC and FEV$_1$/FVC ratio were available in 99.2% of the patients. Data regarding TLC and $D_{LCO_{Hb}}$ were available for 74.5% and 76.4% of the patients, respectively; [+]: oral prednisone doses ranged from 2.5 mg daily to 10 mg daily.

maximum counts (p=0.050) and were more likely to have an eosinophilic phenotype using either definition (table 5).

Discussion

In our study, eosinophilic COPD phenotype was associated with elevated mPAP and an increased likelihood of PH and pre-capillary PH compared to patients with noneosinophilic COPD. On multivariable analyses adjusting for potential confounders, eosinophilic phenotype conferred a seven-fold increase in the likelihood of PH and a three-fold increase in the likelihood of pre-capillary PH. In addition, one-third of the patients with confirmed COPD-PH had eosinophilia, compared to 6% of the COPD-no-PH group.

A growing body of evidence has identified eosinophilic COPD as a distinct phenotype [6, 25] and the use of peripheral eosinophilia as a biomarker to predict steroid responsiveness in COPD patients has been supported by several studies [7, 26–30]. Based on these findings, the GOLD guidelines recommend the use of an absolute eosinophil count $\geqslant$300 cells·µL^{-1} as a cut-off to add or stop inhaled corticosteroids [5]. However, to our knowledge, this is the first study to investigate the pulmonary vascular haemodynamic profile of the eosinophilic COPD phenotype and its association with PH.

TABLE 3 Echocardiographic parameters and right heart catheterisation data of eosinophilic COPD as compared to noneosinophilic COPD[#]

	Eosinophilic COPD	Noneosinophilic COPD	p-value
Patients	26	80	
Echocardiogram			
Ejection fraction %	60 (60–65)	65 (60–65)	0.193
Diastolic dysfunction	5/25 (20)	15/77 (19.5)	0.955
Left ventricular dilation	1 (3.8)	1 (1.3)	0.398
Left ventricular hypertrophy	3 (11.5)	2 (2.5)	0.059
Left atrial dilation	7 (26.9)	8/77 (10.4)	0.039
Right ventricular dilation	8 (30.8)	22 (27.5)	0.748
Right ventricular hypertrophy	0	4 (5.1)	0.314
Right atrial dilation	5 (19.2)	14/75 (18.7)	0.949
RVSP mmHg	56.7±19.9	50.7±24	0.473
Tricuspid regurgitation velocity m·s^{-1}	3.5±0.6	3.2 ±0.7	0.277
TAPSE mm	20 (19–20)	20	0.789
Right heart catheterisation parameters			
Systolic pulmonary artery pressure mmHg	45.5 (35.8–62.5)	37.5 (32–43.8)	0.004
Diastolic pulmonary artery pressure mmHg	20.5 (19.5–30.3)	20 (15–24.8)	0.046
Mean pulmonary artery pressure mmHg	30 (26.8–40.8)	25 (22–30)	0.001
PCWP mmHg	14.7±4.5	13±4.3	0.096
Right atrial pressure mmHg	10.2±4.7	9.4±3.8	0.353
Diastolic pulmonary gradient mmHg	10 (3–13.5)	5 (3–10)	0.064
Pulmonary vascular resistance mmHg	4 (2.8–5.1)	2.9 (2.1–4.1)	0.018
Cardiac output L·min^{-1}	4.3 (4–4.9)	4.2 (3.4–4.9)	0.394
Cardiac index L·min^{-1}·m^{-2}	2.4 (2.2–2.6)	2.2 (2–2.6)	0.760

