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Early View

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Blood eosinophil count and risk of pneumonia hospitalizations in individuals with COPD

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"Take home" message: Eosinophilic COPD with severely impaired lung function is associated with high risk of pneumonia hospitalizations

Abstract

Introduction: Blood eosinophil count in COPD is associated with higher exacerbation rate and favorable response to corticosteroids; however, frequent exacerbations and use of inhaled corticosteroids could also elevate pneumonia risk. We tested the hypothesis that high blood eosinophil counts are associated with high risk of pneumonia in individuals with severe COPD from the general population.

Methods: We included 7,180 individuals with COPD, including 643 with FEV_1<50% of the predicted value from the Copenhagen General Population Study, from 2003-2011. All primary discharge diagnoses of pneumonia during follow-up were recorded.

Results: Among individuals with COPD and FEV_1<50% of the predicted value, the multivariable adjusted incidence rate ratio was 2.17(95% confidence interval, 1.31-3.58) for pneumonia comparing individuals with blood eosinophil counts at or above versus below $0.34 \cdot 10^9$/L. In individuals with clinical COPD, defined by recent exacerbation, ≥10 pack-years, and FEV_1<70% of the predicted value, the corresponding risk was 4.52(2.11-9.72). Risk of pneumonia did not differ by blood eosinophil count in individuals with COPD and FEV_1≥50% of the predicted value.

Conclusion: In individuals with COPD and FEV_1 below 50% percent of predicted value, blood eosinophil count of $0.34 \cdot 10^9$/L or higher was associated with high risk of hospitalization due to pneumonia.

Introduction

The risk of pneumonia is elevated in individuals with chronic obstructive pulmonary disease (COPD)[1,2]; and high age, comorbidity, a history of exacerbations, and severe disease with reduced forced expiratory volume in 1 second (FEV_1) have all been identified as risk factors for the development of pneumonia in COPD[2,3]. Furthermore, therapy with inhaled corticosteroids in COPD is also associated with an elevated risk of pneumonia[4-6] and excess pneumonia deaths[7]. Retrospective analyses have not reported a difference in pneumonia incidence according to the levels of blood eosinophils in patients with COPD[8], but a recent post-hoc meta-analysis of clinical trials found that COPD patients with blood eosinophil counts below 2% had more pneumonia events compared to patients with higher blood eosinophil counts[9]. In addition, some studies suggest that there may exist an inverse relationship between sputum eosinophil count and sputum bacterial count[10].

The peripheral blood eosinophil counts correlate with sputum eosinophil counts in patients with COPD, and thus blood eosinophil count is emerging as a biomarker of eosinophilic airway inflammation[11,12]. Although reports of higher readmission rates in COPD patients with increased blood eosinophil counts at hospitalization are conflicting[13,14], previous studies have found that blood eosinophil counts are associated with future risk of exacerbations. Using a cut-point for eosinophils of 2% or more of total blood leukocytes or a cut-point in absolute number of $0.34 \cdot 10^9$/L, these studies suggest that an elevated blood eosinophil count is associated with a higher risk of COPD exacerbations[8,15,16].

Although controversial[17], high blood eosinophil counts in COPD likely marks a phenotype associated with frequent exacerbations, which could influence the risk of pneumonias, and we

speculated that high blood eosinophil counts may also mark high risk of pneumonia in individuals with COPD. As pneumonias leading to hospitalization are rare in mild to moderate COPD, we tested the hypothesis that a blood eosinophil count of $0.34 \cdot 10^9$/L or higher in individuals with COPD ranging in severity as assessed by FEV_1 in percent of the predicted value, was associated with high risk of being hospitalized due to pneumonia. Furthermore, using the same cut-point in blood eosinophils, we also tested whether a high blood eosinophil count was associated with high risk of pneumonias in individuals with clinical exacerbating COPD[16] defined by recent exacerbation, ≥10 pack-years, and FEV_1 <70% of the predicted value. For this purpose, we studied individuals with COPD from the Copenhagen General Population Study (CGPS), all with baseline measurements of blood eosinophil counts, and followed these up to 8 years for development of pneumonia in a prospective study design.

Methods

Copenhagen General Population Study

The Copenhagen General Population Study (CGPS) is a prospective study of the general population residing in Greater Copenhagen[18-20] with ongoing recruitment begun in 2003. All participants performed a spirometry and we defined COPD as a ratio of FEV_1/FVC under the lower limit of normal and below 0.7, excluding individuals with self-reported asthma. We further subdivided the COPD population according to GOLD 1-2, and GOLD 3-4, that is, FEV_1 above and below 50% of the predicted value. Within the COPD population, we also defined a sub-population with clinical COPD based on the following criteria: at least one exacerbation in the year before baseline, ≥10 pack-years of smoking, and FEV_1 <70% of the predicted value, as done previously[16]. From 81,107 individuals from the CGPS, we identified 7,180 individuals with COPD with full information on spirometry and blood biomarker measurements; of these 4,832 had smoked more than 10 pack-years.

Information on the use of inhaled corticosteroids was obtained by linking CGPS to the national Danish Registry of Medicinal Products Statistics, which records all prescriptions dispensed in Danish pharmacies.

