High eosinophil counts predict decline in FEV_1: results from the CanCOLD study

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This study shows that high blood eosinophil count is significantly related to rapid FEV_1 decline in all people, independent of well-established risk factors such as smoking and age, and may be related to undetected airway abnormalities https://bit.ly/36o8Cc6

Cite this article as: Tan WC, Bourbeau J, Nadeau G, *et al*. High eosinophil counts predict decline in FEV_1: results from the CanCOLD study. *Eur Respir J* 2021; 57: 2000838 [https://doi.org/10.1183/13993003.00838-2020].

ABSTRACT

Introduction: The aim of this study was to examine the association between blood eosinophil levels and the decline in lung function in individuals aged >40 years from the general population.

Methods: The study evaluated the eosinophil counts from thawed blood in 1120 participants (mean age 65 years) from the prospective population-based Canadian Cohort of Obstructive Lung Disease (CanCOLD) study. Participants answered interviewer-administered respiratory questionnaires and performed pre-/post-bronchodilator spirometric tests at 18-month intervals; computed tomography (CT) imaging was performed at baseline. Statistical analyses to describe the relationship between eosinophil levels and decline in forced expiratory volume in 1 s (FEV_1) were performed using random mixed-effects regression models with adjustments for demographics, smoking, baseline FEV_1, ever-asthma and history of exacerbations in the previous 12 months. CT measurements were compared between eosinophil subgroups using ANOVA.

Results: Participants who had a peripheral eosinophil count of $\geqslant$300 cells·μL^{-1} (n=273) had a greater decline in FEV_1 compared with those with eosinophil counts of <150 cells·μL^{-1} (n=430; p=0.003) (reference group) and 150–<300 cells·μL^{-1} (n=417; p=0.003). The absolute change in FEV_1 was −32.99 mL·$year^{-1}$ for participants with eosinophil counts <150 cells·μL^{-1}; −38.78 mL·$year^{-1}$ for those with 150–<300 cells·μL^{-1} and −67.30 mL·$year^{-1}$ for participants with $\geqslant$300 cells·μL^{-1}. In COPD, higher eosinophil count was associated with quantitative CT measurements reflecting both small and large airway abnormalities.

Conclusion: A blood eosinophil count of $\geqslant$300 cells·μL^{-1} is an independent risk factor for accelerated lung function decline in older adults and is related to undetected structural airway abnormalities.

This article has supplementary material available from erj.ersjournals.com

Received: 24 March 2020 | Accepted: 5 Nov 2020

Introduction

A gradual decline in lung function is part of the normal ageing process in adults [1–4], but can be modified by disease states such as COPD [3, 5, 6], risk factors such as developmental disorders of the lungs [1, 7], and environmental exposures, especially cigarette smoking [8]. Individuals with COPD typically have an accelerated rate of decline in lung function compared to individuals without COPD [3, 5, 6], but the individual rate of decline is variable [5, 6]. In patients with COPD, an accelerated decline in lung function is associated with an increase in symptoms, respiratory exacerbations and loss of health status [9], but is difficult to predict [10]. The association of increased concentrations of blood and sputum eosinophils with increased frequency of exacerbations and improved treatment responses to inhaled corticosteroids has been well studied in COPD patients [11, 12]. However, studies on the role of eosinophils on lung function changes in the population are rare [13] and are needed to identify individuals at risk, improve characterisation of the disease and to prevent or reduce disease progression and allow for personalised management of disease [14].

There is little information on the association between eosinophil count and lung function decline in the community. In a population-based birth cohort of young adults with or without asthma followed for up to 38 years, blood eosinophil counts were associated with a greater decline in pre-bronchodilator forced expiratory volume in 1 s (FEV_1) [13], but the underlying mechanism is unclear. In an analysis of a large primary care population-based database of patients stratified by eosinophil level, inhaled corticosteroid (ICS) use was associated with an attenuated rate of lung function decline in patients with COPD, irrespective of blood eosinophil levels [15]. To our knowledge, there is no information on whether raised eosinophil counts could predict subsequent decline in lung function in unselected individuals with and without COPD in the community. The aim of this study was to assess the relationship between blood eosinophil count and decline in lung function over time in individuals with and without chronic airflow limitation in the prospective CanCOLD study [16]. A secondary aim was to explore the structural basis of this association using quantitative airway measurements from computed tomography (CT) images of the lungs.

Methods

Participants

The Canadian Cohort of Obstructive Lung Disease (CanCOLD) is a prospective cohort study built on the original Canadian COPD prevalence study (COLD), which evaluated >6000 subjects (male and female subjects aged ⩾40 years), who were recruited through a random sampling frame [17–20] in nine urban and suburban areas in Canada (ClinicalTrials.gov identifier NCT00920348) [16]. Sampling for CanCOLD consisted of all COPD subjects from the COLD study and an equal number of age- and sex-matched non-COPD peers (defined using a post-bronchodilator FEV_1/forced vital capacity (FVC) ratio >0.70) from the same study. Thus, CanCOLD comprises two balanced COPD subpopulations (mild and moderate–severe) and two matched non-COPD subpopulations including ever-smokers (for those at risk) and never-smokers (for the control subjects). Detailed descriptions of the sampling strategy and assessments can be found in the published protocol [16, 21] and in the supplementary material.

At the time of data extraction in June 2017, the CanCOLD cohort consisted of 1285 individuals, of whom 1120 had provided venous samples for blood counts. Details of participant selection are shown in figure 1.