Data are presented as n, median (interquartile range), n/N (%), n (%) or mean±SD, unless otherwise stated. RVSP: right ventricular systolic pressure; TAPSE: tricuspid annular plane systolic excursion; PCWP: pulmonary capillary wedge pressure. [#]: eosinophilic COPD was defined as having at least three separate absolute blood eosinophil counts ⩾300 cells·µL^{-1}. Five patients did not have at least three eosinophil count values and were not classified based on this definition.
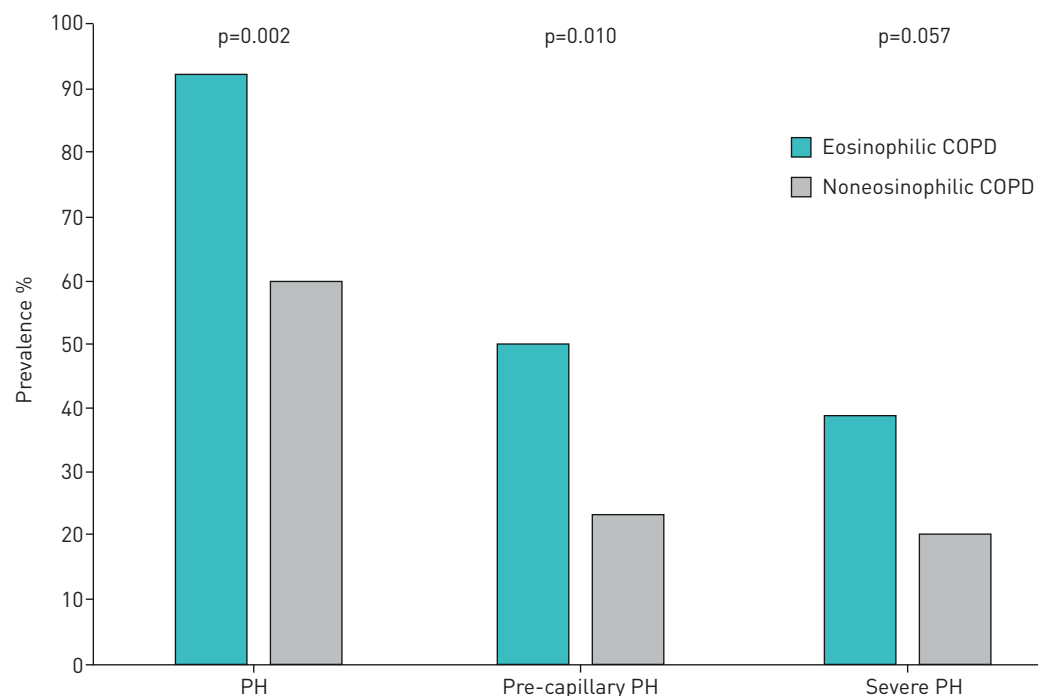

FIGURE 1 Clustered-bar chart demonstrating the prevalence of pulmonary hypertension (PH), pre-capillary PH and severe PH in patients with eosinophilic COPD as compared to noneosinophilic COPD patients.

TABLE 4 Multivariable regression model assessing the association between eosinophilic COPD[#] with pulmonary hypertension (PH), pre-capillary PH and severe PH

	Eosinophilic COPD	Noneosinophilic COPD	p-value	Adjusted p-value	Adjusted OR (95% CI)
Patients	26	80			
PH[¶]	24 (92.3)	48 (60)	0.002	0.018	6.5 (1.4–30.7)
CLD-PH per the WSPH 2018[+]	24 (92.3)	53 (66.3)	0.010	0.041	5.1 (1.1–23.9)
Pre-capillary PH[§]	13 (50)	18/77 (23.4)	0.010	0.027	3.2 (1.1–9)
Severe PH[f]	10 (38.5)	16 (20)	0.057	0.219	2.1 (0.6–7.2)

Data are presented as n, n (%) or n/N (%), unless otherwise stated. CLD-PH: chronic lung disease PH; WSPH: World Symposium on Pulmonary Hypertension definition. [#]: eosinophilic COPD was defined as having at least three separate absolute blood eosinophils count $\geqslant 300$ cells·μL^{-1}. Five patients did not have at least three eosinophil count values and were not classified based on this definition. Regression model adjusted for age, sex, body mass index, forced expiratory volume in 1 s, smoking status (active *versus* former) and the need for supplementary oxygen during right heart catheterisation procedure; [¶]: defined as mean pulmonary arterial pressure (mPAP) $\geqslant 25$ mmHg; [+]: defined per the 6th WSPH as mPAP 21–24 mmHg with pulmonary vascular resistance (PVR) $\geqslant 3$ Wood units (WU), or mPAP $\geqslant 25$ mmHg; [§]: defined as mPAP $\geqslant 25$ mmHg plus PVR $\geqslant 3$ WU with pulmonary capillary wedge pressure (PCWP) $\leqslant 15$ mmHg; [f]: defined as mPAP $\geqslant 35$ mmHg or mPAP 25–34 mmHg with cardiac index <2 L·min^{-1}·m^{-2}.

