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# Association between elevated peripheral blood eosinophil count and respiratory outcomes in adults with cystic fibrosis

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## ABSTRACT

**Background:** Elevated blood eosinophil counts are linked to worse outcomes in asthma and COPD, but have yet to be well characterized in CF. We hypothesized that higher stable visit blood eosinophil counts are associated with increased rates of lung function decline and pulmonary exacerbations (PEX).

**Methods:** We performed a retrospective analysis of adult CF patients ( $\geq 19$  years) enrolled from 2012 to 2018 in a prospective cohort study focused on blood biomarkers. We included individuals with at least one year of follow-up post-stable visit blood draw and compared clinical characteristics by blood eosinophil count ( $< 300$  cells/ $\mu\text{L}$  vs.  $\geq 300$  cells/ $\mu\text{L}$ ). We used multivariate mixed-effects linear regression to estimate annual change in ppFEV<sub>1</sub>. Multivariable poisson and linear regression models were used to estimate rate of PEX requiring IV antibiotics and to compare CF Respiratory Symptom Diary-Chronic Respiratory Infection Symptom Scores (CFRSD-CRISS), respectively.

**Results:** Of 109 patients, 17 (15.6%) had eosinophil counts  $\geq 300$  cells/ $\mu\text{L}$ . After adjustment for age, sex, BMI, and baseline ppFEV<sub>1</sub>, there was no association between high vs. low eosinophil group and rates of lung function decline (difference in slope  $-0.04\%/y$ ; 95% CI  $-1.5$  to  $+1.4$ ) or rates of PEX requiring IV antibiotics (IRR 1.46; 95% CI 0.75 to 2.65). The high eosinophil group had a higher mean CFRSD-CRISS score at stable visit (adjusted mean difference 9.3; 95% CI 2.9 to 16.0).

**Conclusions:** The high eosinophil group experienced increased respiratory symptoms, but the rates of lung function decline and PEX were comparable between groups.

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## 1. Introduction

In cystic fibrosis (CF), chronic progressive lung disease is a key contributor to patient morbidity and is the leading cause of mortality [1]. There are a number of clinical factors that have been associated with lung function decline in CF, including female sex, airway microbiology (*Pseudomonas aeruginosa*, *Burkholderia cepacia*, MRSA), CF-related diabetes, and history of pulmonary exacerbations (PEX) [2]. In terms of biological markers, sputum neutrophil elastase predicts lung function decline and the development of bronchiectasis in CF, which suggests that neutrophil airway inflammation plays a central role in CF lung disease progression [3,4].

Similar to neutrophils, eosinophils are key effector cells of the immune system that can release cytotoxic granules when activated, which can contribute to airway inflammation and destruction [5]. To date, there has been limited evaluation of airway or systemic eosinophilic inflammation in CF. In a study by Koller et al., serum eosinophilic cationic protein, a measure of eosinophil activation, had a negative correlation with lung function and gas exchange parameters in CF [6]. Furthermore, in CF patients with chronic *P. aeruginosa* lung infection, immune responses are predominantly Th2-driven, and a negative correlation has been found between levels of Th2-associated cytokines and ppFEV<sub>1</sub> in stable CF patients [7,8].

Circulating eosinophil count is an attractive biomarker in CF lung disease as it is readily available, extensively studied in other airway diseases, correlates well with airway eosinophilia in other airway conditions, and there is a plausible biological role for

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eosinophils in CF lung injury [6–11]. In this study we explored whether elevated blood eosinophil count would be indicative of lung disease progression in CF patients. Specifically, we hypothesized that elevated blood eosinophil levels, using cut-offs applied in asthma and COPD, would be predictive of an increased rate of lung function decline, pulmonary exacerbations, and more severe respiratory symptoms in adults with CF.

