Prevalence of persistent blood eosinophilia: relation to outcomes in patients with COPD

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The stability of blood eosinophils ⩾300 cells per µL is low in COPD patients and it does not confer a poor prognosis http://ow.ly/TwGX30etVIy

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ABSTRACT The impact of blood eosinophilia in chronic obstructive pulmonary disease (COPD) remains controversial.

To evaluate the prevalence and stability of a high level of blood eosinophils (⩾300 cells·µL^{-1}) and its relationship to outcomes, we determined blood eosinophils at baseline and over 2 years in 424 COPD patients (forced expiratory volume in 1 s (FEV1) 60% predicted) and 67 smokers without COPD from the CHAIN cohort, and in 308 COPD patients (FEV1 60% predicted) in the BODE cohort. We related eosinophil levels to exacerbations and survival using Cox hazard analysis.

In COPD patients, 15.8% in the CHAIN cohort and 12.3% in the BODE cohort had persistently elevated blood eosinophils at all three visits. A significant proportion (43.8%) of patients had counts that oscillated above and below the cut-off points, while the rest had persistent eosinophil levels <300 cells·µL^{-1}. A similar eosinophil blood pattern was observed in controls. Exacerbation rates did not differ in patients with and without eosinophilia. All-cause mortality was lower in patients with high eosinophils compared with those with values <300 cells·µL^{-1} (15.8% *versus* 33.7%; p=0.026).

In patients with COPD, blood eosinophils ⩾300 cells·µL^{-1} persisting over 2 years was not a risk factor for COPD exacerbations. High eosinophil count was associated with better survival.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a complex and heterogeneous disease [1]. It has been proposed that the identification of clinical phenotypes using validated biomarkers may promote the development of targeted treatment strategies directed towards specific biological pathways [2, 3].

In patients with COPD, eosinophilic airway inflammation has been described during stable disease and exacerbations even after the exclusion of patients with any features of asthma [4, 5]. In patients with stable COPD, there is a correlation between blood and sputum eosinophils that is modest; however, blood eosinophil levels have been used as a surrogate maker for airway eosinophils in these patients [6, 7].

Recently, several *post hoc* pharmacological trials have shown that patients with higher blood eosinophil counts had a greater reduction in exacerbation rates with inhaled corticosteroid (ICS) therapy [8–12]. These results suggested that a high blood eosinophil count could represent a simple biomarker in clinical decision making. However, it is unclear what would be the threshold of blood eosinophils that might be relevant. Although some studies have observed a potential biomarker role for blood eosinophils using a low threshold level (similar to the normal range of healthy subjects of $\geqslant$150 cells·μL^{-1}), other studies have only detected potential differences with higher circulating blood values ($\geqslant$280–300 cells·μL^{-1}) [8, 12]. The analysis of two observational cohorts supports the argument that relatively low blood eosinophil counts $\geqslant$150 cells·μL^{-1} are not associated with poor outcomes in COPD [6, 13] and the prevalence of blood eosinophil levels in patients was similar to that observed in healthy subjects [6]. Importantly, all studies used a single cross-sectional measurement taken at baseline without subsequent validation, a concept that is in disagreement with the need to document persistently elevated values to confirm the diagnosis of blood eosinophilia [14].

Given the limited information about the prevalence of persistently elevated eosinophils in patients with COPD and smokers without COPD, and the relationship between persistently elevated eosinophil counts and outcomes in those patients, we assessed blood eosinophil count at baseline and longitudinally (annually over 2 years) in patients participating in the CHAIN (COPD History Assessment In SpaiN) and BODE (body mass index (B), degree of airflow obstruction (O), functional dyspnoea (D) and exercise capacity (E)) cohorts. We hypothesised that persistently elevated blood eosinophil levels would relate to exacerbations, hospitalisations and survival.

Methods

Subjects

CHAIN cohort

CHAIN is an ongoing observational study of 24 COPD cohorts enrolled in Spain [15]. COPD was defined by a smoking history $\geqslant$10 pack-years and a post-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7 after 400 µg albuterol. Patients were stable for at least 6 weeks and received optimal medical therapy. Exclusion criteria were uncontrolled comorbidities such as malignancy or other confounding diseases that could interfere with the study. The recruitment period was January 2010 to March 2012 (ClinicalTrials.gov: identifier NCT01122758). Data analysed in the present study came from the recruitment date up to December 15, 2015. Data were anonymised with hierarchical access

control in order to guarantee that information was secure. All participants signed the informed consent approved by the ethics committees (Comité de Etica de la Investigación, Hospital Universitario la Candelaria, Tenerife; 258/2009).

