



Eosinophilic inflammation in COPD: prevalence and clinical characteristics

Chronic obstructive pulmonary disease (COPD) is a heterogeneous condition, with patients displaying varying clinical and pathophysiological features. The identification of COPD phenotypes with distinct characteristics may allow targeted treatment strategies directed towards specific biological pathways.

Eosinophilic inflammation is thought to be a characteristic feature of asthma rather than COPD. However, studies have shown that a subset of COPD patients with eosinophilic airway inflammation exists, even after the careful exclusion of patients with any features of asthma, such as β -agonist reversibility, bronchial hyperresponsiveness, atopy or a childhood history of asthma [1–4]. Interestingly, these patients exhibit the greatest response to corticosteroid treatment [1–4]. Likewise, sputum eosinophil numbers are increased in a subset of COPD exacerbations [5, 6], and titrating corticosteroid therapy according to sputum eosinophil counts reduces exacerbation rates [7]. Furthermore, there are similar increases in sputum and blood eosinophil numbers during COPD exacerbations [5]; using blood eosinophils as a surrogate maker for airway eosinophils to direct oral corticosteroid therapy for the treatment of COPD exacerbations enhances clinical recovery [8]. Taken together, these observations suggest that eosinophilic airway inflammation in COPD is a predictive biomarker of corticosteroid responsiveness during clinical stability and exacerbations.

The prevalence of eosinophilic inflammation in COPD patients is unknown. We do not know whether patients with sputum or blood eosinophilia represent a stable COPD phenotype over time and, apart from corticosteroid responsiveness, little is known about the other clinical characteristics of this subset of patients.

We analysed samples from the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points) cohort to: 1) determine the prevalence of COPD subjects with eosinophil levels persistently $\geq 2\%$ in blood and sputum over a 3-year follow-up period; and 2) describe their clinical characteristics, both cross-sectionally at baseline and longitudinally during the 3-year follow-up.

The design of the ECLIPSE cohort study has been described elsewhere [9]. COPD subjects aged 40–75 years with a smoking history >10 pack-years, post-bronchodilator forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) ratio <0.7 , FEV₁ $<80\%$ and with no history of asthma were recruited. Control healthy, nonsmoking subjects had an FEV₁/FVC ratio >0.7 and FEV₁ $>80\%$. Both blood and sputum samples were obtained at the start of the study (baseline) and after 1, 2 and 3 years. Blood eosinophils were measured during automated full blood count analysis. Exacerbation severity and frequency, spirometry, 6-min walk test, serum biomarkers and emphysema quantification using low-dose chest computed tomography scan were measured as previously reported [9–11]. Sputum induction was performed in a subset of patients at selected centres [9]. The study protocol was approved by local ethics committees at all 46 participating sites in 12 countries and all participants provided informed consent.

For blood eosinophils, a cut-off level of 2% was used, as this shows high sensitivity for predicting sputum eosinophilia [5]. Differences in clinical measures between eosinophil groups were assessed using ANOVA, Chi-squared test or Kruskal–Wallis test. Spearman correlations assessed the association between sputum and blood eosinophils. Statistical analyses were performed using GraphPad V3 (Graphpad Software Inc, La Jolla, CA, USA) and SAS V9.1.3 on UNIX (SAS Institute, Cary, NC, USA).

A total of 1483 subjects provided blood for eosinophil counts at all visits (table 1). 554 (37.4%) subjects had eosinophil counts persistently $\geq 2\%$ at all visits, 201 (13.6%) had eosinophil counts persistently $<2\%$ at all visits, and an intermittent group of 728 (49%) subjects had variable eosinophil counts that oscillated above and below 2%. The same pattern was also observed in healthy controls, where 73 (36%) out of 203 subjects had eosinophils persistently $\geq 2\%$ at all visits.

COPD subjects with eosinophil counts persistently $\geq 2\%$ were slightly older, had a greater proportion of males and fewer current smokers than the other COPD groups. They were also characterised by a higher FEV₁ % predicted and fat-free mass index, fewer symptoms with lower St George's Respiratory Questionnaire (SGRQ) and modified Medical Research Council (mMRC) scores, and lower BODE (body mass index, airflow obstruction, dyspnoea, exercise capacity) index; other clinical measurements were similar between groups. Serum levels of chemokine ligand 18 were higher and club cell protein 16 and CXCL8 were lower in the group with eosinophils persistently $\geq 2\%$. The differences observed were not

TABLE 1 Baseline cross-sectional characteristics and longitudinal changes in patients defined by peripheral blood eosinophil counts during follow-up