Measurements

Participants were assessed during the initial COLD study visit (visit 0) and over three visits in the CanCOLD longitudinal study: at baseline (visit 1), after 18 months (visit 2) and at 36 months (visit 3). Assessment at these visits included sociodemographic characteristics, respiratory symptoms and health status questionnaires (including the COPD Assessment Test, the St George's Respiratory Questionnaire for COPD, modified Medical Research Council dyspnoea scale and Short Form-36); full lung-function tests including pre- and post-bronchodilator spirometry according to the American Thoracic Society/European Respiratory Society guidelines [22, 23]; CT imaging of the thorax; and incremental maximal cardiopulmonary exercise testing. Details regarding CT imaging are provided in the supplementary material.

Blood eosinophils

Using whole-blood samples collected from participants, complete blood cell counts and differential counts were performed using an automated haematology analyser (ADVIA 2120i Hematology System; Siemens, Erlangen, Germany). Whole-blood samples were taken at the first CanCOLD visit and at 18-month intervals where possible and frozen for later analyses. In a subset of 341 participants, blood count measurements were performed on fresh blood samples before the samples were frozen. Available frozen blood samples (n=1120) for visit 1 were thawed and whole-blood and differential counts were measured. Frozen whole-blood samples were available for 536 participants in visit 2 and 394 participants in visit 3 (figure 1). The eosinophil percentage was multiplied by the white cell count to give the absolute eosinophil

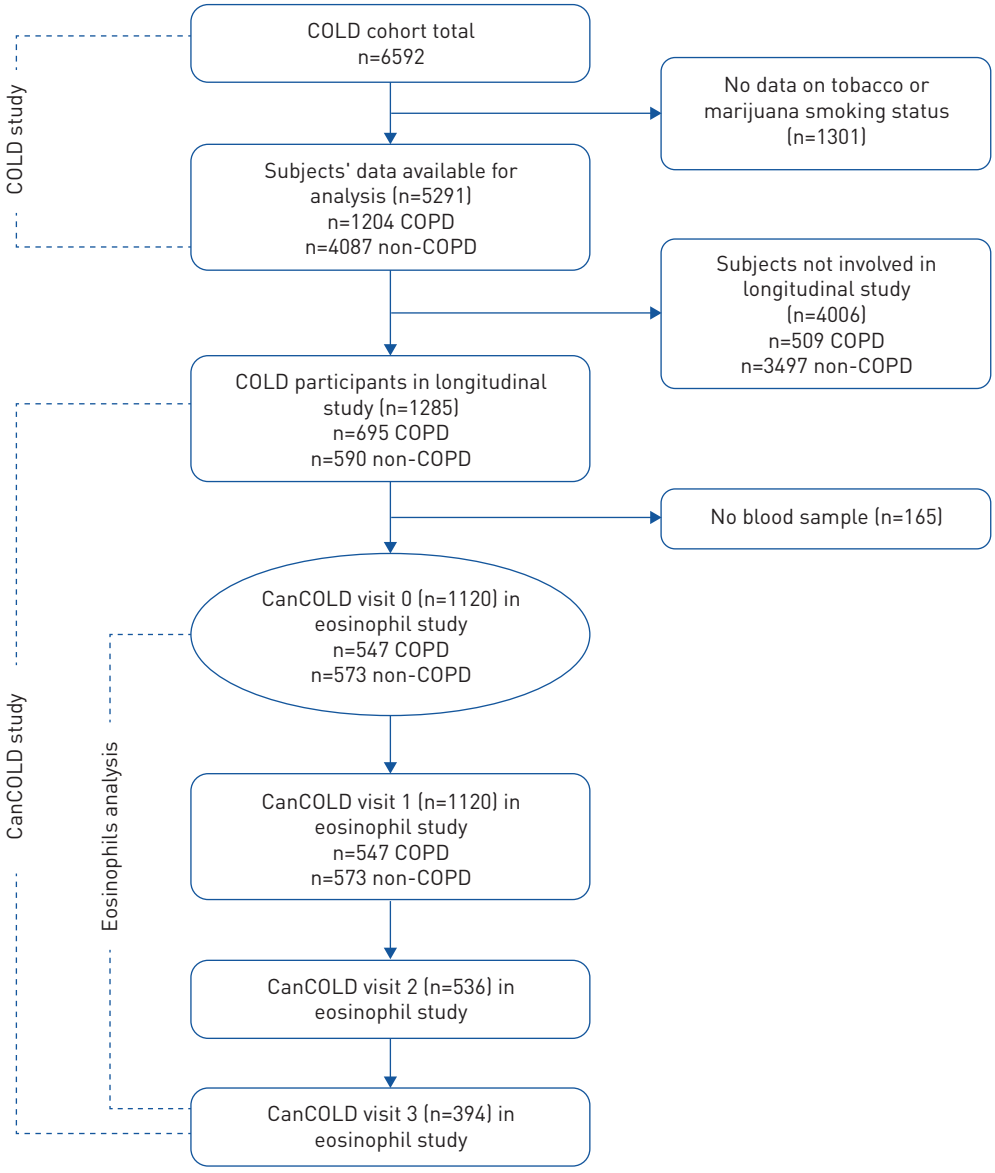

FIGURE 1 CONSORT diagram: study population. COLD: Cohort of Obstructive Lung Disease; CanCOLD: Canadian Cohort of Obstructive Lung Disease.

count. In this study, we used a pre-specified eosinophil cut-off of ⩾300 cells·μL^{-1} [24–27] in addition to two lower measurements of <150 cells·μL^{-1} and 150–<300 cells·μL^{-1}.

Quantitative CT imaging measurements

Because we had previously found that a reduction in total airway count could explain a decline of lung function in the CanCOLD cohort [28], we investigated quantitative CT imaging measurements in the participants with and without COPD stratified by eosinophil subgroups. Refer to the supplementary material for full details of the measurements.

