J Bras Pneumol. 2022;48(6):e20220183
https://dx.doi.org/10.36416/1806-3756/e20220183

Circulating eosinophil levels and lung function decline in stable chronic obstructive pulmonary disease: a retrospective longitudinal study

Marcello Ferrari[1], Michela Pizzini[1], Lucia Cazzoletti[2], Valentina Ermon[1], Francesco Spelta[1], Sergio De Marchi[1], Luca Giuseppe Dalle Carbonare[1], Ernesto Crisafulli[1]

1. Dipartimento di Medicina, Unità di Medicina Interna e Medicina Respiratoria, Università di Verona, Verona (VR), Italia.
2. Dipartimento di Diagnostica e Sanità Pubblica, Università di Verona, Verona (VR), Italia.

Submitted: 27 May 2022.
Accepted: 20 September 2022.

Study carried out in the Unità di Medicina Interna e Medicina Respiratoria, Università di Verona, Verona (VR), Italia.

ABSTRACT

Objective: Whether blood eosinophils (bEOS) in chronic obstructive pulmonary disease (COPD) are associated with disease progression is a topic of debate. We aimed to evaluate whether the differential white blood cell (WBC) count, symptoms and treatment may predict lung function decline and exacerbations in COPD patients. **Methods:** We retrospectively examined stable COPD patients with a minimum follow-up of 3 years at our outpatients' clinic. We collected information about lung volumes (FEV_1, FVC), the total and differential WBC count, acute exacerbations of COPD (number in the 12 months before the beginning of the study=AE-COPD-B, and during the follow-up=AE-COPD-F), smoking status and treatment. FEV_1 decline and AE-COPD-F were described by using a generalized linear model and a 2-level random intercept negative binomial regression, respectively. The models included eosinophil and neutrophil counts as potential predictors and were adjusted by sex, age, smoking status, AE-COPD-B, treatment with bronchodilators and inhaled corticosteroids (ICS). **Results:** Sixty-eight patients were considered, 36 bEOS- (<170 cells/μL, the median value) and 32 bEOS+ (≥170 cells/μL). ΔFEV_1 was higher in bEOS+ than bEOS- (34.86 mL/yr vs 4.49 mL/yr, p=0.029). After adjusting for potential confounders, the eosinophil count was positively (β=19.4; CI 95% 2.8, 36.1; p=0.022) and ICS negatively (β=-57.7; CI 95% -91.5,-23.9; p=0.001) associated with lung function decline. bEOS were not found to be associated with the number of AE-COPD-F. **Conclusion:** In stable COPD patients, a higher level of blood eosinophils (albeit in the normal range) predicts a greater FEV_1 decline, while ICS are associated with a slower progression of airflow obstruction.

Keywords: COPD; Blood eosinophils; FEV_1 decline; Exacerbations; Biomarkers.

INTRODUCTION

Lung function decline is one of the most important features of the natural history in patients with Chronic Obstructive Pulmonary Disease (COPD). It is estimated that mean FEV_1 decline is about 20-40 mL/year and that there are two subgroups of patients, called "faster decliners" and "slower decliners"[1].

Systemic and pulmonary inflammation may contribute to this decline. The primary involvement of neutrophils, whose levels are higher in the airways of COPD patients and are positively associated with airflow obstruction is common knowledge.[2] Moreover, even if eosinophils are the predominant granulocyte population in subjects with asthma, recent evidence suggests the presence of eosinophilic inflammation in a subgroup of COPD patients.[3] Eosinophils in the airways correlate with their blood levels and this correlation, albeit weak, is significant.[4]

COPD patients have more circulating eosinophils than the non-COPD smoker population,[5] even though it is not clear whether this result is clinically relevant, as well as statistically significant. The blood eosinophil count can predict the risk of having an acute exacerbation. In the Copenhagen Study,[6] a blood eosinophil count with more than 340 cells/μL was associated with an almost 2 times higher risk of incurring severe exacerbations. In addition, the greatest risk of having an increased concentration of circulating eosinophils during exacerbations is related to a greater eosinophil count during the stability phase.[7]

The literature supports the notion that blood eosinophils could be a predictor of response to corticosteroid therapy in patients with acute exacerbations of COPD (AE-COPD).[7,8] During an acute exacerbation, COPD patients with an eosinophil count greater than 2% of the total white blood cells (WBC) have a low probability of relapse if they are treated with systemic corticosteroid.[9] However, there are no prospective randomized studies that examine the