	Persistently $\geq 2\%$	Intermittent	Persistently $< 2\%$	ANOVA p-value
Subjects n	554	728	201	
Age years	64 \pm 7	62 \pm 7	62 \pm 7	0.025
Male sex	68	64	56	0.007
Smoking history pack-years	47 \pm 26	47 \pm 26	48 \pm 30	0.810
Current smokers	30	36	42	0.004
Post-bronchodilator FEV1 L	1.45 \pm 0.51	1.37 \pm 0.52	1.33 \pm 0.51	0.003
Post-bronchodilator FVC L	3.20 \pm 0.84	3.05 \pm 0.91	3.01 \pm 0.96	0.005
FEV1 % predicted	51 \pm 15	49 \pm 16	48 \pm 15	0.009
Post-bronchodilator FEV1/FVC %	46 \pm 12	45 \pm 11	45 \pm 11	0.445
BMI kg·m⁻²	27 \pm 5	27 \pm 6	26 \pm 6	0.190
Fat free mass index kg·m⁻²	53 \pm 12	52 \pm 13	50 \pm 13	0.009
6MWD m	395 \pm 116	385 \pm 115	377 \pm 127	0.142
Emphysema by CT (LAA%)	17 \pm 12	17 \pm 12	18 \pm 12	0.486
Oxygen saturation %	94.9 \pm 3.1	94.9 \pm 2.5	94.7 \pm 2.5	0.676
SGRQ total Score	44 \pm 18	47 \pm 18	49 \pm 19	0.002
FACIT fatigue score	37 \pm 10	36 \pm 10	36 \pm 10	0.106
mMRC score	1.4 \pm 1.0	1.6 \pm 1.0	1.7 \pm 1.1	0.006
BODE index	2.6 \pm 1.9	2.9 \pm 2.0	3.2 \pm 2.2	0.001
WBCs $\times 10^9 L^{-1}$	7.5 \pm 2.0	7.9 \pm 2.1	8.1 \pm 2.2	<0.001
Exacerbation rate[#]	0.75 \pm 1.18	0.86 \pm 1.23	0.85 \pm 1.09	0.232
COPD hospitalisation rate[¶]	0.13 \pm 0.47	0.18 \pm 0.53	0.18 \pm 0.57	0.245
Cardiovascular disease	30	33	32	0.679
History of osteoporosis	14	11	11	0.428
Diabetes	11	8	9	0.272
GORD	26	25	27	0.840
Statin use	34	28	27	0.094
Inhaled corticosteroid use	89	92	92	0.362
Oral corticosteroid use	0	1	0	n/a
IL-6 pg·mL⁻¹	1.9 [0.4–4.2]	1.9 [0.4–4.0]	2.2 [0.6–5.1]	0.476
IL-8 pg·mL⁻¹	6.2 [2.8–11.2]	7.0 [3.5–12.4]	8.3 [4.2–14.9]	0.004
Fibrinogen mg·dL⁻¹	443 [384–511]	442 [387–507]	443 [393–510]	0.951
Club cell protein 16 ng·mL⁻¹	5.4 [3.8–7.3]	4.7 [3.4–6.5]	4.7 [3.1–6.4]	<0.001
SP-D ng·mL⁻¹	123 [8–168]	115 [84–162]	122 [87–174]	0.224
CRP mg·L⁻¹	3.0 [1.5–6.1]	3.2 [1.7–7.3]	3.5 [1.6–7.0]	0.147
CCL-18 ng·mL⁻¹	112 [87–140]	102 [79–132]	101 [81–123]	<0.001
Longitudinal Changes				
FEV1 decline mL·year ⁻¹	31 \pm 48	35 \pm 44	30 \pm 42	0.209
COPD exacerbations PPPY ⁺	1.06 \pm 1.18	1.15 \pm 1.27	1.07 \pm 1.31	0.277
COPD hospitalisations PPPY ⁺	0.16 \pm 0.38	0.22 \pm 0.49	0.23 \pm 0.45	0.283
6MWD change over 3 years m	-15 \pm 90	-20 \pm 103	-20 \pm 87	0.626
Emphysema by CT (LAA%) change [§]	1.3 \pm 4.5	1.8 \pm 4.9	2.7 \pm 5.0	0.010
SGRQ total score change ⁺	0.2 \pm 12.5	1.6 \pm 12.8	-1.6 \pm 14.3	0.007

