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Characterisation of Eosinophilic Bronchiectasis: a European Multicohort Study

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Scientific Knowledge on the Subject

Bronchiectasis is a heterogeneous disease. Although classically defined by neutrophilic inflammation, bronchial biopsies have shown increased eosinophil infiltration compared to healthy controls, and eosinophilic inflammation has been observed in the sputum of bronchiectasis patients without a history of asthma. In COPD and asthma, blood eosinophils have been shown to correlate with sputum cells counts allowing them to be used as a surrogate of eosinophilic airway inflammation and inhaled corticosteroid response. No studies to date have investigated the characteristics and outcomes associated with sputum and blood eosinophilic inflammation in bronchiectasis

What this study adds to the field

We show that blood and sputum eosinophil counts in bronchiectasis are correlated in 2 independent cohorts. Around 1 in 5 patients with bronchiectasis are shown to have eosinophilic inflammation, whether defined by sputum or blood eosinophil count. Eosinophilic inflammation was associated with microbiome profiles dominated by *Streptococcus* and *Pseudomonas*. Using a post-hoc analysis of a randomized trial to control for baseline infection status and exacerbation history, we show that elevated blood eosinophil counts are a risk factor for exacerbation in bronchiectasis patients.

Taken together these data suggest that eosinophilic inflammation is common in bronchiectasis and may contribute to an increased risk of exacerbations, identifying a clinically important treatable trait.

Abstract

Rationale: Bronchiectasis is classically considered a neutrophilic disorder but eosinophilic subtypes have recently been described.

Objectives: To use multiple datasets available through the EMBARC consortium to characterise eosinophilic bronchiectasis as a clinical entity focussing on the impact of eosinophils on bronchiectasis exacerbations.

Methods: Patients were included from 5 countries to examine the relationships between blood eosinophil counts and clinical phenotypes after excluded co-existing asthma. 16S rRNA sequencing was used to examine relationships between eosinophil counts and the sputum microbiome. A posthoc analysis of the PROMIS phase 2 trial was used to examine the impact of blood eosinophil counts on exacerbations in patients with *P.aeruginosa* infection.

Measurements and Main *Results:* A relationship between sputum and blood eosinophil counts was demonstrated in 2 cohorts. In analysis of 1007 patients from 5 countries, 22.6% of patients had blood eosinophil counts >300cells/ul. Counts<100cells/ul were associated with higher bronchiectasis severity and increased mortality. There was no clear relationship with exacerbations. Blood eosinophil counts >300cells/ul were associated with both *Streptococcus* and *Pseudomonas* dominated microbiome profiles. To investigate the relationship of eosinophil counts with exacerbations after controlling for the confounding effects of infection, 144 patients were studied in a clinical trial following treatment with antipseudomonal antibiotics. Compared to patients with blood eosinophil counts<100cells(reference), elevated eosinophil counts 100-300cells/ul (HR 2.38 95%CI 1.33-4.25,p=0.003) and >300cells/ul (HR 3.99 95%CI 2.20-7.85,p<0.0001) were associated with shorter time to exacerbation.

Conclusion: Eosinophilic bronchiectasis affects approximately 20% of patients. After accounting for infection status, raised blood eosinophil counts are associated with shortened time to exacerbation.

Introduction

Bronchiectasis is a heterogeneous disease in its aetiology and clinical presentation .(1, 2)Bronchiectasis has historically been considered an exclusively neutrophilic disease, and while markers of neutrophil activation and neutrophil extracellular trap formation predict exacerbations and future outcomes, a subset of patients do not show evidence of neutrophilic inflammation.(3, 4)

Heterogeneity of inflammatory profiles in the airway have been demonstrated in COPD where both neutrophilic and eosinophilic subtypes of the disease are recognised, with the same also observed in severe asthma.(5–8) Given that both neutrophilic and eosinophilic subtypes of these conditions exist, and the well described overlap between these conditions, it is highly likely that both neutrophilic and eosinophilic subtypes of bronchiectasis also exist.(9)