Correspondence to:
Ernesto Crisafulli. Dipartimento di Medicina, Università di Verona, Largo L.A. Scuro, 10, 37134, Verona (VR), Italia.
Tel.: +39 045 8128146. E-mail: ernesto.crisafulli@univr.it
Financial support: None.

role of the blood eosinophil count as a predictor of a response therapy during stable COPD.[10] Only few studies have investigated the association between the progression of airflow obstruction and blood eosinophils, with discordant results.[11-16] Thus, we conducted a longitudinal retrospective study aimed to investigate whether factors such as clinical characteristics, laboratory data and treatment were associated with FEV_1 decline over time, focusing our attention on the differential count of circulating leukocytes.

METHODS

We conducted a retrospective observational study using the last 5-year data in the database of the outpatient clinic at the University Hospital of Verona. A total of 239 patients with COPD were considered. The diagnosis was based on the GOLD criteria.[17] The study was approved by the Ethic Committee of our Institution.

We selected patients with the following characteristics: 1) a stable phase of the disease, i.e., no AE-COPD and no change in the treatment in the previous 3 months; 2) a blood eosinophil count lower than 450 cells/μL (upper normal limit of our laboratory); 3) a minimum follow-up of 3 years. We excluded patients with other lung diseases, such as lung cancer, interstitial lung diseases, asthma, pulmonary resection, and pulmonary infections. We also excluded patients with diseases related to atopy (such as rhinitis) and those with unacceptable spirometry.[18]

Demographic information, including sex, age, height, weight, and smoking habit were collected from the patient's medical record. Medical history and prescribed therapies for the respiratory disease (LABA, Long-acting β_2-Agonists; LAMA, Long-acting Muscarinic Antagonists; Inhaled Corticosteroids, ICS) were also recorded. A patient was on therapy with LABA, LAMA or ICS when he/she took these drugs (alone or in combination) at the beginning of the observation period.

The values of spirometry parameters (Forced Expiratory Volume in 1 second, FEV_1 L; Forced Vital Capacity, FVC L) were registered. Lung function decline (mL/year) was expressed as the FEV_1 value at the beginning minus the FEV_1 value at the end of the observational period, divided by the years of follow-up (ΔFEV_1, mL/yr).

Information on exacerbations, dyspnea (modified MRC scale[19]) were also available. An AE-COPD was defined as the worsening of respiratory symptoms requiring antibiotic or oral corticosteroid treatment.[17] AE-COPD-F was the number of exacerbations at the control visits performed during the follow-up period. AE-COPD-B was the number of exacerbations in the 12-months preceding the beginning of the period of observation.

Statistical analysis

The data are shown as means ± SD, or as median with interquartile range (IQR) as appropriate. The t-test or the Wilcoxon-Mann-Whitney test were used to assess the differences of continuous variables between groups of patients, accordingly. The chi-square test was used for the comparison of data expressed as percentages.

A multivariate generalized linear model was used to evaluate the association between the eosinophil and neutrophil blood count (independent variables) and ΔFEV_1 (mL/yr) (dependent variable), controlling for sex, age, smoking status (active smoker vs former smoker) (Model I). At a later stage, baseline treatment with bronchodilators, ICS and number of AE-COPD-B were included in the model (Model II).

The association between the eosinophil and neutrophil blood count (independent variables) and AE-COPD-F (dependent variable) was assessed by using a 2-level random intercept negative binomial regression, with level 1 units (visits) nested into level 2 units (patients), adjusting for sex and age at baseline (Model III). Another model was fitted to the data adding to the previous model as independent variables treatment with bronchodilators, treatment with ICS at baseline and number of AE-COPD-B (Model IV). The estimated coefficients of the negative binomial regression were expressed as incidence rate ratios (IRR) and 95% confidence intervals were provided. Model III and IV were not adjusted for smoking habits, because the number of current smokers at baseline was very limited (n=5 out of 68 COPD patients) and none of them reported exacerbations in the follow-up.

The goodness-of-fit was assessed using the Akaike information criterion (AIC)[20] and the Bayesian information criterion (BIC):[21] the best performances in predicting FEV_1 decline and the rate of exacerbations during follow-up were identified by the lowest AIC and BIC, when comparing model I with model II and model III with model IV.

The p value less than 0.05 was statistically significant.

The statistical analysis and graphs were processed using STATA/IC 16.1.

