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Blood eosinophil thresholds and exacerbations in chronic obstructive pulmonary disease

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Abstract

Background—Eosinophilic airway inflammation in COPD is associated with exacerbations and responsivity to steroids, suggesting potential shared mechanisms with eosinophilic asthma. However there is no consistent blood eosinophil level that has been used to define the increased exacerbation risk.

Objective—To investigate blood eosinophil levels associated with exacerbation risk in COPD

Methods—Blood eosinophil counts and exacerbation risk were analyzed in moderate-to-severe COPD subjects, using two independent studies of former and current smokers with longitudinal

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data. The COPDGene study was analyzed for discovery (n=1553) and the ECLIPSE study was analyzed for validation (n=1895). A subset of the ECLIPSE subjects were used to assess the stability of blood eosinophil counts over time.

Results—COPD exacerbation risk increased with higher eosinophil counts. An eosinophil threshold of ≥300 cells/μL showed adjusted incidence rate ratios (IRR) for exacerbations of 1.32 in COPDGene (95% confidence interval (CI) 1.10–1.63). The cutoff of ≥300 cells/μL was validated for prospective risk of exacerbation in ECLIPSE with adjusted IRR of 1.22 (95% CI 1.06–1.41) using 3 year follow up data. Stratified analysis confirmed that the increased exacerbation risk associated with an eosinophil count ≥300 cells/μL was driven by subjects with a history of frequent exacerbations in both COPDGene and ECLIPSE.

Conclusions—Patients with moderate to severe COPD and blood eosinophil count ≥ 300 cells/μL had an increased risk exacerbations in the COPDGene Study which was prospectively validated in the ECLIPSE Study.

Keywords

pulmonary disease; chronic obstructive; asthma; eosinophil; exacerbation

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by irreversible airflow limitation and has limited therapeutic options. Patients with COPD are prone to develop exacerbations, which are associated with decline in lung function and increased morbidity and mortality. In COPD, respiratory tract inflammation is thought to be driven by lymphocytes and neutrophils[1], compared to inflammation in asthma which is mediated by Th2 cells and eosinophils. [2] In patients with asthma, eosinophilic inflammation is associated with an increased exacerbation risk and loss of disease control upon inhaled corticosteroid (ICS) withdrawal.[3] Similarly, increasing evidence suggests that 20–40% of patients with stable COPD have eosinophilic airway inflammation measured by sputum eosinophils[4] that is associated with exacerbations[5,6] and response to steroids.[7–9] Since blood eosinophils are more easily assessed than sputum eosinophils, associations between blood eosinophils and COPD phenotypes have been investigated. Prior studies have reported that a blood eosinophil count of 2% can serve as a surrogate for sputum eosinophilia during an exacerbation of COPD,[10] while other studies have reported lack of correlation between sputum and blood eosinophil levels.[11–14]

Furthermore, there is conflicting evidence about whether elevated blood eosinophil levels are associated with increased exacerbations. Some studies reported increased COPD exacerbation risk with varying levels of blood eosinophils in general and clinical populations[15–17] and post hoc analyses of clinical trials,[18–21] while other studies have reported lack of associations between blood eosinophils and COPD exacerbations.[11–13,22,23] Recently, a clinical trial showed that mepolizumab, an anti-interleukin 5 monoclonal antibody, reduced moderate or severe exacerbations in COPD patients with high blood eosinophil counts,[24] demonstrating that blood eosinophils are a useful biomarker to identify eosinophilic inflammation that can be targeted for therapy.

In this study, we aimed to evaluate the relation between blood eosinophil counts and COPD exacerbation risk in two well-phenotyped COPD cohorts, COPDGene[25] and ECLIPSE.[26] We determined blood eosinophil levels associated with COPD exacerbations in COPDGene, and validated the finding prospectively in ECLIPSE.

METHODS

Study populations

The COPDGene (Genetic Epidemiology of COPD) and ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) studies have been described previously.[25,26] Briefly, COPDGene is a multicenter observational study which enrolled 10,192 smokers with and without COPD in Phase 1. Complete blood counts (CBC) were measured at the Phase 2 visit, approximately five years later. Subjects were clinically stable, with at least 30 days since their last exacerbation. The current analysis included 1,553 Phase 2 subjects with GOLD spirometry grade 2–4 COPD (post-bronchodilator ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) <0.7 with FEV_1 < 80% predicted)[27] who had available CBC and clinical data. Of these subjects, 1,113 (71%) participated in a longitudinal follow up program to prospectively assess exacerbations following the Phase 2 visit.

The ECLIPSE study was a multicenter multinational 3-year longitudinal study that enrolled 3,291 subjects. We analyzed 1,895 subjects with GOLD spirometry grade 2–4 with complete CBC and 3 year follow up data. Subjects with an exacerbation within four weeks of enrollment were excluded. We also analyzed 243 smoker controls with repeated CBC measurements to measure eosinophil stability. Subjects taking oral corticosteroids were excluded from both COPDGene and ECLIPSE analyses (Supplemental Figure 1).

Exacerbation ascertainment

In COPDGene and ECLIPSE, exacerbations were self-reported, and defined as an episode of increased dyspnea, cough and/or sputum production that required antibiotic and/or systemic steroid treatment. Severe exacerbations were defined as exacerbations requiring Emergency Department visits or hospitalization.[14,28] In the longitudinal follow up program for COPDGene participants, exacerbation information was collected by telephone call or web-based survey every 6 months.

Statistical analysis

Descriptive analysis was performed with t test, Fisher’s exact test and Kruskal Wallis test as appropriate. Negative binomial multivariate regression was performed for exacerbation analysis and incidence rate ratios (IRRs) were calculated with 95% confidence intervals. Nested models with and without eosinophils were compared using a likelihood ratio test. Logistic regression was performed for binary variables (frequent exacerbations and severe exacerbations). Linear regression was performed for continuous variables, and the residuals were plotted to check normality assumption. Receiver operating characteristic (ROC) curves for multivariate models with different eosinophil counts were plotted for no exacerbations

versus 1 or more exacerbations to evaluate eosinophil cutoffs. Statistical analyses were performed using R version 3.3.1.

