



## Early View

Original article

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**Blood eosinophils on hospital admission for COPD exacerbation do not predict the recurrence of moderate and severe relapses**

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**Take home message:** Shorter time to the next relapse after severe COPD exacerbation is related to the number of prior hospitalizations, smoking history, and more severe airflow limitation. Blood eosinophils are not predictive for the recurrence of moderate or severe relapses.

## **Abstract**

*Background and objective:* The relationship between hospitalization with an eosinophilic acute exacerbation of chronic obstructive pulmonary disease (AE-COPD) and future relapses is unclear. We aimed to explore this association by following 152 patients for 12 months after hospital discharge or until their first moderate or severe flare-up.

*Methods:* Patients hospitalized with AE-COPD were divided into eosinophilic and non-eosinophilic groups based on full blood count results on admission. All patients were treated with a course of systemic corticosteroid. The Cox proportional hazards model was used to study the association with the time to first re-exacerbation, a generalized linear regression model was applied to identify clinical variables related to the recurrence of relapses.

*Results:* We did not find a difference in the time to the next moderate or severe exacerbation between the eosinophilic ( $\geq 2\%$  of total leukocytes and/or  $\geq 200$  eosinophils/ $\mu\text{l}$ , N=51, median /interquartile range/: 21 /10-36/ weeks) and non-eosinophilic groups (N=101, 17 /9-36/ weeks, log-rank test:  $p=0.63$ ). No association was found when other cut-off values ( $\geq 3\%$  of total leukocytes and/or  $\geq 300$  eosinophils/ $\mu\text{l}$ ) were used for the eosinophilic phenotype. However, the higher number of past severe exacerbations, a lower FEV<sub>1</sub> at discharge and higher pack-years were related to shorter exacerbation-free time. According to a subgroup analysis (N=73), 48.1% of patients with initial eosinophilic exacerbations had non-eosinophilic relapses on re-admission.

*Conclusions:* Our data do not support an increased risk of earlier recurring moderate or severe relapses in patients hospitalized with eosinophilic exacerbations of COPD. Eosinophilic severe exacerbations present a variable phenotype.

**Abbreviations:**

AE: acute exacerbation

CI: confidence interval

COPD: chronic obstructive pulmonary disease

CRP: C-reactive protein

FEV<sub>1</sub>: forced expiratory volume in first second

FVC: forced vital capacity

GOLD: global initiative for chronic obstructive lung disease

HR: hazard ratio

ICS: inhaled corticosteroid

IQR: interquartile range

LABA: long-acting  $\beta$ 2-agonist

LAMA: long-acting muscarinic antagonist

PY: pack years

SD: standard deviation

WBC: white blood cell

## **Introduction**

Acute exacerbations (AE) of chronic obstructive pulmonary disease (COPD) are the leading cause of COPD-associated mortality and are contributing at a great extent to the high economic burden of the disease, especially when hospitalization is required. [1-3] According to the global initiative for chronic pulmonary disease (GOLD) recommendations, one of the main goals of the pharmacological therapy is to reduce the number and the severity of acute exacerbations. [4] The best predictor of an exacerbation is a past event, [5] and certain clinical factors have been associated with an early relapse. [6, 7] However, it is less clear if certain inflammatory phenotypes of a flare-up predispose to earlier relapses.

Exacerbations are usually characterized by neutrophilic airway inflammation, but in a subgroup of patients elevated sputum and blood eosinophil granulocyte ratios are observed. [8, 9] Patients with elevated eosinophil counts during exacerbation respond more favourably to systemic corticosteroid treatment and have lower early treatment failure. [10-12] However, this phenotype is also associated with increased COPD-related costs in the year after the relapse, [13] and it is less understood whether an increased blood eosinophil count during AE-COPD is related to re-exacerbations. Few studies investigated the relationship between eosinophil counts or ratios and exacerbation frequency with inconclusive results. [11, 14, 15] In addition, these studies were limited or biased by enrolling patients either with concomitant pneumonia [14] or by previous systemic steroid [11] or antibiotic treatments. [15]

Our aim was to study if the eosinophilic subgroup of COPD exacerbations (defined by  $\geq 2\%$  of blood leukocytes or  $\geq 200$  cells/ $\mu\text{l}$  and also  $\geq 3\%$  of blood leukocytes or  $\geq 300$  cells/ $\mu\text{l}$ ) requiring hospitalizations shows earlier re-exacerbations in 12 months after the index event. We also analysed the associations of clinical factors with the occurrence of future events.

## **Methods**

### *Subjects*

All patients admitted with the primary diagnosis of COPD exacerbation (acute worsening of respiratory symptoms in the last 72 hours including increased dyspnoea, chest tightness, sputum production, sputum purulence) to the Department of Pulmonology, Semmelweis University, Budapest, Hungary between 15 February 2017 and 15 August 2018 were screened (Supplementary Figure S1). COPD had been previously diagnosed by a respiratory specialist according to the GOLD recommendations. [4] Exclusion criteria included asthma or a previous positive reversibility testing ( $>200$  ml or 12% increase in post-bronchodilator FEV<sub>1</sub>), concurrent chronic pulmonary disease other than COPD, history of pulmonary malignancy in the last 3 years, concomitant pneumonia during the current hospitalization, need for invasive ventilatory support and systemic corticosteroid or antibiotic treatment for any reason 4 weeks prior to admission. Corticosteroid use on admission was only allowed when a single dose of methylprednisolone was administered by the ambulance or at the emergency care department ( $<4$  hours before blood sample taking). The choice of hospital treatment was the responsibility of the attending physician, and all patients received systemic steroid during hospitalization. Some patients were prescribed low-dose oral corticosteroids post-discharge, which was tapered, and the treatment stopped within one week after discharge.

All procedures were in accordance with the 1964 Helsinki declaration and its later amendments. The study was approved by the ethics committee at the Semmelweis University (study No. 191/2017) and a written informed consent was signed by all participants.

### *Design*

Our study was an observational prospective cohort clinical study. All patients underwent blood tests on hospital admission, clinical data were collected, and spirometry was done within 48 hours if the patient could perform it.

Based on the full blood picture results on admission, patients were divided into eosinophilic ( $\geq 2\%$  of leukocytes and/or  $\geq 200$  eosinophils/ $\mu\text{l}$ ) and non-eosinophilic subgroups. [15] Additionally, data analysis was also performed using other cut-offs i.e.  $\geq 3\%$  eosinophils of leukocytes and/or  $\geq 300$  eosinophils/ $\mu\text{l}$ . [16]

Patients were followed until the occurrence of the first moderate or severe relapse following the index exacerbation or for 12 months. Moderate exacerbations were defined as the need for out-patient treatment with systemic corticosteroid and/or antibiotics due to worsening symptoms of COPD, while severe exacerbations required hospital treatment. [4] Treatment failure was defined as re-admission to hospital with respiratory symptoms within 4 weeks after discharge following the treatment of the index exacerbation. Patient follow-up was carried out by phone calls every 3 months or by personal interviews upon re-admissions. The time (i.e. weeks after hospital discharge) and the severity of the exacerbations were recorded. Those patients who did not have at least the 3-month follow-up data were categorized as lost to follow-up and were excluded from the analysis.