RESULTS

Two-hundred-and-thirty-nine medical records of COPD patients were analyzed. A total of 171 patients were excluded (Figure 1): 73 patients did not have a follow-up of at least 3 years (among them 11 had died); the blood eosinophil count of 75 patients was missing; 23 were not in a stable phase of the disease.

In total, 68 patients were included in the analysis (age 71.1±6.6 years; 18 (26.5%) females). The mean follow-up was 50.1±16.3 months. The median blood eosinophil count was 170 (IQR:115-260) cells per μL. Based on this value, the patients were divided into two groups, one with less than (36 patients, bEOS- group) and one with at least (32 patients, bEOS+) 170 blood eosinophils/μL.

The demographic and clinical characteristics of the COPD patients in the study are reported in the Table 1. Age, BMI, and male/female distribution were similar in the two groups. Active smokers were present in

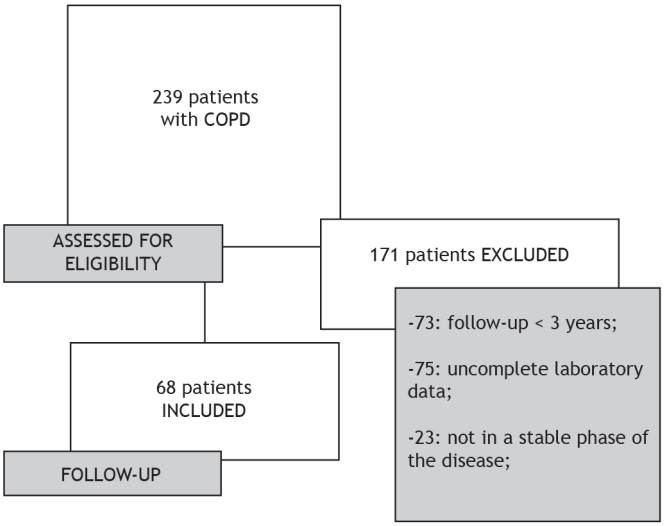

Figure 1. Selection of COPD patients in the study.

Table 1. Characteristics of the study population at baseline.

	Total (N= 68)	EOS – (N= 36)	EOS + (N= 32)	p value
Gender, n (%)				NS
- Male	50 (73.5%)	27 (75.0%)	23 (71.9%)	
- Female	18 (26.5%)	9 (25.0%)	9 (28.1%)	
Mean Age	71.0 (6.6)	71.0 (7.0)	71.1 (6.3)	NS
Median BMI (Kg/m^2)	26.7 (24.6;29.7)	25.3 (24.4;29.5)	27.5 (26.0;29.7)	NS
Median pack years	30.8 (10;48)	27.5 (0;46.5)	32 (16.5;60)	NS
Smoking Habit, n (%)				0.055*
- Ex-smoker	63 (92.6%)	31 (86.1%)	32 (100%)	
- Smoker	5 (7.4%)	5 (13.9%)	0 (0%)	
Median mMRC	1 (1;2)	1 (1;2)	1 (1;2)	NS
Chronic bronchitis, n (%)	26 (41.3%)	13 (39.4%)	13 (43.3%)	NS
Median AE-COPD-B	1 (0;2)	1 (0;2)	1 (0;2)	NS
LABA, n (%)	38 (61.3%)	18 (54.6%)	20 (69.0%)	NS
LAMA, n (%)	27 (43.6%)	14 (42.5%)	13 (44.8%)	NS
ICS, n (%)	22 (35.5%)	13 (39.4%)	9 (31.0%)	NS
Mean FEV_1 (L)	1.55 (0.67)	1.58 (0.72)	1.52 (0.62)	NS
Mean FEV_1 (% pred)	63.79 (21.37)	65.14 (23.14)	62.28 (19.5)	NS
Mean FVC (L)	2.86 (0.80)	2.85 (0.86)	2.89 (0.74)	NS
Mean FVC (% pred)	98.75 (21.23)	99.17 (22.18)	98.29 (20.47)	NS
Mean FEV_1/FVC (L)	52.91 (12.69)	53.90 (13.67)	51.81 (11.62)	NS
Median White blood cell count (10^9/µL)	7030 (5890;8080)	7040 (6030;7830)	6930 (5890;8280)	NS
Median Neutrophil count(10^9/µL)	3990 (3320;4990)	3960 (3430;5020)	4050 (2930;4900)	NS
Median Eosinophil count (10^9/µL)	170 (120;260)	130 (90;160)	270 (220;300)	<0.001
Median Lymphocyte count (10^9/µL)	1960 (1510;2450)	2050 (1500;2610)	1880 (1580;2300)	NS