RESULTS

Eosinophil thresholds and exacerbation risk in cross sectional analysis of COPDGene Of the 1765 COPDGene subjects with GOLD grade 2–4 COPD with CBC data, subjects taking chronic oral steroids or with incomplete information were excluded, leaving 1553 subjects for analysis (Supplemental Figure 1). As there is no consensus definition for eosinophilic COPD, we evaluated a range of thresholds ranging from 2–5% and from 100–400 cells/μL for association with exacerbations. We also included eosinophil cutoff of 340 cells/μL, based on a recent publication.[15] In negative binomial regression analysis adjusting for known COPD exacerbation risk factors including gastroesophageal reflux (GERD), Saint George's Respiratory Questionnaire total score (SGRQ), baseline smoking status, post bronchodilator FEV_1 percent predicted and white blood cell count (WBC), we observed increasing incidence rate ratios (IRR) for exacerbation frequency as the eosinophil cutoff increased (Figure 1). Eosinophil counts showed a consistent linear relationship with exacerbation risk, which was not clearly seen with eosinophil percentages. Multivariable logistic regression models using different eosinophil cutoffs found that a threshold of 300 cells/μL produced the maximal area under the ROC curve, with the highest sensitivity and specificity (Supplementary Figure 2). Compared to the nested model without eosinophils, inclusion of an eosinophil threshold of 300 cells/μL was statistically significant (likelihood ratio test $p = 0.006$).

Subjects with eosinophilic COPD were more likely to be male and non-Hispanic white, and had a greater number of exacerbations per year. Subjects with eosinophil counts ≥ 300 cells/μL had higher WBC counts with lower neutrophil percentages. There was no difference in reported inhaled corticosteroid usage between patients with eosinophil counts above or below 300 cells/μL. (Table 1). In multivariable analysis, eosinophilic COPD was associated with higher SGRQ score (total, impact and activity) and less emphysema measured by quantitative analysis of chest CT scans (low attenuation area at −950HU and the 15^{th} percentile of the lung density histogram) (Supplementary Table 1). There was no difference in spirometric measures (FEV_1, FVC, FEV_1/FVC) or 6 minute walk distance.

In a multivariable model, exacerbation frequency was significantly associated with female sex, white race, GERD, higher SGRQ total score, lower post bronchodilator FEV_1 percent predicted and eosinophil counts ≥ 300 cells/μL (Table 2). Eosinophil counts ≥ 300 cells/μL had IRR of 1.32 (95% CI 1.10–1.61) (Table 2). Furthermore, eosinophilic COPD was associated frequent exacerbations (≥ 2/year) in a logistic regression analysis (OR 1.58, 95% CI 1.07–2.30) (Table 2).

Prospective analysis of COPDGene

Of the 1553 patients in the cross-sectional analysis, 1113 (71.6%) had at least one follow up contact after the COPDGene Phase 2 visit, for a total of 1561 person-years (mean 512 days). There was no difference in follow up duration or proportion lost to follow up between eosinophilic and non-eosinophilic COPD. The same covariates as in the cross sectional

multivariable model were used, with the addition of prior exacerbation history. Subjects with eosinophil counts ≥ 300 cells/μL at the phase 2 visit had an increased prospective exacerbation rate, with a similar risk estimate as the cross-sectional analysis (IRR 1.33, 95% CI 0.91–1.95); however eosinophilic COPD was not statistically significant in multivariable regression (Table 2). When we performed stratified analysis based on the exacerbation frequency in the prior year, we found that the predictive ability of eosinophilic COPD for future exacerbations was driven by the subset of subjects with a history of frequent exacerbations (≥ 2 per year, IRR 1.96, 95% CI 1.21–3.21, p = 0.008). In subjects with 2 or more exacerbations in the prior year, the annual exacerbation rate during follow up was 2.39 in those with elevated eosinophils, compared to 1.42 without elevated eosinophils (Supplementary Table 2). High eosinophil count was not significantly associated with future exacerbations in subjects with a history of one exacerbation or fewer in the previous year (Table 4, Supplementary Table 2).

Validation in ECLIPSE

We prospectively validated the increased exacerbation risk with increased eosinophil counts in the ECLIPSE study. Among 2303 subjects with GOLD grade 2–4 COPD, 1895 with baseline CBC and complete covariate information were included in the analysis. There were 133 subjects who participated in screening visit but did not have follow up visits or had missing laboratory information. There was no difference in the proportion of eosinophilic and non-eosinophilic COPD among these subjects compared to the overall study population. Similar to COPDGene, those with eosinophil counts ≥ 300 cells/μL at the screening visit were more likely to be male. WBC counts were increased and neutrophil percentage was decreased (Table 1). There was no difference in quantitative CT measurements of emphysema and SGRQ score in ECLIPSE, in contrast to COPDGene (Supplementary Table 1). As in COPDGene, there was no association between eosinophil counts ≥ 300 cells/μL and spirometric measures or 6 minute walk distance.

We calculated exacerbation rates by dividing the total number of moderate to severe exacerbations by observed time at two different time points: 1 year follow up and the overall study period (4981 person-years total; mean 959 days). Models were adjusted for age, sex, race, and known risk factors for COPD exacerbations (prior history of exacerbations, GERD, SGRQ total score, baseline smoking status, post bronchodilator FEV_1 percent predicted and WBC count). There was no difference in risk estimates when we accounted for subjects with a change in smoking status, therefore we only included baseline smoking status in the model. An eosinophil count ≥ 300 cells/μL at screening was consistently predictive of future exacerbations (IRR 1.20, 95% CI 1.05–1.36 at 1 year, IRR 1.22, 95% CI 1.06–1.42 for overall study period) (Table 3). Comparable to COPDGene, increasing eosinophil counts were associated with higher risk of exacerbations (Figure 2). Addition of eosinophils was statistically significant based on the likelihood ratio test (p <0.001 for 1 year follow-up and overall study period). Area under the curve in ROC analyses did not differ with different eosinophil count cutoffs (Supplementary Figure 3). Similar to the COPDGene longitudinal follow up study, the exacerbation risk associated with elevated eosinophil counts was driven by the frequent exacerbators with ≥ 2 exacerbations prior to study entry in the stratified analysis(IRR 1.40, 95% CI1.15–1.70, p < 0.001) (Table 4). Subjects with frequent

exacerbation history and eosinophil ≥300 cells/μL had a higher annual exacerbation rate (overall 2.41/year) than those with frequent exacerbation history and lower eosinophil counts (overall 1.61/year, Supplementary Table 2). In the longitudinal analysis, eosinophilic and non-eosinophilic COPD subjects had similar changes in FEV_1, FVC, SGRQ score, and 6 minute walk distance.