### *Measurements*

Venous blood samples were taken to measure white blood cell count and C-reactive protein (CRP) concentration (Sysmex XN-1000, Sysmex Corporation, Kobe, Japan and Beckman Coulter AU680, Beckman Coulter Inc., Indianapolis, IN, USA). Spirometry was performed by 114 subjects according to current guidelines (PDT-111, Piston, Budapest, Hungary). [17] We used the Charlson comorbidity index to assess the burden of comorbidities. [18]

## *Analysis*

Data were analysed by TIBCO Statistica data analysis software system for Windows version 13 (TIBCO Software Inc., Palo Alto, CA, USA). Continuous variables were presented as mean  $\pm$  standard deviation (SD) or median /interquartile range (IQR)/ and were compared using Student t-test and Mann-Whitney U-test. Categorical variables were compared with Chi-square test and two-tailed Fisher exact test. We used Cox proportional hazards model to investigate the association between eosinophil granulocyte count or ratio and the time to first re-exacerbation. Log-rank test was used to compare the time to the next exacerbation between the high and low eosinophilic subgroups. To assess risk factors of the occurrence of relapses, a generalized linear regression model of negative binomial distribution was used. To calculate the required sample size per group, we used the results of the COPD-related readmission rate from a previous study, that divided patients into eosinophilic ( $\geq 2\%$  of leukocytes and/or  $\geq 200$  eosinophils/ $\mu\text{l}$ ) and non-eosinophilic subgroups. [15] We also utilized the findings of the ECLIPSE study, where 47% of patients had  $\geq 1$  moderate or severe exacerbations in the year prior to recruitment. [5] The minimum sample size per group was 39 using the log-rank test ( $1-\beta=0.80$ ,  $\alpha=0.05$ ).

## **Results**

### *Patient characteristics*

152 patients completed the study: 51 patients had an eosinophilic exacerbation as the index event and 101 experienced an initial non-eosinophilic relapse (Table 1). Subjects in the eosinophilic group had less severe airflow limitation on admission as shown by higher FEV<sub>1</sub>/FVC values and lower serum CRP concentration compared to patients with non-



eosinophilic relapses. Other characteristics of the index event including antibiotics therapy, need for non-invasive ventilation, length of hospital stay, and treatment failure were similar between the study groups.

#### *Re-exacerbations in the eosinophilic and non-eosinophilic groups*

More severe exacerbations were recorded than moderate relapses after the index event. The proportion of patients with no relapse or with a moderate and severe re-exacerbations did not differ between the groups (Fisher exact test  $p=0.84$ , Figure 1).

We did not find a difference in the time to the first exacerbation between the eosinophilic ( $\geq 2\%$  and/or  $\geq 200$  eosinophils/ $\mu\text{l}$ ) and non-eosinophilic groups (21 /10-36/ weeks vs. 17 /9-36/ weeks, Mann-Whitney U-test:  $p=0.48$ , log-rank test:  $p=0.63$ ) as shown in Kaplan-Meier function plot (Figure 2). The Cox proportional hazard model did not reveal an altered adjusted hazard ratio (HR) for the time to the first exacerbation for the eosinophilic group (HR:1.10, 95% confidence interval (CI)=0.75-1.61,  $p=0.64$ ). When events were separated into severe and moderate exacerbations, no difference was found in the time of the relapse between the groups (log rank test for severe exacerbations:  $p=0.90$ , for moderate exacerbations  $p=0.51$ ). No significant difference could be detected between the groups when we excluded the patients from the analysis who received systemic corticosteroid before the analysis of full blood count (Supplementary Figure S2).

More patients in the eosinophilic group (N=39) were discharged from the hospital on oral corticosteroids to complete the required course of treatment than in the non-eosinophilic group (N=58,  $p=0.03$ ). However, there was no difference in the time to the first relapse between these subgroups of Eos+ and Eos- patients, either (log-rank test  $p=0.13$ ).

### *Other cut-off values for the eosinophilic phenotype*

We tested our hypothesis using other cut-off values for blood eosinophil ratios and counts. [16] Thirty-nine subjects (26% of all patients) had blood eosinophil granulocytes  $\geq 3\%$  of total leukocytes and/or  $\geq 300$  eosinophils/ $\mu\text{l}$ . Around three quarter of patients had at least one exacerbation during the follow-up period (Eos+: 76.9: %, Eos-: 77.9 %), and in most cases patients required hospitalization (no relapse/moderate exacerbation/severe exacerbation Eos+: N=9/7/23, Eos-: N=25/32/56, Chi-square  $p=0.42$ ). The overall rate of COPD-related readmissions was similar in the two groups (Fisher exact test  $p=0.53$ ). The eosinophilic subjects did not have an elevated risk of re-exacerbation based on Cox proportional hazard model (HR: 0.91, 95% CI=0.60-1.38,  $p=0.66$ ), and the time to the first re-exacerbation was also similar (Eos+: 20 /8-33/ weeks vs. Eos-: 22 /10-38/ weeks, Mann-Whitney U-test  $p=0.54$ ; log-rank test  $p=0.66$ ), as seen on the Kaplan-Meier graph (Figure 3).

We also divided patients into tertiles based on the eosinophil ratios (<0.4% N=50, 0.4-1.8% N=51, >1.8% N=51). No difference was detected among the groups in median exacerbation-free periods (Eos low: 23 /10-52/ weeks, Eos medium: 14 /7-35/ weeks, Eos high: 20 /10-35/ weeks, analysis of variance  $p=0.21$ ; more details in the Supplementary file and Supplementary Figure S3.)

### *Risk factors associated with the time to recurrence of exacerbations*

We explored the risk factors related to the time to the next acute exacerbation including clinical parameters and either blood eosinophil percentage or count as inputs (Table 2 and 3). The higher number of previous severe exacerbations in the past 12 months, a more severe airflow limitation (% predicted post-bronchodilator FEV<sub>1</sub> at discharge) and stronger exposure to cigarette smoke (expressed in pack years) were significantly associated with shorter

exacerbation-free time. However, stepwise regression analysis showed that the previous exacerbation history was the strongest predictor for future events.