Data are presented as mean ± SD or median (IQR). EOS-: eosinophil count < 170 cells/µL; EOS+: eosinophil count ≥ 170/µL; BMI: Body Mass Index; mMRC: modified Medical Research Council dyspnea scale; AE-COPD-B: Number of Acute Exacerbation of Chronic Obstructive Pulmonary Disease in the 12 months prior the beginning of the study; LABA: Long-acting β_2-Agonists; LAMA: Long-acting Muscarinic Antagonists; ICS: Inhaled Corticosteroids; FEV_1, Forced Expiratory Volume in 1 second; FVC, Forced Vital Capacity. *The Fisher test was used for this comparison because the expected counts were not greater than 5 in all cells.

the bEOS- group only, whereas the median value of pack year was higher, although not significantly, in the bEOS+ group. The number of AE-COPD-B tended to be higher in EOS+, even though the difference between

the groups was not statistically significant. The mMRC dyspnea scale and the use of LAMAs, LABAs and ICSs was comparable in bEOS+ and bEOS-. At the beginning of the study, the mean values of FVC, FEV_1 as well as leucocyte, neutrophil and lymphocyte counts were similar in the two groups.

When considering the whole sample, the mean ΔFEV_1 and the mean FVC annual decline resulted in 18.78 ± 58.25 mL/year and 9.5±125 mL/year, respectively. The ΔFEV_1 was significantly higher in bEOS+ (34.86±50.33) than bEOS- (4.49±61.69) (p=0.029). On the contrary, no statistically significant difference was found in terms of the FVC annual change between the two groups (p=0.393). During the follow up, the median number of exacerbations was not significantly different between EOS+ and EOS- (median:0, IQR:0-2 in EOS- and median:0, IQR:0-1 in EOS+; p=0.868).

After adjusting for sex, age and smoking habits at baseline, the eosinophils turned out to be significantly associated with ΔFEV_1 either when only neutrophils and eosinophils were considered as possible determinants (β=16.9; CI 95% 2.0,31.8; p=0.026) (Model I, Table 2) or in the complete model (β=19.4; CI 95% 2.8,36.1; p=0.022) (Model II, Table 2). Figure 2 shows the observed and adjusted mean of ΔFEV_1 by eosinophil count in the complete model: in the case of an increase in the eosinophil count of 100 cells per μL the FEV_1 decline was expected to increase by 19.4 mL/y. ICS treatment was found to be associated with a reduced lung function decline (β=-57.7; CI 95% -91.5,-23.9; p=0.001) (Model II, Table 2).

After adjusting for sex and age at baseline, the neutrophil count was found to be significantly associated with the number of AE-COPD-F when only neutrophils and eosinophils were considered as possible determinants: the estimated rate ratio of exacerbations for an increase in the neutrophil count of 100 cells/μL was 1.03 (CI 95% 1.00,1.07; p=0.032), i.e. holding all other variables in the model constant, the increase in the neutrophil count seems to increase the expected number of exacerbations by 3%. When baseline treatment with bronchodilators and with ICS and AE-COPD-B were included in the model (Model IV, Table 3), neither the eosinophil nor the neutrophil counts were significantly associated with the rate of exacerbations, whereas baseline treatment with bronchodilators resulted in a significant increase in the rate of exacerbations (estimated IRR=15.02, CI 95% 1.65,136.67).

The complete models (Model II and Model IV) had the best fitness, as shown by the lowest AIC and BIC (Table 2, Table 3).

DISCUSSION

The results of this retrospective, longitudinal study in COPD patients show that blood eosinophils are associated with a faster decline in FEV_1. On the contrary, the blood eosinophil count does not predict a higher risk of exacerbations.