An increase in eosinophils in bronchial biopsies from bronchiectasis patients compared to healthy controls has been demonstrated(10) , and elevated blood eosinophil levels have been found in people with bronchiectasis and rhinosinusitis.(11) Sputum cell counts from two studies suggest approximately 20% of patients with bronchiectasis have sputum eosinophilia defined by an eosinophil percentage of 3% or more.(12, 13) Finally, blood eosinophil counts have been used to define the presence of eosinophilic inflammation and in a posthoc analysis of an non blinded trial of inhaled corticosteroids, quality of life improved to a greater extent with inhaled steroids in patients with blood eosinophil counts above 150 cells or 3%.(14) Recognition in clinical practice that an eosinophilic subtype of bronchiectasis exists has led some to use off-label anti-IL5 or anti-IL5 receptor monoclonal antibodies. (15)

These data suggest that clinicians increasingly recognise the presence of an eosinophilic subtype of bronchiectasis, but to date there have been no studies characterising such patients in detail. Blood eosinophil counts are used as a surrogate of airway eosinophilia in COPD and asthma only because studies have shown a statistically significant correlation between blood and sputum eosinophils in these diseases.(16, 17) There is a need to establish this relationship in bronchiectasis. The relationship between eosinophil counts and exacerbations in COPD is complex because of the impact of baseline exacerbation status and treatment with inhaled corticosteroids.(18–20) In studies such as randomized trials, where patient characteristics, exacerbation status and inhaled steroid use are standardized, a clear relationship between eosinophil counts and exacerbations is observed.(18) If therapies are to be used to target eosinophilic disease in bronchiectasis, evidence that eosinophilic inflammation contributes to exacerbations would be important.

Using multiple datasets available through the European Multicentre Bronchiectasis Audit and Research Collaboration (EMBARC) we sought to answer these questions to provide a detailed characterisation of eosinophilic bronchiectasis as a clinical entity.

Methods

We used data from multiple cohorts to answer distinct questions about the role of eosinophils in bronchiectasis. These questions were; 1) Is there a correlation between blood and sputum eosinophil counts and therefore can blood eosinophil counts be used as a surrogate of airway eosinophilic inflammation as in COPD and asthma 2) Are elevated blood eosinophil counts associated with clinical characteristics in bronchiectasis 3) Do patients with raised blood eosinophil counts have a distinct airway infection and microbiome profile compared to patients without raised blood eosinophil counts and 4) After controlling for the effect of airway infection, is blood eosinophilia associated with exacerbations in bronchiectasis.

Question 1: Correlation between blood and sputum eosinophil counts

We examined the relationship between blood and sputum eosinophil counts in the TAYBRIDGE prospective cohort of patients from Dundee, UK(21) and validated the findings in the Inflammaging cohort recruited in Newcastle, UK.

The TAYBRIDGE cohort has been previously described.(21) Patients had sputum cell counts and concurrent blood eosinophil count measurements at baseline. The inflammaging cohort was a single centre study conducted at the Freeman Hospital in Newcastle, UK and also included patients with matched sputum and blood cell count data available. Inclusion criteria in both studies required HRCT confirmed bronchiectasis, age >18 years and ability to give informed consent. Patients with a diagnosis of asthma or active allergic bronchopulmonary aspergillosis were excluded from this and all other subsequent cohorts. Asthma was a clinician diagnosis defined according to the GINA guidelines. ABPA was diagnosed by according to ERS guidelines using specific IgE to aspergillus/total IgE and other supporting laboratory tests as clinically indicated.(22, 23)

In both cohorts, neutrophilia was defined as sputum neutrophil percentage >60% and sputum eosinophilia was defined as >3% cells. To examine the effect of different cut-offs of blood eosinophilia we a-priori used cut-offs that are widely accepted in the COPD literature - <100cells/ul, 100-299 cells/ul and $\geq$300 cells/ul.(24) These are referred to throughout the paper as low, intermediate and high blood eosinophil counts respectively. The relationship between blood and sputum eosinophil counts was assessed by Spearman correlation and the area under the receiver operator characteristic curve (AUC). As blood eosinophil counts have been accepted in asthma and

COPD as a valid surrogate of sputum eosinophil counts, a priori we determined we would regard blood eosinophil counts as a valid surrogate in bronchiectasis if the AUC was similar to those observed in asthma and COPD (Supplementary table 1). Exacerbations were defined in these observational cohorts as an increase in respiratory symptoms leading to a prescription for antibiotics.