#### *Phenotypes of the next exacerbation*

Seventy-three patients had blood eosinophil data of the next exacerbation on hospital admission. By the index exacerbation, 27 patients had an eosinophilic relapse ( $\geq 2\%$  of total leukocytes and/or  $\geq 200$  eosinophils/ $\mu\text{l}$ ) and 46 patients were assigned a non-eosinophilic event ( $< 2\%$  of total leukocytes and  $< 200$  eosinophils/ $\mu\text{l}$ ). On re-admission, 75.3% of these exacerbations (N=55) were of similar type that of the index event. However, 48.1% of patients with an index eosinophilic exacerbation had a non-eosinophilic relapse as the next episode, and 10.9% of patients with an index non-eosinophilic relapse suffered a subsequent eosinophilic exacerbation (Supplementary Table S1). The rate of eosinophilic exacerbations was lower on re-admission than during the first relapse ( $p < 0.001$ , Fisher's exact test).

## **Discussion**

This is the first prospective study to evaluate the relationship between the eosinophilic acute severe COPD exacerbation and future relapses. Our results indicate that patients with an eosinophilic exacerbation, do not have an increased risk of earlier recurrences of moderate or severe relapses. In line with the literature, the history of previous severe exacerbation was the strongest predictor for future relapses.

We explored the recurrence of both severe and moderate exacerbations. [4] Although moderate relapses do not necessitate hospitalization, they have negative effects on health status with similar magnitude as of severe exacerbations. It has been proven that similarly to

reported exacerbations, unreported and moderate exacerbations are associated with increased symptoms, airflow limitation and increased levels of inflammatory markers, although the time to resolution of all symptoms is shorter. [19-21]

Increasing evidence suggests that blood eosinophil level (either count or percentage) can be a signal of corticosteroid response and it can also guide steroid therapy. [10, 22] Blood eosinophil percentage is a surrogate marker of sputum eosinophilia during an exacerbation [8] and it also correlates with small airways inflammation. [9] Strategies directed to normalize sputum eosinophil count improved airflow limitation, reduced symptoms and decreased the number of severe exacerbations, [23-25] also implying a biological role for these cells in COPD.

In our study, the distribution of patients with an eosinophilic exacerbation ( $\geq 2\%$  and /or  $\geq 200/\mu\text{l}$ ) was similar as reported in other investigations. [14, 15] Like others, [14, 26] we also found that patients with an eosinophilic exacerbation present with a lower serum CRP level suggesting lower rate of infections. The eosinophilic type of relapses showed less severe airflow limitation characterized by higher FEV<sub>1</sub>/FVC, which is consistent with the findings of a secondary analysis of the ECLIPSE study, where FEV<sub>1</sub>% predicted was higher in the eosinophilic group. [27] Other authors found no difference in baseline lung function, [11, 14, 15] while Ko et al. reported higher improvement in FEV<sub>1</sub> values (both absolute and % predicted) in patients with blood or sputum eosinophilia. [28]

The time to first moderate or severe exacerbation was similar after hospital treatment of an eosinophilic and non-eosinophilic exacerbation suggesting that the published cut-off values for blood eosinophil count or percentage are not biomarkers for re-admissions as also shown by others. [11, 14] In contrast, Couillard et al. found in a post-hoc analysis that the number of re-admissions was increased and the time to the first re-admission was shorter in patients after an eosinophilic exacerbation. [15] This discrepancy might be explained by the different

outcome parameters (i.e. moderate and severe exacerbations were analysed together in our study), the pre-treatment of patients with systemic steroid before blood tests or the more severe airflow limitation in our patient population compared to the other studies. [11, 15, 28] Of note, in our cohort the rate of corticosteroid therapy on admission was lower than in the study by Bafadhel et al., but higher than in study of Couillard et al, where eosinophil count was determined before the initiation of a systemic steroid therapy. [14, 15]

We did not find a difference in the length of hospital stay or the rate of treatment failure between the eosinophilic and non-eosinophilic groups. This supports the results of Couillard et al., who also reported a similar length of hospitalization, [15] but our findings contradict other studies showing shorter hospital stay in the biomarker positive group. [11, 14, 28] Furthermore, our data are in line with the report of Prins et al., [11] who showed that the rate of late treatment failure (11-30 days post-discharge) was similar after eosinophilic or non-eosinophilic exacerbations.

Our data demonstrated that a subgroup of patients with an increased number of severe COPD exacerbations in the past have a shorter time till the recurrence of the next moderate or severe relapse. These results corroborate the findings of large-scale studies showing that the best predictor of an exacerbation is the positive history for prior events. [5, 29] We observed that exacerbation-free time also shortens with increasing severity of airflow limitation, which is in line with previous studies. [5, 29, 30] However, there is not enough evidence to use FEV<sub>1</sub> alone as a predictor of exacerbation risk in COPD. [31] Interestingly, the stronger exposure to cigarette smoke negatively affected the time to the next flare-up. This may be explained by the relationship between smoking and FEV<sub>1</sub> decline. [32] In addition, the incidence of lower respiratory infections is higher in current smokers, which can precipitate exacerbations [33, 34] and deteriorates lung function. [35]

In an exploratory subgroup analysis, we investigated for the first time the stability of the eosinophilic type of severe exacerbations. Our data demonstrate that the eosinophilic exacerbation is a variable phenotype, while non-eosinophilic exacerbators show a more consistent timeline, which can be, at least partly, explained by the systemic steroid treatment during the first relapse. This observation is also in line with data in stable COPD i.e. patients with higher blood eosinophil counts on enrolment ( $\geq 0.34 \times 10^9$  cells/L) showed less stability within one year than patients with lower eosinophil counts ( $<0.34 \times 10^9$  cells/L). [36]

Our single-centred study has limitations. Approximately one third of patients received a single dose of systemic steroid before blood collection, which could have influenced results on eosinophil count. However, in real life settings treatment is often initiated in severe cases already out of hospital and finding clinically relevant biomarkers is also of importance in this group. In addition, the study has not been powered to analyse the effect of covariates (i.e. previous treatment, post-discharge oral steroid use) on our findings in detail. Furthermore, data on past exacerbations were limited to hospitalizations (46% of all patients had at least one severe relapse in the year prior to study entry). However, as events with moderate severity outnumber severe relapses in a yearly basis, [5] it can be speculated that most patients in our study had a positive exacerbation history.

In summary, eosinophilic exacerbations of COPD as defined by known blood eosinophil cut-off values do not increase the risk of earlier recurrence of moderate and severe relapses. However, the increased number of prior hospitalizations, smoking history and lower FEV<sub>1</sub>% predicted are associated with a shorter exacerbation-free time with the strongest parameter being the previous exacerbation history. Our data add further knowledge on the clinical interpretation of eosinophilic AE-COPD and can facilitate the development of targeted interventions to prevent recurring exacerbations.