We also analyzed the subset of subjects (1839 COPD cases and 243 controls) with repeated eosinophil measurements over the course of the ECLIPSE study. Eosinophil counts were relatively stable over time, with an interclass correlation coefficient (ICC) of 0.57. There was no significant difference in ICC between COPD cases and controls (0.57 and 0.56 respectively). For a subset of 1345 COPD cases (71%) with four CBC measurements over the study (0, 1, 2, and 3 years), 90 (6.7%) had persistently elevated eosinophil levels above 300/μL. The majority (784, 58%) had eosinophil levels below 300/μL for the entire study. The exacerbation risk in subjects with persistently elevated eosinophil counts was higher compared to the subjects with persistently low eosinophil counts and those with fluctuating eosinophil counts (Supplementary Table 3), suggesting that while baseline eosinophil level was predictive of exacerbation risk up to 3 years, patients with persistent eosinophilia are at the greatest risk of exacerbation.

Sensitivity Analysis

To evaluate for confounding by inhaled corticosteroid (ICS) usage, we included use of ICS as covariate in the multivariable model. The association between eosinophilic COPD and exacerbations in COPDGene and ECLIPSE remained (COPDGene IRR 1.34, 95% CI 1.01–1.63, ECLIPSE 1 year follow up IRR 1.18, 95% CI 1.04–1.34, overall study period IRR 1.21, 95% CI 1.05–1.39). In addition we performed a sensitivity analysis focusing on the subgroup of subjects not taking ICS. While increasing eosinophil counts were associated with increased exacerbation risk, the cutoff of 300 cells/μL was no longer statistically significant. This is likely related to the reduced sample size (Supplementary Figure 4) as previously validated risk factors for COPD exacerbations, including GERD and WBC,[14] were no longer significant in the subgroup analysis (data not shown).

Epidemiologic relationship between eosinophilic COPD and Asthma-COPD overlap

We analyzed the relationship between eosinophilic COPD, ACO and exacerbation risk. ACO has previously been shown to be associated with increased exacerbation risk in COPDGene[29,30] and ECLIPSE.[31] COPD subjects with eosinophil counts ≥ 300 cells/μL were more likely to have ACO, using the COPDGene definition of self-report of doctor's diagnosis of asthma before age 40[30,32] (OR 1.51 for COPDGene and OR 1.69 for ECLIPSE) (Supplementary Table 4). However, the overall concordance between eosinophilic COPD and asthma was low (N=54 in COPDGene, N=47 in ECLIPSE) (Figure 3). In negative binomial regression, ACO and eosinophilic COPD were independently associated with exacerbation risk in COPDGene and ECLIPSE (Table 5).

DISCUSSION

In this study of the two multicenter, longitudinal cohorts of moderate to severe COPD subjects, we found that exacerbation risk increased linearly with higher blood eosinophil counts. We identified a threshold blood eosinophil count of ≥300 cells/μL to be associated with exacerbations and then validated this cutoff as a predictor of future exacerbations using prospective data from the ECLIPSE study. We further showed that the increased COPD exacerbation risk associated with elevated eosinophil counts was driven by subjects in both studies with a history of frequent exacerbations, defined as two or more exacerbations per year.

The most commonly used cutoff to define eosinophilic COPD is 2%, which originates from a sentinel study describing increased exacerbation risk with blood eosinophils ≥ 2 % that was predictive of sputum eosinophilia (≥3%) at the time of exacerbation.[10] A previous analysis in ECLIPSE showed 88% concordance between blood eosinophil thresholds of 2% and 150 cells/μL.[33] Many subsequent studies have used these lower thresholds.[11,34–38] In our study, we found that higher baseline eosinophil levels were associated with future exacerbation risk up to three years and that absolute eosinophil counts were consistently associated with increased exacerbations while the eosinophil percentage did not show a linear relationship in either COPDGene or ECLIPSE. The absolute eosinophil count may be more relevant biologically, as WBC count can vary widely and single percentage value may not capture the entire range of blood eosinophils. In contrast, a percentage cutoff has been used for sputum, as sputum differential count is typically based on counting a fixed number of cells (400–600 cells) on a cytospin slide.[13,39–42]

Our finding is in line with the recently published Copenhagen General Population Study, where an eosinophil cutoff of 340 cells/μL was associated with COPD exacerbations.[15] Two studies based on health care utilization data also demonstrated increased exacerbation risk with blood eosinophil counts ≥ 300 cells/μL[17] and ≥ 450 cells/μL.[16] In common with our study, these studies included large numbers of subjects with moderate to severe COPD (FEV_1 <80% or GOLD C and D) and applied higher eosinophil cutoffs defined by absolute counts. Other studies reporting a lack of association between blood eosinophils and COPD exacerbations had differences compared to our study. Most of these studies were smaller. [11,12,22,23] AERIS [11], BPCO [22] and a recent meta analysis used only percentage cutoffs for eosinophils.[38] The importance of higher, count based eosinophil cutoff is demonstrated by the lack of association between eosinophil cutoff of 2% and exacerbation risk in a subset of the ECLIPSE study.[33] No association was found in SPIROMICS, which included GOLD 0 subjects (37% of the study population), while moderate to severe COPD comprised 22% of the subjects.[13] As low lung function is a risk factor for COPD exacerbations, selection of moderate to severe COPD patients may enrich for subjects prone to exacerbations. These observations suggest that patient population, disease severity and eosinophil cutoffs all matter in understanding the significance of eosinophils in COPD.