Question 2: Relationship between blood eosinophil counts, clinical characteristics and exacerbations in bronchiectasis

This study utilised the FRIENDS cohort of the EMBARC bronchiectasis registry.(25, 26) Patients from 5 countries (UK, Italy, Israel, Spain and Belgium) were included with standardised data collection between centres. The inclusion criteria were HRCT confirmed bronchiectasis, age >18 years, and a clinical history consistent with bronchiectasis. Patients were followed-up in this dataset for up to 5 years and endpoints were frequency of exacerbations, hospitalizations and mortality.

Question 3: Relationship between blood eosinophil counts and airway infection in bronchiectasis

Patients enrolled in the EMBARC BRIDGE study(4) (NCT03791086) from 3 centres in the UK, Spain and Italy were included in a study of the relationship between the sputum microbiome and blood eosinophil counts. DNA was extracted from sputum as previously described and the V3 and V4 region of the bacterial 16S rRNA gene was sequenced, and the resulting data quality checked for sequencing and contamination errors as described in the online supplement. Alpha diversity was measured by determining the Shannon-Wiener Diversity Index (SWDI) and the Chao index, whilst beta diversity (between sample diversity) was calculated by the Bray Curtis distance. Permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences. Linear discriminant analysis Effect Size (LEFSE) was used to identify taxa that were significantly different between groups defined by blood eosinophil count.

Question 4: Relationship between blood eosinophil counts and exacerbations after controlling for infection status

The PROMIS phase 2 randomized controlled trial(ISRCTN49790596) was a double blind placebo controlled trial of inhaled colistin for 6 months in patient with bronchiectasis.(27) Patients had at least 2 positive sputum samples for *P. aeruginosa* in the 12 months prior to the study and were randomized within 21 days of a course of antipseudomonal antibiotics with a positive baseline sputum sample for *P. aeruginosa*. The trial was conducted at 35 sites in the UK, Russia and Ukraine.

The objective of this posthoc analysis was to examine whether blood eosinophil count which was performed on all subjects at the randomization visit, was associated with the primary outcome of time to first exacerbation. This cohort consists exclusively of patients with *P. aeruginosa* infection. Antibiotic treatment at study baseline reduced bacterial load. Therefore as far as possible infection status was standardised in all subjects. We hypothesised that this cohort would be able to demonstrate an independent effect of eosinophilic inflammation on exacerbations after removing the potential confounding effect of infection. Exacerbations were defined as the presence of three or more of the following signs or symptoms for at least 24 hours: increased cough, increased sputum volume, increased sputum purulence, hemoptysis, increased dyspnea, increased wheezing, fever (≥38°C) or malaise, and the treating physician agreed that antibiotic therapy was required.(27) Within this study participants had eosinophil counts measured at the start and end of treatment. This data was used to investigate the stability of the eosinophilic phenotype in bronchiectasis patients.

Statistical analysis

Data are presented as mean with standard deviation of median with interquartile range according to their distribution. Comparisons of 3 groups were performed by ANOVA or Kruskal-Walis test as appropriate. Correlations were performed using Spearman correlation. Exacerbation frequency and frequency of severe exacerbations requiring hospital admission over time was studied using a negative binomial model with time in study as an offset. Mortality analysis utilised Cox proportional hazards regression. Adjusted analyses incorporated age, sex, and country as covariates. The relationship between two eosinophil measurements 6 months apart was determined by Spearman correlation and the Kappa statistic was used to examine the agreement between eosinophilic status (eosinophil count >300cells/ul) at baseline and end of study. All analyses utilised SPSS version 25 or Graphpad prism, with the exception of the microbiome analyses which were performed in R v3.5.1.

Results

A summary of the study cohorts and the questions being addressed is shown in figure 1.

Correlation between blood and sputum eosinophil counts

Overall across two cohorts 64 patients (27.2%) had blood eosinophil counts <100cells/ul, 123 (52.3%) had eosinophil counts 100-300cells/ul, with 48 (20.4%) having eosinophil counts ≥300 cells/ul. Supplementary table 2 describes the cohorts according to baseline blood eosinophil counts. The proportions were consistent between the two cohorts with 22 (19.6%) and 26 (21.1%) classified as eosinophilic in the inflammaging and Taybridge cohorts respectively.