Blood eosinophil counts

Blood eosinophil counts were measured on fresh blood samples using the ADVIA^{TM}120 Hematology system and reported in total numbers ($\cdot 10^9$/L). We defined high versus low eosinophil counts in individuals with COPD as blood eosinophil counts of $0.34 \cdot 10^9$/L or higher versus those below, as determined in our previous work on blood eosinophil counts and COPD

exacerbations[16]. However, in sensitivity analyses we used blood eosinophil cut-points in percentage of all leukocytes of 2%, which has been widely used[15], and of 3.3%, also determined in our previous work[16].

Pneumonia

Risk of pneumonia was analyzed prospectively. We defined pneumonia events as a hospital admission with a primary discharge diagnosis of pneumonia (World Health Organization International Classification of Diseases code J12-J18). Information on diagnoses was drawn from the national Danish Patient Registry, which records all hospital contacts in Denmark, and linked to the CGPS. Prior pneumonia events were defined in the same manner as pneumonias during follow-up, but with an admission date before the date of examination in the CGPS.

Statistical Analysis

Analyses were done using STATA/SE version 14.1 software.

A negative binomial regression model with 95% confidence intervals was used to compare risk of pneumonias during follow-up. Analyses were multivariable adjusted for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% of predicted value. Potential confounders were pre-specified and included irrespectively of their contribution to the model. A nested model with and without blood eosinophil counts was tested using a likelihood ratio test. Additionally, we included use of inhaled corticosteroids in the year prior to baseline, and inflammatory biomarkers high sensitivity C-reactive protein, fibrinogen, and total leukocytes as confounders, as these have previously been associated with the risk of pneumonia in COPD[21]. Follow-up began at study entry and ended at death (n=583), emigration (n=8), or end of follow-up

December 31st, 2011, whichever came first. We tested for interaction of blood eosinophil counts and inhaled corticosteroids use and risk of pneumonias using the likelihood-ratio test.

Imputation of missing covariates at baseline for adjustments in the multivariable adjusted analyses was done by multivariable regression. However, analyses only including individuals with full information on covariates gave similar results to those reported.

Additional details on the methods are provided in the online supplementary material.

Results

We included 7,180 individuals with COPD (Figure 1). Median follow-up time was 3.7 years (interquartile range 1.5-5.8). Among individuals with COPD and $FEV_1 \geq 50\%$ of the predicted value, 271 were hospitalized due to pneumonia during follow-up; of these 51 individuals were hospitalized due to pneumonia more than once. In the COPD population with $FEV_1 < 50\%$ of the predicted value, 114 were hospitalized due to pneumonia during follow-up; of these 36 individuals were hospitalized more than once.

Baseline characteristics according to eosinophil count of $0.34 \cdot 10^9$/L or higher versus those below in the COPD population divided according to FEV_1 of the predicted value are shown in Table 1. A higher proportion of individuals with COPD and a blood eosinophil count of $0.34 \cdot 10^9$/L or higher had elevated markers of low-grade systemic inflammation regardless of FEV_1 in percent of the predicted value. Among individuals with COPD and $FEV_1 < 50\%$, we found no difference in the percentage of patients treated with ICS according to blood eosinophil count and the distribution of those treated with fluticasone versus budesonide did not differ significantly (supplementary table S1).

Blood eosinophil counts and risk of pneumonia

Among individuals with COPD and $FEV_1 < 50\%$ of the predicted value, we found a multivariable adjusted incidence rate ratio of 2.17 (95% confidence interval 1.31-3.58) for pneumonia comparing individuals with blood eosinophil count of $0.34 \cdot 10^9$/L or higher versus those below (Figure 2, top half). The risk remained significant although attenuated with a risk ratio of 1.81 (1.11-2.94) after

further adjustment for the use of inhaled corticosteroids, inflammatory biomarkers, and prior pneumonia event. Compared to the nested full model without blood eosinophil counts, including eosinophils at a cut-point of $0.34 \cdot 10^9$/L improved the model (likelihood ratio test P=0.02). Further adjusting the model for exacerbations in the year prior to baseline gave an incidence rate ratio of 1.80 (1.13-2.86). Among individuals with COPD and FEV_1 ≥50% of the predicted value, the multivariable adjusted incidence rate ratio for pneumonias was 0.80 (0.55-1.15) and further adjustment for the use of inhaled corticosteroids, inflammatory biomarkers, and prior pneumonia event did not change the results. Among individuals with COPD and with ≥10 pack-years of smoking, repeating these analyses gave similar results (Figure 2, bottom half). Further, in a model not taking multiple pneumonias into account, main findings were similar (supplementary figure S1). Risk of pneumonia using a multivariable adjusted Cox proportional hazard model excluding individuals with a hospitalization due to pneumonia before baseline also gave similar although not significant results when comparing individuals with a blood eosinophil count of $0.34 \cdot 10^9$/L or higher to those below (supplementary figure S2). Only including individuals with COPD and no use of inhaled corticosteroids prior to baseline gave similar although not significant results (supplementary figure S3). In both forward and backward stepwise regression models, significant covariates were prior pneumonia events, number of high inflammatory biomarkers, and blood eosinophil count (supplementary table S2).

Using cut-points of 2% and 3.3% in blood eosinophils, individuals with FEV_1 <50% of the predicted value and higher percentages of blood eosinophils had multivariable adjusted incidence rate ratios of 1.98 (95% CI 1.27-3.02) and 1.50 (0.99-2-27), respectively, compared to individuals with lower percentages of blood eosinophils (supplementary figure S4).