Statistical analysis

Comparison of the eosinophil counts in participants with COPD and in those without COPD were made using t-tests. For testing the associations between baseline values of both thawed and fresh blood eosinophil counts (expressed as categorical variables of three groups <150 cells·μL^{-1}, 150–<300 cells·μL^{-1} and ⩾300 cells·μL^{-1}) with the decline in FEV_1, random mixed-effects multivariable regression models were constructed using all time-points (visit 1, visit 2, visit 3), with adjustments for age, sex, body mass index (BMI), baseline FEV_1, smoking history (yes *versus* no), ever-asthma (yes *versus* no), history of

exacerbations (at least two *versus* one or fewer) in the previous year, presence of COPD (yes *versus* no) and the use of any ICS (yes *versus* no). All participants had at least two (median four) FEV_1 measurements at separate visits. The lung function decline over time (up to 10 years) was projected using β-coefficients from the model and right-censored at 10 years of follow-up in the figures. Sensitivity analyses were conducted by repeating the regression models after excluding participants with 1) a reported history of doctor-diagnosed asthma in the whole cohort, and again in the subset of participants with COPD; 2) allergic rhitinits from the whole cohort; and 3) significant bronchodilator response from the whole cohort. Additional analyses for the decline in FVC and in FEV_1/FVC ratio were performed (supplementary material). The Akaike information criterion [29] was used for testing the goodness-of-fit and model selection for the regression methods. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC, USA). ANOVA was used for statistical comparison of CT measurements between all groups (<150 cells·μL^{-1}, 150–<300 cells· μL^{-1}, ⩾300 cells· μL^{-1}) for participants with and without COPD.

Results

Baseline demographics and clinical characteristics, including spirometric measurements and eosinophil counts for all visits are presented in table 1. During the study period, the mean age increased from 65 to 70 years over a mean±SD follow-up period of 5.5±1.4 years. The proportion of participants with different levels of eosinophil count was relatively constant between visits (supplementary tables E1 and E2), with the lowest eosinophil group being the most stable. The mean, median and interquartile range (IQR) for the number of measurements of FEV_1 per participant was three, three and two to four, respectively (data not shown).

The demographic and clinical characteristics of participants with COPD were similar in the cross-sectional and longitudinal phases: the COLD cohort, the CanCOLD cohort and finally the CanCOLD cohort with available eosinophil data between the groups, except for the proportion of males, which was increased in the COPD-CanCOLD eosinophil subgroup (supplementary table E3). Compared with COPD subgroup, the non-COPD subgroup had a lower proportion of individuals with positive bronchodilator response (⩾12% change and ⩾200 mL increase in FEV_1): 5.2% *versus* 11.8% and a lower proportion with self-reported asthma: 5.9% *versus* 14.8% (supplementary table E4).

TABLE 1 Demographic and clinical characteristics of participants by visit

	Visit 0	Visit 1	Visit 2	Visit 3
Participants	1120	1120	536	394
Age years	65±9.79	68±9.66*	69±9.32*	70±9.42*
Male	629 (56.2)	629 (56.2)	281 (52.4)	204 (51.8)
BMI kg·m^{-2}	27.81±10.29	27.63±5.14	28.14±5.26*	27.87±5.55
Years of follow-up		2.36±1.62*	4.40±1.24*	5.52±1.39*
Post-BD FEV_1 L	2.59±0.84	2.52±0.81	2.43±0.81*	2.34±0.76*
Post-BD FVC L	3.73±1.09	3.65±1.07	3.52±1.08*	3.50±1.07*
Post-BD FEV_1/FVC %	69.43±9.92	69.22±10.50	69.17±10.58	67.15±10.75*
Post-BD FEV_1 % predicted	90.62±20.47	91.53±20.54	90.88±21.03	89.32±21.42
Post-BD FVC % predicted	98.13±17.37	98.94±17.24	97.98±17.30	98.96±18.39
Post-BD−Pre-BD FEV_1 L	0.12±0.20	0.11±0.16	0.11±0.14	0.11±0.15
(Post-BD−Pre-BD FEV_1)/Pre-BD FEV_1 %	5.48±8.95	5.41±7.76	5.28±7.27	5.91±8.23
Proportion with bronchodilator response#	136 (12.1)	132 (11.8)	53 (9.9)	47 (11.9)
Ever-tobacco smoker¶	673 (60.1)	673 (60.1)	286 (53.4)*	215 (54.6)
Reported physician-diagnosed asthma	115 (10.3)	115 (10.3)	54 (10.1)	41 (10.4)
⩾2 exacerbations in the previous year	14 (1.3)	20 (1.8)	14 (2.6)	6 (1.5)
COPD FEV_1/FVC <0.7	547 (48.8)	547 (48.8)	261 (48.7)	240 (60.9)*
Absolute eosinophil count cells·μL^{-1}		0.23±0.21	0.28±0.41	0.28±0.38
Eosinophil subgroups				
<150 cells·μL^{-1}		430 (38.4)	213 (39.7)	151 (38.3)
150–<300 cells·μL^{-1}		417 (37.2)	198 (36.9)	146 (37.1)
⩾300 cells·μL^{-1}		273 (24.4)	125 (23.3)	97 (24.6)

Data are presented as n, mean±SD or n (%). BMI: body mass index; BD: bronchodilator; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity. #: ⩾12% change in FEV_1 ([post-BD−pre-BD FEV_1]/pre-BD FEV_1 %) and post-BD−pre-BD FEV_1 ⩾200 mL; ¶: 56% of ever-tobacco smokers had COPD. *: statistically significant difference (p<0.05) compared to visit 0.