There was a relationship between blood and sputum eosinophils in both datasets. Combining the two datasets together, patients with blood eosinophil counts 100-300cells/ul and >300cells/ul had higher sputum eosinophil counts (figure 2A, p=0.0005). Differences in sputum eosinophil % between these groups was statistically significant in both individual datasets (p=0.01 in Inflammaging and p=0.03 in Taybridge). The area under the receiver operator characteristic curve for blood eosinophils, where the dependent variable was a sputum eosinophil count >3%, was 0.68 95% CI 0.56-0.79,p=0.003. The corresponding AUC values were 0.67 in the Dundee cohort and 0.72 in the Newcastle cohort. The correlation between blood and sputum eosinophils was also statistically significant, r=0.31,p<0.0001, figure 2B. To predict an eosinophil count >3%, a blood eosinophil count >300 cells per ul was 44.4% sensitive and 82.0% specific, while using a count >100 cells per ul was 85.2% sensitive and 23.8% specific.

Based on these data we subsequently use blood eosinophils >300 cells/ ul and >100 cells/ul as surrogates of airway eosinophilia in subsequent analyses.

Relationship between blood eosinophil counts, clinical characteristics and exacerbations in bronchiectasis

1007 patients were included from 5 countries. 56 patients were excluded due to current asthma or allergic bronchopulmonary aspergillosis leaving 951 patients for analysis. 263 patients from the UK, 231 patients from Italy, 207 from Israel, 178 from Belgium and 72 from Spain were included in the FRIENDS cohort. The eosinophilic subgroup (blood eosinophils >300 cells/ul) was present in all 5 cohorts (UK= 50 (19.0%), Italy 50 (21.6%), Israel 61 (29.5%), Belgium 41 (23.0%) and Spain 13 (18.1%). Figure 3 shows the percentage of patients with eosinophils >300cells/ul across all the cohorts included in this study (18.1-29.5%).

Characteristics	Blood eosinophil counts			p-value
	<100 cells/ul	100-299 cells/ul	≥300cells/ul	
N	218	518	215	
Age	66.8 (15.3)	66.0 (14.4)	65.1 (16.1)	0.53
Sex	125 (57.3%)	270 (52.1%)	106 (49.3%)	0.23
BMI	24.5 (5.5)	25.4 (5.4)	26.4 (5.9)	0.013
Aetiology				
Idiopathic	106 (48.6%)	246 (47.5%)	102 (47.4%)	
Postinfective	32 (14.7%)	109 (21.0%)	34 (15.8%)	
TB	5 (2.3%)	18 (3.5%)	7 (3.3%)	
Immunodeficiency	8 (3.7%)	17 (3.3%)	12 (5.6%)	
CTD	14 (6.4%)	9 (1.7%)	2 (0.9%)	
COPD	16 (7.3%)	29 (5.6%)	21 (9.8%)	
NTM	2 (0.9%)	4 (0.8%)	0 (0%)	
PCD	7 (3.2%)	8 (1.5%)	5 (2.3%)	
Others	28 (12.8%)	78 (15.1%)	32 (14.9%)	
BSI (median-IQR)	8 (5-11)	6 (4-9)	6 (4-9)	<0.0001
mMRC (median-IQR)	1 (0-2)	1 (0-2)	1 (0-2)	0.071
FEV_1 litres	1.64 (0.69)	1.93 (0.84)	1.92 (0.78)	0.003
FEV_1% predicted	73.2 (26.8)	78.1 (26.1)	77.9 (25.9)	0.061
Pseudomonas aeruginosa	32 (14.7%)	80 (15.4%)	42 (19.5%)	0.3
NTM infection	13 (6.0%)	25 (4.8%)	15 (7.0%)	0.08
Inhaled corticosteroid use	124 (56.4%)	214 (41.2%)	94 (43.6%)	0.0004
Eosinophil count (cells/ul) (median-IQR)	60 (20-90)	170 (120-200)	400 (310-540)	<0.0001

Table 1. Characteristics of the patients across 5 European cohorts stratified by blood eosinophil count. All values are mean with standard deviation unless otherwise stated.

Next we investigated with relationship between blood eosinophil counts and exacerbations. There were 2101 exacerbations within 12 months of eosinophil measurement. Using a negative binomial model there was no relationship between eosinophil counts and exacerbations. Compared to those with blood eosinophil counts <100 cells/ul (reference), patients with 100-299cells/ul had a rate ratio 1.06 95% CI 0.85-1.33,p=0.61, and those with counts ≥300cells/ul had a rate ratio of 0.93 (0.77-1.13,p=0.46).