The previously reported prevalence of eosinophilic COPD ranges from ~20% to 70%, depending on the threshold used and the patient population studied [31]. In a *post hoc* analysis of the WISDOM trial, 20% of the COPD patients had eosinophils $\geqslant 300$ cells·μL^{-1} [7], which is close to what we found in our study using the same cut-off (24.5%). However, we chose to use three eosinophil counts, as these tend to be unstable and using one measurement to define the eosinophilic COPD phenotype has been questioned before [6, 32]. In addition, the slightly higher BMI we found in our cohort is similar to that which has been reported in eosinophilic COPD, as is the similarity in GOLD airflow limitation between the two groups [31]. The eosinophilic patients had a significantly lower TLC, which could indicate that they had less hyperinflation and emphysema, although we did not quantify the degree of emphysema on computed tomography. This finding might be in line with a report by SINGH *et al.* [26], who found that patients with persistent eosinophil counts <2% had more emphysema progression, which is biologically plausible as neutrophils are known to cause more emphysema. We chose not to include TLC in the multivariable model as it was unfortunately not available in a quarter of the patients. Lung hyperinflation is known to be associated with impaired left ventricular filling [33] and given that TLC values were generally lower in the eosinophilic group, including it in the model might have strengthened the association between eosinophilic COPD and PH.

Unlike asthma, the role of eosinophils in the pathophysiology of COPD is not fully clear [34], but they are associated with more airway remodelling and hyperresponsiveness [35]. The association we found between the eosinophilic COPD phenotype and PH is novel and remained significant despite adjusting for multiple confounders, using a different cut-off to define eosinophilia and limiting the analysis to the pre-transplant subgroup. Furthermore, patients with COPD-PH had higher blood eosinophil counts than COPD patients

TABLE 5 Eosinophils count in patients with and without COPD-pulmonary hypertension (PH)[#]

	COPD-PH	COPD no PH	p-value
Patients	77	34	
Max. absolute blood eosinophil count cells·μL^{-1}	263 (180–375)	220 (157–292.5)	0.050
Max. blood eosinophil percentage %	3.4 (2–5)	2.4 (2–4.1)	0.146
Eosinophil absolute count closest to RHC	190 (140–270)	160 (117.5–230)	0.046
At least three eosinophil counts $\geqslant 300$ cells·μL^{-1}	24/72 (33.3)	2/34 (5.9)	0.002
Max. eosinophil count $\geqslant 340$ cells·μL^{-1}	26 (33.8)	4 (11.8)	0.016

Data are presented as n, median (interquartile range), n/N (%) or n (%), unless otherwise stated. Max: maximum; RHC: right heart catheterisation. [#]: defined as haemodynamic measurement of mean pulmonary artery pressure $\geqslant 25$ mmHg.

without PH. There is no reason to suspect that this finding was due to hypoxia, as the eosinophilic group was less likely to require long-term supplemental oxygen.

Although the eosinophilic patients had more left atrial dilation on echocardiogram, it is unlikely that the difference in mPAP and PH prevalence was driven primarily by more pulmonary venous congestion, as there was no difference in diastolic dysfunction on echocardiogram or PCWP on RHC. Additionally, pre-capillary PH was more prevalent in the eosinophilic COPD group. Of note, on subgroup analysis including pre-transplant patients only, pre-capillary PH was numerically more in the eosinophilic COPD group, but was not statistically significant (39% *versus* 21%, p=0.108). This could be due to the drop in sample size on subgroup analysis or that the difference in pre-capillary PH might be driven by nontransplant candidates.