Among individuals with COPD and FEV_1 <50% of the predicted value, the multivariable adjusted hazard ratio of all-cause mortality after hospitalization due to pneumonia was 2.65 (95% CI 1.08-6.52) comparing individuals with an eosinophil count of $0.34 \cdot 10^9$/L or higher to those below (supplementary figure S5). Although the risk of all-cause mortality remained significant after additional adjustment for inhaled corticosteroids, it was insignificant in the full model additionally adjusted for inflammatory biomarkers.

Blood eosinophil counts and risk of pneumonia in individuals with clinical COPD

To further examine the risk of pneumonia among individuals with COPD, we used a previously defined sub-population with clinical COPD based on recent exacerbation, ≥10 pack-years, and FEV_1 <70% of the predicted value. Among these individuals, we found a multivariable adjusted incidence rate ratio of 4.52 (95% CI 2.11-9.72) comparing individuals with a blood eosinophil count of $0.34 \cdot 10^9$/L or higher to those below (Figure 3). Further adjustments for inflammatory biomarkers, the use of inhaled corticosteroids, and prior pneumonia events resulted in a multivariable adjusted incidence rate ratio of 3.14 (1.46-6.72).

As for the total COPD population, using cut-points of 2% and 3.3% in blood eosinophils in percentage of total leukocytes gave similar results to those reported for the absolute count (supplementary figure S6).

Use of inhaled corticosteroids and risk of pneumonia stratified by blood eosinophil count

As previous studies have indicated a better response of inhaled corticosteroids in COPD patients with higher blood eosinophil counts, and because the use inhaled corticosteroids in COPD is also associated with an elevated risk of pneumonia, we investigated whether the risk of pneumonia in users of inhaled corticosteroids differed according to blood eosinophil count. Among all individuals with COPD and FEV_1 <50% of the predicted value, the use of inhaled corticosteroids at baseline was associated with a higher risk of pneumonia with an incidence rate ratio of 1.62 (95% CI 1.03-2.57) (Figure 4). In analyses stratified by blood eosinophil count, the risk of pneumonia was elevated in users of inhaled corticosteroids compared to non-users in both strata, although this was not statistically significant in each stratum. There was no difference in risk of pneumonias according to the use of inhaled corticosteroids in strata based on eosinophil count (P for interaction=0.78).

In the sub-group with clinical COPD and less statistical power, the use of inhaled corticosteroids was not significantly associated with a higher risk of pneumonias with an incidence rate ratio of 1.72 (95% CI 0.81-3.67) comparing the use of inhaled corticosteroids at baseline with no use. In stratified analyses, the risk of pneumonias was higher in individuals with a blood eosinophil count of $0.34 \cdot 10^9$/L or higher than in those with an eosinophil count below, however, confidence intervals overlapped and there was no significant interaction of the use of inhaled corticosteroids by blood eosinophil counts on risk of exacerbation (P for interaction=0.34).

Repeated measurements of blood eosinophil counts

For 890 individuals with COPD, we had repeated measurements of blood eosinophil counts measured with a median of 10 years apart (interquartile range 8.5-10.6) (Figure 5). Only 41 (6.4% of the individuals included in the main analyses) of these had FEV_1<50% of the predicted value. In the COPD population with FEV_1<50% of the predicted value, 12% with eosinophil counts above the cut-point of $0.34 \cdot 10^9$/L had lower values at second measurements, or *vice versa*. The corresponding percentage in the COPD population with $FEV_1 \geq 50\%$ was 13%.

Discussion

In this study of 7,180 individuals with COPD from the general population, blood eosinophil count of $0.34 \cdot 10^9$/L or higher in individuals with COPD and FEV_1 below 50% of the predicted value was associated with a higher risk of future hospitalizations with pneumonia, compared with individuals with the same degree of airflow limitation but lower eosinophil count. The high risk was, however, driven by few individuals in the whole COPD cohort. Only few studies have investigated the association between blood eosinophil count and risk of pneumonias, but knowledge of this relationship becomes relevant, as COPD patients with high blood eosinophil counts are candidates for ICS-treatment and as ICS as such elevate pneumonia risk in COPD. However, as we were only able to include pneumonias treated in a hospital setting, we cannot conclude on the association between blood eosinophil count and less severe pneumonias. Moreover, we cannot exclude that some severe exacerbations of COPD might have been misdiagnosed as pneumonias, which could potentially bias our results.

High blood eosinophil counts in COPD likely marks a phenotype with frequent exacerbations although the underlying mechanism remains unknown. Eosinophilic activation during viral infections has been reported[22] and eosinophils might also have an anti-bacterial role although this role is controversial and only reported in vitro[23]. The elevated sputum and blood eosinophil

counts reported in both stable COPD[12,24,25] and at exacerbations of the disease[11,13] might reflect a persistent eosinophilic inflammation with high recruitment rate of eosinophils to the airways as part of chronic airway inflammation in individuals with severe lung function impairment and increased susceptibility to pneumonias. In our study, the high risk of pneumonias appeared not to be mediated through ICS use, prior exacerbations, or prior repeated events of pneumonia, but rather related to the specific eosinophilic phenotype with severe lung impairment.

