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Blood Eosinophil Count and Exacerbation Risk in Patients with COPD

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'Take home' message: Blood eosinophil counts may serve as a biomarker of exacerbation risk in subgroups of patients with COPD.

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ABSTRACT

Blood eosinophil counts are a promising biomarker to guide therapy for chronic obstructive pulmonary disease (COPD). We examined the association between blood eosinophil counts in stable COPD and exacerbations in the following year in real-life patients, and assessed whether the relationship differs in specific patient subgroups.

We conducted a historical follow-up study using anonymized medical records for patients ≥40 years with spirometrically confirmed COPD and a smoking history. We estimated the association between eosinophil counts and the number of exacerbations during 1 follow-up year, adjusted for confounders assessed in the preceding year.

Of 8,318 patients with COPD (56% male; mean [SD] age 70 [10] years), 87.7% had eosinophil counts ranging from 0.05 to $<0.45 \times 10^9$/L (reference), 8.9% had elevated counts ($\geq 0.45 \times 10^9$/L), and 3.4% low counts ($<0.05 \times 10^9$/L). Patients with elevated counts had an overall 13% higher rate of exacerbations during follow-up than the reference group (RR 1.13; 95% CI 1.01–1.26). This increased exacerbation rate was restricted to ex-smokers (RR 1.32; 1.15–1.51) and not found in current smokers (RR 0.86; 0.71–1.05). Low counts were not associated with exacerbation rates.

Elevated blood eosinophil counts may potentially serve as a biomarker of COPD exacerbation risk, yet only in some subgroups of COPD.

Study registration: European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP, study no. 4922)

Key words: Chronic obstructive pulmonary disease, exacerbation, eosinopenia, eosinophilia, biomarker, eosinophils

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a complex and heterogeneous disease, therefore non-invasive reliable biomarkers are needed to stratify patients in subgroups with specific risk of adverse outcomes and therapeutic requirements [1]. Sputum eosinophilia has been reported in 10–40% of patients with COPD [2-5] and is associated with greater corticosteroid responsiveness [2, 3, 6]. Moreover, ≥3% eosinophils in induced sputum predicts an increased exacerbation risk after withdrawal of inhaled corticosteroids (ICS) in COPD [7]. However, measuring sputum eosinophils is not routinely available in practice, is time consuming, and requires relatively advanced laboratory techniques [8]. Because there is some evidence of concordance between blood and sputum eosinophil counts both in stable COPD [9] and during an exacerbation [10], blood eosinophil counts, as easily measured in general practice, have been considered a promising alternative to identify patients with COPD who respond to treatment with ICS [11].

Post-hoc analyses from randomized controlled trials (RCTs) suggest that elevated blood eosinophil counts in stable COPD predict positive response to maintenance ICS [12, 13]. Low eosinophil counts are considered a clinical marker of acute infection and a predictor of mortality in patients hospitalized with a COPD exacerbation [14, 15]. However, the association between blood eosinophil counts measured at stable disease and COPD exacerbations in the subsequent year is not fully understood. A recent general population study [16] showed only a relevant

increased risk of moderate exacerbations with elevated blood eosinophils in patients with more severe airflow limitation but did not investigate the role of other factors that may affect this association. For instance, active cigarette smoking has been reported to reduce lung and systemic eosinophil counts in experimental conditions [17]. Moreover, sex-related differences in eosinophil counts have not been studied in COPD, although healthy men have higher eosinophil counts than healthy women [18], possibly because of differing biological and sociocultural factors [19, 20] and differing type of inflammatory response elicited by tobacco smoking [21]. Furthermore, disease severity should be taken into account, given that the numbers of inflammatory cells in the airways seem to increase in proportion to the severity of airflow limitation in COPD [5].

Based on these areas of uncertainty, we performed a historical follow-up study using longitudinal medical record data from large electronic databases in the United Kingdom (UK). Specifically, we first evaluated the potential of blood eosinophil counts as a biomarker of exacerbation risk by assessing the association between blood eosinophil levels measured in stable COPD and the number of exacerbations in the subsequent year, in real-life COPD patients. Then, we investigated whether the association significantly differed in specific patient subgroups defined by smoking status, sex, disease severity (as defined by the four GOLD groups, A to D), or ICS treatment. Some results from this study have been previously reported in the form of an abstract [22].

METHODS

Data Source

This historical follow-up study analyzed data recorded from March 1994 to February 2014 in the Optimum Patient Care Research Database (OPCRD)[23]. The OPCRD is a bespoke database that contains anonymous, longitudinal data, including routine medical record data extracted from primary care practices for chronic respiratory service reviews, as well as patient-reported outcomes collected via questionnaires. At the time of data extraction, it contained data from over 400 UK primary care practices across England, Scotland, Wales, and Northern Ireland.

Study Design

We evaluated patient characteristics during 1 baseline year before the index date – defined as the date of the most recent eosinophil count at stable COPD – and study outcomes during 1 follow-up year after the index date.

Patients

Eligible patients were aged ≥40 years, had a recorded diagnosis code for COPD, no other chronic respiratory disease, and two or more valid blood eosinophil counts recorded during the registration period (ever-recorded), with at least one measured at stable disease. Stable disease was defined as no COPD exacerbations within 4 weeks before and after the index date. The most recent eosinophil count at stable COPD (defined as being on the index date) was used in the analyses. Other eligibility criteria were a forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) <0.70 recorded within 5 years of index date, a history of cigarette smoking (both current smokers and ex-smokers were included) and ≥1 year of data before and after the index date.

In addition, in order to capture the broad spectrum of patients with COPD seen by clinicians in primary care, we performed secondary analyses on a broader population called 'full population'. This additionally included patients with a COPD diagnosis who had FEV_1/FVC ratio <0.70 recorded more than 5 years prior to the index date, patients with asthma-related Read codes (see Supplement for complete definition) ever-recorded, and patients without a history of smoking (Figure E1). Results of these secondary analyses are reported in the online Supplement.

Study outcomes and definitions

The study outcome was the number of COPD exacerbations in the follow-up year. We defined COPD exacerbations as any of the following: unscheduled hospital admission or emergency department (ED) attendance, and/or an acute course of oral corticosteroids (OCS), and/or antibiotics prescribed at a lower respiratory consultation (see Supplement for complete definition).

Eosinophil counts were recorded at 10^9 cells per liter (L) with 1 decimal place accuracy in most of the practices. Eosinophil counts ranging from 0.05 to $<0.45 \times 10^9$/L were defined as the reference category, low counts were defined as $<0.05 \times 10^9$/L, and elevated counts as $\geq 0.45 \times 10^9$/L. We chose these eosinophil cut-points based on the reference values (0.04–0.40 $\times 10^9$/L for adults) applied in UK laboratories [24].

Patients were allocated into The Global Initiative for Chronic Obstructive Lung Disease (GOLD) groups A–D. These are defined based on a combined assessment of symptoms, spirometry and exacerbation risk [25] (see online Supplement for more details) and are calculated for patients

with available modified Medical Research Council (mMRC) scores from questionnaire data, spirometry recorded within 5 years, and FEV_1/FVC ratio <0.7 at this measurement. Because primary health care professionals do not always report independent spirometry results along with the FEV_1/FVC ratio (and, in addition, mMRC scores are not available for all patients), data on GOLD groups could not be calculated for all patients. We also reported data on GOLD grades of airflow obstruction, which were defined using the most recent available FEV_1 % predicted data (mild, moderate, severe, and very severe airflow limitation).

Statistical Analyses

All analyses were performed using R version 3.0.2 (The R Project for Statistical Computing; https://www.r-project.org/). Baseline patient characteristics and outcomes for the three eosinophil categories (low, reference, and elevated count) were compared using descriptive statistics. The unadjusted rates of exacerbations were compared for patients with low or elevated count versus those of patients in the reference category using the χ^2 test. In order to evaluate our choice of blood eosinophil cut-points, we repeated the analyses at different cut-points of eosinophil counts (see Results).

The association of blood eosinophil counts with the subsequent rate of exacerbations was analyzed by estimating rate ratios (RRs) with 95% confidence intervals (CIs) using a quasi-Poisson regression model allowing for overdispersion [26]. RRs adjusted for potential confounders (see online Supplement) were estimated in the overall population and stratified by smoking habits, sex, treatment with ICS, and GOLD groups A–D. Whether associations were significantly different between strata was statistically tested by including an interaction term of the stratification variable with low / elevated count in the full model (see online Supplement).