Blood eosinophils

There was a good correlation between fresh and thawed absolute blood eosinophil count (r=0.649) and relative eosinophil percentages (r=0.659) and a good agreement in the Bland–Altman plots (supplementary figures E1 and E2). The distribution of fresh blood eosinophil counts was similar between the non-COPD and COPD participants (data not shown). The eosinophil counts expressed as a geometric mean, median and IQR were 139 cells·μL^{-1}, 150 cells·μL^{-1} and 89–240 cells·μL^{-1}, respectively, for non-COPD participants and 142 cells·μL^{-1}, 150 cells·μL^{-1} and 90–234 cells·μL^{-1}, respectively, for participants with COPD (data not shown).

Blood eosinophil counts and decline in lung function

Table 2 shows the estimated annual rate of change in FEV_1 (mL·year^{-1}) of the individuals in the subgroups 150–<300 cells·μL^{-1} and ⩾300 cells·μL^{-1} compared with the reference subgroup <150 cells·μL^{-1}.

Participants with an eosinophil count ⩾300 cells·μL^{-1} had a statistically significantly greater annual decline in FEV_1 compared with those with a blood eosinophil count <150 cells·μL^{-1} (reference group) (p=0.004) and those with an eosinophil count 150–<300 cells·μL^{-1} (p=0.01); however, the annual FEV_1 decline in participants with an eosinophil count 150–<300 cells·μL^{-1} was not statistically significantly different compared with the reference group (table 2, figure 2a). The absolute change in FEV_1 was −32.99 mL·year^{-1} for participants with blood eosinophil counts <150 cells·μL^{-1}, −38.78 mL·year^{-1} for those with eosinophil counts 150–<300 cells·μL^{-1} and −67.30 mL·year^{-1} for participants with eosinophil counts ⩾300 cells·μL^{-1}.

For the other covariates, age, female sex, increased BMI, tobacco smoking, a higher baseline FEV_1 and presence of COPD, but not patient-reported asthma, were independently associated with an accelerated decline in FEV_1 (table 2). The association between an eosinophil count ⩾300 cells·μL^{-1} and an accelerated rate of change was statistically significant for FVC (mL·year^{-1}) and FEV_1 (mL·year^{-1}) but not for FEV_1/FVC (supplementary table E5).

Using data from a smaller subset of participants with available fresh blood eosinophil counts (n=341), the associations between the decline of FEV_1 and eosinophil levels were similar to those for individuals with thawed blood. Participants with an eosinophil count ⩾300 cells·μL^{-1} had a statistically significantly greater annual decline in FEV_1 compared with those with a blood eosinophil count <150 cells·μL^{-1} (reference

TABLE 2 Results from the mixed-effects multivariable regression model showing the lung function decline for two levels of eosinophil count subgroups (150–<300 cells·μL^{-1} and ⩾300 cells·μL^{-1}) compared with the reference subgroup (<150 cells·μL^{-1}) and other covariates (n=1120)

	Participants	Annual rate of change in FEV_1 mL·year^{-1}
Eosinophil count subgroups		
<150 cells·μL^{-1} (reference)	430/1120	
150–<300 cells·μL^{-1}	417/1120	−5.79 (−25.46–13.88)
⩾300 cells·μL^{-1}	273/1120	−34.31 (−57.84–−10.78)$^{\#,*}$
Female *versus* male	491/1120	−116.50 (−152.70–−80.39)*
Follow-up time years	1120	−14.13 (−29.82–1.55)
Age years	1120	−7.23 (−8.97–−5.48)*
BMI kg·m^{-2}	1120	−3.70 (−6.34–−1.06)*
Tobacco smoking status (yes *versus* no)	673/1120	−57.00 (−87.55–−26.45)*
Baseline FEV_1 L	1120	−7.93 (−13.70–−2.17)*
Physician-diagnosed asthma (yes *versus* no)	115/1120	−17.21 (−52.57–18.15)
⩾2 exacerbations in the previous year (yes *versus* no)	20/1098	−52.83 (−121.00–15.33)
COPD, by spirometry (yes *versus* no)	547/1120	−104.10 (−127.20–−81.00)*
ICS (yes *versus* no)	163/1120	3.16 (−31.09–37.41)

Data are presented as n/N or estimate (95% CI). The computed equation (follow-up time+age+basal metabolic index, body mass index (BMI)+baseline forced expiratory volume in 1 s (FEV_1)) for the reference group (<150 cells·μL^{-1}) provides the value for the absolute reference decline, which is −32.99 mL·year^{-1}. For participants with eosinophil counts 150–<300 cells·μL^{-1} the absolute rate of change was −32.99–5.79= −38.78 mL·year^{-1}; for participants with eosinophil counts ⩾300 cells·μL^{-1} the absolute rate of change was −32.99–34.31=−67.30 mL·year^{-1}. ICS: inhaled corticosteroid. $^{\#}$: the β-coefficient (estimates) is relative to both the reference and the 150–<300 cells·μL^{-1} group. *: p<0.05 is considered significant.