BODE cohort

The study comprised most of the original 625 patients recruited for the BODE study between July 1997 and December 2011 and followed until December 2015 in the USA and Spain. The study was approved by the human review board at each site and all patients provided informed consent. The definition of COPD was similar to that of CHAIN, as published previously [16]. All patients were clinically stable and receiving therapy according to international guidelines [1].

Patients were not included in the study if they had a history of asthma or were being treated for asthma at the time of the clinical evaluation. In addition, patients were not included if they had >15% increase in their baseline FEV_1 after administration of albuterol. No selection was made based on any other biological marker, including eosinophil count.

Blood eosinophils

All participants had a minimum of three blood eosinophil measurements separated by at least 1 year. Blood eosinophils were measured using automated blood count analysis and were reported as cells per microlitre. A threshold value of 300 cells·μL^{-1} was used to define blood eosinophilia, based on the relationship between that level and poor outcomes in cohort [13] and clinical studies [8, 12]. In addition, we quantified the levels of IgE in patients in the CHAIN cohort.

Based on the eosinophil count, the cohort was divided into three subgroups: patients with a persistently high blood eosinophil count ($\geqslant$300 cells·μL^{-1}) in the three measurements, patients with an intermittently variable eosinophil count (fluctuating between $\geqslant$300 and <300 cells·μL^{-1}) in the three measurements and patients with a persistently low eosinophil count (<300 cells·μL^{-1}) in the three measurements.

Clinical and physiological measurements

Trained staff obtained information on age, sex and body mass index at baseline and subsequent visits. A questionnaire was used to determine smoking status (current or ex-smoker) and smoking history (in pack-years).

Pulmonary function tests were performed following the American Thoracic Society guidelines [17]. Transfer coefficient of the lung for carbon monoxide was determined with the single-breath technique following international guidelines [18]. Arterial blood gases were measured while sitting and breathing room air. The 6-min walk distance (6MWD) was measured as the better of two walks separated by at least 30 min [19]. Dyspnoea was evaluated with the modified Medical Research Council (mMRC) scale [20]. FEV_1, body mass index, 6MWD and mMRC values were integrated into the BODE index [16]. In CHAIN, the COPD Assessment Test questionnaire was self-administered with the supervision of the interviewer. Comorbidities were scored using the Charlson index [21].

Outcomes

Exacerbations were defined as a worsening of respiratory symptoms (dyspnoea, cough or sputum) that required the use of antibiotics, systemic corticosteroids or both, or necessitated emergency room visit or hospital admission [1]. Exacerbations were evaluated only in the CHAIN cohort.

All-cause mortality was recorded using information obtained from the family and then confirmed by reviewing medical records, as published previously [16].

Statistical analysis

Data are summarised as frequency for categorical variables, median (5th–95th percentile) for ordinal or nonnormal scale variables and mean±SD for normally distributed scale variables. Comparisons were made between groups using Pearson's Chi-squared test, the Kruskal–Wallis H-test or the Mann–Whitney U-test and one-way ANOVA or the t-test as appropriate. Kaplan–Meier analysis for survival due to all causes was performed with an eosinophil value of 300 cells·μL^{-1}. Finally, to predict the risk of death we performed Cox proportional hazard regression analyses with the same eosinophil threshold. Significance was established as two-tailed $p<0.05$. Calculations were performed using SPSS version 21.0 (IBM, Armonk, NY, USA).

Results

Characteristics of the participants

We identified 424 COPD patients and 67 smokers without COPD from the CHAIN cohort and 308 COPD patients from the BODE cohort with three eosinophil measurements over 2 years (figure 1).

The baseline characteristics for the CHAIN patients are shown in table 1. There were no differences between groups except for the IgE levels, which were higher in patients with persistently elevated eosinophil counts. The population included a broad range of airflow limitation and consisted mostly of men. A similar proportion of patients used inhaled anticholinergics, β_2-agonists and ICSs in both groups. On average, the patients reported relatively few symptoms, and scored low in the BODE index and Charlson index.

Table 1 also shows the baseline characteristics of the BODE cohort patients. There were no differences in any of the clinical and physiological variables in patients with and without persistently elevated blood eosinophils.