Our findings highlight the utility of measuring eosinophil counts in patients with frequent exacerbations, as eosinophilic COPD is a significant risk factor for future exacerbations in this subgroup. In the prospective follow up study of both COPDGene and ECLIPSE,

frequent exacerbators with eosinophil counts ≥ 300 cells/μL had an average of one additional exacerbation episode per year compared to frequent exacerbators with lower eosinophil counts. While the most significant predictive factor for future exacerbation is a prior history of exacerbation in COPD patients,[14] we showed that measurement of eosinophil counts in a high risk group may serve as a biomarker for additional risk stratification. Measurements of eosinophil counts may not have much utility in COPD in subjects with few exacerbations. Furthermore, eosinophil measurements in patients with frequent exacerbations may identify subjects who can be treated with anti-eosinophilic therapy, based on recent mepolizumab trials.[24] METREX and METREO were phase 3, randomized, placebo-controlled, double blind trials that enrolled patients with two or more moderate exacerbations or one or more severe exacerbations on triple inhaled therapy.[24] Eosinophilic COPD phenotype based on blood eosinophil count was part of the enrollment criteria in METREO but not for METREX.[24] In line with our study, these parallel trials also showed that exacerbation risk can be further stratified based on blood eosinophil counts even in patients with significant prior exacerbation history.[24]

Subjects with a history of 2 or more exacerbations in the past year included 14% of moderate to severe COPD subjects in COPDGene and 22% in ECLIPSE. Based on the current guidelines, frequent exacerbators on maximal inhaled therapy would be considered for long-term azithromycin or roflumilast to prevent future exacerbations.[43,44] Eosinophilic frequent exacerabators comprised 2.8% of moderate to severe COPD subjects in COPDGene and 5% of ECLIPSE, corresponding to 20% of the frequent exacerbator group. Further studies are required to determine the effects of anti-inflammatory therapy vs. anti-eosinophilic therapy, in eosinophilic and non-eosinophilic exacerbation-prone subjects.

At a single time point, both the COPDGene and ECLIPSE studies had approximately 20% of moderate to severe COPD patients with eosinophil count ≥ 300 cells/μL. However, in ECLIPSE only 6.7% had persistently elevated eosinophil levels above 300 cells/μL which carried the greatest risk of exacerbations. A recent study of the UK Clinical Practice Research Datalink showed that blood eosinophil counts are more variable in COPD, particularly for patients with higher baseline eosinophil levels.[45] While we did not observe differences in eosinophil stability between COPD patients and controls, we found that about 60% of COPD patients never had eosinophil counts ≥ 300 cells/μL.

In COPDGene, eosinophilic COPD was associated with an approximately 30% increased risk of exacerbations in both cross-sectional and prospective data. However, eosinophil level was not statistically significant in the prospective analysis. The data collection for the longitudinal follow up program was different than the main COPDGene study, since exacerbations were assessed every six months using a web and phone based telecommunication system.[46] It is possible that the longitudinal follow up data may not represent the same type of exacerbation information collected in person at the study visits.

We have also found differences between COPDGene and ECLIPSE. For example, current smoking was negatively associated with exacerbation frequency in the COPDGene study, while it was associated with an increased exacerbation rate in ECLIPSE. This likely reflects indication bias in the COPDGene population; subjects with more severe disease may

preferentially quit smoking.[47] ECLIPSE subjects had a higher proportion of bronchodilator reversibility, which may be due to the withdrawal of bronchodilators prior to assessment, which was not performed in COPDGene. Individuals with COPD and blood eosinophil counts ≥ 300 cells/μL had higher SGRQ scores in COPDGene, but not in ECLIPSE.

As eosinophilic inflammation is classically regarded as a feature of allergic asthma,[48] we examined the relationship between eosinophilic COPD and asthma. Both COPDGene and ECLIPSE did not exclude subjects with asthma history. In univariate analysis there was no significant associations between eosinophilic COPD and asthma-COPD overlap, defined as asthma diagnosis before the age of 40 in the COPDGene study.[30] The limited concordance between eosinophilic COPD and asthma could be due to the incomplete definition of ACO, as we relied on patient report of a diagnosis of asthma, or due to the heterogeneous nature of ACO itself. ACO can include asthmatics with fixed obstruction or smokers with COPD and features of asthma or a Th2 immune response that may develop independent of asthma.[49]

A count-response relation between blood eosinophil counts and asthma-related outcomes has well been described in patients with asthma; blood eosinophil counts of 290–400 cells/μL have been associated with increased asthma exacerbations.[50–53] However, eosinophilic COPD subjects in our study had an average of fifty pack-year smoking history, while studies of eosinophilic asthma typically exclude greater than ten pack-year smokers or subjects with COPD. Therefore, even if a subset of COPD patients had prior asthma that progressed to COPD in our cohorts, these subjects still had significant cigarette smoke exposure that is much higher than populations in asthma studies. Despite the differences in smoking exposure, eosinophilic asthma and eosinophilic COPD patients show similar relationships between blood eosinophil counts and frequent exacerbations, which points to a shared role of eosinophilic inflammation. This is in line with a recently published study demonstrating asthma-like airway remodeling and inflammation in eosinophilic COPD patients without allergies or asthma history.[54]

Response to therapeutics in eosinophilic COPD also provides clues to a potential shared mechanism with eosinophilic asthma. Previous studies have shown that elevated blood eosinophil counts in COPD patients predict favorable response to oral[55,56] and inhaled corticosteroids,[18–20,57] which have been cornerstones for asthma therapy. In a recent trial, benralizumab (anti IL5 receptor α monoclonal antibody) reduced exacerbations in COPD subjects with blood eosinophils greater than 300 cells/μL.[58] As mentioned above, mepolizumab reduced exacerbations in COPD subjects with elevated blood eosinophils.[24] The blood eosinophil threshold for targeting intervention requires additional investigation and is an effort in the COPD Biomarker Qualification Consortium.[59]