In contrast to high eosinophil counts, peripheral eosinopenia can be observed in response to acute inflammation or infection[26] and has previously been identified as a strong predictor of mortality in COPD patients hospitalized with acute exacerbations complicated by pneumonia[27]. In the general population however, elevated blood eosinophil counts during stable condition have been associated with a higher risk of mortality from COPD in a study with up to 30 years of follow-up[28] although retrospective data from a cohort of COPD patients with shorter follow-up time did not support this[29]. Our group has previously shown that the increased COPD exacerbation frequency associated with a higher blood eosinophil count was more pronounced, when restricted to severe exacerbations requiring hospitalization[16]. In the present study, we found an association between a blood eosinophil count of $0.34 \cdot 10^9$/L or higher and high risk of pneumonias in individuals with COPD and severely impaired lung function, that is, a FEV_1 % of the predicted value below 50%. Using cut-points of blood eosinophils in percentage, we found similar results. Lange et al., using data from another Copenhagen cohort, have previously shown that age and FEV_1 in percent of the predicted value are the most important risk factor for hospitalization due to pneumonia in the general population[30]. In the present study, we found no association between high blood eosinophil counts and pneumonia in individuals with COPD and less severe lung function impairment. We were able to demonstrate an association in a subgroup with clinical exacerbating COPD with low

FEV_1 and smoking history, similar to COPD patients included in clinical trials. Our findings are, however, in contrast to a recent post-hoc meta-analysis of GlaxoSmithKline-funded clinical trials including 10,861 patients with COPD from ten clinical trials, which reported a small increase in risk of pneumonias in patients with blood eosinophil counts <2%[9]. The discrepancy may be due to different populations studied as individuals with COPD identified from the general population as in our study might differ from patients included in clinical trials in terms of treatment. Another difference relates to the fact that our study had longer follow-up time than most clinical trials, which enabled us to capture more pneumonia events. The post-hoc meta-analysis found no increased risk of pneumonia in COPD patients with blood eosinophil count less than 2% in the trials of shorter duration, with less severe COPD patients, and with low incidence of pneumonia[9]. Previous post-hoc analyses of randomized trials found no difference in pneumonia incidence according to subgroups divided by a blood eosinophil count of 2%[15,31] or the median blood eosinophil count[8]. The same analyses, however, did find higher exacerbations rates with increasing levels of blood eosinophil counts and a better response to inhaled corticosteroids among COPD patients with an eosinophil counts of 2% or more although recent data from the FLAME study, a randomized trial of a combination of long-acting beta-agonist and long-acting muscarinic antagonist compared with a combination of long-acting beta-agonist and inhaled corticosteroid, did not confirm blood eosinophils as a potential biomarker of ICS response[32]. In the present study, we found that use of inhaled corticosteroids was associated with an elevated risk of pneumonia in individuals with COPD and severely impaired lung function. Although our data show a trend towards higher risk of pneumonias among individuals with high eosinophil count, this risk did not vary significantly according to blood eosinophil count suggesting that the use of inhaled corticosteroids among individuals with COPD and high blood eosinophil counts might not increase the risk of pneumonias

further. This was also true for individuals with clinical COPD and a history of exacerbations in which treatment with inhaled corticosteroids is relevant. Although we used a cut-point in absolute blood eosinophil count, our findings are in accordance with the FLAME study, which reported a higher incidence of pneumonia in patients on inhaled corticosteroids regardless of their blood eosinophil count[33].

Strengths of the present study include the large sample size and the fact that we were able to follow all individuals through the Danish registries with no loss to follow-up. A limitation in this study is, however, that we could only capture severe pneumonias treated in a hospital setting, as pneumonias treated in primary care by the general practitioners are not captured by the Danish registries[34]. Therefore, we cannot conclude on the association between blood eosinophil count and less severe pneumonia. Moreover, the present study included individuals with COPD from the general population and thus the association between blood eosinophil count at stable state and risk of pneumonia may differ in a population of COPD patients recruited in a pulmonary department. As we rely on pneumonia diagnoses drawn from the national Danish Patient Registry we cannot exclude that some severe exacerbations of COPD may be reported as pneumonias instead as the differential diagnosis between these two events can be difficult. We are also limited by the lack of information on pneumococcal or influenza vaccination. Furthermore, it can be debated, whether single measurements of blood eosinophil count during stable COPD can be used as a marker of eosinophilic airway inflammation[17,35]. Yet, a study of 141 stable COPD patients found that a peripheral blood eosinophil count of $0.30 \cdot 10^9$/L or higher had a specificity of 76% and a sensitivity of 60% to identify sputum eosinophilia[12]. Data from the UK Clinical Practice Research Datalink have shown that blood eosinophil counts are more variable in COPD, especially for patients with higher baseline eosinophil levels[36] however, this would tend to bias our result towards the null

hypothesis. In our study we had repeated measurements of blood eosinophil counts in 890 individuals with COPD; of these, 13% had varying blood eosinophil counts around the cut-point of $0.34 \cdot 10^9$/L. Although repeated measurements were done with a median of 10 years apart in only 6.5% of the individuals included in our main analyses, this suggests certain stability of the blood eosinophil count in COPD. Lastly, as we defined COPD according to the lower limit of normal and ratio of FEV_1/FVC based on spirometry measurements without the use of a bronchodilator we cannot exclude that some individuals had asthma and not COPD although we excluded individuals with self-reported asthma from our definition of COPD. In our analyses, we adjusted for inflammation using established markers of low-grade inflammation such as C-reactive protein, fibrinogen, and leukocytes. Adjustments for these confounders may be problematic as they may be intermediate variables or a descending proxy for an intermediate variable and thus adjusting for these may bias results toward the null hypothesis [37] which might be the case with mortality after pneumonia.