Incorporating country as a random effect we found no effect on exacerbations rate ratio 1.08 95% CI 0.86-1.35,p=0.53 and RR 0.97 95% CI 0.80-1.18,p=0.77. No statistically significant relationship with exacerbations was observed in any of the individual country datasets.

There was similarly no relationship between blood eosinophil counts and hospital admissions, with eosinophil counts 100-299cells/ul (rate ratio 0.74 95% CI 0.48-1.13,p=0.16) and ≥300cells/ul (rate ratio 0.71 95% CI 0.50-1.01,p=0.054) not significantly associated with hospital admissions. With no significant effect seen after adjustment for country as a random effect, and no statistically significant effects were seen in individual country datasets.

To analyse mortality, there were 135 deaths (14.2%), with a median follow-up time of 1147 days (3.1 years, IQR 730-2001 days). 44 patients (20.2%) in the low, 68 patients (13.1%) in the intermediate and 23 patients (10.7%) in the high eosinophil groups died. Mortality was significantly lower in both intermediate and high blood eosinophil groups HR 0.67 95% CI 0.45-0.98,p=0.037, and HR 0.48 95% CI 0.29-0.79,p=0.004, respectively. The relationship between high blood eosinophil counts and improved survival persisted after adjustment for country, age and sex HR 0.53 95% CI 0.32-0.88,p=0.014, while the relationship with intermediate eosinophil counts was not significant HR 0.70 95% CI 0.48-1.03,p=0.072. Similar results were observed after adjustment for FEV_1 (HR 0.72 95% CI 0.49-1.05,p=0.091 and HR 0.47 95% CI 0.28-0.80,p=0.005 for intermediate and high eosinophil groups respectively). The survival curve is shown in supplementary figure 1.
We did not observe any statistically significant interaction between blood eosinophil count and inhaled corticosteroid use or COPD for any of the reported outcomes (p>0.05 for all tested interactions).

Relationship between blood eosinophil counts and airway infection in bronchiectasis

We next hypothesised that blood eosinophil counts would be associated with distinct microbiome profiles in bronchiectasis. To investigate this we utilised the BRIDGE cohort which has been previously described(4). This analysis included 210 patients, in whom eosinophil counts >300cells/ul were present in 19.5% (figure 3). For microbiome analysis we included 198 patients from the UK, Italy and Spain who provided baseline sputum samples. 16S rRNA sequencing was used to characterise the sputum microbiome. We found no correlation between blood eosinophil counts and measures of alpha diversity include the Shannon-Wiener index (r=0.1,p=0.4) and the Chao index (r=0.06,p=0.9). Examining beta-diversity, however, statistically significant differences were observed between the 3 eosinophil groups (p=0.012 by PERMANOVA). Beta-diversity analysis based on the Bray Curtis difference is strongly influenced by the most abundant taxa, and ellipses showed in figure 4A, indicated a potential association between elevated eosinophil counts ≥300cells/ul and *Pseudomonas* and *Streptococcus* dominated microbiome profiles, while the *Haemophilus* dominated cluster was more associated with eosinophil counts <100cells/ul.

Using LEFSE, eosinophil counts 100-300cels/ul were associated with increased relative abundance of *Streptococcus, Veillonella, Prevotella, Granulicatella* and *Megaphaera* (all p<0.05 after FDR adjustment). *Haemophilus* and *Moraxella* had the strongest effect sizes associated with low blood eosinophil counts (figure 4B). Higher blood eosinophil counts were associated with increased relative abundance of *Neisseria, Rothia* and *Pseudomonas*.

Patients were then classified by the dominant organism as previously described.(28) Clear differences across 4 groups (p=0.024 by Chi square test) with a higher proportion of eosinophilic patients in the *Streptococcus* and *Pseudomonas* dominated profiles and lower proportion of eosinophilic patients in those infected with *Haemophilus* and *Moraxella (*figure 4C*).*

We therefore show a relationship between eosinophil counts and infection status. Elevated blood eosinophil counts (>300cells/ul) were associated with both *Streptococcus* and *Pseudomonas* dominated profiles, this may confound the relationship between eosinophilic inflammation and exacerbations, since *Pseudomonas* infection is a strong predictor of exacerbation and mortality(24, 28), while *Streptococcus* is typically associated with a lower frequency of exacerbations.(30) We therefore identified a dataset where we could test the association of blood eosinophil counts with exacerbations while controlling for infection status.