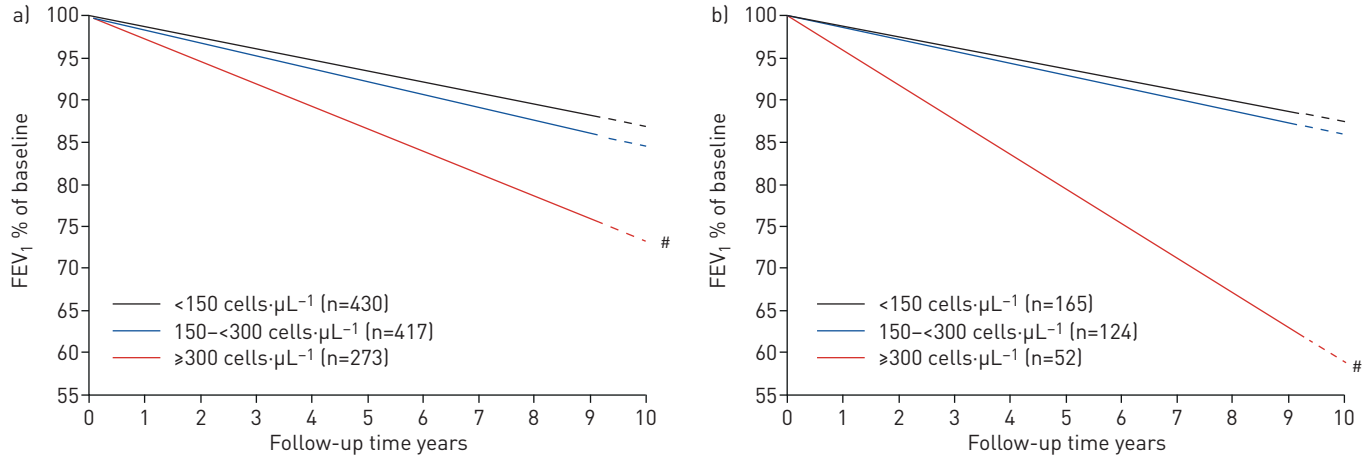

FIGURE 2 Longitudinal forced expiratory volume in 1 s (FEV_1) decline trajectories by eosinophil subgroups in a) thawed and b) fresh blood. The solid line represents the linear regression line for the actual measurements and the dashed line represents the projected FEV_1 decline based on the regression equation. #: the annual decline in FEV_1 for the subgroup of participants with eosinophil ⩾300 cells·μL^{-1} was significantly greater than for the subgroup with eosinophil <150 cells·μL^{-1} (a) p=0.004; b) p=0.003) and was also significantly greater than in the eosinophil subgroup 150–<300 cells·μL^{-1} (a) p=0.01; b) p=0.003).

group) and those with 150–<300 cells·μL^{-1} (p=0.003 for both comparisons) (figure 2b). Covariates of age, female sex, tobacco and marijuana smoking and presence of COPD were independently associated with an accelerated decline in FEV_1 (supplementary table E6). The absolute change in FEV_1 was −31.59 mL·year^{-1} for participants with fresh eosinophil counts <150 cells·μL^{-1} (reference group), −35.56 mL·year^{-1} for participants with fresh eosinophil counts 150–<300 cells·μL^{-1} and −103.22 mL·year^{-1} for participants with fresh eosinophil counts ⩾300 cells·μL^{-1}.

Similar results were obtained from all the sensitivity analyses conducted by repeating the regression models after excluding participants with 1) a history of reported physician-diagnosed asthma in the whole cohort of participants, and again in the subset of participants with COPD (figure 3, supplementary tables E7, E8 and E9); 2) allergic rhinitis from the whole cohort; and 3) significant bronchodilator response from the whole cohort (supplementary tables E10 and E11).

CT measurements comparison in eosinophil subgroups

Quantitative CT imaging measurements were compared between eosinophil subgroups in the participants with and without COPD. The results from thawed and fresh blood for participants with and without

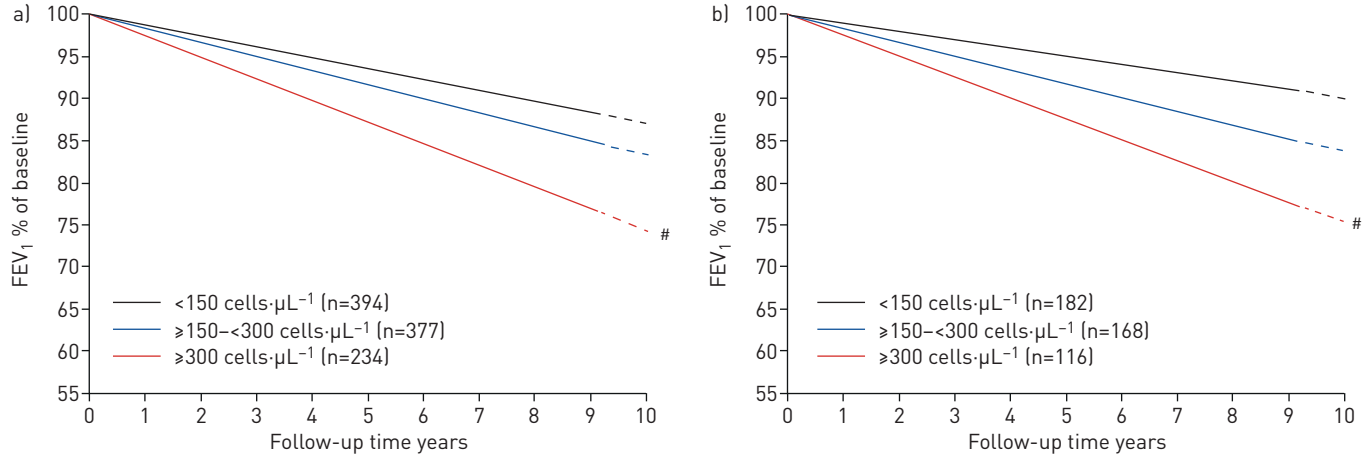

FIGURE 3 Longitudinal forced expiratory volume in 1 s (FEV_1) decline trajectories by eosinophil subgroups in thawed blood a) for the whole cohort with asthma excluded and b) for the subgroup of participants with COPD with asthma excluded. The solid line represents the linear regression line for the actual measurements and the dotted line represents the projected FEV_1 decline based on the regression equation. #: the annual decline in FEV_1 for the subgroup of participants with eosinophils ⩾300 cells·μL^{-1} was significantly greater (a) p=0.011; b) p=0.0437) than for the subgroup with eosinophils <150 cells·μL^{-1}, but was not significantly greater than in the eosinophil subgroup 150–<300 cells·μL^{-1} for both a) and b).