## 2. Methods

### 2.1. Study design and patient population

We conducted a retrospective analysis of adult CF patients ( $\geq 19$  years) followed at the St. Paul's Hospital Adult CF Clinic (Vancouver, Canada) who were prospectively enrolled in a biomarker study ("Biomarkers in CF") between July 2012 and April 2018, specifically designed to identify blood biomarkers of exacerbations and lung disease progression [12]. Follow-up data were recorded until April 2019. Patients must have provided written informed consent, had a confirmed CF diagnosis based on sweat chloride testing and/or genotyping, provided at least 1 blood sample during a stable clinic visit, and must have had at least one year of follow-up post-stable visit blood sampling. We excluded patients who had a previously documented diagnosis of allergic bronchopulmonary aspergillosis (ABPA) or received oral antibiotics and/or systemic corticosteroids within two weeks prior to stable visit as this could alter eosinophil count.

The study was approved by the Research Ethics Board at St Paul's Hospital, Vancouver, British Columbia, Canada (PHC-H18-03,015).

### 2.2. Study variables

Baseline clinical data was extracted from a combination of paper and electronic medical records. Data collected included: age, sex, CF genotype, weight, height, lung function and PEx in the year prior to the stable visit, sputum microbiology, CF-related comorbidities (i.e., pancreatic insufficiency, diabetes, sinus disease, and liver disease), medications, and respiratory symptoms 24 h prior to the stable visits measured with the CF Respiratory Symptom Diary-Chronic Respiratory Infection Symptom Scale (CFRSD-CRISS) [13]. A change in CFRSD-CRISS score of  $\geq 11$  represents a minimal clinically important difference [14].

Blood eosinophil counts were obtained from a stable clinic visit and each patient was categorized into high ( $\geq 300$  cells/ $\mu\text{L}$ ) or low ( $< 300$  cells/ $\mu\text{L}$ ) eosinophil groups based on a cut-off established in the asthma and COPD literature and often used as a criterion to predict response to Th2 biologic therapy [15–17]. A stable clinic visit was defined as the patient not meeting the modified Fuchs criteria for an exacerbation and not currently receiving or requiring IV or oral antibiotic therapy [18]. When multiple stable clinic visits existed for an individual patient, the earliest visit that maximized follow-up time was chosen.

Lung function, based on Forced Expiratory Volume in one second ( $\text{FEV}_1$ ) and expressed as a percent predicted (pp $\text{FEV}_1$ ) using the GLI equation, was measured from stable visit to a minimum follow-up of one year and up to a maximum of three years. Lung function measurements calculated using the Hankinson equation were recalculated with the GLI equation using the rspiro package in R [19]. Measurements after lung transplantation were excluded.

Patients were deemed to have a PEx if they required IV antibiotics (hospital or home) within one-year post-stable visit for increased respiratory symptoms and/or decreased lung function. To capture milder exacerbations, we used a separate composite outcome combining both PEx treated with IV and oral antibiotics. Both outcomes were expressed as counts.

### 2.3. Blood sample collection

As part of the "Biomarkers in CF" study, patients had peripheral venous blood samples drawn on stable clinic visits. Each whole blood sample was analyzed for complete blood counts (CBC) using the Abbott CELL-DYN Hematology Analyzer (Abbott, Illinois, USA) until 2015 and the ADVIA 2120i Hematology System (Siemens, Erlangen, Germany) afterwards.

### 2.4. Statistical analysis

Baseline clinical characteristics were summarized using simple descriptive statistics (i.e., median, counts, and proportions). Chi-squared test of independence, Fisher's exact test (when expected counts less than 5), or the Kruskal-Wallis test were used for comparisons stratified by high and low eosinophils groups.