Prevalence and longitudinal follow-up of blood eosinophil levels

The distribution of blood eosinophil count was similar in smokers with and without COPD in the CHAIN cohort (figure 2a). Over time, the proportion of COPD patients and controls with a persistent blood eosinophil count $\geqslant$300 cells·μL^{-1} was similar (15.8 and 14.9%, respectively) (figure 2b). The same distribution and persistence was observed using thresholds of 150 or 350 cells·μL^{-1} (supplementary table S1). This analysis was not performed in the BODE cohort because there were no control subjects in this cohort. However, the proportion of patients who remained with high levels of blood eosinophils between the first and third measurements was similar in the CHAIN and BODE cohorts (45.6% *versus* 46.3%, respectively) (figure 1). Therapy with ICSs did not influence these results as the proportion of users of ICSs was similar in patients with persistently high, intermittently variable or persistently low blood eosinophils (supplementary table S2).

Outcomes in patients with COPD

There were no significant differences in baseline clinical and physiological characteristics between the COPD patients with persistently high blood eosinophil counts ($\geqslant$300 cells·μL^{-1}), compared with those with persistently low or intermittently variable eosinophil counts (tables 2 and 3). We observed similar results when we used different thresholds of $\geqslant$250 and 350 cells·μL^{-1} (data not shown).

In the CHAIN cohort, the median follow-up time after the eosinophils longitudinal pattern was established was 17 (14–22) months. During the first 12 months of follow-up, the proportions of patients with one or more moderate exacerbations and the distribution for persistently low, intermittently variable and persistently high blood eosinophil patterns were 53 (30.1%), 31 (24.2%) and 29 (22.8%), respectively (p=0.302). Among the exacerbators, only 39 (9%) patients had two or more moderate exacerbations. The proportion of patients hospitalised was 7.4% for the persistently low eosinophil group, 6.3% for the intermittently variable eosinophil group and 2.4% for the persistently high eosinophil group (p=0.157).

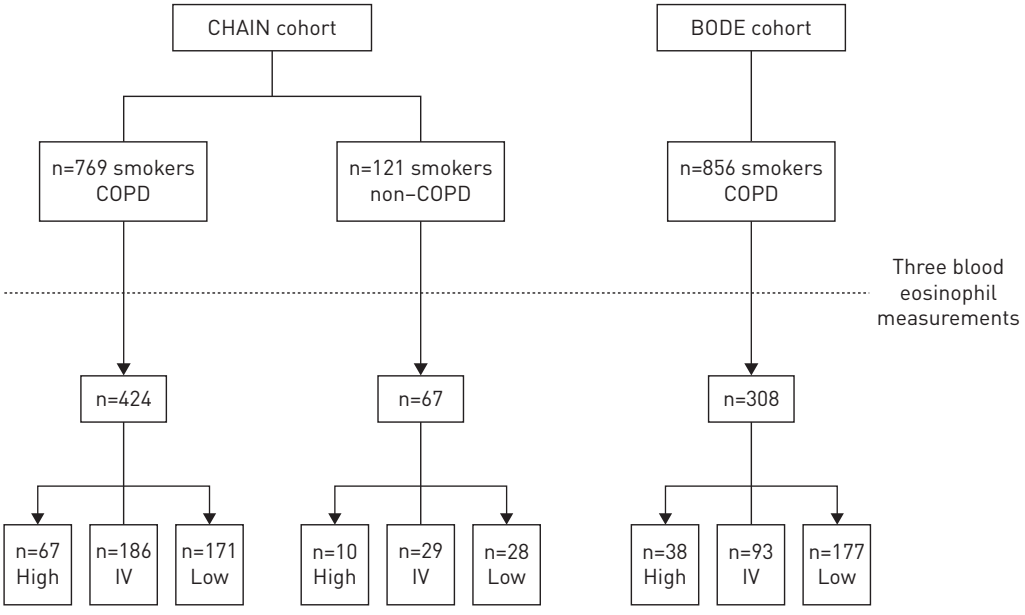

FIGURE 1 Flow diagram of subjects from the CHAIN and BODE cohorts. COPD: chronic obstructive pulmonary disease; high: persistently high eosinophil count ($\geqslant$300 cells·μL^{-1}); IV: intermittently variable eosinophil count (fluctuating between $\geqslant$300 and <300 cells·μL^{-1}); low: persistently low eosinophil count (<300 cells·μL^{-1}).

There were 37 (8.7%) deaths, of which 17 (9.9%) had a persistently low eosinophil count, 17 (9.1%) had an intermittently variable eosinophil count and three (4.5%) had a persistently high eosinophil count.