COPD are shown in table 3. In the participants without COPD, low attenuation area <−856 HU (LAA_{856}) was increased (indicating gas trapping) in both the ⩾300 cells·μL^{-1} eosinophils subgroup and the 150–<300 cells·μL^{-1} eosinophils subgroup relative to the <150 cells·μL^{-1} eosinophils subgroup ($p<0.05$) for the thawed cells. In the COPD participants, LAA_{856} was also increased in the ⩾300 cells·μL^{-1} eosinophils subgroup relative to the <150 cells·μL^{-1} eosinophils subgroup ($p<0.05$) for the thawed cells. For the fresh cells, the ⩾300 cells·μL^{-1} eosinophils subgroup had significantly increased estimated airway wall thickness for an idealised airway with an internal perimeter of 10 mm (Pi10) ($p<0.05$) (indicating airway wall thickening) and decreased total airway count ($p<0.05$) relative to the <150 cells·μL^{-1} eosinophils subgroup; Pi10 was also increased in the 150–<300 cells·μL^{-1} eosinophils subgroup relative to the <150 cells·μL^{-1} eosinophils subgroup. These findings in the COPD participants suggest that those with elevated eosinophil counts also have thickened central airway walls and reduction in the total number of visible airways, indicating airway remodelling.

Discussion

The present study shows that approximately one in four adults aged ⩾40 years have a baseline blood eosinophil count ⩾300 cells·μL^{-1} and that these individuals demonstrate a faster decline in lung function compared with the rest of the population, by an average of 34 mL·year^{-1}, irrespective of the presence of COPD. Furthermore, from the analyses of the quantitative CT imaging measurements we showed that in the non-COPD subjects, the subgroup with the highest eosinophil had increased gas trapping compared to those with lower eosinophil counts, while in the COPD participants, those with elevated eosinophil counts had thickened central airway walls and reduction in the total number of visible airways, indicating increased airway remodelling. To our knowledge, these findings are novel and provide a potential pathogenetic explanation for the association of high eosinophils with rapid decline in FEV_1.

To date, most of the studies on eosinophil count have focused on it as a biomarker for increased exacerbations and treatment responses to inhaled corticosteroid in patients with moderate-to-severe COPD recruited in clinical therapeutic trials [11, 12, 15, 27, 30, 31]. There is little information on the association

TABLE 3 Comparison of imaging measurements between eosinophil subgroups

	<150 cells·μL^{-1}	150–<300 cells·μL^{-1}	⩾300 cells·μL^{-1}
No COPD			
Thawed			
Participants	202	186	109
LAA_{950} %	3.2±3.8	3.2±3.6	2.5±2.0
LAA_{856} %	18.2±14.2	22.3±15.9*	23.0±18.8*
Pi10 mm	3.93±0.16	3.91±0.13	3.93±0.13
TAC L^{-1}	51.1±18.9	49.8±16.4	49.8±17.9
Fresh			
Participants	75	44	22
LAA_{950} %	4.0±3.5	3.7±2.9	2.4±1.8
LAA_{856} %	32.3±20.2	30.5±20.2	35.0±21.4
Pi10 mm	3.92±0.10	3.92±0.11	3.92±0.07
TAC L^{-1}	49.5±17.1	50.7±15.0	48.7±17.7
COPD			
Thawed			
Participants	166	166	132
LAA_{950} %	5.9±5.4	5.8±5.5	6.0±6.1
LAA_{856} %	28.6±16.2	33.1±18.5	34.1±19.3*
Pi10 mm	3.91±0.13	3.91±0.12	3.92±0.13
TAC L^{-1}	35.9±13.5	37.9±15.1	35.1±1.5
Fresh			
Participants	77	61	25
LAA_{950} %	6.3±6.2	7.1±6.9	7.5±7.6
LAA_{856} %	38.5±18.5	40.9±22.2	39.1±21.0
Pi10 mm	3.93±0.11	3.91±0.08*	3.99±0.14*
TAC L^{-1}	37.8±12.1	36.5±14.2	31.6±12.9*

Data are presented as n or mean±SD. LAA_{950}: low attenuation area <−950 HU; LAA_{856}: low attenuation area <−856 HU; Pi10: estimated airway wall thickness for an idealised airway with an internal perimeter of 10 mm; TAC: total airway count. *: significantly different ($p<0.05$) from <150 cells·μL^{-1} group.

of eosinophil levels with decline in lung function in individuals with and without COPD. In a *post hoc* analysis of the ISOLDE clinical trial, Barnes *et al.* [31] showed that higher blood eosinophil count was associated with a reduction in the rate of annual decline in post-bronchodilator FEV_1 in patients with moderate and severe COPD treated with fluticasone propionate compared with placebo. In the community-based Dunedin Multidisciplinary Health and Development Study, Hancox *et al.* [13] showed that among adults aged ⩽38 years with and without asthma, individuals with blood eosinophil counts of ⩾400 cells·μL^{-1} experienced a slightly increased rate of FEV_1 decline, independent of asthma. Similarly, in the present CanCOLD study we evaluated older adults, who were randomly selected from the general population, and found that individuals with high eosinophil counts had a more rapid decline in FEV_1 compared to individuals with lower eosinophil counts, independent of underlying COPD status. Of those participants with COPD, the majority had mild disease (Global Initiative for Chronic Obstructive Lung Disease grade 1 and 2), which is associated with a faster decline in FEV_1 compared to patients with more advanced disease [32, 33]. Thus, disease severity and differences in study population may explain in part the previous findings [10, 26], which failed to demonstrate a significant relationship between blood eosinophil counts and the rate of change in FEV_1 in patients with moderate-to-severe COPD who were treated with various therapies, including ICS, which may have further modified this relationship [31, 34].