In conclusion, in this population study of COPD defined as a ratio of FEV_1/FVC under the lower limit of normal and below 0.7 determined without the use of a bronchodilator, we found that a blood eosinophil count of $0.34 \cdot 10^9$/L or higher in individuals with COPD and FEV_1 <50% of the predicted value was associated with a higher risk of being hospitalized due to pneumonia. Among individuals with high blood eosinophil counts and FEV_1<50% of the predicted value, the use of inhaled corticosteroids did not elevate the risk of pneumonia further. As we were not able to capture less severe pneumonias, our study cannot conclude on the association between blood eosinophil count and pneumonias treated outside a hospital setting.

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Table 1. Baseline characteristics of individuals with chronic obstructive pulmonary disease according to blood eosinophil count and FEV_1 in percent of the predicted value.

	COPD, N=7,180					
	FEV_1%<50			**FEV_1%≥50**		
	Blood eosinophils < $0.34 \cdot 10^9$/L, n=521	Blood eosinophils ≥ $0.34 \cdot 10^9$/L, n=122	**P-value**	Blood eosinophils < $0.34 \cdot 10^9$/L, n=5,646	Blood eosinophils ≥ $0.34 \cdot 10^9$/L, n=891	**P-value**
Men	261 (50%)	91 (75%)	<0.001	2,700 (48%)	531 (60%)	<0.001
Age, years	71 (64-77)	71 (66-78)	0.32	63 (53-71)	64 (55-73)	0.02
Current smokers	224 (43%)	49 (40%)	0.57	2,045 (36%)	367 (41%)	0.004
Pack-years of smoking	40 (26-55)	43 (28-63)	0.05	28 (15-42)	30 (18-45)	<0.001
≥10 pack-years of smoking	445 (85%)	108 (89%)	0.37	3,641 (64%)	638 (72%)	<0.001
Body mass index, kg/m^2	25.5 (22.6-28.5)	25.8 (22.9-29.0)	0.24	24.9 (22.7-27.6)	25.3 (23.1-28.1)	0.003
Low level of education	346 (66%)	80 (66%)	0.86	2,977 (53%)	478 (54%)	0.61
FEV_1 % of predicted	42 (36-46)	43 (37-46)	0.26	81 (69-91)	79 (67-90)	0.003
Users of Inhaled corticosteroids	148 (28 %)	33 (27%)	0.76	250 (4%)	61 (7%)	0.002
Ischemic heart disease prior to baseline	71 (14%)	33 (27%)	<0.001	424 (8%)	891 (13%)	<0.001
Frequency of exacerbations (per individual per year during follow-up)	0.59 (1.28)	1.06 (1.83)	0.03	0.11 (0.50)	0.12 (0.45)	0.01
Number of high biomarkers*			<0.001			<0.001
0	3,623 (59%)	433 (43%)		3,425 (61%)	404 (45%)	
1	1,569 (25%)	311 (31%)		1,408 (25%)	274 (31%)	
2	728 (12%)	176 (17%)		610 (11%)	145 (16%)	
3	247 (4%)	93 (9%)		203 (4%)	68 (8%)	

Definition of abbreviations: COPD = Chronic obstructive pulmonary disease. FEV_1 % = FEV_1 as a percentage of the predicted value.

Data are number and percentage for categorical variables and mean and standard deviation (SD) for continuous variables. Baseline characteristics were recorded at baseline except for the use of inhaled corticosteroids, which was recorded up to one year prior to baseline. Pack-years were calculated for current and former smokers only. Low level of education was less than three years following the mandatory primary school. *Number of high biomarkers was defined as C-reactive protein above 3 mg/L, leukocytes above $9 \cdot 10^9$/L, and/or fibrinogen above 14 μmol/L, as done previously[18,21].

Figure legends

Figure 1. Study population.

COPD: chronic obstructive pulmonary disease; FEV_1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; LLN: Lower limit of normal.

Figure 2. Risk of pneumonia in individuals with COPD according to blood eosinophil count.

Individuals with COPD were grouped based on their FEV_1% according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) spirometric classification of COPD severity using FEV_1 in percent of the predicted value. Analyses were repeated in a COPD population restricted to ≥10 pack-years of smoking.

N is the number of individuals, events is the total number of count events. IRR is incidence rate ratio given with 95% confidence interval (CI). Multivariable adjusted was for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% predicted. Inflammatory biomarkers were high sensitive C-reactive protein, leukocyte count, and fibrinogen.

COPD: Chronic Obstructive Pulmonary Disease; FEV_1%: Forced expiratory volume in 1 second in percent of the predicted value; IRR: Incidence Rate Ratio; CI: Confidence Interval.

Figure 3. Risk of pneumonia in the clinical COPD population according to blood eosinophil count.

Clinical COPD was defined as individuals with at least one exacerbation in the year before baseline, ≥10 pack-years of smoking, and FEV_1 <70% of the predicted value. N is the number of individuals, events is the total number of count events. IRR is incidence rate ratio given with 95% confidence interval (CI). Multivariable adjusted was for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% predicted. Inflammatory biomarkers were high sensitive C-reactive protein, leukocyte count, and fibrinogen.