The association between eosinophilic inflammation and lung function decline has been investigated in few studies with contrasting results. One study, in agreement with our results, found that an eosinophil blood count greater than 2% was associated with

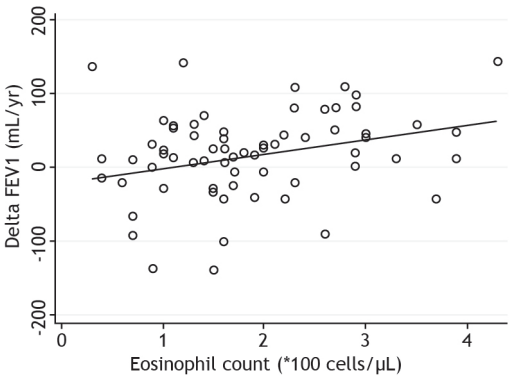

Figure 2. Observed (hollow circles) and adjusted mean (line) of delta FEV_1 by Eosinophil count (*100 cells/μL). The means are adjusted for age at baseline, sex, smoking status (active smoker vs former smoker), bronchodilator use, ICS use and baseline number of exacerbations.

Table 2. Multivariate Linear Regression Model, considering FEV_1 decline over time (ΔFEV_1, mL/yr) as the outcome.

	Model I			Model II		
Dependent variable: Δ FEV_1 (mL/yr)	β	CI 95%	p value	β	CI 95%	p value
Eosinophils (100 cells/μL)	16.9	2.0,31.8	0.026	19.4	2.8,36.1	0.022
Neutrophils (100 cells/μL)	0.2	-1.0,1.3	0.795	0.8	-0.4,1.9	0.192
Bronchodilator				23.6	-14.7,61.9	0.227
ICS				-57.7	-91.5,-23.9	0.001
AE-COPD-B				1.7	-11.4,14.9	0.797
Goodness of fit measures						
AIC		10.968			10.881	
BIC		193171.6			126148.3	

Model adjusted for age at baseline, sex, smoking status (active smoker vs former smoker).

Table 3. Multivariate Linear Regression Model, considering the number of exacerbations in the previous 12 months during follow up period (AE-COPD-F, N°/yr) as the outcome.

	Model III			Model IV		
Dependent variable: Number of exacerbations during follow up	IRR	CI 95%	p value	IRR	CI 95%	p value
Eosinophils (100 cells/μL)	1.28	0.87,1.90	0.214	1.15	0.75,1.74	0.524
Neutrophils (100 cells/μL)	1.03	1.00,1.07	0.032	1.01	0.98,1.04	0.393
Bronchodilator				15.02	1.65,136.67	0.016
ICS				1.06	0.45,2.52	0.889
AE-COPD-B				1.33	0.94,1.88	0.113
Goodness of fit measures						
AIC		295.852			257.894	
BIC		315.066			284.338	

Model adjusted for age at baseline and sex.

a faster decline of FEV_1.[11] Also, in a more recent paper,[15] it was shown that a blood eosinophil count of ≥300 cells/μL was an independent risk factor for accelerated lung function decline in a large Canadian cohort of older adults from the general population. On the contrary, in the ECLIPSE study,[13] COPD patients with a blood eosinophil count higher or lower than 2% had a similar FEV_1 decline in the 3-year follow-up. The HOKKAIDO COPD cohort study[14] reported that COPD patients with higher levels of circulating eosinophils maintained FEV_1 levels that were substantially stable over a period of 5 years, whereas a higher emphysema level and a greater circulating neutrophil count were predictors for a more rapid FEV_1 decline. Ethnic and environmental differences between our and the Japanese study may account for the contrasting results. More recently, one study[22] demonstrated that in COPD patients with a high blood eosinophil count (≥350 cells/μL), the exacerbations were associated with the subsequent acceleration of FEV_1 decline. In addition, the association between eosinophils in the sputum and lung function decline is uncertain. One study[23] found that both the neutrophil and eosinophil count in the sputum were related to FEV_1 decline in COPD. Another study[24] reported the association between eotaxin-1 (a chemokine inducing eosinophil activation) in lung lavage and a faster progression of the disease in COPD patients.[11]

We failed to find an association between the blood eosinophil count and the risk of exacerbation, in agreement with two group of authors,[25,26] but in contrast to other studies reporting a role of circulating eosinophils in predicting the onset of AE-COPD.[27-30] In the present study, the circulating neutrophil count was associated with exacerbations during the follow-up, although this was true only in the model in which sex and age, but not treatments, were considered as confounders. This result partially supports a recent study suggesting that a high blood neutrophil count may provide a useful indicator of the risk of exacerbations in COPD patients.[31]

Our retrospective study does not reach reliable conclusions regarding the possible effect of inhaled drugs on the decline of respiratory function and exacerbations. However, it is worth noting the finding of a negative association between ICS and FEV_1 decline, which may suggest a slowing-down effect of inhaled corticosteroids on the disease progression. The positive association between treatment with bronchodilators and the rate of exacerbations during the follow-up has no clear explanation. In our study, almost all patients were treated with a combination of drugs and only a minority of them were being treated with either LAMA or LABA alone, so that we are unable to evaluate the separate effect of the two types of bronchodilators. While keeping in mind this limit, we hypothesize that patients with more frequent exacerbations at baseline, and consequently with a higher risk of developing exacerbations in the follow-up, are more frequently treated. The use of medications may also explain the lack of an association between AE-COPD-B and AE-COPD-F. We are aware that only prospective, randomized studies are necessary to definitively clarify this point.