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## Tables

**Table 1.** Patient characteristics

	Total (N=152)	Eosinophilic exacerbation (N=51)	Non-eosinophilic exacerbation (N=101)	P value
Male	71 (46.7)	23 (45.1)	48 (47.5)	0.86
Age, years	66.2 ± 8.7	65.9 ± 9.1	66.3 ± 8.6	0.81
Charlson Comorbidity Index	2 [1-3]	2 [1-3]	2 [1-3]	0.93
Current smoker	98 (64.5)	32 (62.7)	66 (65.3)	0.56
Tobacco smoke exposition, PY	40 [30-50]	40 [30-50]	40 [30-50]	0.94
FEV <sub>1</sub> (postbronchodilator), %predicted	35.52 ± 14.07	35.89 ± 11.59	35.35 ± 15.18	0.85
FEV <sub>1</sub> /FVC	0.47 ± 0.11	0.51 ± 0.10	0.45 ± 0.11	<b>&lt;0.01</b>
Time since COPD diagnosis, years	7 [3-12]	7 [2-11]	6.5 [3.5-12.5]	0.52
Hospital admission due to COPD in the previous year, N	0 [0-1]	0 [0-1]	0 [0-1]	0.67
Admission corticosteroid use, N (%)	43 (37.0)	17 (41.4)	26 (34.7)	0.55
Baseline ICS use, N (%)	93 (61.2)	29 (56.9)	64 (63.4)	0.38
Baseline LAMA use, N (%)	118 (77.6)	38 (74.5)	80 (79.2)	0.40
Baseline LABA use, N (%)	117 (77.0)	38 (74.5)	79 (78.2)	0.53

Post-discharge ICS use, N (%)	105 (69.1)	33 (64.7)	72 (71.3)	0.46
WBC count on admission, 10 <sup>9</sup> /L	10.88 ± 4.17	11.20 ± 4.10	10.72 ± 4.22	0.50
Eosinophil count, cell/μL	91 [19-261]	362 [251-524]	37 [4-89]	<b>&lt;0.0001</b>
Eosinophil count, % of WBC	0.80 [0.20-2.45]	3.50 [2.40-5.20]	0.40 [0.03-0.80]	<b>&lt;0.0001</b>
C-reactive protein, mg/L	9.7 [3.0-27.0]	7.8 [2.6-17.5]	14.4 [4.2-37.9]	<b>0.02</b>
Antibiotic use, N (%)	116 (77.9)	37 (72.5)	79 (80.6)	0.30
Need for non-invasive ventilation, N (%)	24 (15.8)	5 (9.8)	19 (18.8)	0.24
Length of hospitalization, days	7 [5-10]	7 [5-12]	7 [6-10]	0.89
Treatment failure, N (%)	31 (20.6)	13 (26.0)	18 (18.0)	0.29

Data are shown as mean ± SD and compared with t-test or shown as median [interquartile range] and analysed with Mann-Whitney U-test. Categorical variables were analysed with Fisher exact test. CRP C-reactive protein, ICS inhaled corticosteroid, FEV<sub>1</sub> forced expiratory volume in the first second, FVC forced vital capacity, ICS inhaled corticosteroid, LABA long-acting β<sub>2</sub>-agonist, LAMA long-acting muscarinic antagonist, N number, PY pack-year, WBC white blood cell

**Table 2.** Generalized linear model of risk factors and time to first re-exacerbation including blood eosinophil percentage

Effect	Regression coefficient	Standard error	Lower CI (95%)	Upper CI (95%)	P value
Eosinophil (%)	-0.034	0.041	-0.115	0.046	0.40
% Predicted post-bronchodilator FEV <sub>1</sub> at discharge	0.015	0.006	0.003	0.028	<b>0.01</b>
Post-bronchodilator FEV <sub>1</sub> /FVC ratio in % at discharge	-1.320	0.764	-2.818	0.177	0.08
Charlson-index	0.010	0.068	-0.123	0.143	0.89
Smoking history, PY	-0.008	0.003	-0.014	-0.001	<b>0.03</b>
No. of severe exacerbations in the past 12 months	-0.103	0.047	-0.195	-0.010	<b>0.03</b>
Time since COPD diagnosis, years	0.001	0.014	-0.026	0.028	0.92
Age	0.003	0.010	-0.016	0.022	0.74
Gender (male)	0.019	0.084	-0.145	0.183	0.82
Smoking habit (current smoker)	-0.130	0.088	-0.302	0.043	0.14
Need for noninvasive ventilation	-0.011	0.111	-0.229	0.206	0.92

**Table 3.** Generalized linear model of risk factors and time to first re-exacerbation including blood eosinophil count

Effect	Regression coefficient	Standard error	Lower CI (95%)	Upper CI (95%)	P value
Absolute eosinophil count	-0.001	0.000	-0.001	0.000	0.12
% Predicted post-bronchodilator FEV <sub>1</sub> at discharge	0.016	0.006	0.004	0.028	<b>0.01</b>
Post-bronchodilator FEV <sub>1</sub> /FVC ratio in % at discharge	-1.288	0.758	-2.773	0.197	0.09
Charlson-index	0.001	0.067	-0.131	0.133	0.99
Smoking history, PY	-0.008	0.003	-0.014	-0.001	<b>0.03</b>
No. of severe exacerbations in the past 12 months	-0.102	0.046	-0.193	-0.011	<b>0.03</b>
Time since COPD diagnosis, years	<0.001	0.014	-0.026	0.027	0.98
Age	0.002	0.010	-0.016	0.021	0.80
Gender (male)	0.021	0.083	-0.142	0.183	0.80
Smoking habit (current smoker)	-0.137	0.088	-0.308	0.035	0.12
Need for non-invasive ventilation	-0.020	0.110	-0.235	0.195	0.85