COPD: Chronic Obstructive Pulmonary Disease; FEV_1%: Forced Expiratory Volume in 1 second in percent of the predicted value; IRR: Incidence Rate Ratio; CI: Confidence Interval.

Figure 4. Risk of pneumonia according the use of inhaled corticosteroids and blood eosinophil count.

Top panel is the risk of pneumonias comparing individuals using inhaled corticosteroids to individuals not using inhaled corticosteroids in the COPD population with FEV_1 in percent of the predicted value below 50%. Below are the risks of pneumonias associated with the use of inhaled corticosteroids stratified by levels of blood eosinophil count below and at or above $0.34 \cdot 10^9$/L.

Bottom panel shows the same analyses for the clinical COPD population.

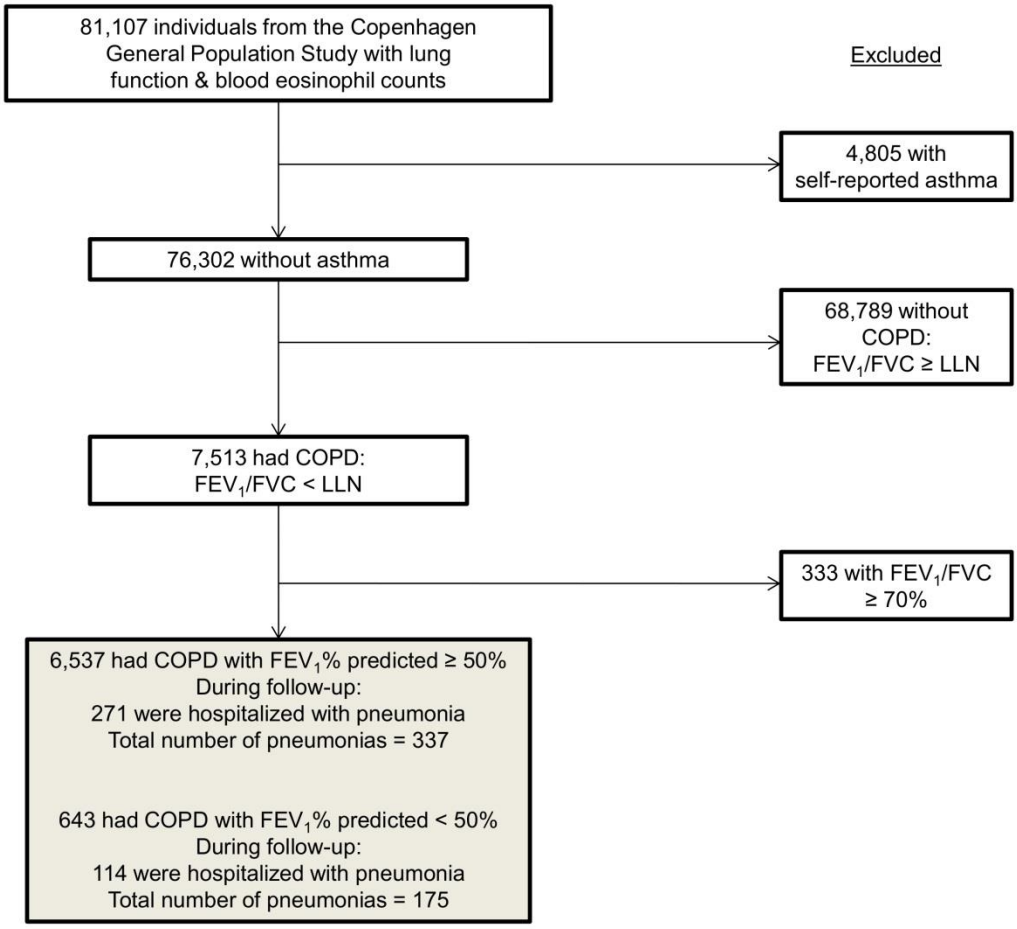
N is the number of individuals, events is the total number of count events. IRR is incidence rate ratio given with 95% confidence interval (CI). Multivariable adjusted was for sex, age, smoking status,

pack-years of smoking, body mass index, education, and FEV_1% predicted. Inflammatory biomarkers were high sensitive C-reactive protein, leukocyte count, and fibrinogen. P for interaction was for use of inhaled corticosteroids by blood eosinophil counts on risk of exacerbation.