The same methods were applied to the full population. Complete study definitions and additional details on the methods are provided in the online Supplement.

RESULTS

Patients and Eosinophil Counts

A total of 64,847 patients with COPD were identified of whom 8,318 were included in the primary study population after applying all eligibility criteria (Figure E1). Of these 8,318 patients, 6,660 (80%) had mMRC scores available to determine GOLD groups A–D.

The median year of the last blood eosinophil count for patients with COPD with 1 full outcome year available was 2009 (interquartile range [IQR], 2007–2010). Overall, 56% of patients were male, and the mean (SD) patient age was 70 (10) years. Table 1 summarizes the baseline characteristics of the primary study population and of patients in the three eosinophil categories, i.e. reference count (87.7%), elevated count (8.9%), and low count (3.4% of all patients). The median eosinophil count was 0.20 (IQR: 0.11–0.30) $x10^9$/L. Eosinophil counts above the median were more frequently found in men than in women (Figure 1). Low eosinophil counts were less frequent, while elevated counts were more frequent, in men than in women (Table E1).

1

2 **Table 1**. Baseline characteristics of patients with COPD by eosinophil count category

		All patients (*n=8,318*)	**Reference count (0.05–0.45x10^9/L)** (*n=7,291*)	**Elevated count (≥0.45x10^9/L)** *(n=743)*	**Low count (<0.05x10^9/L)** (n=284)	***P* value**[*]
Sex,	Male	4,695 (56.4)	4,012 (55.0)	543 (73.1)	140 (49.3)	<0.001 / 0.057
	Female	3,623 (43.6)	3,279 (45.0)	200 (26.9)	144 (50.7)	
Age, mean (SD)		70.1 (9.9)	70.0 (9.9)	70.8 (9.4)	70.4 (10.1)	0.035 / 0.653
Smoking status						0.510 / 0.205
	Ex-smoker	4,708 (56.6)	4,128 (56.6)	430 (57.9)	150 (52.8)	
	Current smoker	3,610 (43.4)	3,163 (43.4)	313 (42.1)	134 (47.2)	
Body mass index[†]						0.597 / <0.001
	Underweight	480 (5.8)	396 (5.4)	45 (6.1)	39 (13.7)	
	Normal weight	3,121 (37.5)	2,729 (37.4)	264 (35.5)	128 (45.1)	
	Overweight	2,757 (33.1)	2,419 (33.2)	260 (35.0)	78 (27.5)	
	Obese	1,960 (23.6)	1,747 (24)	174 (23.4)	39 (13.7)	
Comorbidities[‡]						
	Rhinitis					0.619 / 0.482
	Allergic[§]	398 (4.8)	350 (4.8)	35 (4.7)	13 (4.6)	
	Non-allergic	425 (5.1)	371 (5.1)	44 (5.9)	10 (3.5)	
	Eczema	1,552 (18.7)	1,369 (18.8)	146 (19.7)	37 (13.0)	0.562 / 0.015
	Nasal polyps	124 (1.5)	98 (1.3)	24 (3.2)	2 (0.7)	<0.001 / 0.354
	Anxiety / depression	2,615 (31.4)	2,298 (31.5)	216 (29.1)	101 (35.6)	0.171 / 0.151
Drug treatment						<0.084 / 0.245

None	1,514 (18.2)	1,345 (18.4)	121 (16.3)	48 (16.9)	
Short-acting bronchodilator	1,587 (19.1)	1,419 (19.5)	125 (16.8)	43 (15.1)	
LABA and/or LAMA	1,126 (13.5)	978 (13.4)	113 (15.2)	35 (12.3)	
ICS without LABA / LAMA	544 (6.5)	473 (6.5)	52 (7.0)	19 (6.7)	
ICS + (LABA or LAMA)	1,756 (21.1)	1,513 (20.8)	176 (23.7)	67 (23.6)	
ICS + LABA + LAMA	1,732 (20.8)	1,514 (20.8)	148 (19.9)	70 (24.6)	
ICS + LTRA	50 (0.6)	43 (0.6)	6 (0.8)	1 (0.4)	
LTRA + bronchodilator	7 (0.1)	4 (0.1)	2 (0.3)	1 (0.4)	
Other	2 (0.0)	2 (0)	0 (0)	0 (0)	
Any ICS during 2 study years	4,082 (49.1)	3,543 (48.6)	382 (51.4)	157 (55.3)	0.143 / 0.027
FEV_1, available data	7,417 (89.2)	6,526 (89.5)	642 (86.4)	249 (87.7)	
FEV_1 % predicted, mean (SD)	58.0 (18.7)	58.2 (18.7)	57.5 (18.2)	54.3 (18.6)	0.386 / <0.002
GOLD grades of airflow limitation[¶]					0.015 / 0.048
GOLD 1: Mild	850 (11.5)	764 (11.7)	60 (9.3)	26 (10.4)	
GOLD 2: Moderate	3,975 (53.6)	3,503 (53.7)	356 (55.5)	116 (46.6)	
GOLD 3: Severe	2,145 (28.9)	1,870 (28.7)	189 (29.4)	86 (34.5)	
GOLD 4: Very severe	447 (6.0)	389 (6.0)	37 (5.8)	21 (8.4)	
GOLD groups, available data[#]	6,660 (80.1)	5,875 (80.6)	571 (76.9)	214 (75.4)	0.297 / 0.002
GOLD A	2,357 (35.4)	2,100 (35.7)	193 (33.8)	64 (29.9)	
GOLD B	1,364 (20.5)	1,210 (20.6)	120 (21)	34 (15.9)	
GOLD C	1,379 (20.7)	1,222 (20.8)	109 (19.1)	48 (22.4)	
GOLD D	1,560 (23.4)	1,343 (22.9)	149 (26.1)	68 (31.8)	
COPD exacerbations in baseline year					0.296 / 0.168
0	5,257 (63.2)	4,641 (63.7)	451 (60.7)	165 (58.1)	
1	1,879 (22.6)	1,628 (22.3)	183 (24.6)	68 (23.9)	
2	712 (8.6)	626 (8.6)	59 (7.9)	27 (9.5)	
3	306 (3.7)	258 (3.5)	32 (4.3)	16 (5.6)	
≥4	164 (2)	138 (1.9)	18 (2.4)	8 (2.8)	

The table shows baseline characteristics of patients with COPD and of those with low eosinophil count (<0.05x10^9/L), reference count (0.05 to <0.45x10^9/L), and elevated count (≥0.45x10^9/L) at the last recorded blood eosinophil count during a time of stable COPD. Data shown are n (%) unless otherwise stated.

Abbreviations: FEV_1 = forced expiratory volume in 1 sec; ICS = inhaled corticosteroids; GOLD = Global initiative for chronic Obstructive Lung Disease; LABA = long-acting β_2 agonist; LAMA = long-acting muscarinic antagonist; LTRA = leukotriene receptor antagonist.

*P-values of χ^2 test for comparison between patients with elevated (left p-value) or low (right p-value) eosinophil count and patients with a reference eosinophil count, respectively. For variables with more than one category, p-values indicate whether there are differences in the overall distribution of the variable between the groups.

†BMI cut-off points in kg/m^2: underweight, <18.5; normal weight, ≥18.5 to <25.0; overweight, ≥25.0 to <30.0; obese, ≥30.

‡Comorbidities were captured as an ever-recorded diagnostic Read code (see Supplement for complete definition) in the database.

§Allergic rhinitis was defined as a Read code diagnosis of allergic rhinitis, hay fever, perennial rhinitis, or seasonal rhinitis.

¶GOLD grades 1–4 were defined as FEV_1 % predicted: GOLD 1, ≥80; GOLD 2, ≥50 to <80; GOLD 3, ≥30 to <50; GOLD 4, <30 [25]

#GOLD groups A–D were defined as follows: GOLD A, FEV_1 % predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC 0–1; GOLD B, FEV_1 % predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC ≥2; GOLD C, FEV_1 % predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year and mMRC 1–2; GOLD D, (FEV_1 % predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year and mMRC ≥2

Blood Eosinophil Counts and Rate of COPD Exacerbations

During the follow-up year, 40% of patients with reference eosinophil counts, 42% of those with elevated counts, and 43% of those with low counts experienced ≥1 COPD exacerbations, while 16%, 18%, and 17%, respectively, experienced ≥2 COPD exacerbations (Table 2).