In the BODE cohort, the median follow-up time after the three measurements of eosinophils was 130 (91–170) months. During this period, there were 97 deaths: 62 (34.4%) in patients with a persistently low eosinophil count, 29 (30.5%) in patients with an intermittently variable eosinophil count and six (15.4%)

TABLE 1 Clinical and physiological characteristics at baseline of the chronic obstructive pulmonary disease (COPD) patients (patients with at least three blood eosinophils measurements were classified using ⩾300 cells·μL^{-1} as the cut-off point)

	Eosinophils ⩾300 cells·μL^{-1}	Eosinophils <300 cells·μL^{-1}	p-value
CHAIN cohort			
Patients n	147	277	
Male	125 (85)	230 (83)	0.595
Age year	67±9	67±9	0.715
Pack-years	53±28	58±30	0.142
Active smoking	32	26	0.192
BMI kg·m^{-2}	28.9±7.4	28.1±4.7	0.171
FEV_1 L	1.68±0.65	1.68±0.63	0.920
FEV_1 % pred	60±19	60±19	0.517
P_{aO_2} mmHg	68.9±8.1	68.0±9.4	0.443
FVC L	3.14±0.91	3.16±0.94	0.796
FVC % pred	85±23	87±23	0.404
FEV_1/FVC	53±11	53±10	0.663
6MWD m	444±98	445±92	0.732
Dyspnoea mMRC	1 (0–3)	1 (0–3)	0.682
BODE index	2 (0–6)	1 (0–5)	0.396
IC/TLC	0.35±0.09	0.35±0.09	0.994
K_{CO} % pred	78±24	73±24	0.102
Charlson index	1 (0–5)	1 (0–4)	0.105
Inhaled anticholinergic	70	73	0.548
Inhaled β_2-agonist	76	75	0.334
Inhaled corticosteroid	66	68	0.472
CAT	11 (2–27)	10 (2–26)	0.305
History of asthma	2.5	3.1	0.755
IgE total U·L^{-1}	57 (9–1121)	36 (6–506)	0.003
BODE cohort			
Patients n	82	226	
Male	70 (85)	180 (80)	0.256
Age years	65±9	65±9	0.611
Pack-years	60±31	61±33	0.876
Active smoking	34	31	0.597
BMI kg·m^{-2}	27.1±5.3	27.1±5.1	0.988
FEV_1 L	1.58±0.59	1.54±0.64	0.555
FEV_1 % pred	61±20	59±22	0.899
FVC L	2.92±0.71	3.01±0.84	0.478
FVC % pred	83±19	85±24	0.342
FEV_1/FVC	53±13	50±13	0.071
6MWD m	391±134	409±119	0.269
Dyspnoea mMRC	1 (0–4)	1 (0–3)	0.066
BODE index	1 (0–5)	1 (0–6)	0.116
IC/TLC	0.34±0.06	0.33±0.11	0.976
K_{CO} % pred	74±22	73±20	0.510
Charlson index	2 (0–6)	1 (0–4)	0.216
Inhaled anticholinergic	62	64	0.760
Inhaled β_2-agonist	62	63	0.824
Inhaled corticosteroid	59	57	0.773

Data presented as n (%), mean±SD, % or median (5th–95th percentile), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; P_{aO_2}: arterial oxygen tension; FVC: forced vital capacity; 6MWD: 6-min walk distance; mMRC: modified Medical Research Council; IC/TLC: inspiratory capacity/total lung capacity; K_{CO}: transfer coefficient of the lung for carbon monoxide; CAT: COPD Assessment Test.