To estimate the rate of lung function decline of the blood eosinophil groups, an interaction between eosinophil group and time was included in the multivariate linear mixed-effects model. The multivariate model was adjusted for *a priori* selected covariates including age, sex, BMI, and baseline pp $\text{FEV}_1$ . Mixed-effects regression models account for the correlated nature of repeated pp $\text{FEV}_1$  measures per patient by incorporating random intercepts following an unstructured correlation pattern using the lme4 R package [20]. In addition, we performed a sensitivity analysis for our lung function model using an eosinophil count cut-off of 400 cells/ $\mu\text{L}$ , also commonly used in the eosinophilic asthma literature [16,17]. A multivariable linear regression model was used to compare CFRSD-CRISS scores at stable visits between high and low eosinophil groups, adjusting for the aforementioned covariates. A multivariable poisson regression model was employed to examine the relationship between eosinophil grouping and rate of PEx in the year following stable clinic visits, adjusted for the same covariates as well as number of PEx in the year prior to stable visit (treated with IV antibiotics only or with either IV or oral antibiotics to match the outcome variable).

We performed a secondary analysis with eosinophil count as a continuous predictor and rate of lung function decline as our outcome. We compared a linear regression model with our previous model treating eosinophil count as a categorical variable. Model fit was assessed using Akaike (AIC) and Bayesian information criteria (BIC).

Missing data was minimal and therefore complete case analysis was used for regression analyses. The level of statistical significance was set at  $P < 0.05$  for all statistical analyses, and all reported  $P$ -values reflect two-tailed tests. All analyses were conducted using R 4.1.0 statistical programming [21].

## 3. Results

### 3.1. Distribution of blood eosinophil counts

In total, 109 patients were included in the study. Of these patients, 17 (15.6%) had stable visit blood eosinophil counts  $\geq 300$  cells/ $\mu\text{L}$  and 92 (84.4%) had counts  $< 300$  cells/ $\mu\text{L}$  (Fig. 1). The median blood eosinophil count was 147 cells/ $\mu\text{L}$  (IQR 123). Using a higher cut-off, 9 (8.3%) had eosinophil counts  $\geq 400$  cells/ $\mu\text{L}$  and 100 (91.7%) had counts  $< 400$  cells/ $\mu\text{L}$ .

### 3.2. Baseline clinical characteristics

Compared to the low eosinophil group, the high eosinophil group was older (31.0 vs. 29.5 years), had more females (47.1% vs. 40.2%), but had less severe CF lung disease with a higher median baseline pp $\text{FEV}_1$  (80.7% vs. 78.2%; Table 1). The proportion of patients with *P. aeruginosa* lung infection was similar between groups

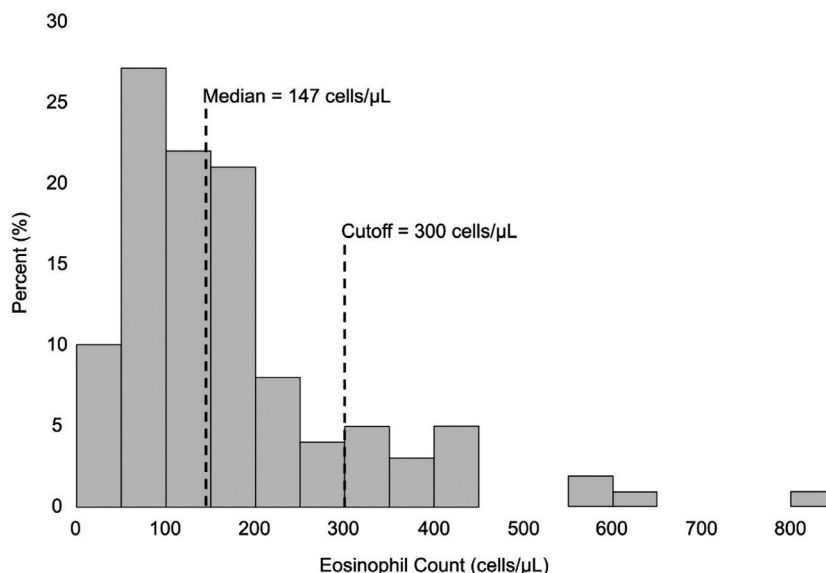


Fig. 1. Distribution of blood eosinophil count.

Table 1

Baseline demographics and clinical characteristics. *P*-values refer to comparisons between high and low eosinophil count groups. PEx IV - Pulmonary exacerbation treated with IV antibiotics. MRSA - methicillin resistant staphylococcus aureus. CFTR - Cystic fibrosis transmembrane conductance regulator.