There are few reports in humans linking eosinophils to PAH. A previous report from Sri Lanka found that >75% of patients with primary PAH had eosinophilia which was significantly higher than the control groups [11]. Similarly, in humans with schistosomiasis-related PH, high levels of interleukin (IL)-5 and subsequent recruitment of eosinophils are thought to contribute to the development of PH [12]. In addition, there have been a handful of reports of PH associated with hypereosinophilic disorders [36–38]. Finally, in a single-centre study from Germany, HARBAUM *et al.* [13] explored the CBC differential in patients with PAH and found that >50% had elevated blood eosinophils. However, they used a much lower cut-off to define eosinophilia ($\geqslant$100 cells·μL^{-1}). Interestingly, the morphology of the vascular lesions noted in explanted lungs of patients with COPD-PH were comparable to those noted in idiopathic PAH in a report by CARLSEN *et al.* [39]. Whether eosinophils are biomarkers for PH or act as a direct vascular modulator in patients with PH is unclear. DALEY *et al.* [8] have shown previously that prolonged intermittent airway challenge with extrinsic antigens induced muscularisation of small- to medium-sized pulmonary arteries that was significantly ameliorated by the depletion of IL-13. Furthermore, in a mouse model of PH, WENG *et al.* [9] demonstrated that eosinophils were necessary to induce pulmonary vascular remodelling. Specifically, they compared the degree of pulmonary arterial muscularisation in eosinophil-deficient and wild-type mice and found that eosinophil-deficient mice have significantly less pulmonary arterial wall thickening. In addition, they found that mice treated with anti-IL-5 antibodies had markedly lower bronchoalevolar lavage eosinophilia and, more importantly, pulmonary arterial muscularisation compared to mice treated with control antibodies. Additionally, the treatment of pulmonary arterial smooth muscle cells with eosinophilic granule extracts led to two-fold higher proliferation compared to the controls. They also found higher phosphorylation rates of protein kinase B (Akt)1 and extracellular signal-regulated kinase (ERK) in these cells, suggesting that the mechanism linking eosinophil and PH might be due to activation of Akt1 and ERK, both of which are downstream mediators of pulmonary arterial smooth muscle cell proliferation [9]. In another animal model, anti-IL-5 effectively suppressed IL-33 induced pulmonary arterial hypertrophy [10]. Together, these studies suggest that eosinophils may contribute to the development of PH. However, it is more likely that the pathogenesis of PH in eosinophilic COPD is multifactorial, as PH has not been reported in eosinophilic asthma, for example. We suspect that other processes such as chronic hypoxaemia, respiratory acidosis, mechanical factors and loss of pulmonary vascular beds due to parenchymal destruction interact together to cause PH in COPD patients [4].

Our findings may have therapeutic implications, potentially opening the door to study the use of eosinophil-depleting biologics to treat or prevent PH in patients with eosinophilic COPD. In addition, screening for PH might be more warranted in patients with eosinophilic COPD, but further research is needed. We acknowledge the limitations inherent in retrospective chart review studies. However, we meticulously reviewed the patients' charts and had strict inclusion criteria regarding COPD diagnosis, eosinophilic COPD definition and the need for RHC to define PH. Second, our study subjects were mainly Caucasians with severe and very severe COPD, hence the generalisability of our results to other races/ethnicities and to those with less severe disease might be limited. The severity of airway obstruction perhaps explains the low rate of bronchodilator response detected in our cohort even in eosinophilic COPD patients. Third, the referral bias perhaps explains the high prevalence of PH and severe PH in our patients. However, previous studies have shown that up to 90% of GOLD IV COPD patients can have mPAP >20 mmHg, which is close to our study [2–4]. Lastly, 17.5% of the noneosinophilic COPD patients were on and off chronic oral prednisone therapy, which could have caused falsely low eosinophil counts [40]. However, these patients were on low doses ($\leqslant$10 mg daily), which have been shown to result in no or mild suppression of peripheral eosinophilia [41].

In summary, we found a significant association between eosinophilic COPD and PH. Patients with eosinophilic COPD had higher mPAP and PVR than noneosinophilic COPD. More studies are needed to reproduce these results, investigate the pathophysiological role of eosinophils in COPD-PH and to explore the role of eosinophil-depleting therapy in this patient population.

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