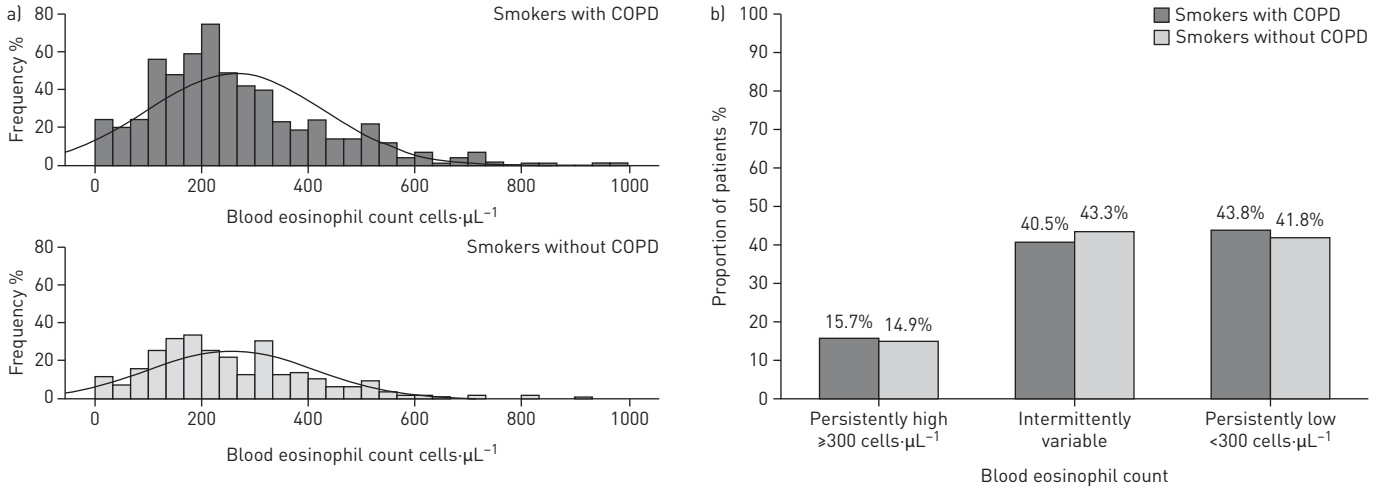

FIGURE 2 a) Distribution of blood eosinophil levels in smoker subjects with and without chronic obstructive pulmonary disease (COPD) in the CHAIN cohort at baseline. b) Longitudinal distribution of blood eosinophil levels in smoker subjects with and without COPD in the CHAIN cohort.

in patients with a persistently high eosinophil count (p=0.06) (figure 3). The statistical difference increased if the analysis performed compared patients with persistently high eosinophils *versus* the rest (15.8% *versus* 33.7%; p=0.026). When the analysis was repeated using ⩾250 cells·μL^{-1} as the threshold, the difference persisted (14% *versus* 34.3%; p=0.008). Once the eosinophil threshold was raised to

TABLE 2 Baseline characteristics of blood eosinophil longitudinal patterns in patients with chronic obstructive pulmonary disease (COPD) (CHAIN cohort) using ⩾300 cells·μL^{-1} as the cut-off point

	Persistently high ⩾300 cells·μL^{-1}	Intermittently variable	Persistently low <300 cells·μL^{-1}	p-value
Patients n	67	186	171	
Male	60 (90)	156 (84)	139 (81)	0.298
Age years	66±8	68±9	67±9	0.148
Pack-years	55±29	55±28	57±31	0.779
Active smoking	31	29	27	0.729
BMI kg·m^{-2}	29.8±5.5	28.2±4.9	28.2±4.7	0.114
FEV$_1$ L	1.80±0.63	1.63±0.64	1.69±0.62	0.150
FEV$_1$ % pred	61±18	60±20	60±19	0.949
***P*aO_2 mmHg**	69.6±7.4	67.5±9.0	68.9±9.3	0.446
FVC L	3.37±0.94	3.08±0.92	3.15±0.93	0.094
FVC % pred	87±22	86±24	86±22	0.974
FEV$_1$/FVC	54±11	53±11	54±10	0.704
6MWD m	461±95	438±99	451±89	0.175
Dyspnoea mMRC	1 (0–3)	1 (0–4)	1 (0–3)	0.212
BODE index	1 (0–5)	2 (0–6)	1 (0–5)	0.346
IC/TLC	0.36±0.09	0.34±0.09	0.35±0.09	0.091
***K*co % pred**	82±25	73±23	74±24	0.059
Charlson index	1 (0–5)	1 (0–4)	1 (0–4)	0.158
Inhaled anticholinergic	64	76	71	0.172
Inhaled β_2-agonist	73	75	70	0.483
Inhaled corticosteroid	64	68	59	0.233
CAT	11 (1–27)	12 (2–27)	10 (2–24)	0.297
IgE total U·L^{-1}	50 (8–1158)	62 (7–690)	26 (5–504)	<0.001

Data presented as n (%), mean±SD, % or median (5th–95th percentile), unless otherwise stated. BMI: body mass index; FEV$_1$: forced expiratory volume in 1 s; *P*aO_2: arterial oxygen tension; FVC: forced vital capacity; 6MWD: 6-min walk distance; mMRC: modified Medical Research Council; IC/TLC: inspiratory capacity/total lung capacity; *K*CO: transfer coefficient of the lung for carbon monoxide; CAT: COPD Assessment Test.