More than 40 years ago, Fletcher and Peto [3] described the normal rate of FEV_1 decline in an ageing population and the accelerated rate of FEV_1 decline in tobacco smokers. Consistent with this observation, the current study found that tobacco smoking, the presence of COPD and female sex were risk factors for accelerated FEV_1 decline independent of eosinophilia, with smokers experiencing, on average, a 57 mL·$year^{-1}$ faster decline than nonsmokers. Other non-smoking-related risk factors were highlighted by Agusti and Faner [35], who described different lung function decline trajectories unrelated to smoking, including immature lung development *in utero* or in early childhood, repeated exacerbations and a history of uncontrolled asthma leading to the development of COPD. While these early-life events have an impact in adulthood, it is unclear whether they continue to impact lung function decline in older adults. In the present study, the cohort consisted of Canadians aged ⩾40 years and the rate of lung function decline was calculated from their entry in the cohort and therefore did not account for earlier decline. The present population-derived cohort did not find a statistically significant association between asthma and FEV_1 decline, although asthma-like features were paradoxically associated with better clinical course in predominantly male patients with COPD receiving appropriate treatment [26].

DiSantostephano [36] described blood eosinophil distribution in participants with and without COPD from the National Health and Nutrition Examination Survey database and found that the distribution of blood eosinophil count was similar in both groups. The results presented here concur with these findings, as blood eosinophil counts were similarly distributed in the reference group and in the participants with COPD. It is therefore unlikely that these findings are driven by higher blood eosinophil counts in the participants with COPD.

Strengths

A key advantage of the CanCOLD study is that it uses the same sampling methodology as in the multinational BOLD [17] and PLATINO [37] studies, which was applied worldwide across >40 studies and five studies, respectively. This allows for interpretation of results not only across Canada but also for their extrapolation to other countries. As opposed to more traditional cohorts built on convenience samples of patients seen in a clinical setting, CanCOLD is the first population-derived cohort that has characterised the spectrum of FEV_1 decline as a function of blood eosinophils in the general community including never-smokers, individuals at risk from smoking exposure, and those who had spirometrically defined mild-to-moderate COPD. Another strength of this study is the new findings from quantitative CT measurements which provide a possible structural explanation for the association of high eosinophils with a rapid decline in FEV_1 in individuals with and without COPD.

Limitations

The present analysis has several limitations. CanCOLD is a cohort of participants of never-smokers, smokers with normal lung function and those with COPD who were identified in a random sample of the population, with age- and sex-matched participants without COPD. By design, it was enriched for participants with COPD and this enrichment of the longitudinal cohort could have caused a potential bias towards a more rapid decline in FEV_1. However, the current findings, based both on statistical adjustments and sensitivity analyses, showed that high eosinophil levels (⩾300 cells·μL^{-1}) were associated with a rapid rate of decline in FEV_1 independent of the presence of COPD.

Another caveat was the concern that because the composition of the CanCOLD longitudinal cohort did not fully reflect the relative proportions of the population-based COLD study, our findings may not be

extrapolated to the general population. It should be noted that all participants with COPD detected in the population-based COLD study were included in the CanCOLD cohort [19]. As highlighted in a previous study [38], this suggests that the findings in this subgroup with mild-to-moderate and largely undiagnosed COPD have characteristics of COPD found in the general population.

Frozen blood samples were used in the current analysis because of the small sample size of available fresh eosinophil counts. Using frozen blood samples is usually not recommended, as some cells may be damaged during the freezing/thawing process. However, the data from the fresh and thawed blood samples in a subset of the CanCOLD population demonstrated good concordance, which suggests that the use of frozen samples did not materially affect the results of the analyses. Any residual confounding by this process probably led to a nondifferential bias (resulting in dilution of effects) because samples were frozen regardless of COPD status, lung function, smoking status or FEV_1 decline data.

Lastly, in this study we did not test for atopy or bronchial hyperresponsiveness, which are hallmarks of asthma and which could potentially explain part of the observed accelerated decline in FEV_1 associated with blood eosinophilia in non-COPD subjects. However, we believe this effect is unlikely for several reasons: 1) in the modelling for lung function decline, unlike eosinophilia, patient-reported asthma was not an independent risk factor for increased decline in FEV_1 in the whole cohort; and 2) the association between blood eosinophilia and increased decline in FEV_1 persisted in both COPD and non-COPD subjects even after excluding self-reported asthmatics from the cohort. Likewise, exclusion of hay fever/rhinitis or significant bronchodilator response from the cohort had no material impact on the findings. Importantly, these findings in older adults concur with those from another longitudinal population-based birth cohort study comprising younger adults with and without asthma and which concluded that blood eosinophil count was associated with airflow obstruction and enhanced decline in lung function, independently of asthma [13].