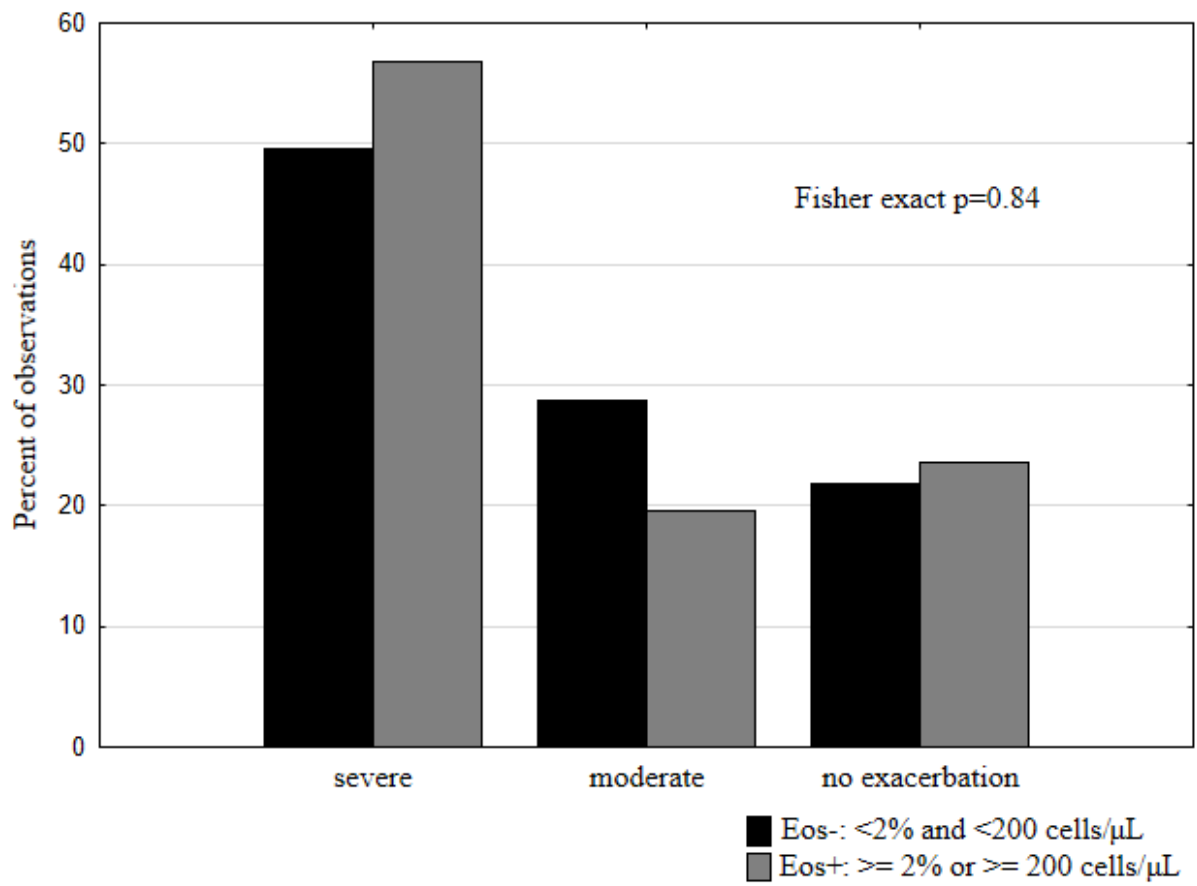
## **Figure legends**

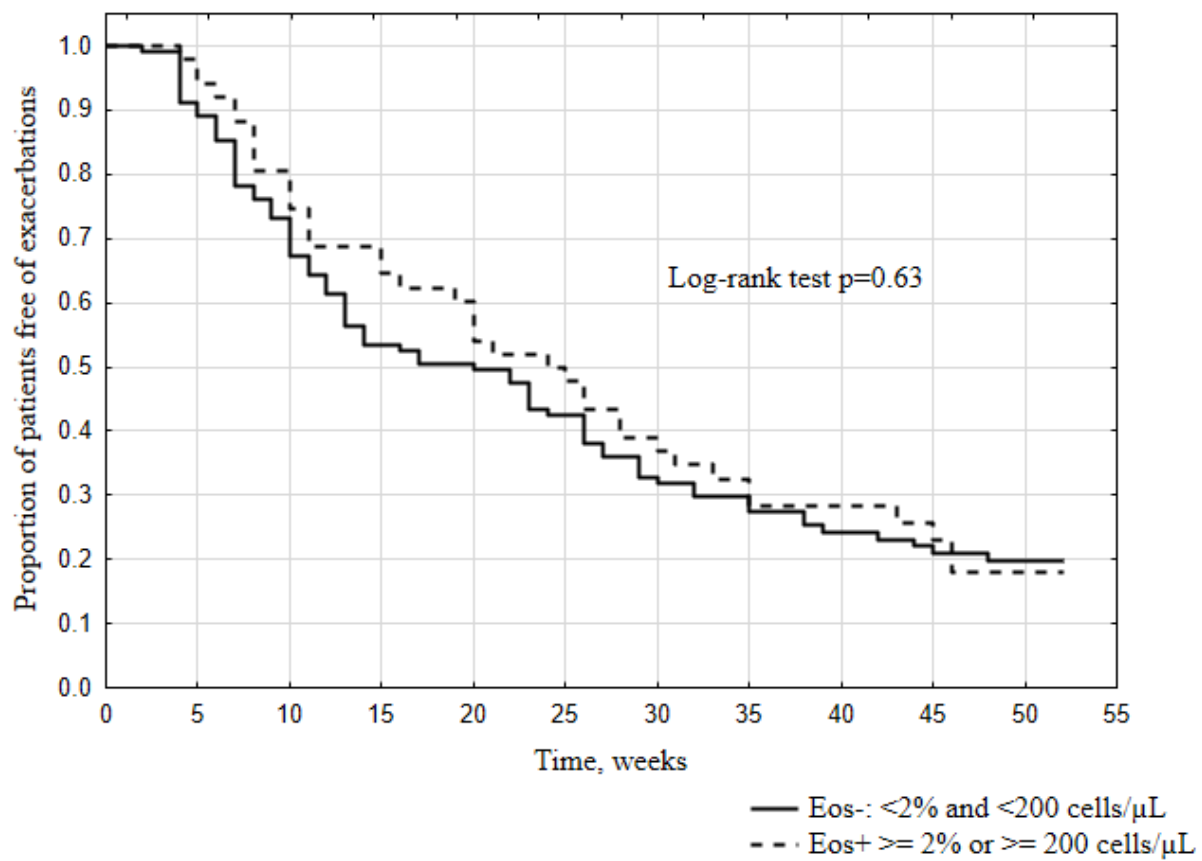
**Figure 1.** Patients with and without exacerbations in the eosinophilic and noneosinophilic groups during the 12 months of the follow-up