Relationship between eosinophil inflammation and exacerbations after controlling for infection status

144 patients with *P. aeruginosa* infection were included in the PROMIS phase 2 randomized controlled trial. The characteristics of the patients have been previously reported. Baseline blood eosinophil counts were available for 126 out of 144 patients. 47 (37.3%) were less than 100cells/ul, 57 (45.2%) had eosinophil counts 100-300cells/ul, and 22 (17.5%) had eosinophil counts greater than 300 cells per ul. Baseline and end of study eosinophil counts showed moderate correlation (Spearman correlation 0.46 95% CI 0.29-0.60,p<0.0001). 7 patients who had raised eosinophil counts as baseline had eosinophil counts <300cells/ul at follow-up, and 6 patients who were not eosinophilic at baseline, were eosinophilic at follow-up, supplementary figure 2. The other participants remained within their groups, giving an agreement of 88.1% and Cohen's kappa statistic of 0.58 indicating moderate agreement.

There were no significant differences between the groups in age, BMI, Symptoms measured using the SGRQ, FEV1 or *Pseudomonas aeruginosa* CFU/g at baseline (all p>0.05). Characteristics of the patients according to baseline blood eosinophil counts is shown in supplementary table 3.

Time to first exacerbation was significantly decreased with increasing baseline blood eosinophil counts. Compared to patients with blood eosinophil counts <100cells (reference) eosinophil counts 100-299cells/ul (HR 2.38 95% CI 1.33-4.25,p=0.003) and $\geq$300cells/ul (HR 3.99 95% CI 2.20-7.85,p<0.0001) were associated with shorter time to first exacerbation (figure 5). After adjustment for age, sex, baseline CFU/g, FEV1, symptoms and treatment with placebo or colistin, the relationship between blood eosinophil counts and time to first exacerbation persisted for eosinophil counts 100-299 cells/ul (HR 2.38 95% 1.32-4.30,p=0.004) and $\geq$300cells/ul (HR 3.17 95%CI 1.58-6.39,p=0.001). Supplementary table 4 online shows that these relationships were observed in both patients randomized to placebo or colistin.

Discussion

In this study we show that blood eosinophil counts >300cells/ul are common in bronchiectasis and are found in a subgroup of ~20% patients. Patients with eosinophilic inflammation cannot be identified immediately from clinical features as there is a large overlap in lung function, aetiology and infection status between patients with high and low blood eosinophils. Since patients cannot be easily identified by clinical features and eosinophil counts in sputum are not simple to conduct in the clinic we sought to validate blood eosinophils as a measure eosinophilic inflammation in the airways. We demonstrate a relationship between blood and sputum eosinophil counts in bronchiectasis that is imperfect but similar to that observed in COPD and asthma. We conclude that elevated blood eosinophil counts may be used as a surrogate of airway eosinophilic inflammation in bronchiectasis and that low blood eosinophils make eosinophilic disease unlikely.(16, 17)

Our initial studies to tease out the relationship between eosinophil counts and disease severity indicated that low eosinophil counts are associated with an increased bronchiectasis severity index score and an increased mortality rate. This is consistent with similar data in patients with COPD where those with the most severe neutrophilic disease have relatively low blood eosinophil counts.(19, 31) Bronchiectasis has historically been regarded as a neutrophilic disease and markers of neutrophilic inflammation are associated with severity of disease and disease progression(4, 32) It is therefore not surprising to find a similar relationship between low eosinophil counts and mortality as has been observed in COPD.

The inverse relationship between eosinophilic and neutrophilic inflammation, and the interaction between inflammatory status and infection complicates any attempts to find crude associations between blood eosinophil counts and exacerbations. Such associations have been inconsistently observed in COPD and asthma.(20) Exacerbations are heterogeneous events that may be caused by multiple triggers. A subset of exacerbations may involve eosinophilic inflammation, while other risk factors such as infection status, bacterial load, neutrophilic inflammation and exposure to viruses may be present in others.(33–35) Any analysis of the impact of eosinophils therefore needs to account for these other confounders.