The blood eosinophil count is influenced by several factors, such as diurnal, seasonal, and hormonal variations.[32,33] Moreover, fluctuation in the disease and treatment may increase this variability.[32] Therefore, it has been pointed out that a single estimation of the eosinophil count, as in the case of our study, would be unlikely to reflect the overall pattern of blood eosinophilia.[34] However, a group of authors have shown that the proportion of COPD patients with a stable eosinophil count in a six-month time interval is high (93%) for subjects with a mean age of 70 years.[5] The variability is even more limited for an absolute eosinophil count lower than 340 cells/μL, which is similar to that found in our patients.[5] Finally, in the present study the eosinophil count was measured in a stable phase of the disease, at the same hour of the day, after an overnight fasting, all factors that may have further reduced the count variability.

Our study has some limitations. Other than the ones connected to its retrospective observational design, the choice of selecting patients in a stable phase of disease and the numerous exclusion criteria prevent from extending the findings to COPD patients with

different characteristics. Another limitation is the small number of the patients, that may explain the lack of some associations, such as between eosinophils and exacerbations. However, the sample size was large enough to support the hypothesis of a relationship between eosinophils and FEV_1 decline.

We considered an exacerbation to be ongoing when the patient reported the use antibiotics or corticosteroid after the worsening of symptoms, regardless of admission to an emergency room or hospitalization. Therefore, our results cannot be applied to the severe exacerbations (i.e., requiring hospitalization) or to the mild ones (i.e., those producing only a slight variation of the usual treatment).

We acknowledge that FEV_1 decline was calculated on only two points at the beginning and at the end of the observational period. A higher number of measurements might have provided a more reliable value of functional change. However, lung function tests were performed during a stable phase of disease, adopting the criteria of the ATS technical statement,[16] a fact that enabled us to obtain high quality data.

In conclusion, our results suggest that in patients with stable COPD, a higher level of blood eosinophils (albeit in the normal range) predicts a greater decline in FEV_1 over time, but not a higher number of exacerbations. Furthermore, ICS therapy seems to attenuate the progression of airflow obstruction, even though prospective randomized studies are needed to confirm these results.

AUTHOR CONTRIBUTIONS

MF, MP, LC: conception and design of the study. MF, MP, LC, VE, FS, SDM, LGDC, EC: acquisition, analysis, or interpretation of data. MF, MP, LC, LGDC, EC: drafting the work or revising it critically for important intellectual content. MF, LC, EC: approval of the final version of the manuscript.