Patients with COPD who had elevated blood eosinophil counts had an overall 13% higher rate of exacerbations during the following year than patients in the reference group (adjusted RR 1.13; 95% CI 1.01–1.26; Table 3). When investigating this association in different patient subgroups defined by patient characteristics – i.e. sex, smoking status, ICS use, and GOLD group – we found a significant (P=0.0002) difference between ex-smokers and current smokers; the higher rate was restricted to ex-smokers (RR 1.32; 1.15–1.51).

To investigate this further, we compared the exacerbation rates in 4 different patient subgroups defined by smoking status and eosinophil counts, the reference rate being that of ex-smokers with eosinophil counts in the reference range (0.05 to $<0.45 \times 10^9$/L). Figure 2 illustrates that ex-smokers with elevated eosinophil counts had the highest exacerbation rate (RR 1.30; 1.14–1.48) (Figure 2); by contrast, current smokers with elevated eosinophil counts had the lowest exacerbation rate (RR 0.89; 0.73–1.08; Figure 2).

When studying patient subgroups defined by other characteristics, we found significant associations between elevated eosinophil counts and exacerbation rates in men (RR 1.21; 1.06–1.38), patients treated with ICS (RR 1.17; 1.02–1.35), and patients with GOLD group B (RR 1.33; 1.02–1.73) (Table 3). However, no significant effect modification by any of these characteristics was found (Table 3).

Elevated blood eosinophil count and exacerbation rates in ex-smokers

As the observed increased exacerbation rate with elevated blood eosinophil counts was restricted to ex-smokers, we further studied associations within this subgroup.

A significant increased rate of exacerbations with elevated eosinophil counts was observed in male ex-smokers (RR 1.40; 1.20–1.64), ex-smokers treated with ICS (RR 1.37; 1.17–1.62) and ex-smokers with GOLD groups B-D (Table 4). However, no significant effect modification by these characteristics was observed (Table 4).

Low eosinophil counts

For patients with COPD and low eosinophil counts, the exacerbation rate was not significantly different from that of patients with a reference count (RR 1.06; 0.89–1.27).

Real-life data

When studying the broader population of 24,089 patients (online Supplement), the results were similar to those reported in the primary population (Table E5), showing increased rates of exacerbations with elevated eosinophil counts only in patients who were ex-smokers and never smokers (investigated as one subgroup, see Supplement for more details). In this subgroup, we found a significant sex difference in association (P=0.02; Table E6).

Table 2. Number of COPD exacerbations during the follow-up year for patients with COPD by eosinophil category

	All patients (*n=8,318*)	**Reference count (0.05–0.45x10^9/L)** (*n=7,291*)	**Elevated count (≥0.45x10^9/L)** (*n=743*)	**Low count* (<0.05x10^9/L)** (*n=284*)
COPD exacerbations /year				
Mean (SD)	0.69 (1.12)	0.68 (1.10)	0.77 (1.25)	0.76 (1.19)
0	4,992 (60.0)	4,402 (60.4)	428 (57.6)	162 (57.0)
1	1,969 (23.7)	1,714 (23.5)	182 (24.5)	73 (25.7)
2	774 (9.3)	680 (9.3)	67 (9.0)	27 (9.5)
3	310 (3.7)	263 (3.6)	37 (5.0)	10 (3.5)
≥4	273 (3.3)	232 (3.2)	29 (3.9)	12 (4.2)

Data are expressed as n (%).

*There were no statistically significant differences in outcomes when comparing the low or elevated eosinophil count outcomes with the reference count cohort ($P \geq 0.05$, χ^2 test).

Table 3 Rate Ratios (95% CI) of the association between elevated blood eosinophil count and COPD exacerbations during the follow-up year in the total population of patients with COPD and in subgroups of patients defined by sex, smoking status, ICS therapy and GOLD groups.

Study population and subgroups		**Patients with COPD (*n=8,318*)**				
		Prevalence of elevated eosinophil counts (%)	RR* (95% CI)	*P* value	N	*P-value interaction*
Total population of patients with COPD		8.9	**1.13 (1.01-1.26)**	**0.03**	8,318	
Male pts		11.6	**1.21 (1.06-1.38)**	**0.005**	4,695	0.11
Female pts		5.5	0.98 (0.80-1.21)	0.88	3,623	
Current smokers		8.7	0.86 (0.71-1.05)	0.14	3,610	**0.0002**
Ex-smokers		9.1	**1.32 (1.15-1.51)**	**<0.0001**	4,708	
Pts on ICS‡		9.4	**1.17 (1.02-1.35)**	**0.03**	4,082	0.29
Pts not on ICS		8.5	1.02 (0.84-1.24)	0.82	4.236	
GOLD groups§					6,600	
	GOLD A	8.2	0.99 (0.77-1.29)	0.97	2,357	reference
	GOLD B	8.8	**1.33 (1.02-1.73)**	**0.04**	1,364	0.07
	GOLD C	7.9	1.27 (0.99-1.63)	0.06	1,379	0.12
	GOLD D	9.6	1.17 (0.95-1.44)	0.13	1,560	0.24

The table shows the rate of COPD exacerbations in patients with elevated eosinophil counts ($\geq 0.45 \times 10^9$/L) relative to patients with reference eosinophil counts (0.05 to $<0.45 \times 10^9$/L) in each subgroup shown in column one. Differences between subgroups were tested by including an interaction term of elevated eosinophil count

and the variable used to define the categories of the subgroup in a multiple regression model (e.g. interaction term of elevated eosinophil count (yes/no)*sex (male/female) had a P-value of 0.11)

Abbreviations: FEV_1 = forced expiratory volume in 1 sec; FVC = forced vital capacity; ICS = inhaled corticosteroids; GOLD = Global initiative for chronic Obstructive Lung Disease; MRC = Medical Research Council; Pts = patients.
[*]Rate Ratio adjusted for potential confounders (see online Supplement)
[‡]Maintenance treatment with inhaled corticosteroids.
[§]GOLD groups for patients with $FEV_1/FVC < 0.70$, defined based on MRC score ≥2 (yes, B or D; no, A or C), number of baseline exacerbations ≥2 or leading to hospitalization ≥1 or FEV_1% predicted <50% (yes, C or D; no, A or B). Obstruction defined as $FEV_1/FVC < 0.70$ at spirometry measurement closest to index date within ≤5 years.

Table 4 Rate Ratios (95% CI) of the association between elevated blood eosinophil count and COPD exacerbations during the follow-up year in subgroups of ex-smokers defined by sex, ICS therapy and GOLD groups.

Study subgroups	**Total patients with COPD (*n=8,318*)**				
	Prevalence of elevated eosinophil counts (%)	RR* (95% CI)	*P* value	N	*P-value interaction*
Male ex-smokers	11.7	**1.40 (1.20-1.64)**	**<0.0001**	2,914	0.13
Female ex-smokers	5.0	1.08 (0.81-1.46)	0.60	1,794	
Ex-smokers on ICS‡	9.8	**1.37 (1.17-1.62)**	**0.0001**	2,545	0.18
Ex-smokers, no ICS	8.4	1.14 (0.89-1.47)	0.30	2,163	
Ex-smokers at different GOLD groups§,				3,881	
GOLD A	7.0	1.06 (0.74-1.51)	0.76	1,302	reference
GOLD B	9.8	**1.38 (1.01-1.89)**	**0.04**	813	0.26
GOLD C	8.9	**1.42 (1.06-1.90)**	**0.02**	816	0.17
GOLD D	10.1	**1.41 (1.11-1.79)**	**0.005**	950	0.26

The table shows the rate of COPD exacerbations in patients with elevated eosinophil counts ($\geq 0.45 \times 10^9$/L) relative to patients with reference eosinophil counts (0.05 to $<0.45 \times 10^9$/L) in each subgroup shown in column one. Differences between subgroups were tested by including an interaction term of elevated eosinophil count and the variable used to define the categories of the subgroup in a multiple regression model (e.g. interaction term of elevated eosinophil count (yes/no)*sex (male/female) had a P-value of 0.13).

Abbreviations: FEV_1 = forced expiratory volume in 1 sec; FVC = forced vital capacity; ICS = inhaled corticosteroids; GOLD = Global initiative for chronic Obstructive Lung Disease; MRC = Medical Research Council.