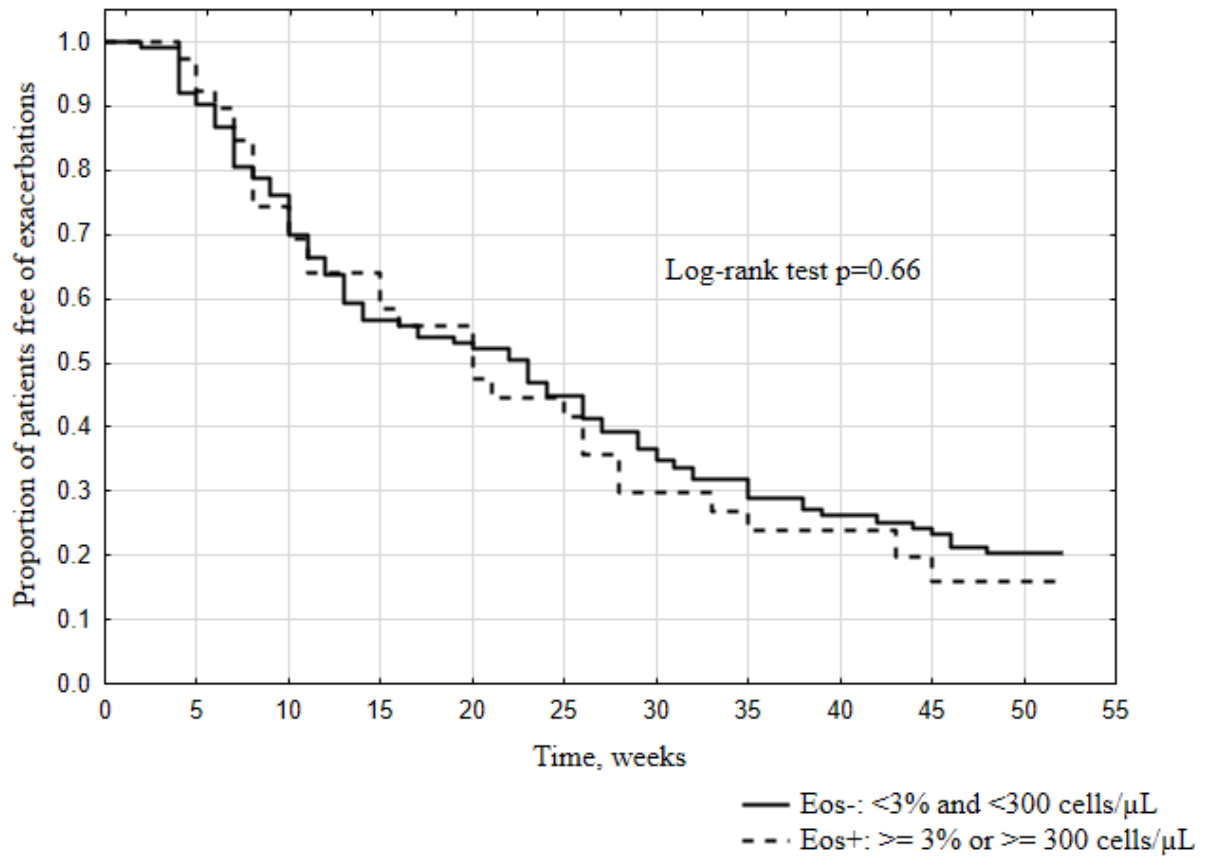
**Figure 2.** Recurrence of moderate or severe exacerbations in the eosinophilic and non-eosinophilic groups

**Figure 3.** Recurrence of moderate or severe exacerbations in the eosinophilic and non-eosinophilic groups









## **Supplementary information**

### **Blood eosinophils on hospital admission for COPD exacerbation do not predict the recurrence of moderate and severe relapses**

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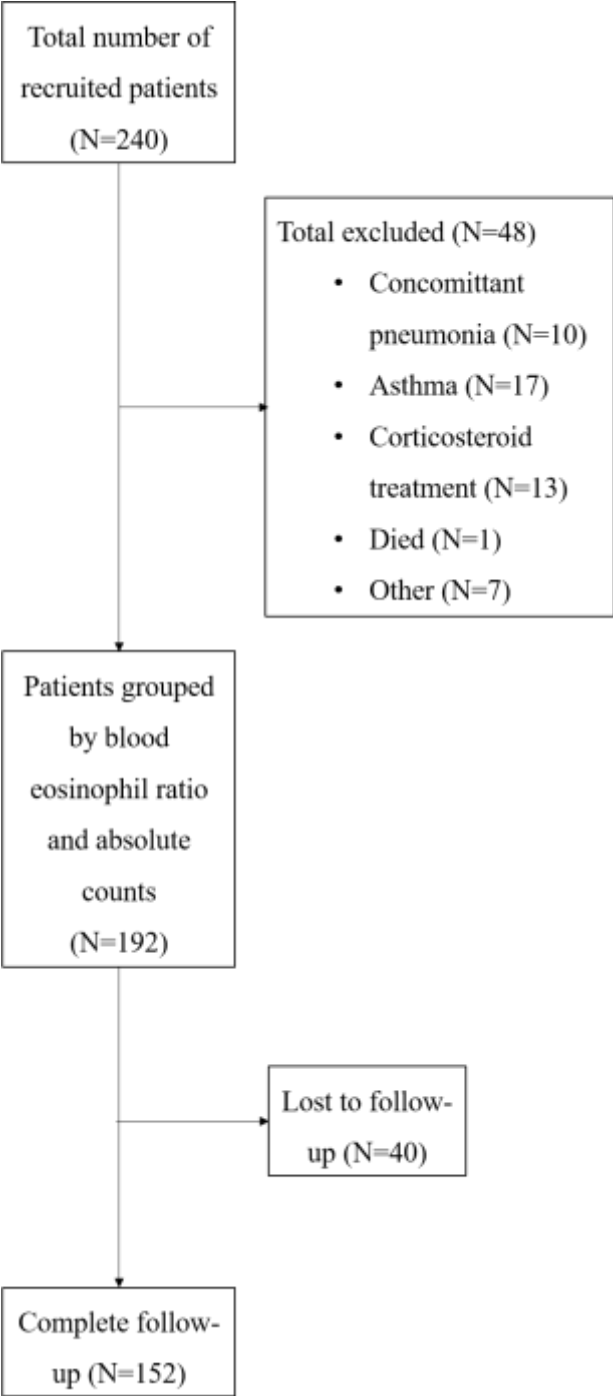
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## **Methods**

### *Subjects*

All patients admitted with the primary diagnosis of COPD exacerbation (acute worsening of respiratory symptoms in the last 72 hours including increased dyspnoea, chest tightness, sputum production, sputum purulence) to the Department of Pulmonology, Semmelweis University, Budapest, Hungary between 15 February 2017 and 15 August 2018 were screened (Supplementary Figure S1). COPD had been previously diagnosed by a respiratory specialist according to the GOLD recommendations.<sup>1</sup> Exclusion criteria included a history of asthma or a previous positive reversibility testing (>200 ml or 12% increase in post-bronchodilator FEV<sub>1</sub>), concurrent chronic pulmonary disease other than COPD, history of pulmonary malignancy in the last 3 years, concomitant pneumonia during the current hospitalization, need for invasive ventilatory support and systemic corticosteroid or antibiotic treatment for any reason 4 weeks prior to admission. Admission corticosteroid use was only allowed when a single dose of methylprednisolone was administered by the ambulance or at the emergency care department (<4 hours before blood sample taking).