REFERENCES

1. Vestbo J, Edwards LD, Scanlon PD, Yates JC, Agusti A, Bakke P, et al. Changes in forced expiratory volume in 1 second over time in COPD. N Engl J Med. 2011;365(13):1184-92. http://dx.doi.org/10.1056/NEJMoa1105482. PMid:21991892.
2. Stockley JA, Walton GM, Lord JM, Sapey E. Aberrant neutrophil functions in stable chronic obstructive pulmonary disease: the neutrophil as an immunotherapeutic target. Int Immunopharmacol. 2013;17(4):1211-7. http://dx.doi.org/10.1016/j.intimp.2013.05.035. PMid:23994347.
3. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2016;138(1):16-27. http://dx.doi.org/10.1016/j.jaci.2016.05.011. PMid:27373322.
4. Hastie AT, Martinez FJ, Curtis JL, Doerschuk CM, Hansel NN, Christenson S, et al. Association of sputum and blood eosinophil concentrations with clinical measures of COPD severity: an analysis of the SPIROMCSI cohort. Lancet Respir Med. 2017;5(12):956-67. http://dx.doi.org/10.1016/S2213-2600(17)30432-0. PMid:29146301.
5. Oshagbemi OA, Burden AM, Braeken DCW, Henskens Y, Wouters EFM, Driessen JHM, et al. Stability of blood eosinophils in patients with chronic obstructive pulmonary disease and in control subjects, and the impact of sex, age, smoking, and baseline counts. Am J Respir Crit Care Med. 2017;195(10):1402-4. http://dx.doi.org/10.1164/rccm.201701-0009LE. PMid:28165763.
6. Vedel-Krogh S, Nielsen SF, Lange P, Vestbo J, Nordestgaard BG. Blood eosinophils and exacerbations in chronic obstructive pulmonary disease. the copenhagen general population study. Am J Respir Crit Care Med. 2016;193(9):965-74. http://dx.doi.org/10.1164/rccm.201509-1869OC. PMid:26641631.
7. Bafadhel M, McKenna S, Terry S, Mistry V, Pancholi M, Venge P, et al. Blood eosinophils to direct corticosteroid treatment of exacerbations of chronic obstructive pulmonary disease: a randomized placebo-controlled trial. Am J Respir Crit Care Med. 2012;186(1):48-55. http://dx.doi.org/10.1164/rccm.201108-1553OC. PMid:22447964.
8. Brightling CE, McKenna S, Hargadon B, Birring S, Green R, Siva R, et al. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. Thorax. 2005;60(3):193-8. http://dx.doi.org/10.1136/thx.2004.032516. PMid:15741434.
9. Bafadhel M, Davies L, Calverley PM, Aaron SD, Brightling CE, Pavord ID. Blood eosinophil guided prednisolone therapy for exacerbations of COPD: a further analysis. Eur Respir J. 2014;44(3):789-91. http://dx.doi.org/10.1183/09031936.00062614. PMid:24925917.
10. Bafadhel M, Peterson S, de Blas MA, Calverley PM, Rennard SI, Richter K, et al. Predictors of exacerbation risk and response to budesonide in patients with chronic obstructive pulmonary disease: a post-hoc analysis of three randomised trials. Lancet Respir Med. 2018;6(2):117-26. http://dx.doi.org/10.1016/S2213-2600(18)30006-7. PMid:29331313.
11. Rogliani P, Puxeddu E, Ciaprini C, Ora J, Onorato A, Pezzuto G, et al. The time course of pulmonary function tests in copd patients with different levels of blood eosinophils. BioMed Res Int. 2016;2016:4547953. http://dx.doi.org/10.1155/2016/4547953. PMid:27822474.
12. Singh D, Kolsum U, Brightling CE, Locantore N, Agusti A, Tal-Singer R. Eosinophilic inflammation in COPD: prevalence and clinical characterist. Eur Respir J. 2014;44(6):1697-700. http://dx.doi.org/10.1183/09031936.00162414. PMid:25323230.
13. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, et al. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). Eur Respir J. 2008;31(4):869-73. http://dx.doi.org/10.1183/09031936.00111707. PMid:18216052.
14. Nishimura M, Makita H, Nagai K, Konno S, Nasuhara Y, Hasegawa M, et al. Annual change in pulmonary function and clinical phenotype in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;185(1):44-52. http://dx.doi.org/10.1164/rccm.201106-0992OC. PMid:22016444.
15. Tan WC, Bourbeau J, Nadeau G, Wang W, Barnes N, Landis SH, et al. High eosinophil counts predict decline in FEV1: results from the CanCOLD study. Eur Respir J. 2021;57(5):2000838. http://dx.doi.org/10.1183/13993003.00838-2020. PMid:33303555.
16. Park HY, Chang Y, Kang D, Hong YS, Zhao D, Ahn J, et al. Blood eosinophil counts and the development of obstructive lung disease: the Kangbuk Samsung Health Study. Eur Respir J. 2021;58(4):2003823. http://dx.doi.org/10.1183/13993003.03823-2020. PMid:33737406.
17. GOLD: Global Initiative for Chronic Obstructive Lung Disease [Internet]. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (2021 Report). [cited 2021 Dec 19]. Available from: https://goldcopd.org/wp-content/uploads/2020/11/GOLD-REPORT-2021-v1.1-25Nov20_WMV.pdf
18. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med. 2019;200(8):e70-88. http://dx.doi.org/10.1164/rccm.201908-1590ST. PMid:31613151.
19. Ong KC, Earnest A, Lu SJ. A multidimensional grading system (BODE index) as predictor of hospitalization for COPD. Chest. 2005;128(6):3810-6. http://dx.doi.org/10.1378/chest.128.6.3810. PMid:16354849.
20. Akaike H. A new look at the statistical model identification. IEEE Trans Automat Contr. 1974;19(6):716-23. http://dx.doi.org/10.1109/TAC.1974.1100705.
21. Schwarz G. Estimating the dimension of a model. Ann Stat [serial on the Internet]. 1978 [cited 2021 Dec 19];6(2):461-64. Available from: http://www.jstor.org/stable/2958889
22. Kerkhof M, Voorham J, Dorinsky P, Cabrera C, Darken P, Kocks JWH, et al. Association between COPD exacerbations and lung function