The strength of our study is the inclusion of two large cohorts of well-characterized subjects with COPD that were prospectively analyzed as discovery and validation populations to identify an eosinophil threshold increasing exacerbation risk. We are also aware of limitations of this study. We measured blood eosinophils and not sputum or lung tissue eosinophils, which may be more closely related to the disease process. We excluded patients taking oral steroids, however approximately 50% of the COPDGene and 70% of the ECLIPSE subjects were using ICS. While ICS use has minimal impact on blood eosinophil

counts,[60] eosinophilic COPD patients are known to be more responsive to ICS therapy and have less exacerbations on ICS, which may reduce the impact of the exacerbation risk. The analysis restricted to non-users of ICS showed increasing eosinophil counts were associated with increased risk of exacerbation but the cutoff of 300 cells/μL was no longer significant likely due to the reduced number of subjects. In the COPDGene study, CBC were only obtained at the Phase 2 visit, so we could not assess changes in eosinophil counts over time or use the full longitudinal data from Phase 1 to Phase 2. Self-report of exacerbation history may be a limitation, though this definition has been used in multiple prior studies in both COPDGene[28] and ECLIPSE.[14] Finally, as patients with asthma history were enrolled in the cohorts, there could be misclassification between eosinophilic COPD and asthma. We did not have skin testing results or IgE to independently assess allergic component. However as there is no gold standard definition of ACO, we aimed to assess the relationship of ACO and eosinophilic asthma utilizing this dataset.

In conclusion, we showed that blood eosinophil counts greater than or equal to 300 cells/μL were associated with increased exacerbation frequency in two large COPD studies. This threshold identified an eosinophilic subgroup of 20% of subjects with moderate to severe COPD, and 20% of subjects with frequent exacerbations in whom blood eosinophils could further stratify exacerbation risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACO	asthma-COPD overlap
BDR	bronchodilator reversibility
BMI	body mass index
CBC	complete blood count
COPD	chronic obstructive pulmonary disease
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
GERD	gastroesophageal reflux
HU	Hounsfield units
ICC	interclass correlation coefficient
ICS	inhaled corticosteroid
IRR	incidence rate ratio
LAA950	percent of lung with attenuation less than −950 Hounsfield units
Perc15	15th percentile of the lung density histogram
ROC	receiver operating characteristics
SGRQ	Saint George's Respiratory Questionnaire
Th2	T helper type 2
WBC	white blood cell

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Key Messages

- Patients with moderate to severe COPD and elevated blood eosinophil counts have increased exacerbation risk compared to those with blood eosinophil count of less than 300 cells per μL.
- Our study supports measurement of eosinophils in patients with frequent COPD exacerbations which may have utility in identifying a population for treatments targeting eosinophilic inflammation.

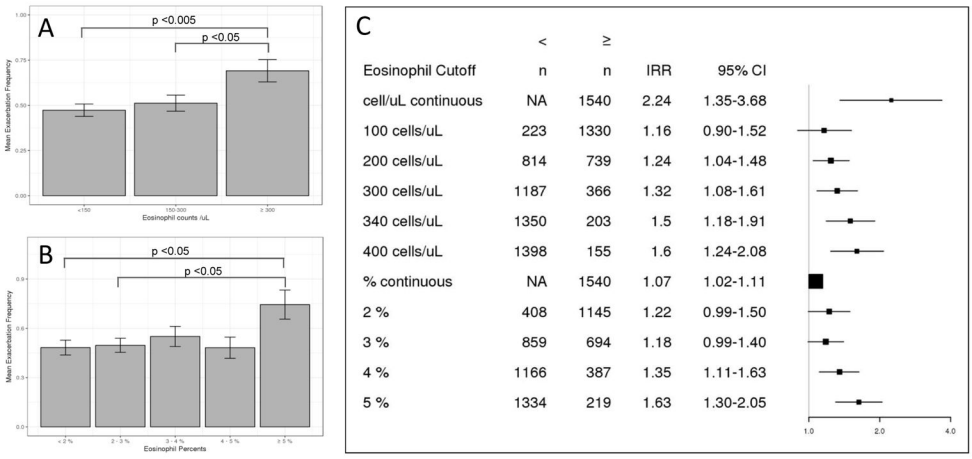

Figure 1.

Risk of COPD exacerbations with increasing blood eosinophil levels in COPDGene. (A) Mean 1 year exacerbation frequency reported at phase 2 visit per blood eosinophil count range in moderate to severe COPD subjects. Error bars indicate standard errors. Difference between group means determined by one-way ANOVA and Tukey honestly significant difference test. (B) Mean 1 year exacerbation frequency reported at phase 2 visit per blood eosinophil percent range in moderate to severe COPD subjects. Error bars indicate standard errors. Difference between group means determined by one-way ANOVA and Tukey honestly significant difference test. (C) Adjusted incidence rate ratios for COPD exacerbations with different eosinophil cutoff values. Risk estimates are derived from negative binomial regression models adjusted for age, sex, race, gastroesophageal reflux, Saint George's Respiratory Questionnaire score, post bronchodilator forced expiratory volume at 1 second percent predicted, and white blood cell count. IRR: incidence rate ratio. CI: confidence interval.