Characteristics	Total sample (n = 109)	Eos count <300 cells/μliter (n = 92) Count (%), Median [IQR]	Eos count ≥300 cells/μliter (n = 17)	<i>P</i> value
Age (yrs)	30.0 [14.0]	29.5 [14.0]	31.0 [15.0]	0.634
Sex M	64 (58.7)	55 (59.8)	9 (52.9)	0.796
F	45 (41.3)	37 (40.2)	8 (47.1)	
BMI (kg/m <sup>2</sup> )	22.5 [4.2]	22.7 [4.2]	21.9 [4.2]	0.418
F508del homozygous	47 (43.1)	43 (46.7)	4 (23.5)	0.107
F508del heterozygous	42 (38.5)	35 (38.0)	7 (41.2)	1.000
Other genotype	18 (16.5)	13 (14.1)	5 (29.4)	0.152
Baseline ppFEV <sub>1</sub> (%)	80.0 [40.0]	78.2 [42.6]	80.7 [26.0]	0.947
PEx IV in year prior to stable visit	42 (38.5)	37 (40.2)	5 (29.4)	0.569
CF-related diabetes	22 (20.4)	17 (18.7)	5 (29.4)	0.332
Pancreatic insufficiency	75 (69.4)	66 (72.5)	9 (52.9)	0.186
Liver disease	20 (18.5)	18 (19.8)	2 (11.8)	0.734
CF-related arthritis	7 (6.48)	5 (5.49)	2 (11.8)	0.303
<i>Pseudomonas aeruginosa</i>	35 (39.3)	30 (39.5)	5 (38.5)	1.000
MRSA	13 (14.8)	10 (13.3)	3 (23.1)	0.399
<i>Burkholderia cepacia</i>	11 (10.1)	9 (9.8)	2 (11.8)	0.625
Peripheral neutrophil count (cells/μL)	5120 [2730.0]	5120 [3690.0]	5070 [1910.0]	0.850
Peripheral monocyte count (cells/μL)	480 [230.0]	480 [280.0]	510 [90.0]	0.469
Inhaled corticosteroid use	60 (55.0)	48 (52.2)	12 (70.6)	0.256
CFTR modulator use	6 (5.5)	5 (5.4)	1 (5.9)	1.000

(39.5% vs. 38.5%). More patients in the high eosinophil group were actively using inhaled corticosteroids at stable visit compared to the low eosinophil group (70.6% vs. 52.2%). One patient in each group was documented to have newly diagnosed ABPA during follow-up.

### 3.3. Baseline symptoms

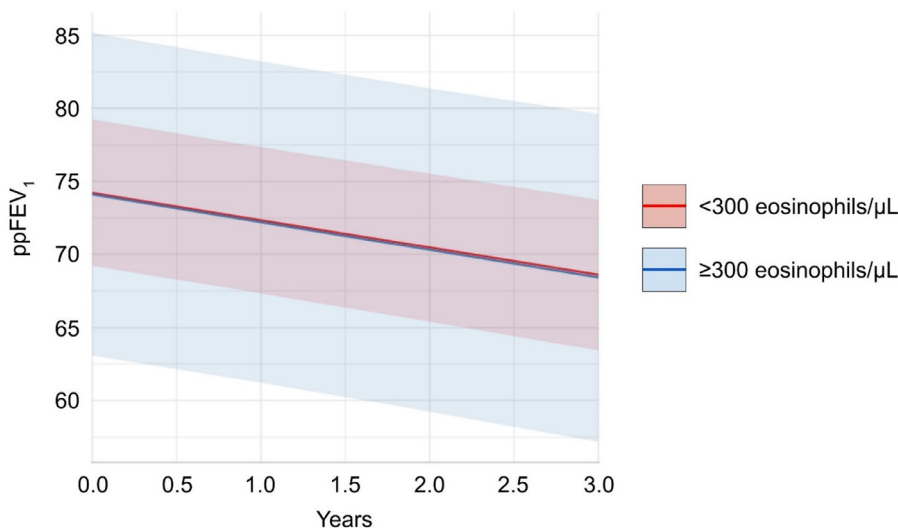
Comparing symptoms noted by patients 24 h prior to stable visit (Table 2), the median CFRSD-CRISS score was higher in the high vs. low eosinophil group (41.0 vs. 29.0). Four patients in the low eosinophil group did not fully complete the self-reported questionnaires. The high eosinophil group had a higher proportion of individuals with difficulty breathing (47.1% vs. 14.1%), feeling tired (70.6% vs. 32.6%), chills or sweats (29.4% vs. 4.3%), and wheezing (35.3% vs. 9.8%). The relationship between eosinophil group and CFRSD-CRISS score was similar after adjustment (adjusted mean difference 9.3; 95% CI 2.9 to 16.0; *p* = 0.005).