Date are presented as mean \pm SD, % or median (interquartile range), unless otherwise stated. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; BMI: body mass index; 6MWD: 6-min walking distance; CT: computed tomography; LAA%: % low-attenuation areas; SGRQ: St George's Respiratory Questionnaire; FACIT: Functional Assessment of Chronic Illness Therapy; mMRC: modified Medical Research Council; BODE: BMI, airflow obstruction, dyspnoea, exercise capacity; WBCs: white blood cells; COPD: chronic obstructive pulmonary disease; GORD: gastro-oesophageal reflux disease; IL: interleukin; SP-D: surfactant protein D; CRP: C-reactive protein; CCL-18: chemokine ligand 18; PPPY: per person per year; n/a: not applicable. [#]: in the year prior to entry; [¶]: in the prior year; ⁺: over 3 years; [§]: change in LAA% reflects year 3 value minus baseline value.

attributed to the imbalance in current smokers across the groups, as similar patterns were observed when only ex-smokers were analysed (data not shown).

Emphysema progression during follow-up appeared enhanced in subjects with persistent eosinophil counts $< 2\%$. There was an improvement in SGRQ scores in this group that was statistically significant but small in magnitude (1.6 units).

Using an alternative blood eosinophil cut-off level (absolute numbers $\geq 150 \mu L^{-1}$) showed a similar pattern of differences to the 2% eosinophil cut-off. The correlation between eosinophil absolute numbers and

percentages was strong ($\rho=0.92$; $p<0.001$), with 88% concordance between samples classified using 2% and 150 μL^{-1} cut-off values.

An evaluable sputum sample was obtained on at least one visit from 543 different subjects. However, only 138 subjects produced an evaluable sputum count for ≥ 3 visits; five (4%) subjects demonstrated sputum eosinophils $\geq 2\%$ at baseline or year 1 and at every subsequent visit. Blood and sputum eosinophil percentages were moderately, but significantly, correlated (ρ values ranging from 0.24–0.40; $p<0.05$).

Higher sputum and blood eosinophil counts are associated with increased corticosteroid responsiveness in COPD patients [1–4, 8]. The novelty of the current analysis is the evaluation of blood and sputum eosinophils over 3 years to determine the prevalence of persistently raised eosinophils in COPD subjects. A significant proportion of COPD subjects in ECLIPSE (37.4%) had peripheral blood eosinophil counts persistently $\geq 2\%$ over 3 years. Similar findings were observed in healthy controls in ECLIPSE. Repeated induced sputum sampling was less successful in identifying persistently eosinophilic subjects, due to practical difficulties in obtaining sufficient repeated samples.

Subjects with blood eosinophil counts persistently $\geq 2\%$ had some evidence of better clinical characteristics at baseline, including higher FEV₁, and lower SGRQ and mMRC scores. However, the difference between groups in FEV₁ % predicted was small ($\sim 3\%$), and, therefore, of debateable clinical relevance. Overall, these data provide evidence suggesting that subjects with blood eosinophils persistently $>2\%$ have less severe COPD, but this requires confirmation in other cohorts.

There was an increased rate of emphysema progression in subjects with eosinophil counts persistently $<2\%$. This finding is biologically plausible, as lower eosinophil numbers in this group implicate other immune cell types in disease pathophysiology, such as neutrophils which are known to cause emphysema. However, there was no difference in emphysema scores at baseline, arguing against the association between blood eosinophil counts and emphysema.

The proportion of COPD and healthy control subjects with blood eosinophils persistently $>2\%$ was similar, suggesting normal eosinophil recruitment into the blood in COPD. However, lung eosinophil numbers are increased in COPD subjects compared with controls [12], indicating altered eosinophil recruitment into the pulmonary compartment in COPD. We observed a weak correlation between sputum and blood eosinophil counts. Perhaps eosinophils persistently $>2\%$ in COPD patients' blood is a simple predictor of more lung eosinophils, and hence increased likelihood of corticosteroid responsiveness. Targeted therapies against eosinophilic inflammation, such as anti-interleukin-5, may have greater benefits in COPD patients with persistent blood eosinophilia [13].

In summary, we show that 37% of COPD patients have blood eosinophil counts persistently $\geq 2\%$. Previous studies suggest that higher eosinophil levels in COPD are associated with increased corticosteroid responsiveness [1–5, 7, 8]. Blood eosinophil measurements may be useful for selecting patients for different therapeutic approaches.



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Blood eosinophil measurements may be useful for selecting COPD patients for different therapeutic approaches <http://ow.ly/BY19D>

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