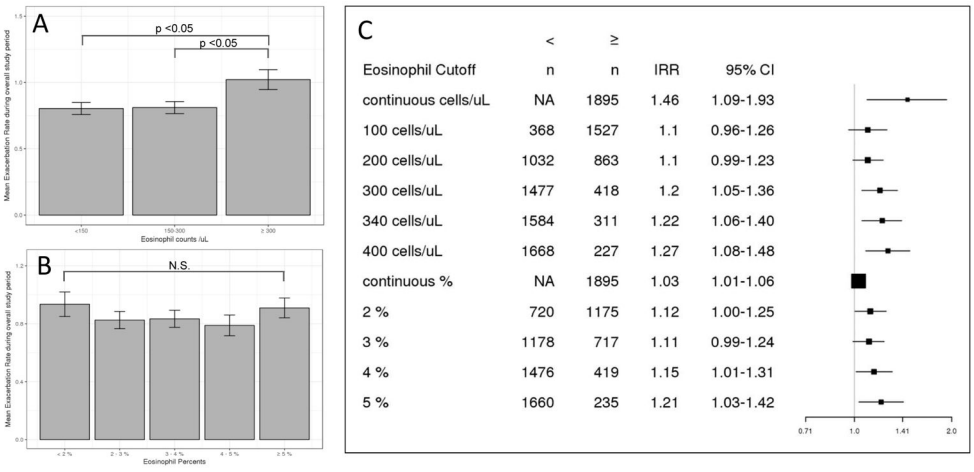

Figure 2.

Risk of prospective COPD exacerbations with increasing blood eosinophil levels in ECLIPSE. (A) Mean exacerbation rate during overall study period per blood eosinophil count range in moderate to severe COPD subjects. Error bars indicate standard errors. Difference between group means determined by one-way ANOVA and Tukey honestly significant difference test. (B) Mean exacerbation rate during overall study period per blood eosinophil percent range in moderate to severe COPD subjects. Error bars indicate standard errors. Difference between group means determined by one-way ANOVA and Tukey honestly significant difference test. (C) Adjusted incidence rate ratios for COPD exacerbations with different eosinophil cutoffs. Risk estimates are derived from negative binomial regression models adjusted for age, sex, race, previous exacerbations, gastroesophageal reflux, Saint George's Respiratory Questionnaire score, post bronchodilator forced expiratory volume at 1 second percent predicted, and white blood cell count. IRR: incidence rate ratio. CI: confidence interval.

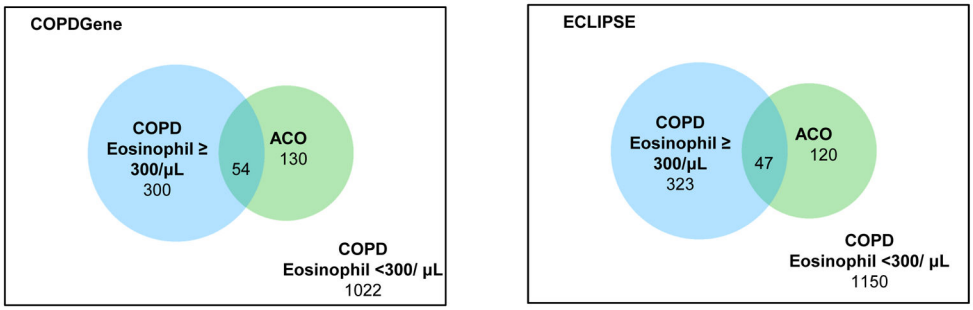

Figure 3.

Venn Diagrams of the number of subjects with asthma-COPD overlap (defined by asthma diagnosis before the age of 40[30]) and COPD subjects with blood eosinophil counts ≥ 300 cells/μL in COPDGene and ECLIPSE.

Table 1

Baseline characteristics of subjects with high vs. low blood eosinophils.

	COPDGene			ECLIPSE		
	Eosinophil < 300 cells/μL (n=1187)	**Eosinophil ≥ 300 cells/μL (n=366)**	**p value**	**Eosinophil < 300 cells/μL (n=1477)**	**Eosinophil ≥ 300 cells/μL (n=418)**	**p value**
Age (mean(SD))	67.88 (8.19)	68.27 (8.29)	0.43	63.23 (7.1)	63.86(6.8)	0.11
Gender (Female) (%)	**567 (47.8)**	**130 (35.5)**	**<0.001**	**542 (36.7)**	**114 (27.3)**	**<0.001**
Race (White) (%)	**911 (76.7)**	**305 (83.3)**	**0.009**	1446 (97.9)	407 (97.4)	0.64
post-bronchodilator FEV_1 % predicted(mean(SD))	53.11 (17.0)	51.43 (17.2)	0.10	47.96 (15.5)	48.72 (15.9)	0.38
GOLD grade (%)			0.55			0.77
2	695 (58.6)	204 (55.7)		644 (43.6)	179 (42.8)	
3	360 (30.3)	115 (31.4)		631 (42.7)	186 (44.5)	
4	132 (11.1)	47 (12.8)		202 (13.7)	53 (12.7)	
FEV_1 decline ml/year (mean(SD))	NA	NA	NA	34.12 (45.0)	30.0 (50.0)	0.18
DLCO (ml/min/mmHg)[¶] [*]	14.70 (6.4)	15.17 (6.4)	0.26	NA	NA	NA
CT Emphysema % at −950 HU [*] (mean(SD))	12.59 (12.8)	11.69 (11.7)	0.31	17.77 (12.4)	17.43 (11.8)	0.65
CT lung density 15th Percentile [*] (mean(SD))	−936.30 (27.3)	−933.32 (29.9)	0.13	−948.37 (27.07)	−949.35(25.41)	0.54
SGRQ total score (mean(SD))	35.21(20.3)	37.41 (21.8)	0.07	49.97 (20.26)	47.98 (20.23)	0.08
6 min walk distance, meters (mean(SD)) [*]	345.9 (128)	340.5(138)	0.50	370.9 (120)	372.2 (122.8)	0.85
Any asthma history (%)	299 (25.2)	101 (27.6)	0.51	326 (24.0)	102 (26.3)	0.39
Asthma-COPD overlap (%)	130 (11.3)	54 (15.3)	0.06	120 (9.4)	47 (12.7)	0.09
Bronchodilator reversibility [†] (%)	189 (15.9)	65 (17.8)	0.46	**318 (21.5)**	**120 (28.7)**	**0.003**
Exacerbations/year [‡] (mean(SD))	**0.49 (0.9)**	**0.69 (1.2)**	**<0.001**	**1.15 (1.3)**	**1.37 (1.6)**	**0.004**