Conclusion

In summary, the current study highlights that in a population-derived cohort, a baseline blood eosinophil count $\geqslant$300 cells·μL^{-1} is a significant and independent risk factor for accelerated decline in lung function, increasing the rate of FEV_1 decline by approximately two-fold compared to the rest of the population. This finding is independent of exacerbation status; occurs in individuals with and without COPD; and is related to gas trapping, airway wall thickening and reduction of total airway count based on thoracic CT scans. Despite the utility of blood eosinophil as an emerging biomarker in COPD, its pathogenic role remains to be established [39]. Further studies are warranted to validate the use of eosinophil as a biomarker for lung function decline.

Acknowledgements: The authors would like to thank the men and women who participated in the study, and individuals in the CanCOLD Collaborative Research Group not listed as authors: Jonathon Samet (the Keck School of Medicine of USC, Los Angeles, CA, USA); Milo Puhan (John Hopkins School of Public Health, Baltimore, MD, USA); Qutayba Hamid, Carolyn Baglole, Palmina Mancino, Pei-Zhi Li, Zhi Song, Dennis Jensen, Benjamin Mcdonald Smith (McGill University, Montreal, QC, Canada); Yvan Fortier and Mina Dligui (Sherbrooke University, Sherbrooke, QC, Canada); Kenneth Chapman, Jane Duke, Andrea S. Gershon (University of Toronto, Toronto, ON, Canada); J. Mark Fitzgerald, Mohsen Sadatsafavi (University of British Columbia, Vancouver, BC, Canada); Christine Lo, Sarah Cheng, Elena Un, Michael Cheng, Cynthia Fung, Nancy Haynes, Liyun Zheng, LingXiang Zou, Joe Comeau, Harvey Coxson, Miranda Kirby, Jonathon Leipsic, Cameron Hague (UBC James Hogg Research Center, Vancouver, BC, Canada); Brandie L. Walker, Curtis Dumonceaux, (University of Calgary, Calgary, AB, Canada); Paul Hernandez, Scott Fulton, (University of Dalhousie, Halifax, NS, Canada); Shawn Aaron, Kathy Vandemheen, (University of Ottawa, Ottawa, ON, Canada); Denis O'Donnell, Matthew McNeil, Kate Whelan (Queen's University, Kingston, ON, Canada); Francois Maltais, Cynthia Brouillard (University of Laval, Quebec City, QC, Canada); Darcy Marciniuk, Ron Clemens, Janet Baran (University of Saskatchewan, Saskatoon, SK, Canada).

Author contributions: W.C. Tan, J. Bourbeau, D.D. Sin, G. Nadeau, S.H. Landis, N. Barnes and J.C. Hogg contributed to the design and implementation of the study; W.C. Tan, J. Bourbeau and D.D. Sin contributed to the collection of data and the analysis; W.C. Tan, J. Bourbeau, D.D. Sin and G. Nadeau contributed to the interpretation of the data and the writing of the manuscript; S.H. Landis, N. Barnes, M. Kirby and J.C. Hogg contributed to the interpretation of data, and the revision of the manuscript; W. Wang contributed to the analysis and interpretation of the data and revision of the manuscript; W.C. Tan and J. Bourbeau had full access to all data in this study and had final responsibility for the decision to submit this manuscript for publication. All authors approved the final version of the manuscript.

Conflict of interest: W.C. Tan reports grants from Canadian Institute of Heath Research (CIHR/Rx&D Collaborative Research Program, operating grants 93326) with industry partners AstraZeneca Canada Ltd, Boehringer Ingelheim Canada Ltd, GlaxoSmithKline Canada Ltd, Merck, Novartis Pharma Canada Inc., Nycomed Canada Inc. and Pfizer Canada Ltd, during the conduct of the study. J. Bourbeau reports funding for the current study from GlaxoSmithKline; grants from CIHR, Canadian Respiratory Research Network (CRRN), Foundation of the MUHC and Aerocrine, personal fees for consultancy and lectures from Canadian Thoracic Society and Chest, grants and personal fees for advisory board work and lectures from AstraZeneca, Boehringer Ingelheim, Grifols, GlaxoSmithKline, Novartis and Trudell, outside the submitted work. G. Nadeau was an employee of and held shares in a pharmaceutical company at

the time of the study. W. Wang has nothing to disclose. N. Barnes was an employee of and held shares in a pharmaceutical company at the time of the study. S.H. Landis held stocks and shares in GlaxoSmithKline, during the conduct of the study. M. Kirby is a consultant for VIDA Diagnostics Inc., outside the submitted work. J.C. Hogg has nothing to disclose. D.D. Sin reports grants from Merck, personal fees for advisory board work from Sanofi-Aventis and Regeneron, grants and personal fees for research from Boehringer Ingelheim, grants and personal fees for advisory board work and lectures from AstraZeneca, personal fees for advisory board work and lectures from Novartis, outside the submitted work.

Support statement: The Canadian Cohort Obstructive Lung Disease (CanCOLD; NCT00920348) study is currently funded by the Canadian Respiratory Research Network and the industry partners AstraZeneca Canada Ltd, Boehringer Ingelheim Canada Ltd, GlaxoSmithKline Canada Ltd, and Novartis. Researchers at RI-McGill University Health Centre Montreal and iCAPTURE Centre Vancouver lead the project. Previous funding partners were the Canadian Institutes of Health Research (CIHR; CIHR/Rx&D Collaborative Research Program Operating Grants 93326), the Respiratory Health Network of the Fonds de la recherche en santé du Québec (FRQS), and industry partners: Almirall; Merck Nycomed; Pfizer Canada Ltd; and Theratechnologies. The eosinophil analyses were sponsored by GlaxoSmithKline plc. (study number PRJ2824). With the exception of the GlaxoSmithKline authors (please see author contributions), the funders had no role in the study design, data collection, and analysis, or preparation of the manuscript. Funding information for this article has been deposited with the Crossref Funder Registry.

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