As expected therefore, we found no association between blood eosinophil counts and exacerbations in the FRIENDS cohort. Exploring the microbiome profiles, however, we identified that the microbiome is significantly different in high, medium and low eosinophil groups with eosinophilic patients generally being associated with both *Streptococcus* and *Pseudomonas* dominated profiles. *Pseudomonas* infection is usually associated with severe disease, increased exacerbations and mortality(24), whereas *Streptococcus* is associated with less exacerbations which may explain some

of the complexity in our large cohort studies. Recent evidence suggests a strong relationship between reduced microbiome diversity and mortality and our observations that eosinophil counts are associated with both microbial dysbiosis and mortality risk may be linked.(36)

The PROMIS phase 2 randomized trial(27) therefore represented an ideal cohort in which to test the impact of blood eosinophil status on exacerbations. These patients are had the same infection (with *P. aeruginosa*) and had baseline bacterial load standardised through the administration of antibiotics prior to randomization. In this cohort we can show a clear relationship between the presence of eosinophilic inflammation by blood eosinophils and time to next exacerbation. Our data suggest eosinophilic inflammation is a risk factor for exacerbations in patients with *P. aeruginosa* infection and could therefore be a treatable trait. This study adds to recent data showing that quality of life was improved with inhaled corticosteroid treatment in bronchiectasis patients with blood eosinophil counts >3%(14) and a case series describing clinical improvements with anti-IL-5 or anti-IL5 receptor monoclonal antibody treatment.(15) We also observed relative stability in blood eosinophil counts from baseline to 6 months in the same cohort.

Our study has important limitations. Firstly in our large cohort studies we excluded individuals with typically eosinophilic disease such as physician diagnosed asthma and ABPA, but we did not seek to systematically exclude this with methacholine or alternative challenge testing or markers of fungal sensitisation. There is no single perfect diagnostic test for asthma. We did not exclude individuals with COPD (7%). However, rather than seeing these conditions as separate entities, bronchiectasis-asthma and COPD overlap disease is increasingly recognised and we suggest the importance here is in identifying treatable traits in patients with a label of bronchiectasis within clinical practice.(9) . We have not looked at other markers such as fractional exhaled nitric oxide or IgE levels in this study. The value of other markers of Th2 driven inflammation in bronchiectasis needs to be established. We had no control group against which to compare the levels of blood eosinophils in bronchiectasis. A recent meta-analysis found median values of 130-140cells/ul in the healthy general populations compared to 160-235cells/ul in asthma. Our median value of 170cells/ul in bronchiectasis suggests bronchiectasis may be higher than the general population but lower than asthma.(37)

The strengths of this study are the utilisation of multiple international cohorts and large numbers of patients to validate our findings and the post hoc analysis of the PROMIS trial allowing us to remove infection as a confounder in our studies.

Taken together, these observations suggest that eosinophilic airway inflammation in bronchiectasis occurs in a subset of ~ 20% individuals. Eosinophilic bronchiectasis is associated with a distinct

airway microbiome. However once infection has been controlled for, patients with higher blood eosinophils suffer reduced time to next exacerbation supporting the concept that alongside other triggers, eosinophils contribute to bronchiectasis exacerbations.

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Figure legends

Figure 1. Summary flow chart of the questions addressed and cohorts used in this study

Figure 2. A: Relationship between eosinophil counts in blood and sputum in combined data from two cohorts, using cut-offs of 100cells/ul and 300cells/ul. Data are displayed as mean with standard deviation. B: correlation between blood and sputum eosinophil counts ($r=0.31, p<0.0001$).

Figure 3. Map depicting the percentage of patients with eosinophils >300cells/ul across all the cohorts included in this study (ranging from 18.1-29.5%)

Figure 4. The relationship between the sputum microbiome and blood eosinophil counts in bronchiectasis. A: Principal components analysis based on the Bray Curtis distance. B: Linear discriminant analysis Effect Size *indicates taxa that are statistically significant after FDR adjustment. Colour indicates which eosinophil subgroup is enriched for each taxa. C: Proportion of patients in each group with different blood eosinophil counts. Groups are defined by the dominant taxa (40% of OTUs).

Figure 5. Kaplain Meier survival curve showing percentage exacerbation event free survival from randomization.