TABLE 3 Baseline characteristics of blood eosinophil longitudinal patterns in patients with chronic obstructive pulmonary disease (BODE cohort) using ⩾300 cells·μL^{-1} as the cut-off point

	Persistently high ⩾300 cells·μL^{-1}	Intermittently variable	Persistently low <300 cells·μL^{-1}	p-value
Patients n	38	93	177	
Male	30 (79)	76 (82)	143 (81)	0.930
Age years	63±8	66±9	65±9	0.386
Pack-years	64±33	59±30	61±33	0.701
Active smoking	42	33	29	0.298
BMI kg·m^{-2}	28.2±6.1	28.2±4.9	27.8±4.7	0.655
FEV$_1$ L	1.58±0.53	1.52±0.56	1.56±0.68	0.877
FEV$_1$ % pred	60±18	60±22	59±22	0.959
FVC L	2.92±0.76	3.02±0.80	2.98±0.85	0.839
FVC % pred	84±18	84±22	85±24	0.874
FEV$_1$/FVC	53±12	51±13	50±13	0.289
6MWD m	397±131	379±132	418±115	0.058
Dyspnoea mMRC	1 (0–4)	1 (0–4)	1 (0–3)	0.340
BODE index	1 (0–7)	1 (0–6)	1 (0–6)	0.384
IC/TLC	0.34±0.05	0.34±0.11	0.33±0.11	0.913
***K*CO % pred**	74±23	74±20	72±20	0.718
Charlson index	2 (0–4)	1 (0–5)	1 (0–4)	0.624
Inhaled anticholinergic	60	65	65	0.853
Inhaled β_2-agonist	65	58	65	0.529
Inhaled corticosteroid	65	55	58	0.563

Data presented as n (%), mean±SD, % or median (5th–95th percentile), unless otherwise stated. BMI: body mass index; FEV$_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; 6MWD: 6-min walk distance; mMRC: modified Medical Research Council; IC/TLC: inspiratory capacity/total lung capacity; *K*CO: transfer coefficient of the lung for carbon monoxide.

350 cells·μL^{-1}, the proportion was similar (14.3% *versus* 32.3%), but it was no longer significant (p=0.239) due to the low number of patients above this threshold.

The Kaplan–Meier analysis for all-cause mortality showed that an eosinophil count <300 cells·μL^{-1} was associated with a shorter survival time (174 months, 95% CI 165–183) than an eosinophil count ⩾300 cells·μL^{-1} (201 months, 95% CI 183–219) (p=0.037) (figure 4). Using Cox regression analysis with eosinophils <300 cells·μL^{-1} as the cut-off value, the hazard ratio for all-cause mortality was 2.344 (95% CI 1.026–5.355; p=0.043), and 7.287 (95% CI 1.004–52.891) (p=0.050) after adjusting for sex, age, body mass index, FEV$_1$ % pred and Charlson index (table 4).

Discussion

This longitudinal study of blood eosinophil levels in two observational cohorts of COPD patients attending pulmonary clinics and a group of smokers without COPD as controls yielded several important

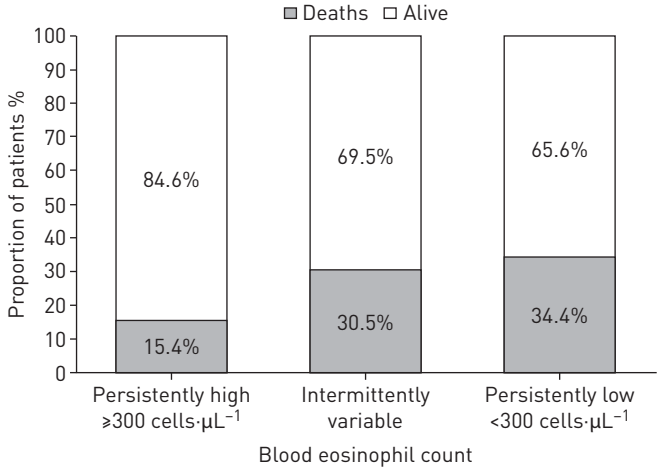

FIGURE 3 Blood eosinophil longitudinal pattern and mortality in the BODE cohort.