[*]Rate Ratio adjusted for potential confounders (see online Supplement)
[‡]Maintenance treatment with inhaled corticosteroids.
[§]GOLD groups for patients with FEV_1/FVC <0.70, defined based on MRC score ≥2 (yes, B or D; no, A or C), number of baseline exacerbations ≥2 or leading to hospitalization ≥1 or FEV_1% predicted <50% (yes, C or D; no, A or B).
Obstruction defined as FEV_1/FVC <0.70 at spirometry measurement closest to index date within ≤5 years.

Evaluation of Blood Eosinophil Cut-Points

We evaluated the choice of study cut-points by repeating the analyses in ex-smokers using different values to define elevated eosinophil counts (from $\geq 0.15 \times 10^9$/L to $\geq 0.55 \times 10^9$/L, with increments of 0.10). We found that the association between eosinophil count and exacerbation rate was significant when using ≥ 0.45 as the cut-point, was not significant when using ≥ 0.35 and most pronounced when using ≥ 0.55 (Figure 3), confirming the appropriateness of our choice.

DISCUSSION

We conducted a large-size historical follow-up study evaluating the relationship between blood eosinophil counts (determined during stable disease) and rate of exacerbations during follow-up in real-life patients with COPD. Our findings show that elevated blood eosinophil count (above the reference value of 0.45×10^9/L [24]) is associated with a higher risk of having an exacerbation in the following year. In particular, we made the following specific and novel observations: *(1)* the increased rate of exacerbations with elevated eosinophil count (reported in previous studies[10, 16]) was restricted to ex-smokers; *(2)* this association was significant in male ex-smokers (but not in female ex-smokers) and was also found in patients who were on ICS treatment; *(3)* patients with elevated eosinophil count who were currently smoking showed the lowest rate of exacerbations; and, *(5)* there was no association between low eosinophil counts and COPD exacerbations. These results were confirmed in a broader population of 24,089 patients (online Supplement) who represent the broad spectrum of patients with COPD seen in clinical practice, suggesting that our findings can be translated into real-life clinical settings.

Previous studies and interpretation of findings

In a recent observational study (n=7,225; median 3.3 years' follow-up), Vedel-Krogh et al [16] reported that blood eosinophil counts >0.34x10^9/L are associated with increased risk of both moderate (short course OCS) and severe (hospitalizations) COPD exacerbations in patients with spirometrically confirmed COPD. However, they did not investigate (as we did) other patient characteristics that may potentially affect the association between blood eosinophils and exacerbations. Our findings suggest that elevated blood eosinophil count present in ex-smokers (but not in current smokers) at a time of stable COPD may identify patients with an increased susceptibility to exacerbations in the near future. These patients may constitute a target population for more specific treatment of their eosinophilic inflammation beyond ICS therapy, since the increased risk was even more pronounced in patients receiving therapy with ICS. This may seem in contrast with the abovementioned data from controlled clinical trials showing an association between elevated sputum [2, 3, 6] and blood [12] eosinophil count and response to ICS, however it may be explained by indication bias – namely, ICS being prescribed more frequently to patients at higher risk of exacerbations, a group that likely had eosinophilic inflammation.

Our finding that a higher percentage of men than women had elevated eosinophil counts is in keeping with findings reported by Vedel-Krogh et al [16]. Sex-related differences in dimension, structure, and function of the airways, together with variations in pathophysiologic and, more specifically, inflammatory mechanisms elicited by tobacco smoking, may lead to differences in clinical manifestations of airway disease [19, 21, 27]. In our study population, we observed an increased rate of exacerbations with elevated eosinophil count only in male ex-smokers, although

this sex difference was only significant in the full population (described in the online supplement). Therefore, further research is needed to fully elucidate the influence of sex-related differences on the interaction between eosinophilic airway inflammation and exacerbation risk in COPD.

We can only speculate about a possible mechanism behind the absence of association between elevated blood eosinophil count and exacerbations in current smokers. The rate of COPD exacerbations among patients with elevated eosinophil count who were current smokers was relatively low. This finding is inconsistent with the well-recognized predisposition of smokers for development of respiratory infections [28], but it could be explained by the "healthy smoker effect," namely, the tendency of people who tolerate cigarettes to continue smoking, whereas those who experience serious health problems tend to quit [29]. There is also some evidence that active tobacco smoking has a suppressive effect on eosinophils and inflammatory cells or cytokines [17], observed not only in patients with asthma [30] or chronic rhinosinusitis [31], but also in healthy intermittent smokers [32], which may explain why some patients with elevated eosinophil count continue smoking despite their diagnosis of COPD.

We acknowledge that the study populations were broader than the "classical COPD populations" included in clinical trials. In particular, the full population included patients with an ever-recorded diagnosis of asthma. This may be considered a confounding factor, given the well-known link between elevated eosinophil count and exacerbation in asthma [33]. However, we found comparable results of increased exacerbation risk with elevated blood eosinophil count in both populations indicating that the association is independent of the presence of an asthma

diagnosis. Real-life patients include a diverse spectrum of individuals with a physician's diagnosis of COPD. Many of these patients would not meet eligibility criteria for randomized controlled COPD trials [34, 35] but they are representative of the diversity of patients seen by clinicians and relate our findings to the broad spectrum of patients with COPD.

Strengths and limitations

Study strengths include the large size of the patient populations (n =8,318) drawn from geographically and socioeconomically diverse primary care practices throughout the UK. The recent timeline of the present study (median index date 2009) likely improved the capture and accuracy of the data, as the UK Quality and Outcomes Framework (QOF), instituted in 2005, requires the recording of current smoking status and FEV_1 for patients with COPD [36, 37]. In addition, we had access to recent spirometry results for 85% of patients and were able to calculate GOLD groups A–D for 80% of patients. Finally, results of the count-response analyses supported our cut-point of $0.45x10^9$/L, as the rate of exacerbations in ex-smokers started to increase at levels of $0.45x10^9$/L and $0.55x10^9$/L.

There are, however, some study limitations that deserve discussion. Firstly, although we used eosinophil counts at stable disease, the indications for doctors to run a full blood count may have affected the outcome of blood differential counts, and thus the associations of eosinophils and exacerbations may have been underestimated. Moreover, we studied only the association with exacerbation rate in the subsequent year and did not assess whether there is a sustained increased risk over time. Secondly, while laboratory and prescribing information in UK electronic medical records is considered reliable, the quality of spirometry in primary care practice may be inconsistent [38, 39], and miscoding of smoking status or other data entry errors are possible.

Moreover, hospitalizations and ED attendances may be under-recorded, and additional patient data, such as smoking pack-years, were not available in the database but would have been of interest. Finally, the present study used the mMRC dyspnea scale score to assign GOLD groups A–D, however results may have been different had we used the COPD assessment test (CAT) [40].

Conclusions

This study shows that elevated blood eosinophil counts may predict COPD exacerbation risk in some patient subgroups. This analytical strategy may eventually help in targeting therapy to specific patients (precision medicine) so benefit is maximized and risk minimized for individual patients.

ACKNOWLEDGMENTS

We wish to thank Anna Rigazio for her assistance with data extraction and Alessandra Cifra for support with medical writing and manuscript review. Their work was funded by Observational and Pragmatic Research Institute Pte Ltd.

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Figure Legends

Figure 1. Distribution of blood eosinophil counts in the study population, stratified by patient sex.

Values are rounded to the nearest decimal place.

Figure 2. Effect of smoking status and blood eosinophil count on COPD exacerbations.

The figure shows rate ratios (RRs; [95% CI]) of COPD exacerbations obtained from a regression model adjusted for confounders with different combinations of smoking status and blood eosinophil count as a categorical variable. Ex-smoking patients with blood eosinophil counts in the reference range (0.05 to <0.45x10^9/L) are the reference group.

Figure 3. Evaluation of blood eosinophil cut-points. The figure shows adjusted rate ratios (RRs; [95% CI]) of COPD exacerbations for ex-smoking patients at different cut-points used to define elevated blood eosinophil counts.