### 3.4. Rate of lung function decline

Median lung function follow-up was 2.0 years (IQR 2.0) among all patients and included a total of 1014 lung function measurements. Overall, the mean change in ppFEV<sub>1</sub> for the study population was -1.9% per year (95% CI -1.4 to -2.4). With unadjusted regression analysis, we observed that the rate of lung function decline in the high eosinophil group was similar compared to the low eosinophil group (difference in slope between high and low eosinophil group -0.30%/y; 95% CI -1.8 to +1.2). After adjustment, the rates of lung function decline remained similar between groups (difference in slope -0.04%/y; 95% CI -1.5 to +1.4; Fig. 2). When a cut-off of 400 cells/μliter was employed, the high eosinophil group again had a similar rate of lung function decline compared to the low eosinophil group (difference in slope -0.31%/y; 95% CI -2.3 to +1.7). In a linear model, eosinophil count as a continuous variable was not associated with rate of lung function decline after adjustment (Supplementary Table 1).

**Table 2**  
Reported symptoms (based on CFRSD-CRISS) at stable visit.

Symptoms in last 24h	Eos count <300 cells/μliter (n = 88) Count (%), Median [IQR]	Eos count ≥300 cells/μliter (n = 17)	P value
CFRSD-CRISS score	29.0 [14.0]	41.0 [10.0]	0.002
Difficulty breathing	13 (14.1)	8 (47.1)	0.005
Feverish	1 (1.1)	0 (0.0)	1.000
Tired	30 (32.6)	12 (70.6)	0.009
Chills or sweats	4 (4.3)	5 (29.4)	0.005
Cough	78 (84.8)	15 (88.2)	1.000
Cough up mucus	70 (76.1)	15 (88.2)	0.515
Chest tightness	11 (12.0)	5 (29.4)	0.128
Wheeze	9 (9.8)	6 (35.3)	0.018



**Fig. 2.** Predicted ppFEV<sub>1</sub> measurements over time from the adjusted linear mixed-effect model for high and low eosinophil groups. All other variables in the model were held constant at their means for continuous covariates, and categorical covariates at their proportions.

### 3.5. Pulmonary exacerbations

In total, 43 out of 109 (46%) patients experienced at least one PEx treated with IV antibiotics and 77 (70.6%) experienced at least one PEx treated with IV or oral antibiotics during a year of follow-up. On unadjusted poisson regression analysis, both eosinophil groups had comparable rates of PEx requiring IV antibiotics in the first year (Incidence rate ratio 1.22; 95% CI 0.66 to 2.12;  $p = 0.498$ ). This relationship was similar after adjusting for age, sex, BMI, baseline ppFEV<sub>1</sub>, and number of PEx requiring IV antibiotics in the year prior to stable visit (IRR 1.46; 95% CI 0.75 to 2.65;  $p = 0.237$ ). The results were again similar if we used PEx treated with IV or oral antibiotics as the outcome (adjusted IRR 1.17; 95% CI 0.77 to 1.74;  $p = 0.439$ ).