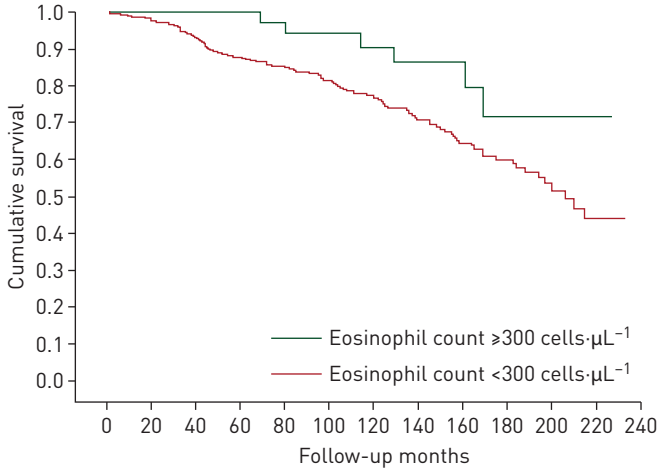

FIGURE 4 Kaplan–Meier analysis for all-cause mortality. An eosinophil count <300 cells·μL^{-1} was associated with a shorter survival time than an eosinophil count ⩾300 cells·μL^{-1} ($p_{log\text{-}rank}$=0.037) in the BODE cohort.

findings. First, the pattern and distribution of eosinophils over time were similar in patients with COPD and smoking subjects without disease. The proportion of patients and smoker subjects without COPD with persistent high blood eosinophils (⩾300 cells·μL^{-1}) was ~15% in both groups. Second, in the COPD population, there was an important annual variability in the eosinophil count. Third, there were no significant differences in clinical and physiological characteristics among the patients with and without persistent blood eosinophilia. A persistently high blood eosinophil level (⩾300 cells·μL^{-1}) was not associated with an increased exacerbation rate, but rather with a lower risk of death.

The identification of biomarkers in COPD has generated interest in an attempt to develop personalised management of this disease. So far, no biomarker alone or in combination has proven reliable enough to use in clinical practice [22]. Recently, circulating eosinophils have emerged as a potential biomarker in selected patients, based on the observation that the level of a single blood eosinophil count appears to be associated with subsequent risk for exacerbation and to predict the response to ICS therapy in clinical trials [8–12]. This is in contrast to the findings of the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points) observational study which found that 36% of the patients had blood eosinophil counts persistently ⩾2%, and these patients had higher FEV_1, and lower St George's Respiratory Questionnaire and mMRC scores. Interestingly, the proportion of patients ⩾2% was similar to that seen in the healthy subjects in the same cohort [6]. The stratification of patients using 2% and ⩾150 cells·μL^{-1} cut-off values has been justified because this threshold seemed to identify patients more likely to exacerbate in the *post hoc* analysis of clinical trials [5, 9–11, 23].

Prevalence and longitudinal levels of eosinophils

We selected the level of 300 cells·μL^{-1} to anchor this study because cohort reports [13, 24] and analysis from trials [8, 12] and asthma–COPD overlap consensus [25] support the use of higher thresholds in patients with COPD. We found that blood eosinophil count at baseline and its longitudinal variability over 2 years were similar between smokers with and without COPD (figure 2a and b). As a healthy control population was not included in the design of CHAIN, we cannot interpret whether the eosinophilia observed is associated with smoking itself, but our results suggest that the circulating levels and

TABLE 4 Cox regression analysis[#] for risk of death in patients with chronic obstructive pulmonary disease with eosinophils (300 cells·μL^{-1} cut-off value), adjusted for longitudinal eosinophil pattern, age, sex, body mass index (BMI), forced expiratory volume in 1 s (FEV_1) % pred and Charlson index

	Hazard ratio (95% CI)	p-value
Eosinophils <300 cells·μL^{-1}[¶]	7.287 (1.004–52.891)	0.050
Age (each 1 year more)	1.073 (1.043–1.105)	<0.001
BMI (each 1 kg·m^{-2} lower)	1.100 (1.038–1.162)	0.001
FEV_1 % pred (each 1% lower)	1.015 (1.003–1.029)	0.017

[#]: backward stepwise method and Wald's criteria/three iterations; [¶]: persistently lower eosinophil pattern.

distribution of eosinophils are not associated with the diagnosis of COPD. Furthermore, using a threshold of eosinophilia at 150 cells·μL^{-1} did not change the results reported (supplementary table S2).