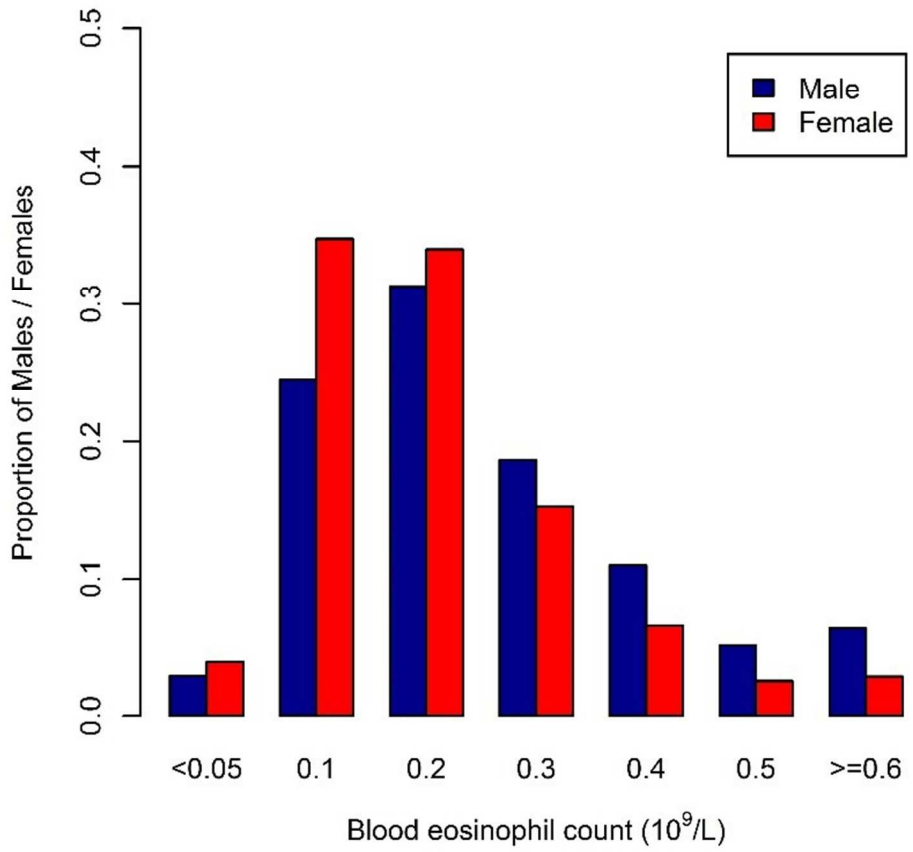

Figure 1. Distribution of blood eosinophil counts in the study population, stratified by patient sex.

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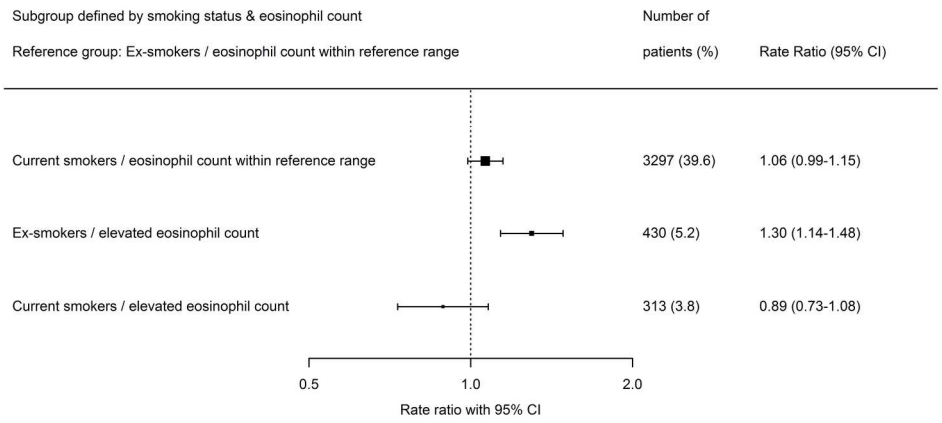


Figure 2. Effect of smoking status and blood eosinophil count on COPD exacerbations.

99x49mm (600 x 600 DPI)

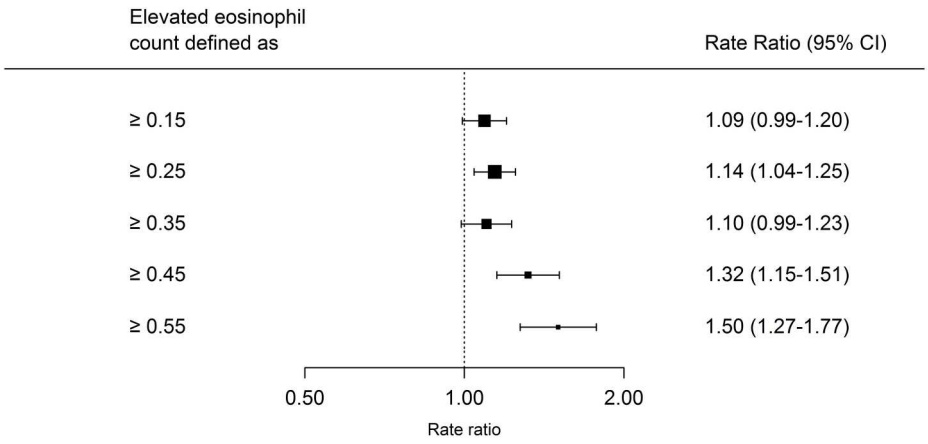


Figure 3. Evaluation of blood eosinophil cut-points.

99x49mm (600 x 600 DPI)

Online Supplementary material

Blood Eosinophil Count and Exacerbation Risk in Patients with COPD

Marjan Kerkhof, Dirkje S Postma, Guy Brusselle, Alvar Agusti, Antonio Anzueto, Rupert Jones, Alberto Papi, Ian Pavord, Emillio Pizzichini, Todor Popov, Nicolas Roche, Dermot Ryan, Mike Thomas, Claus Vogelmeier, Alison Chisholm, Daryl Freeman, Mona Bafadhel, Elizabeth V Hillyer, David B Price on behalf of the Respiratory Effectiveness Group

Supplementary Methods

Data Source

The use of the Optimum Patient Care Research Database (OPCRD [1]) for clinical research has been approved by the Health Research Authority of the UK NHS for clinical research use (REC reference: 15/EM/0150), and the protocol for this study was approved by the Anonymised Data Ethics Protocols and Transparency (ADEPT) committee, the independent scientific advisory committee for the OPCRD.

The study was conducted according to accepted standards for observational research [2], including an *a priori* analysis plan, study registration with commitment to publish, and an independent steering committee not remunerated for participation. The study protocol was registered with the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP, study no. 4922) [3].

Additional study definitions

Read codes are the standard coding system used in primary care practices in the United Kingdom. They are used to record patient data including diagnosis, procedures, and prescribed therapies.

Acute oral corticosteroids (OCS) courses associated with chronic obstructive pulmonary disease (COPD) exacerbation treatment were defined as all courses that were definitely not maintenance therapy, and/or all courses where dosing instructions suggested exacerbation treatment (e.g. 6,5,4,3,2,1 reducing, or 30 mg as directed), and/or all courses with no dosing instructions, but unlikely to be maintenance therapy because of prescription strength or frequency of prescriptions. Where ≥1 OCS course/hospitalization/antibiotics prescription occurred within 2 weeks of another, these events were considered to be the result of the same exacerbation (and were counted only once).

Lower respiratory consultations were defined as any of the following:

a) Lower respiratory Read codes (including asthma, COPD and lower respiratory tract infection Read codes);

b) Asthma/COPD review codes excluding any monitoring letter codes;

c) Lung function and/or asthma monitoring

d) Any additional respiratory examinations, referrals, chest x-rays, or events

COPD-related hospitalizations were defined as either a definite COPD-related emergency department (ED) attendance or a definite COPD-related hospital admission, or a generic hospitalization Read code that was recorded on the same day

as a lower respiratory consultation (see above (a) – (c) only and excluding where the only lower respiratory code recorded on that day was for a lung function test).