decline during maintenance therapy. Thorax. 2020;75(9):744-53. http://dx.doi.org/10.1136/thoraxjnl-2019-214457. PMid:32532852.

23. Donaldson GC, Seemungal TA, Patel IS, Bhowmik A, Wilkinson TM, Hurst JR, et al. Airway and systemic inflammation and decline in lung function in patients with COPD. Chest. 2005;128(4):1995-2004. http://dx.doi.org/10.1378/chest.128.4.1995. PMid:16236847.
24. D'Armiento JM, Scharf SM, Roth MD, Connett JE, Ghio A, Sternberg D, et al. Eosinophil and T cell markers predict functional decline in COPD patients. Respir Res. 2009;10(1):113. http://dx.doi.org/10.1186/1465-9921-10-113. PMid:19925666.
25. Singh D, Wedzicha JA, Siddiqui S, de la Hoz A, Xue W, Magnussen H, et al. Blood eosinophils as a biomarker of future COPD exacerbation risk: pooled data from 11 clinical trials. Respir Res. 2020;21(1):240. http://dx.doi.org/10.1186/s12931-020-01482-1. PMid:32943047.
26. Miravitlles M, Monteagudo M, Solntseva I, Alcázar B. Blood eosinophil counts and their variability and risk of exacerbations in COPD: a population-based study. Arch Bronconeumol (Engl Ed). 2021;57(1):13-20. http://dx.doi.org/10.1016/j.arbres.2019.12.015. PMid:32061402.
27. Cheng SL, Lin CH. Effectiveness using higher inhaled corticosteroid dosage in patients with COPD by different blood eosinophilic counts. Int J Chron Obstruct Pulmon Dis. 2016;11:2341-8. http://dx.doi.org/10.2147/COPD.S115132. PMid:27703344.
28. Tashkin DP, Wechsler ME. Role of eosinophils in airway inflammation of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2018;13:335-49. http://dx.doi.org/10.2147/COPD.S152291. PMid:29403271.
29. Prins HJ, Duijkers R, Lutter R, Daniels JM, van der Valk P, Schoorl M, et al. Blood eosinophilia as a marker of early and late treatment failure in severe acute exacerbations of COPD. Respir Med. 2017;131:118-24. http://dx.doi.org/10.1016/j.rmed.2017.07.064. PMid:28947018.
30. Zeiger RS, Tran TN, Butler RK, Schatz M, Li Q, Khatry DB, et al. Relationship of blood eosinophil count to exacerbations in chronic obstructive pulmonary disease. J Allergy Clin Immunol Pract. 2018;6(3):944-954.e5. http://dx.doi.org/10.1016/j.jaip.2017.10.004. PMid:29153881.
31. Lonergan M, Dicker AJ, Crichton ML, Keir HR, Van Dyke MK, Mullerova H, et al. Blood neutrophil counts are associated with exacerbation frequency and mortality in COPD. Respir Res. 2020;21(1):166. http://dx.doi.org/10.1186/s12931-020-01436-7. PMid:32611352.
32. Bafadhel M, Pavord ID, Russell REK. Eosinophils in COPD: just another biomarker? Lancet Respir Med. 2017;5(9):747-59. http://dx.doi.org/10.1016/S2213-2600(17)30217-5. PMid:28601554.
33. Spector SL, Tan RA. Is a single blood eosinophil count a reliable marker for "eosinophilic asthma?". J Asthma. 2012;49(8):807-10. http://dx.doi.org/10.3109/02770903.2012.713428. PMid:22900679.
34. Hamad GA, Cheung W, Crooks MG, Morice AH. Eosinophils in COPD: how many swallows make a summer? Eur Respir J. 2018;51(1):1702177. http://dx.doi.org/10.1183/13993003.02177-2017. PMid:29371387.