	COPDGene			ECLIPSE		
	Eosinophil < 300 cells/μL (n=1187)	**Eosinophil ≥ 300 cells/μL (n=366)**	**p value**	**Eosinophil < 300 cells/μL (n=1477)**	**Eosinophil ≥ 300 cells/μL (n=418)**	**p value**
Severe exacerbations[§] ((%), mean(SD))	190 (16)	69 (18.9)	0.23	0.27 (0.6)	0.29 (0.7)	0.55
BMI, kg/m^2 (mean(SD))	28.06 (6.4)	28.52(6.4)	0.22	26.42 (5.7)	26.65 (5.5)	0.45
Pack-Years of smoking (mean(SD))	51.96 (24.3)	51.43 (25.8)	0.72	47.91 (26.6)	50.53 (28.0)	0.08
Current smokers (%)	404 (34.0)	116 (31.7)	0.44	926 (62.7)	266 (63.6)	0.77
Inhaled corticosteroid use (%) [*]	568 (48.4)	187 (51.1)	0.40	1047 (70.9)	310 (74.2)	0.21
WBC (mean(SD))	**7.32 (2.2)**	**8.32 (1.9)**	**<0.001**	**7.70 (2.3)**	**8.76 (2.4)**	**<0.001**
eosinophil % (mean(SD))	**2.01 (1.1)**	**5.15 (2.3)**	**<0.001**	**2.10 (1.2)**	**5.56 (2.7)**	**<0.001**
neutrophil % (mean(SD))	**62.06 (10.2)**	**59.61 (9.7)**	**<0.001**	**65.29 (8.9)**	**62.99 (8.4)**	**<0.001**
lymphocyte % (mean(SD))	27.08 (9.3)	26.30 (8.9)	0.16	**26.06 (8.1)**	**24.91 (7.3)**	**0.009**
monocyte % (mean(SD))	8.26 (2.5)	8.26 (2.2)	1.00	6.22 (2.3)	6.15 (2.2)	0.57
basophil % (mean(SD))	**0.60 (0.6)**	**0.68 (0.6)**	**0.02**	**0.33 (0.2)**	**0.38 (0.2)**	**<0.001**

[¶]Diffusion capacity of the lung for carbon monoxide (DLCO), adjusted for altitude and hemoglobin.

[*]subsets of subjects with complete information were used. DLCO: Eosinophil < 300 cells/μL n = 1003, Eosinophil ≥ 300 cells/μL n= 304, Chest CT data: COPDGene (Eosinophil < 300 cells/μL n = 863, Eosinophil ≥ 300 cells/μL n= 256), ECLIPSE (Eosinophil < 300 cells/μL n= 1263, Eosinophil ≥ 300 cells/μL n= 347). 6 minute walk distance: COPDGene (Eosinophil < 300 cells/μL n = 1166, Eosinophil ≥ 300 cells/μL n= 355), ECLIPSE(Eosinophil < 300 cells/μL n = 1457, Eosinophil ≥ 300 cells/μL n= 414). Asthma: COPDGene (Eosinophil <300 cells/μL 1322, Eosinophil ≥ 300 cells/μL n= 184), ECLIPSE (Eosinophil <300 cells/μL 1170, Eosinophil ≥ 300 cells/μL n= 370).)

[†]Defined as change in FEV_1 at least 200ml and 12% after bronchodilator. 2 subjects from COPDGene had missing data.

[‡]Number of exacerbations in the prior year to Phase 2 study visit is reported for COPDGene and exacerbation rate during the overall study period is reported for ECLIPSE.

[§]Severe exacerbations defined by Emergency Room visits or hospitalization was a binary variable in COPDGene. In ECLIPSE, severe exacerbation rate during the overall study period is reported.

Table 2

Multivariable models of blood eosinophil count ≥ 300 cells/μL and exacerbation risk in COPDGene.

	COPDGene: Year Prior to Visit 2 (Cross sectional)				COPDGene: Longitudinal Follow Up	
	Exacerbation Frequency* (n=1553)		Frequent Exacerbations† (n=1281)		Exacerbation Rate* (n=1113)	
Factors	**IRR* (95% CI)**	**p value**	**OR† (95% CI)**	**p value**	**IRR* (95% CI)**	**p value**
Age	**0.98 (0.97–1.00)**	**0.007**	**0.96 (0.94–0.99)**	**0.003**	0.99(0.97–1.01)	0.48
Female	**1.43 (1.20–1.71)**	**<0.001**	**1.83(1.30–2.58)**	**<0.001**	0.87(0.65–1.15)	0.31
Non White Race	**0.73 (0.57–0.92)**	**0.008**	**0.63(0.40–0.99)**	**0.05**	**1.73 (1.19–2.55)**	**0.005**
SGRQ total score‡	**1.02 (1.02–1.03)**	**<0.001**	**1.04(1.03–1.05)**	**<0.001**	**1.02(1.01–1.03)**	**<0.001**
post-bronchodilator FEV_1 % predicted §	**0.98 (0.98–0.99)**	**<0.001**	**0.97(0.96–0.98)**	**<0.001**	**0.99(0.98–1.00)**	**0.003**
GERD	**1.33 (1.11–1.59)**	**0.002**	**1.35(0.95–1.91)**	**0.09**	1.08(0.81–1.45)	0.57
Current smoking	**0.71(0.57–0.89)**	**0.002**	**0.56(0.37–0.84)**	**0.006**	**0.63(0.45–0.90)**	**0.01**
Previous Exacerbations	NA	NA	NA	NA	**2.51(1.87–3.37)**	**<0.001**
WBC	1.00 (0.96–1.04)	0.97	1.02(0.94–1.10)	0.66	1.03(0.96–1.10)	0.44
Eosinophil ≥300	**1.32 (1.08–1.61)**	**0.006**	**1.58(1.07–2.30)**	**0.019**	1.33(0.92–1.95)	0.13

*Risk estimate from negative binomial regression.

†Odds ratio from logistic regression comparing subjects with 2 or more exacerbations per year to subjects with less than 1 exacerbation per year.