The Global initiative for chronic Obstructive Lung Disease (GOLD) 2011 groups A–D, calculated for patients with available spirometry results and a modified Medical Research Council (mMRC) score within 5 years of the index date, were defined based on the record of ≥2 (yes/no) COPD exacerbations or ≥1 (yes/no) hospitalizations for COPD in the baseline year or forced expiratory volume in 1 second (FEV_1) <50% of predicted with FEV_1/FVC <0.70 combined with mMRC score ≥2 (yes/no) [4], as follows:

- GOLD A: FEV_1 % predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC 0–1
- GOLD B: FEV_1 % predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC ≥2
- GOLD C: FEV_1 % predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year) and mMRC 0–1
- GOLD D: FEV_1 % predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year) and mMRC ≥2

The GOLD grades of airflow limitation were based on FEV_1 % predicted as follows:

- GOLD 1, mild: FEV_1 % predicted ≥80%
- GOLD 2, moderate: FEV_1 % predicted ≥50% to <80%
- GOLD 3, severe: FEV_1 % predicted ≥30% to <50% (severe)
- GOLD 4, very severe: FEV_1 %predicted <30%

Smoking status in patients of the *full population*

The full population included both patients with a history of smoking (current smokers and ex-smokers) and patients with no history of smoking (never-smokers). However, we elected to pool the results for never/ex-smokers because our initial separate

analyses of the three groups (current, ex-, and never-smokers) found that the association was similar in ex- and never-smokers and there was no reason to distinguish them (data not shown). In contrast, the results were highly significantly different in current smokers. Moreover, there are theoretical arguments to distinguish current smokers from non-current smokers, as cigarette smoking may have short-term effects on airways inflammation and/or eosinophils [5, 6].

Potential Confounders used in the statistical analyses

We adjusted the analyses for the following potential confounding factors: patient sex, age, last recorded smoking status (never, ex-smoker, current smoker), and body mass index (BMI as normal, underweight, overweight, or obese) within a period of 1 year before the last eosinophil count. Comorbidities included as potential confounding factors were defined as those recorded at any time (ever-recorded) during the registration period and included, allergic and non-allergic rhinitis, eczema, nasal polyps, and anxiety/depression. We also included COPD treatment during the baseline and follow-up years. This applies also to the full population, with additional inclusion of presence of asthma diagnosis as potential confounder.

Subgroup analyses

Heterogeneity of the association between eosinophil counts and COPD exacerbations was studied by stratified analyses. Potential effect modifiers to define strata were selected *a priori*. Observed differences in associations between strata were statistically tested for significance by including an interaction term of the stratification variable and elevated blood eosinophil count / low count into the full regression to evaluate whether observed differences were likely to be caused by random error.

Supplementary Results

Results in the *full population* of patients with COPD

The full population of patients with COPD numbered 24,089 (Figure E1). Tables E2 and E3 present baseline characteristics and eosinophil counts of these patients, of whom 10.2% had elevated eosinophil count. During the follow-up year, 44% of patients with reference eosinophil counts, 47% of those with elevated counts, and 47% of those with low counts experienced ≥1 COPD exacerbations, while 20%, 23%, and 22%, respectively, experienced ≥2 COPD (Table E4).

As in the primary study population, also in this population patients with COPD who had elevated blood eosinophil count had a higher rate of exacerbations during the follow-up year compared with patients with reference counts (adjusted RR 1.10; 95% CI 1.04–1.17; Table E5).

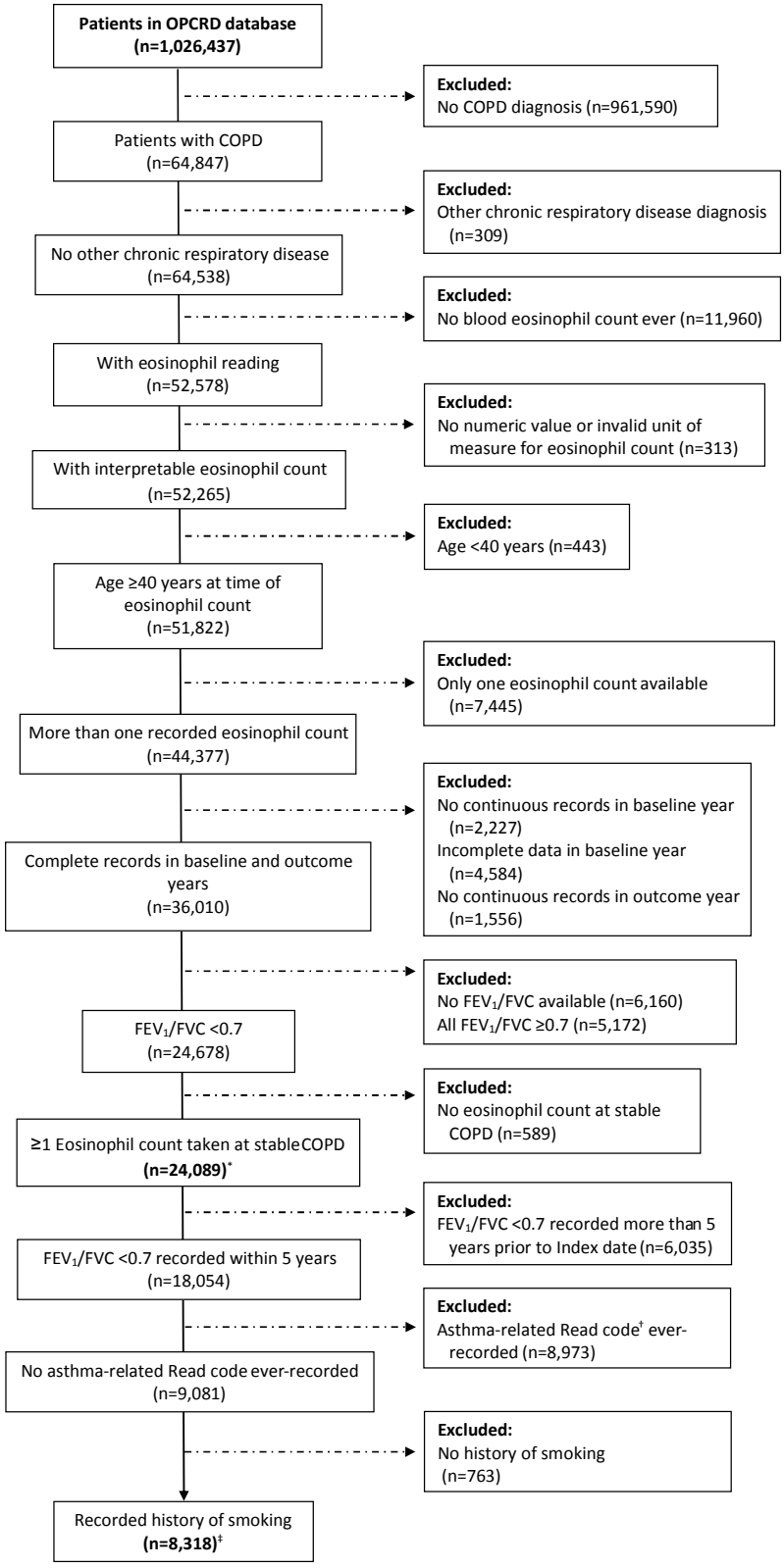
Similarly, when investigating this association in different patient subgroups defined by patient characteristics – i.e. sex, smoking status, ICS use, and GOLD group – we found a significant (P=0.00001) difference only between patients not currently smoking (ex- and never-smokers were pooled together, see Supplementary methods above) and current smokers; the higher rate was restricted to patients not currently smoking (RR 1.19; 1.11–1.27, Table E5). When looking at other characteristics, we found significant associations between elevated eosinophil counts and exacerbation rates in men (RR 1.15; 1.07–1.24), patients treated with ICS (RR 1.11; 1.04−1.18), and patients with GOLD groups B and C (RR 1.21, 1.02−1.44 and 1.17, 1.02−1.34, respectively) (Table E5). However, no significant effect modification by any of these characteristics was found (Table E5).

As the observed increased exacerbation rate with elevated blood eosinophil counts was restricted to patients not currently smoking, we further studied associations within this subgroup (Table E6).

A significant increased rate of exacerbations with elevated eosinophil counts was observed in male patients not currently smoking (RR 1.26; 1.15−1.37), patients not currently smoking treated with ICS (RR 1.21; 1.12−1.30), and patients not currently smoking with GOLD groups B and C (RR 1.32, 1.09−1.61 and 1.28, 1.10−1.49, respectively). However, a significant effect modification was observed only for sex (p=0.02; Table E6).

No increased risk of exacerbations with elevated eosinophil count was observed in current smokers (RR 0.89; 0.79–1.00) (Table E5). Finally, there was no association between low eosinophil count and exacerbation rate (RR 1.07; 0.97–1.18).