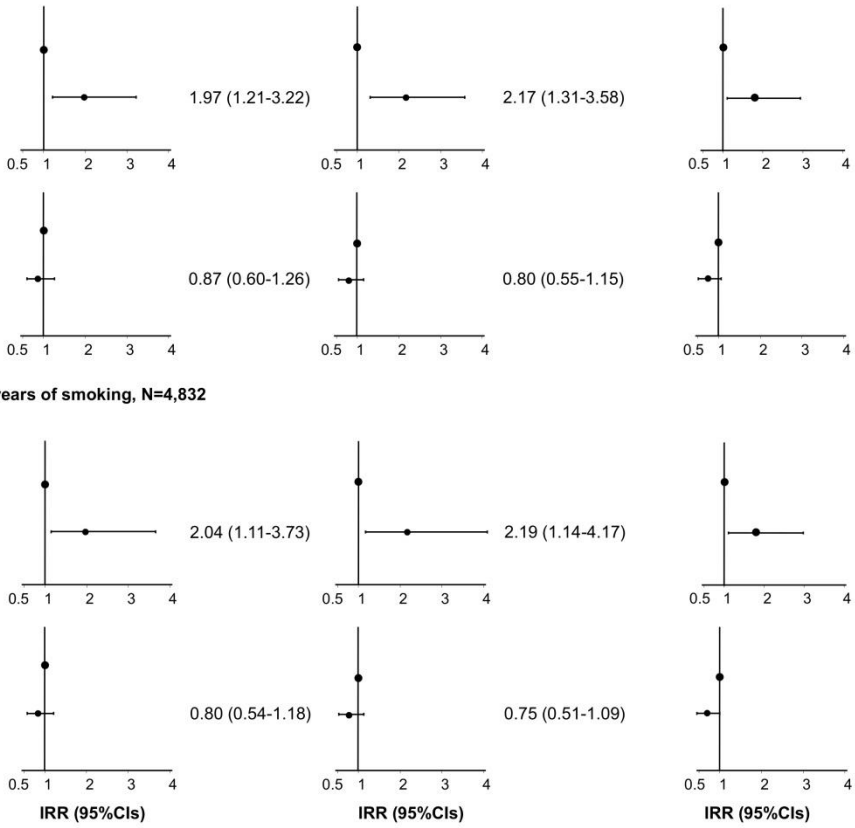
COPD: Chronic Obstructive Pulmonary Disease; FEV_1%: Forced Expiratory Volume in 1 second in percent of the predicted value; IRR: Incidence Rate Ratio; CI: Confidence Interval.

Figure 5. Repeated measurements of blood eosinophil counts 10 years apart in individuals with COPD grouped according to FEV_1 in percent of the predicted value.

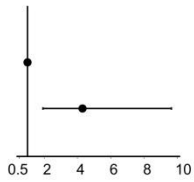
Individuals are shown along the x-axis. The horizontal line represents the cut-off at 0.34 109/L. Each individual has two measurements, with each dot representing a single measurement.


81,107 individuals from the Copenhagen General Population Study with lung function & blood eosinophil counts
Excluded
4,805 with self-reported asthma
76,302 without asthma
68,789 without COPD: FEV1/FVC ≥ LLN
7,513 had COPD: FEV1/FVC < LLN
333 with FEV1/FVC ≥ 70%
6,537 had COPD with FEV1% predicted ≥ 50%
During follow-up:
271 were hospitalized with pneumonia
Total number of pneumonias = 337
643 had COPD with FEV1% predicted < 50%
During follow-up:
114 were hospitalized with pneumonia
Total number of pneumonias = 175

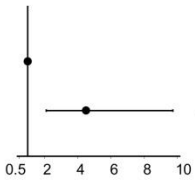
Blood eosinophils	N/ Pneumonias	Sex and age adjusted model	IRR (95%CIs)	Multivariable adjusted model	IRR (95%CIs)	Multivariable adjusted model incl. inflammatory biomarkers, inhaled corticosteroids, and prior pneumonia events	IRR (95%CIs)
COPD population, N=7,180							
COPD, FEV_1 <50% of predicted							
< 0.34 · 10^9/L (ref)	521/125						
≥ 0.34 · 10^9/L	122/50		1.97 (1.21-3.22)		2.17 (1.31-3.58)		1.81 (1.11-2.94)
COPD, FEV_1 ≥50% of predicted							
< 0.34· 10^9/L (ref)	5,646/290						
≥ 0.34· 10^9/L	891/47		0.87 (0.60-1.26)		0.80 (0.55-1.15)		0.73 (0.51-1.06)
COPD population with ≥ 10 pack-years of smoking, N=4,832							
COPD, FEV_1 <50% of predicted							
< 0.34 · 10^9/L (ref)	445/96						
≥ 0.34 · 10^9/L	108/42		2.04 (1.11-3.73)		2.19 (1.14-4.17)		1.82 (1.10-3.02)
COPD, FEV_1 ≥50% of predicted							
< 0.34· 10^9/L (ref)	3,641/238						
≥ 0.34· 10^9/L	638/39		0.80 (0.54-1.18)		0.75 (0.51-1.09)		0.69 (0.47-1.00)



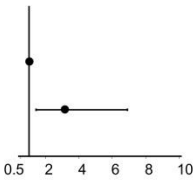
Blood eosinophils	N/ Pneumonias	Sex and age adjusted model	IRR (95%CIs)	Multivariable adjusted model	IRR (95%CIs)	Multivariable adjusted model incl. inflammatory biomarkers, inhaled corticosteroids, and prior pneumonia events	IRR (95%CIs)
Clinical COPD							
< 0.34 · 10^9/L (ref)	164/31						
≥ 0.34 · 10^9/L	38/27		4.31 (1.93-9.64)		4.52 (2.11-9.72)		3.14 (1.42-6.91)