‡per 1 point increase in score,

§per percentage point increase in FEV_1

Table 3

Multivariable models of blood eosinophil count ≥ 300 cells/μL and exacerbation risk in ECLIPSE.

ECLIPSE				
	1 year		overall study period	
Factors associated with Exacerbation Rate	IRR (95% CI)	p value	IRR (95% CI)	p value
Age	**1.01 (1.00–1.02)**	**0.008**	**1.01 (1.00–1.02)**	**0.01**
Female	**1.23 (1.10–1.38)**	**<0.001**	**1.31 (1.15–1.49)**	**<0.001**
Non White race	0.82(0.56–1.18)	0.29	0.71(0.45–1.08)	0.12
SGRQ total score *	**1.01 (1.01–1.01)**	**<0.001**	**1.01 (1.01–1.01)**	**<0.001**
Post-bronchodilator FEV_1 % predicted †	**0.99 (0.98–0.99)**	**<0.001**	**0.98 (0.97–0.98)**	**<0.001**
GERD	**1.39 (1.24–1.56)**	**<0.001**	**1.36 (1.19–1.56)**	**<0.001**
Current smoking	1.10(0.98–1.24)	0.11	**1.21(1.06–1.39)**	**0.005**
previous exacerbations	**2.45 (2.18–2.74)**	**<0.001**	**2.87 (2.51–3.29)**	**<0.001**
WBC	**1.04 (1.01–1.06)**	**0.001**	**1.04 (1.02–1.07)**	**<0.001**
Eosinophil ≥300 at screening	**1.20 (1.05–1.36)**	**0.005**	**1.22 (1.06–1.42)**	**0.006**

Risk estimate from negative binomial regression.

* per 1 point increase in score,

† per percentage point increase in FEV_1

Table 4

Risk of elevated blood eosinophils on future exacerbations, stratified by prior exacerbation history. (A) Incidence rate ratio (IRR) associated with eosinophil counts ≥ 300 cells/μL derived from multivariable analysis for exacerbation rate in COPDGene Longitudinal Follow-up Study, (B) IRR associated with eosinophil counts ≥ 300 cells/μL derived from multivariable analysis for exacerbation rate in ECLIPSE overall study period. Multivariable model adjusted for age, sex, race, Saint George Respiratory Questionnaire score, gastroesophageal reflux disease, current smoking, and white blood cell count.

(A)						
COPDGene Longitudinal Follow Up Stratified Analysis						
	No exacerbations in the prior year (666 vs. 98)		**1 exacerbation in the prior year (165 vs. 28)**		**≥2 exacerbations in the prior year (125 vs. 31)**	
(Non-eosinophilic vs. eosinophilic)	**IRR (95% CI)**	**p value**	**IRR (95% CI)**	**p value**	**IRR (95% CI)**	**p value**
Eosinophil ≥300 at phase 2 visit	1.12 (0.57–2.28)	0.73	1.02 (0.48–2.20)	0.97	**1.96 (1.21–3.21)**	**0.008**

(B)						
ECLIPSE Stratified Analysis on overall study period						
	No exacerbations in the prior year (788 vs. 223)		**1 exacerbation in the prior year (373 vs. 99)**		**≥2 exacerbations in the prior year (316 vs. 96)**	
(Non-eosinophilic vs. eosinophilic)	**IRR (95% CI)**	**p value**	**IRR (95% CI)**	**p value**	**IRR (95% CI)**	**p value**
Eosinophil ≥300 at screening	1.03 (0.85–1.24)	0.77	1.15(0.87–1.51)	0.33	**1.40(1.15–1.70)**	**<0.001**

Table 5

Asthma-COPD overlap, defined by asthma diagnosis before age 40, and elevated eosinophils (≥ 300 cells/μL) independently predict exacerbations.

	COPDGene		ECLIPSE			
			Prospective Exacerbation Rate			
	Cross-sectional Exacerbation Frequency		1yr		overall study period	
Factors	IRR (95% CI)	p value	IRR (95% CI)	p value	IRR (95% CI)	p value
Age	**0.99 (0.97–1.00)**	**0.02**	**1.01 (1.00–1.02)**	**0.006**	**1.01 (1.00–1.02)**	**0.009**
Non White race	0.69 (0.54–0.88)	0.003	0.83(0.54–1.25	0.37	0.69 (0.41–1.12)	0.15
Female	**1.39 (1.16–1.66)**	**<0.001**	**1.20 (1.06–1.36)**	**0.004**	**1.29 (1.11–1.49)**	**<0.001**
SGRQ score *	**1.02 (1.02–1.03)**	**<0.001**	**1.01 (1.01–1.01)**	**<0.001**	**1.01(1.01–1.01)**	**<0.001**
post-bronchodilator FEV1 % predicted †	**0.98 (0.98–0.99)**	**<0.001**	**0.99 (0.98–0.99)**	**<0.001**	**0.98(0.98–0.99)**	**<0.001**
GERD	**1.37 (1.14–1.64)**	**<0.001**	**1.40 (1.23–1.59)**	**<0.001**	**1.39(1.20–1.61)**	**<0.001**
Current smoking	**0.73(0.58–0.90)**	**0.004**	**1.10 (0.97–1.25)**	**0.12**	**1.25(1.08–1.44)**	**0.003**
previous exacerbations	**NA**	**NA**	**2.35 (2.08–2.65)**	**<0.001**	**2.77(2.40–3.21)**	**<0.001**
WBC	1.01(0.97–1.05)	0.70	**1.03 (1.01–1.06)**	**0.006**	**1.04(1.01–1.07)**	**0.004**
Asthma COPD overlap	**1.33 (1.04–1.71)**	**0.02**	**1.29 (1.08–1.54)**	**0.005**	**1.33(1.08–1.63)**	**0.006**
Eosinophils ≥300	**1.26 (1.03–1.54)**	**0.02**	**1.21 (1.06–1.39)**	**0.005**	**1.22(1.04–1.42)**	**0.01**

Results from negative binomial regression analysis are shown.

*per 1 point increase in score,

†per percentage point increase in FEV_1