Figure E1. Patient flowchart



Definition of abbreviations: COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in 1 sec; FVC = forced vital capacity; OPCRD = Optimum Patient Care Research Database

*'Full population' on which we conducted secondary analyses; results reported in this Supplement

‡Read codes are the standard coding system used in primary care practices in the United Kingdom. They are used to record patient data including diagnosis, procedures, and prescribed therapies.

‡Primary study population

Table E1. Distribution of blood eosinophil counts ($x10^9$/L) in patients with COPD from the primary study population stratified by sex

Blood eosinophil counts: cut points for 2 (or 1) decimals*	**All patients (*n=8,318*)**	**Male Patients (*n=4,695*)**	**Female Patients (*n=3,623*)**
Median (IQR)	0.20 (0.11-0.30)	0.20 (0.13-0.32)	0.19 (0.10-0.26)
Categorical distribution			
<0.05†	284 (3.4)	140 (3.0)	144 (4.0)
0.05-0.24	2,410 (29)	1,152 (24.5)	1,258 (34.7)
0.25-0.34	2,698 (32.4)	1,468 (31.3)	1,230 (33.9)
0.35-0.44	1,428 (17.2)	876 (18.7)	552 (15.2)
0.45-0.54‡	337 (4.1)	243 (5.2)	94 (2.6)
≥0.55‡	406 (4.9)	300 (6.4)	106 (2.9)

Abbreviations: IQR = interquartile range

Data are n (%) unless otherwise stated.

*Approximately 50% of measurements were recorded at 1 decimal place.

†Defined as low blood eosinophil count.

‡Defined as elevated blood eosinophil count.

Table E2. Baseline characteristics of patients from the *full population* by eosinophil category

		All patients (*n=24,089*)	**Reference count (0.05–0.45x10^9/L)** (*n=20,849*)	**Elevated count (≥0.45x10^9/L)** *(n=2,449)*	**Low count (<0.05x10^9/L)** (*n=791*)	***P* value**[*]
Sex,	Male	12,601 (52.3)	10,687 (51.3)	1,552 (63.4)	362 (45.8)	<0.001 / 0.0024
	Female	11,488 (47.7)	10,162 (48.7)	897 (36.6)	429 (54.2)	
Age, mean (SD)		70.6 (10.3)	70.6 (10.3)	71.2 (10.3)	71.4 (10.6)	0.002 / 0.027
Smoking status						<0.001 / 0.144
	Never-smoker	3,057 (12.7)	2,576 (12.4)	372 (15.2)	109 (13.8)	
	Ex-smoker	12,975 (53.9)	11,211 (53.8)	1,366 (55.8)	398 (50.3)	
	Current smoker	8,057 (33.4)	7,062 (33.9)	711 (29.0)	284 (35.9)	
Body mass index[†]						0.026 / <0.001
	Underweight	1,162 (4.8)	955 (4.6)	126 (5.1)	81 (10.2)	
	Normal weight	8,515 (35.3)	7,258 (34.8)	898 (36.7)	359 (45.4)	
	Overweight	8,129 (33.7)	7,073 (33.9)	835 (34.1)	221 (27.9)	
	Obese	6,283 (26.1)	5,563 (26.7)	590 (24.1)	130 (16.4)	
Comorbidities[‡]						
	Asthma Read code ever	12,152 (50.4)	10,364 (49.7)	1,400 (57.2)	388 (49.1)	<0.001 / 0.716
	Rhinitis					<0.001 / 0.460
	Allergic[§]	1,883 (7.8)	1,588 (7.6)	243 (9.9)	52 (6.6)	
	Non-allergic	1,533 (6.4)	1,309 (6.3)	178 (7.3)	46 (5.8)	
	Eczema	5,288 (22.0)	4,527 (21.7)	602 (24.6)	159 (20.1)	0.001 / 0.280
	Nasal polyps	642 (2.7)	449 (2.2)	174 (7.1)	19 (2.4)	<0.001 / 0.637
	Anxiety / depression	7,936 (32.9)	6,920 (33.2)	735 (30.0)	281 (35.5)	0.002 / 0.172

Drug treatment					<0.001 / 0.015
None	2,618 (10.9)	2,315 (11.1)	224 (9.1)	79 (10.0)	
Short-acting bronchodilator	3,105 (12.9)	2,761 (13.2)	259 (10.6)	85 (10.7)	
LABA and/or LAMA	2,294 (9.5)	2,015 (9.7)	220 (9.0)	59 (7.5)	
LTRA + bronchodilator	45 (0.2)	36 (0.2)	5 (0.2)	4 (0.5)	
ICS without LABA / LAMA	2,208 (9.2)	1,907 (9.1)	227 (9.3)	74 (9.4)	
ICS + (LABA or LAMA)	7,055 (29.3)	6,018 (28.9)	792 (32.3)	245 (31.0)	
ICS + LABA + LAMA	6,116 (25.4)	5,266 (25.3)	628 (25.6)	222 (28.1)	
ICS + LTRA	639 (2.7)	525 (2.5)	92 (3.8)	22 (2.8)	
Other	9 (0.0)	6 (0.0)	2 (0.1)	1 (0.1)	
Any ICS during 2 study years	16,018 (66.5)	13,716 (65.8)	1,739 (71.0)	563 (71.2)	0.002 / <0.001
Spirometry, available data	20,480 (85.0)	17,746 (85.1)	2,070 (84.5)	664 (83.9)	
FEV_1 %predicted, mean (SD)	59.1 (19.6)	59.2 (19.6)	58.7 (19.3)	56.4 (19.9)	0.270 / <0.001
GOLD grades of airflow limitation‖					0.363 / 0.005
GOLD 1: Mild	2,809 (13.7)	2,463 (13.9)	261 (12.6)	85 (12.8)	
GOLD 2: Moderate	10,705 (52.3)	9,290 (52.3)	1,101 (53.2)	314 (47.3)	
GOLD 3: Severe	5,770 (28.2)	4,964 (28.0)	595 (28.7)	211 (31.8)	
GOLD 4: Very severe	1,196 (5.8)	1,029 (5.8)	113 (5.5)	54 (8.1)	
GOLD groups, available data¶	14,466 (60.1)	12,567 (60.3)	1,451 (59.2)	448 (56.6)	0.557 / 0.001
GOLD A	4,737 (32.7)	4,153 (33.0)	460 (31.7)	124 (27.7)	
GOLD B	2,845 (19.7)	2,496 (19.9)	280 (19.3)	69 (15.4)	
GOLD C	3,172 (21.9)	2,727 (21.7)	333 (22.9)	112 (25.0)	
GOLD D	3,712 (25.7)	3,191 (25.4)	378 (26.1)	143 (31.9)	
COPD exacerbations in baseline year					<0.001 / <0.001
0	13,805 (57.3)	12,090 (58.0)	1,299 (53.0)	416 (52.6)	
1	5,725 (23.8)	4,927 (23.6)	606 (24.7)	192 (24.3)	
2	2,601 (10.8)	2,225 (10.7)	284 (11.6)	92 (11.6)	

3	1,139 (4.7)	948 (4.5)	146 (6.0)	45 (5.7)
≥4	819 (3.4)	659 (3.2)	114 (4.7)	46 (5.8)

The table shows baseline characteristics of total patients in the *full population* and of those with low eosinophil count (<0.05x10^9/L), reference count (0.05 to <0.45x10^9/L), and elevated count (≥0.45x10^9/L) at the last recorded blood eosinophil count during a time of stable COPD. Data shown are n (%) unless otherwise stated.

Abbreviations: FEV_1 = forced expiratory volume in 1 sec; ICS = inhaled corticosteroids; GOLD = Global initiative for chronic Obstructive Lung Disease; LABA = long-acting β_2 agonist; LAMA = long-acting muscarinic antagonist; LTRA = leukotriene receptor antagonist.

*P-values of χ^2 test for comparison between patients with elevated (left p-value) or low (right p-value) eosinophil count and patients with a reference eosinophil count, respectively. For variables with more than one category, p-values indicate whether there are differences in the overall distribution of the variable between the groups.