**Figure S1.** Flow chart of patient enrolment



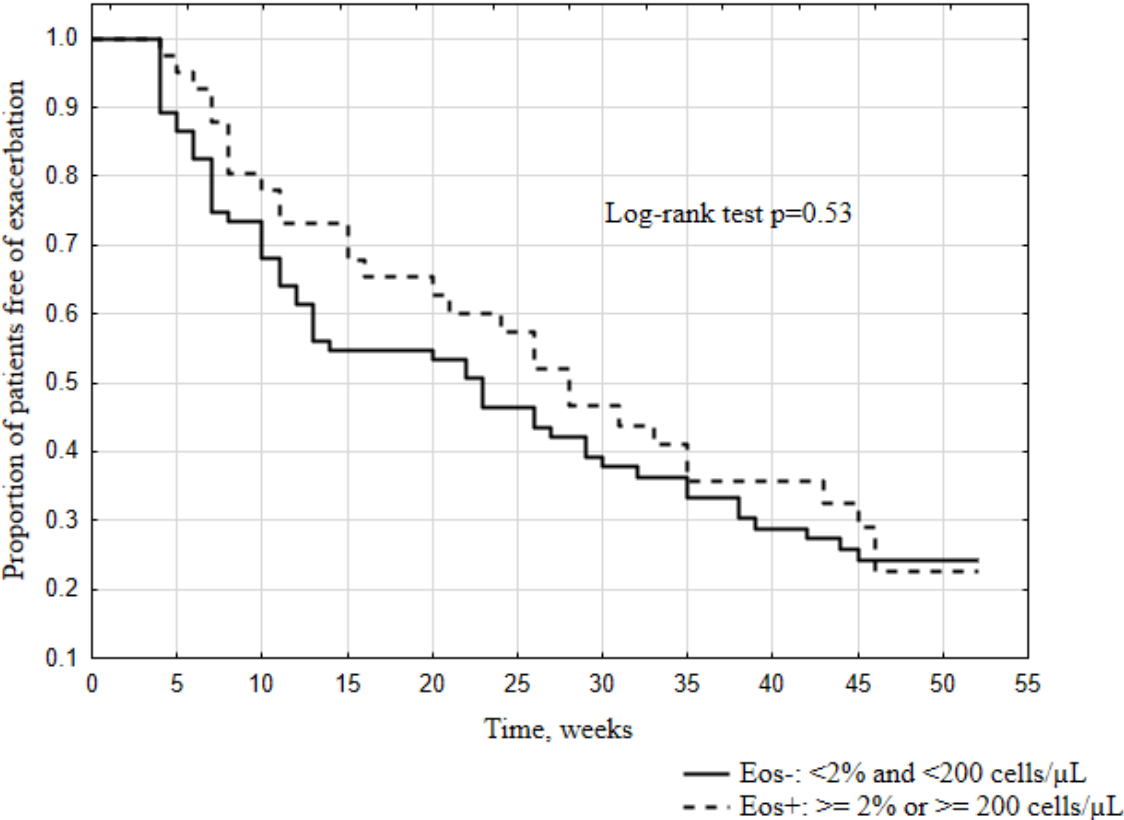
## Results

*Re-exacerbations in the eosinophilic and non-eosinophilic groups without the patients who received systemic corticosteroid prior to the blood test*

One hundred and sixteen patients did not receive systemic corticosteroid before the analysis of full blood count, and 41 patients had an eosinophilic exacerbation ( $\geq 2\%$  of leukocytes and/or  $\geq 200$  eosinophils/ $\mu\text{l}$ ). The proportion of patients with no relapse or with a moderate to severe re-exacerbations did not differ between the groups (Fisher exact test  $p=0.83$ ).

The time to the first relapse was similar between the groups (Eos+: 26 /11-43/ weeks vs. Eos-: 22 /7-42/ weeks, Mann-Whitney U-test:  $p=0.38$ , log-rank test:  $p=0.53$ ; Supplementary Figure S2), and there was no difference in the Cox proportional hazard ratio, either (HR of the eosinophil group: 1.15, 95% confidence interval (CI)=0.73-1.80,  $p=0.55$ )

**Figure S2.** Time to re-exacerbation based on blood eosinophil ratio without the patients who received systemic corticosteroid prior to the analysis of full blood count