The few studies that have evaluated the levels of eosinophils in COPD patients over time have observed significant instability [24, 26]. In our study, only a small proportion (15%) of the patients had persistently high eosinophils over the period of observation, while 41% of the patients fluctuated above and below this threshold, a proportion that is relatively high to use as a single measurement of eosinophils as a clinical biomarker. Indeed, experts in the field of eosinophilic syndromes require repeated elevated measurements over 6 months to meet the criteria for the diagnosis of eosinophilic diseases [14]. Unlike Oshagbemi *et al.* [26], we did not observe differences with the control group of smokers without COPD (figure 2b). The differences between study results could be explained by the fact that the report by Oshagbemi *et al.* [26] was population-based (diagnosis of COPD without spirometry) and 50% of the subjects were never-smokers.

Eosinophils and outcomes in COPD

The lung function tests, arterial blood gases, 6MWD, health status, perception of dyspnoea, comorbidities and BODE index were similar in the patients irrespective of eosinophil level or longitudinal eosinophil level behaviour (tables 1–3). After the observation period used to establish the stability of the signal, there were no differences among the groups in exacerbations or hospitalisation rates due to exacerbations in the subsequent median period of 12 months of follow-up. This was not due to the potential differential effect of uneven use of medication, because the proportion of medications documented was similar in all groups (table 2). In addition, it has been shown that the use of ICSs does not affect blood levels of eosinophils at the pharmacological doses used [27, 28]. Furthermore, in the CHAIN and BODE cohorts, the prevalence of ICS use was similar in patients with persistently high, intermittently variable or persistently low eosinophil counts (supplementary table S2), thus negating an effect of use of ICSs on the findings reported here. The lack of a relationship between eosinophil level and outcomes is similar to the results reported by Zysman *et al.* [29], who followed 458 patients with COPD for an average of 3 years. They observed no difference in exacerbation rates of asthma or COPD exacerbations irrespective of the threshold of eosinophils used for the analysis.

The longitudinal observation of patients in the BODE cohort allowed us to evaluate the relationship between eosinophil levels and survival. To our surprise, patients with persistently elevated eosinophils over 2 years had a significantly lower risk of death than those whose levels were lower than the predetermined threshold of 300 cells·μL^{-1}. We repeated the analysis using thresholds of 250 and 350 cells·μL^{-1} and the findings remained unchanged, providing evidence that this was not due to the arbitrary threshold chosen. These findings are in agreement with those of Suzuki *et al.* [24], who reported that COPD patients without clinically diagnosed asthma who had two or more asthma-like features had better outcome and lower mortality than those without that asthma phenotype. In the Suzuki *et al.* [24] study, blood eosinophils $\geqslant$300 cells·μL^{-1} by itself failed to reach statistical significance, likely due to the small sample size of that cohort. Our study, larger in size, was capable of detecting a significant signal. There is no easy explanation for this finding, but a recent *post hoc* meta-analysis showed that patients with COPD with lower blood eosinophil counts (<2%) had more pneumonias than those with higher counts [30]. Together, this information suggests that caution should be taken to determine the appropriate pathological threshold of eosinophils in COPD before we use the eosinophil count as a biomarker of poor prognosis and/or for specific therapeutic interventions.

This study has several strengths. It included a large number of well-characterised patients being treated for COPD in pulmonary clinics in two countries and its "real-life" nature allowed the sequential measurement of blood counts in regular laboratories, as is available to clinicians. Finally, the long follow-up time provides invaluable information on outcomes, not usually available in most pharmacological trials. Our study also had several limitations. First, the CHAIN and BODE cohorts are observational studies not constituted of patients recruited from a general medical practice or a population-based study. Therefore, they may not represent the distribution of COPD severity in the general population. However, both cohorts included a broad range of disease severity. Second, most of the participants were men, although over 130 women were included in the analysis and the distribution of women was similar in the different eosinophil longitudinal patterns. Third, the lack of a healthy (nonsmoker) control group did not allow us to evaluate if blood eosinophils could be related to the smoking habit itself. However, this was not the main objective of this study. Finally, the BODE cohort did not include repeated eosinophil blood measurements in all of the patients recruited. However, this cohort is large and well characterised, recruited by a team with experience in COPD and a long-term follow-up providing an adequate number of patients with three measurements and enough deaths to enhance the mortality analysis.

In summary, data on two large cohorts of well-characterised COPD patients show that in three measurements of blood eosinophils measured over 2 years, there is important variability in blood levels of

eosinophils, with only 15% of patients showing a persistently high blood eosinophil count (⩾300 cells·μL^{-1}). The prevalence and stability is similar to that seen in smokers without COPD. A persistent high eosinophil level did not confer an increased risk of exacerbations or hospitalisations in the patients with COPD, but was rather associated with a survival benefit. Further studies in other populations should validate our results.

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