†BMI cut-off points in kg/m^2: underweight, <18.5; normal weight, ≥18.5 to <25.0; overweight, ≥25.0 to <30.0; obese, ≥30

‡Comorbidities were captured as an ever-recorded diagnostic Read code in the database

§Allergic rhinitis was defined as a Read code diagnosis of allergic rhinitis, hay fever, perennial rhinitis, or seasonal rhinitis

‖GOLD stages 1-4 were defined as FEV_1 % predicted: GOLD 1, ≥80; GOLD 2, ≥50 to <80; GOLD 3, ≥30 to <50; GOLD 4, <30

¶GOLD groups A–D were defined as follows: GOLD A, FEV_1 %predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC 0–1; GOLD B, FEV_1 %predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC ≥2; GOLD C, FEV_1 %predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year and mMRC 1–2; GOLD D, (FEV_1 %predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year and mMRC ≥2

Table E3. Distribution of blood eosinophil counts ($x10^9$/l) in patients with COPD from the *full population* stratified by sex

Blood eosinophil counts: cut points for 2 (or 1) decimals*	**All patients (*n=24,089*)**	**Male Patients (*n=12,601*)**	**Female Patients (*n=11,488*)**
Median (IQR)	0.20 (0.12-0.30)	0.20 (0.13-0.33)	0.20 (0.10-0.29)
Categorical distribution			
<0.05†	791 (3.3)	362 (2.9)	429 (3.7)
0.05-0.24	14,353 (59.6)	6,872 (54.5)	7,481 (65.1)
0.25-0.34	4,276 (17.8)	2,431 (19.3)	1,845 (16.1)
0.35-0.44	2,220 (9.2)	1,384 (11.0)	836 (7.3)
0.45-0.54‡	1,081 (4.5)	679 (5.4)	402 (3.5)
≥0.55‡	1,368 (5.7)	873 (6.9)	495 (4.3)

Abbreviations: IQR = interquartile range

Data are n (%) unless otherwise stated

*Approximately 50% of measurements were recorded at 1 decimal place

†Defined as low eosinophil count

‡Defined as elevated eosinophil count

Table E4. Number of COPD exacerbations during the follow-up year for the *full population* of patients with COPD by eosinophil category

	All patients (*n=24,089*)	**Reference count (0.05–0.45x10^9/L)** (*n=20,849*)	**Elevated count (≥0.45x10^9/L)** (*n=2,449*)	**Low count* (<0.05x10^9/L)** (*n=791*)
COPD exacerbations /year				
0	13,305 (55.2)	11,592 (55.6)	1,293 (52.8)†	420 (53.1)
1	5,833 (24.2)	5,049 (24.2)	590 (24.1)‡	194 (24.5)
2	2,689 (11.2)	2,310 (11.1)	296 (12.1)§	83 (10.5)
3	1,177 (4.9)	994 (4.8)	134 (5.5)¶	49 (6.2)
≥4	1,085 (4.5)	904 (4.3)	136 (5.6)$^\#$	45 (5.7)

Data are expressed as n (%).

*There were no statistically significant differences in outcomes between the low count and reference count cohorts ($P \geq 0.10$, χ^2 test).

P-value (χ^2 test) comparing the elevated count cohort with the reference count cohort: $^\dagger P=0.006$; $^\ddagger P<0.0001$; $^\S P=0.03$; $^\P P=0.42$; $^\# P=0.76$.

Table E5. Rate Ratios (95% CI) of the association between elevated blood eosinophil count and COPD exacerbations during the follow-up year in the *full population* of patients with COPD and in subgroups of patients defined by sex, smoking status, ICS therapy and GOLD groups.

Study population and subgroups	**Patients with COPD (*n=24,089*)**				
	Prevalence of elevated eosinophil count (%)	RR* (95% CI)	*P* value	N	*P-value interaction*
Total population of patients with COPD	10.2	**1.10 (1.04−1.17)**	**0.001**	24,089	
Male pts	12.3	**1.15 (1.07−1.24)**	**0.002**	12,601	0.052
Female pts	7.8	1.03 (0.95−1.13)	0.46	11,488	
Pts currently smoking	8.8	0.89 (0.79−1.00)	0.054	8,057	**0.00001**
Pts not currently smoking†	10.8	**1.19 (1.11−1.27)**	**<0.0001**	16,032	
Pts on ICS‡	10.9	**1.11 (1.04−1.18)**	**0.002**	16,018	0.49
Pts not on ICS	8.8	1.05 (0.92−1.20)	0.44	8,071	
GOLD groups§					
GOLD A	9.7	1.09 (0.94−1.27)	0.26	4,737	reference
GOLD B	9.8	**1.21 (1.02−1.44)**	**0.03**	2,845	0.17
GOLD C	10.5	**1.17 (1.02−1.34)**	**0.02**	3,172	0.30
GOLD D	10.2	1.06 (0.94−1.20)	0.36	3,712	0.72

The table shows the rate of COPD exacerbations in patients with elevated eosinophil counts (≥0.45x10^9/L) relative to patients with reference eosinophil counts (0.05 to <0.45x10^9/L) in each subgroup shown in column

one. Differences between subgroups were tested by including an interaction term of elevated eosinophil count and the variable used to define the categories of the subgroup in a multiple regression model (e.g. interaction term of elevated eosinophil count (yes/no)*sex (male/female) had a P-value of 0.052).

Abbreviations: FEV_1 = forced expiratory volume in 1 sec; FVC = forced vital capacity; ICS = inhaled corticosteroids; GOLD Global initiative for chronic Obstructive Lung Disease = MRC = Medical Research Council; Pts = patients.

*Rate Ratio adjusted for potential confounders
†Includes ex-smokers and never-smokers
‡Maintenance treatment with inhaled corticosteroids
§GOLD groups A–D were defined as follows: GOLD A, FEV_1 %predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC 0–1; GOLD B, FEV_1 %predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC ≥2; GOLD C, FEV_1 %predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year and mMRC 1–2; GOLD D, (FEV_1 %predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year and mMRC ≥2

Table E6. Rate Ratios (95% CI) of the association between elevated blood eosinophil count and COPD exacerbations during the follow-up year in subgroups of patients not currently smoking (from the *full population*) defined by sex, ICS therapy and GOLD groups.

Study subgroups	**Patients with COPD (*n=24,089*)**				
	Prevalence of elevated eosinophil count (%)	RR* (95% CI)	*P* value	N	*P-value interaction*
Male pts not currently smoking†	12.6	**1.26 (1.15−1.37)**	**<0.0001**	8,851	**0.02**
Female pts not currently smoking	8.6	1.08 (0.97−1.20)	0.17	7,181	
Pts on ICS‡ not currently smoking	11.7	**1.21 (1.12−1.30)**	**<0.0001**	11,304	0.16
Pts not on ICS not currently smoking	8.8	1.06 (0.89−1.25)	0.52	4,728	
pts not currently smoking at different GOLD groups§					
GOLD A	9.8	1.20 (1.00−1.44)	0.05	3,074	reference
GOLD B	10.6	**1.32 (1.09−1.61)**	**0.006**	1,879	0.22
GOLD C	11.4	**1.28 (1.10−1.49)**	**0.001**	2,113	0.31
GOLD D	10.9	1.13 (0.98−1.30)	0.09	2,577	0.95

The table shows the rate of COPD exacerbations in patients with elevated eosinophil counts ($\geq 0.45 \times 10^9$/L) relative to patients with reference eosinophil counts (0.05 to $<0.45 \times 10^9$/L) in each subgroup shown in column one. Differences between subgroups were tested by including an interaction term of elevated eosinophil count and the variable used to define the categories of the subgroup in a multiple regression model (e.g. interaction term of elevated eosinophil count (yes/no)*sex (male/female) had a P-value of 0.02).

Abbreviations: FEV_1 = forced expiratory volume in 1 sec; FVC = forced vital capacity; ICS = inhaled corticosteroids; GOLD Global initiative for chronic Obstructive Lung Disease = MRC = Medical Research Council; Pts = patients.

*Rate Ratio adjusted for potential confounders

†Includes ex-smokers and never-smokers

‡Maintenance treatment with inhaled corticosteroids

§GOLD groups A–D were defined as follows: GOLD A, FEV_1 %predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC 0–1; GOLD B, FEV_1 %predicted ≥50 and ≤1 COPD exacerbation and no hospitalization for COPD in baseline year and mMRC ≥2; GOLD C, FEV_1 %predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year and mMRC 1–2; GOLD D, (FEV_1 %predicted <50 or ≥2 COPD exacerbations or ≥1 hospitalization for COPD in baseline year and mMRC ≥2

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