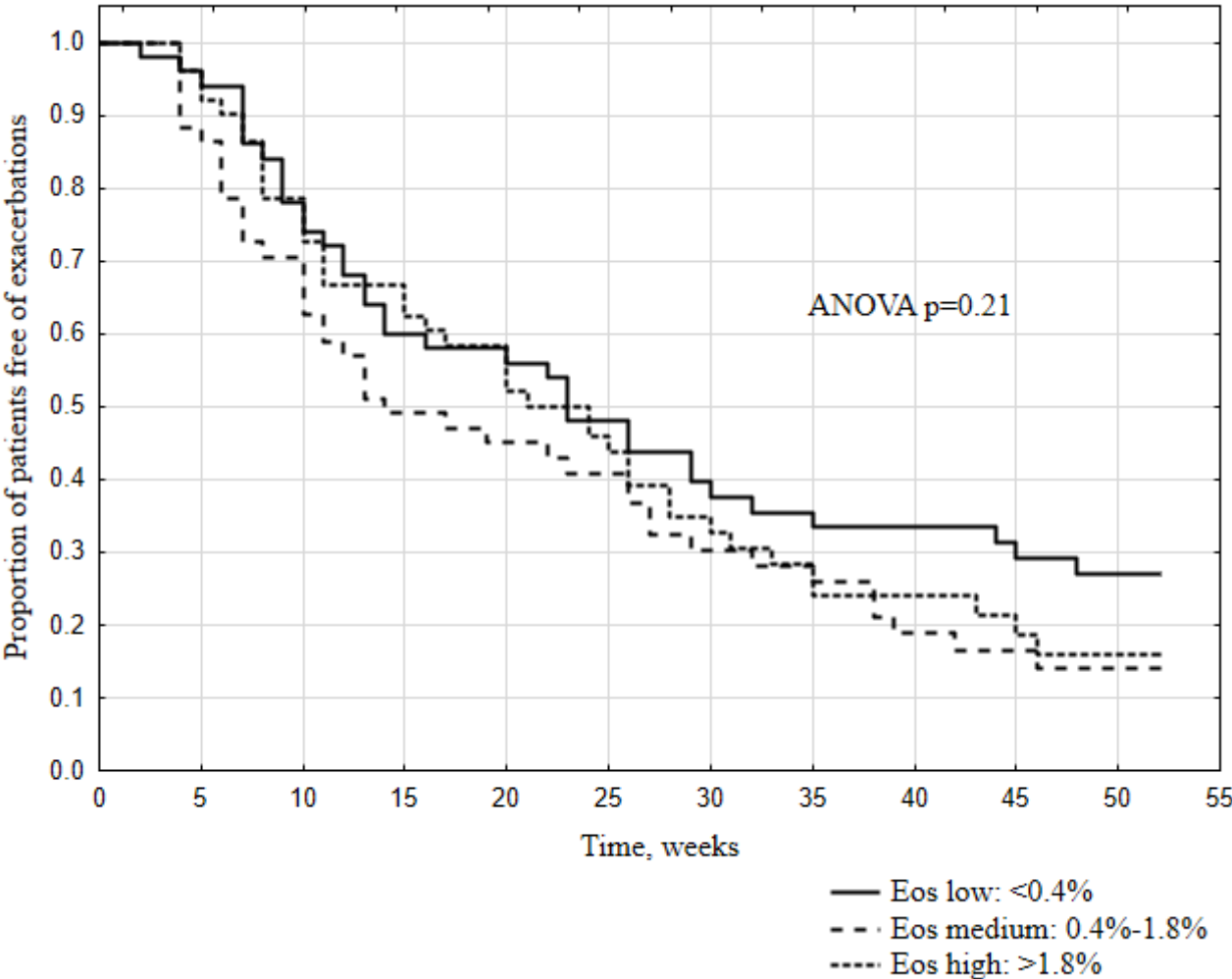




### *Comparing tertiles*

We also divided the patients into 3 groups using the tertiles of the eosinophil ratios (<0.4% N=50, 0.4-1.8% N=51, >1.8% N=51). No differences could be detected among the groups in the occurrence of the next exacerbations (no relapse/moderate exacerbation/severe exacerbation Eos low: N=14/11/25, Eos medium: N=9/16/26, Eos high: N=11/12/28, Chi-square  $p=0.67$ ) or in median exacerbation-free periods (Eos low: 23 /10-52/ weeks, Eos medium: 14 /7-35/ weeks, Eos high: 20 /10-35/ weeks, analysis of variance (ANOVA)  $p=0.21$ ) as also shown by the Kaplan-Meier curves in Supplementary Figure S3.

**Figure S3.** Time to re-exacerbation in tertiles of patients based on blood eosinophil ratio



### *Stability of the types of exacerbations*

We determined the type of the exacerbations on recurrence during hospitalization in 73 patients using the same cut-off values as in the original manuscript (eosinophilic:  $\geq 2\%$  of total leukocytes and/or  $\geq 200$  eosinophils/ $\mu\text{l}$ , non-eosinophilic:  $< 2\%$  of total leukocytes and  $< 200$  eosinophils/ $\mu\text{l}$ ). 75.3% of these exacerbations were of similar type that of the index event. However, 48.1% (13 out of 27) of patients with an index eosinophilic exacerbation had a non-eosinophilic relapse as the next episode, and 10.9% (5 out of 46) of patients with an index non-eosinophilic relapse suffered a subsequent eosinophil exacerbation. The rate of eosinophilic exacerbations was lower on re-admission than during the first relapse ( $p < 0.001$ , Fisher's exact test, Supplementary Table S1).

**Table S1.** Variability of the rate of eosinophilic (Eos+) and non-eosinophilic (Eos-) exacerbations between the index hospitalization and re-admission by the next relapse

	Re-admission Eos+	Re-admission Eos-	Total
Index exac Eos+	14	13	27
Index exac. Eos-	5	41	46
Total	19	54	73

### *Adjustment of inhaled drug therapy during hospitalization*

Inhaled drug therapy was recorded during hospital admission and discharge and shown in the Eos+ and Eos- groups separately (Supplementary Table S2 and Supplementary Table S3).

Twenty-five patients had not been using inhaled therapy regularly before enrolment to the study (Eos+ N=10 /19.6%/ vs. Eos-: N=15 /14.6%/,  $p=0.49$ ). In both groups, LABA+LAMA or triple therapy was initiated in most cases (Eos+: 6/10, Eos-: 11/15). During hospital stay, 5 patients in the Eos+ group and 17 patients in the Eos- group had a change in inhaled regimen ( $p=0.33$ ). In the Eos+ group ICS was retrieved from 2 patients and added to 1 patient, while 4 patients received a second bronchodilator. Among Eos- patients, 7 received add-on ICS and it was withdrawn from 6 patients, and 6 patients were prescribed a second bronchodilator. In addition, during hospital treatment the inhaled drug was switched within class in some patients (Eos+ N=4, Eos- N=4). Importantly, there was no difference in the inhaled drug combinations at discharge between Eos+ and Eos- subgroups ( $p=0.90$ ).

We also compared the number of inhalers and inhaler types (dry powder inhaler, metered dose inhaler) between patient groups. On admission to hospital, there was no difference neither in the inhaler types ( $p=0.54$ ), nor in the number of inhalers between patients with Eos+ and Eos- exacerbations ( $p=0.25$ ).

At discharge from hospital, there was no difference between the study groups in the inhaler types ( $p=0.44$ ) or in the number of inhalers ( $p=0.29$ ), either. The inhaler type was changed in 3 patients with Eos+ exacerbations and in 14 patients with Eos- exacerbations during hospitalization ( $p=0.26$ ).

**Table S2.** Inhaled drug therapy on hospital admission and at discharge in the eosinophilic (Eos+) group.

	Patients with Eos+ exacerbations	Discharge					Total
		LAMA	LABA	LAMA+LABA	LABA+ICS	LAMA+LABA+ICS	
Admission	no therapy	<b>2</b>	0	<b>3</b>	<b>2</b>	<b>3</b>	10
	LAMA	1	0	<b>1</b>	0	<b>1</b>	3
	LABA	0	0	0	0	0	0
	LAMA+LABA	0	0	9	0	0	9
	LABA+ICS	0	0	<b>1</b>	1	<b>1</b>	3
	LAMA+LABA+ICS	0	0	<b>1</b>	0	25	26
	Total	3	0	15	3	30	51

**Table S3.** Inhaled drug therapy on hospital admission and at discharge in the non-eosinophilic (Eos-) group.

	Patients with Eos- exacerbations	Discharge					Total
		LAMA	LABA	LAMA+LABA	LABA+ICS	LAMA+LABA+ICS	
Admission	no therapy	<b>3</b>	0	<b>5</b>	<b>1</b>	<b>6</b>	15
	LAMA	4	0	<b>2</b>	0	0	6
	LABA	0	0	0	0	<b>1</b>	1
	LAMA+LABA	0	0	9	0	<b>6</b>	15
	LABA+ICS	0	0	<b>1</b>	2	<b>2</b>	5
	LAMA+LABA+ICS	<b>2</b>	0	<b>3</b>	0	54	59
	Total	9	0	20	3	69	101

## References

1. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. *Eur Respir J.* 2017;49(3).