## 4. Discussion

This study is the first to examine the association between blood eosinophil count and longitudinal disease outcomes in CF lung disease. We observed no association between high and low eosinophil groups with lung function decline (based on ppFEV<sub>1</sub>) or rates of pulmonary exacerbations. However, high eosinophil count was independently associated with higher CFRSD-CRISS scores during stable visits.

Based on our findings, there may be a stronger role for neutrophilic inflammation and a weaker role for eosinophilic inflammation in CF lung injury compared to sub-phenotypes of COPD and asthma. However, we observed that the high eosinophil group self-

reported significantly more respiratory and constitutional symptoms. Specifically, they were more likely to experience difficulty breathing, wheezing, chills or sweats, and feeling tired 24 h before their stable visits. This does not seem to be explained by worse lung disease severity or history of PEx as the high eosinophil group had milder lung disease based on baseline FEV<sub>1</sub> and a lower proportion of patients who experienced a PEx in the year prior to their stable visit.

It is difficult to ascertain whether eosinophilic inflammation is contributing to more respiratory and constitutional symptoms or if both the observed symptoms and elevated eosinophil counts are indicative of a common subacute process. Two such contributing processes may be undiagnosed ABPA and asthma as both are associated with elevated peripheral blood eosinophil counts and can contribute to increased respiratory symptoms, including difficulty breathing and wheezing [22,23]. Supportive of this, there was a higher proportion of patients prescribed inhaled corticosteroids in the high eosinophil group compared to the low eosinophil group. However, based on chart review, only 1 patient in each group was diagnosed with ABPA in follow-up. ABPA and asthma are very challenging to diagnose in CF due to considerable overlap in clinical presentation with CF lung disease. Although we did not specifically explore the prevalence of asthma in our sample given the lack of standardized diagnostic criteria, it remains possible that undiagnosed asthma or ABPA may be responsible for increased symptom scores in the high eosinophil group.

Limitations to our study include a modest sample size, particularly for the high eosinophil group, which could affect the

power and precision of our statistical models. In addition, using eosinophil count from a single time point may lead to misclassification. Ideally, patients should be classified as having eosinophilia based on multiple measurements over time. Patients in our study had too few subsequent stable visits for this to be feasible, but this should be evaluated in future studies. A limitation regarding the CFRSD-CRISS tool is that it has generally been validated for use in exacerbation settings and as a measure of change in symptoms over time rather than as a single outcome point. Another limitation is that we used peripheral blood eosinophil count as a surrogate for airway eosinophilia. Studies in COPD and asthma have demonstrated that peripheral blood eosinophils are well correlated with sputum eosinophils but it will be worth confirming this correlation specifically in the CF population [9,10].

The generalizability of our findings is limited by our single center design and focus on adult CF patients. Furthermore, few patients in our study used CFTR modulators, which may limit our study's applicability to populations in which these therapies are becoming more widely employed.

## 5. Conclusion

Our study found that CF patients with elevated blood eosinophil counts at stable visits reported more respiratory symptoms. However, we did not identify an association between elevated blood eosinophil counts and a greater rate of lung function decline or pulmonary exacerbations. Future studies should investigate these relationships in a larger cohort and if confirmed examine the effect of therapies targeting peripheral blood eosinophilia to reduce respiratory symptoms.

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## Declaration of Competing Interest

There are no financial disclosures or conflicts of interest for any of the authors related to this work.

## CRediT authorship contribution statement

**Si Cong Ye:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Sameer Desai:** Methodology, Formal analysis, Writing – review & editing. **Emma Karlsen:** Data curation, Project administration, Writing – review & editing. **Eugenie Kwong:** Data curation, Project administration, Writing – review & editing. **Pearce G. Wilcox:** Project administration, Writing – review & editing. **Bradley S. Quon:** Supervision, Project administration, Conceptualization, Methodology, Writing – review & editing.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.jcf.2022.03.009](https://doi.org/10.1016/j.jcf.2022.03.009).

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