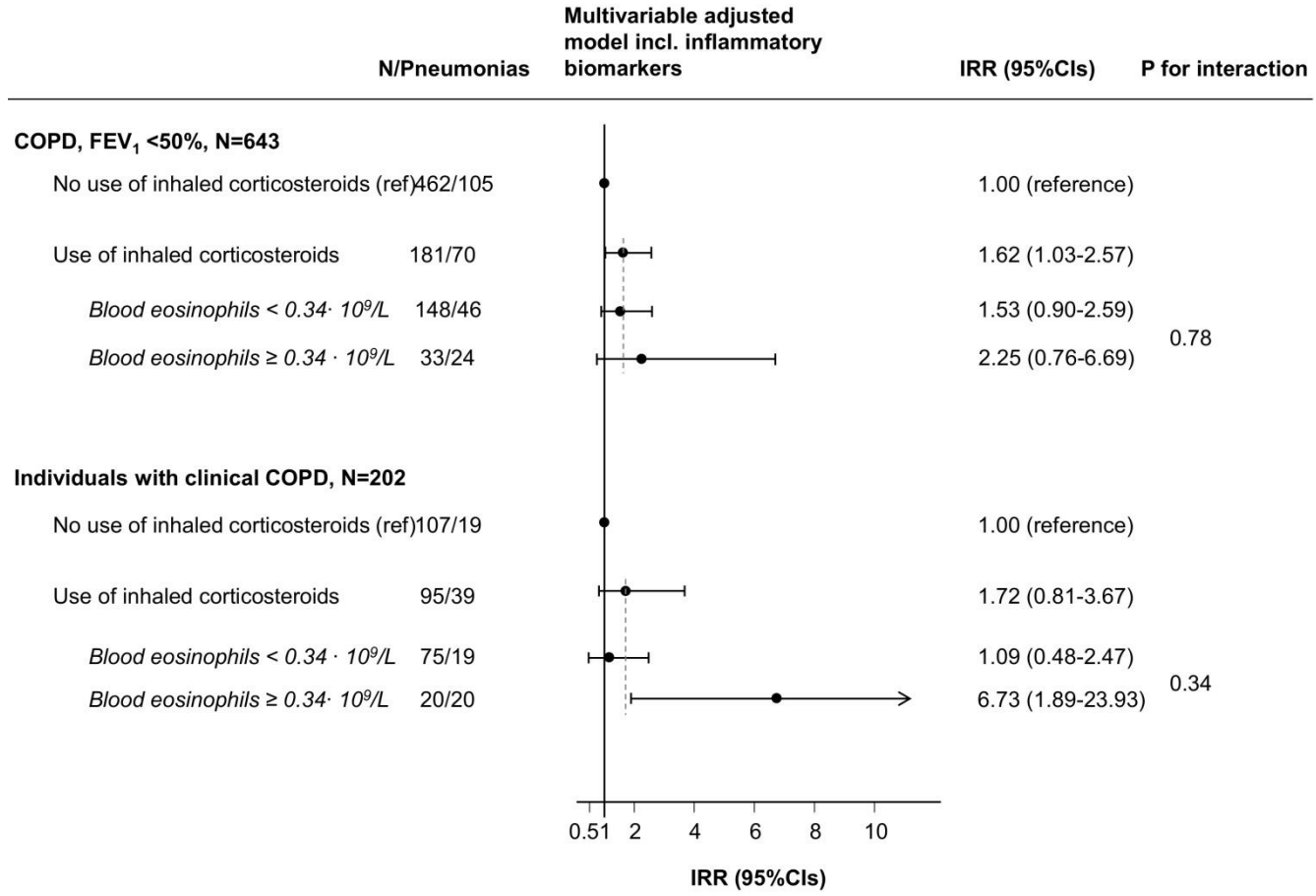
IRR (95%CIs)



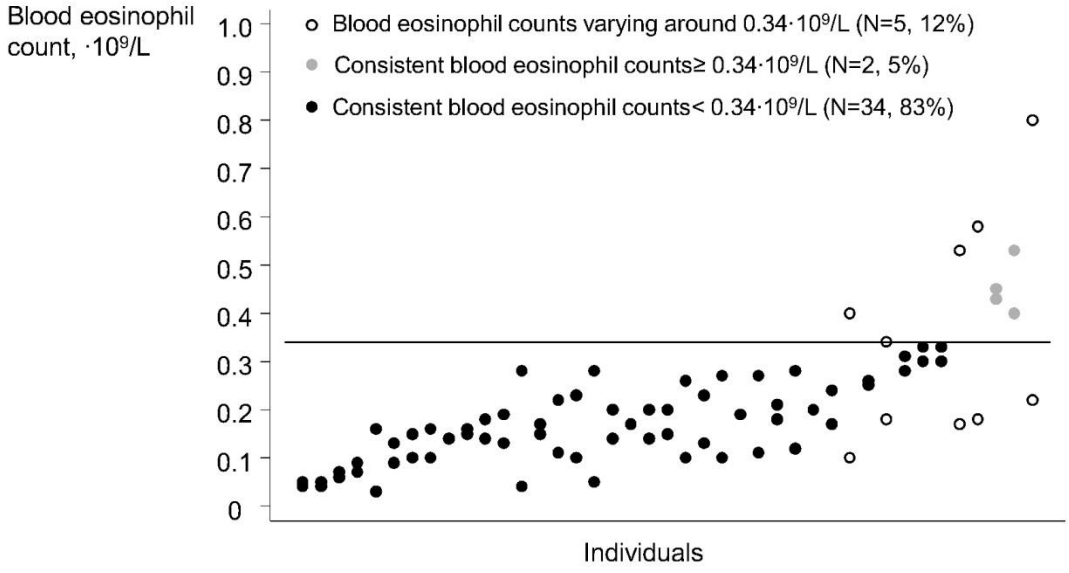
IRR (95%CIs)



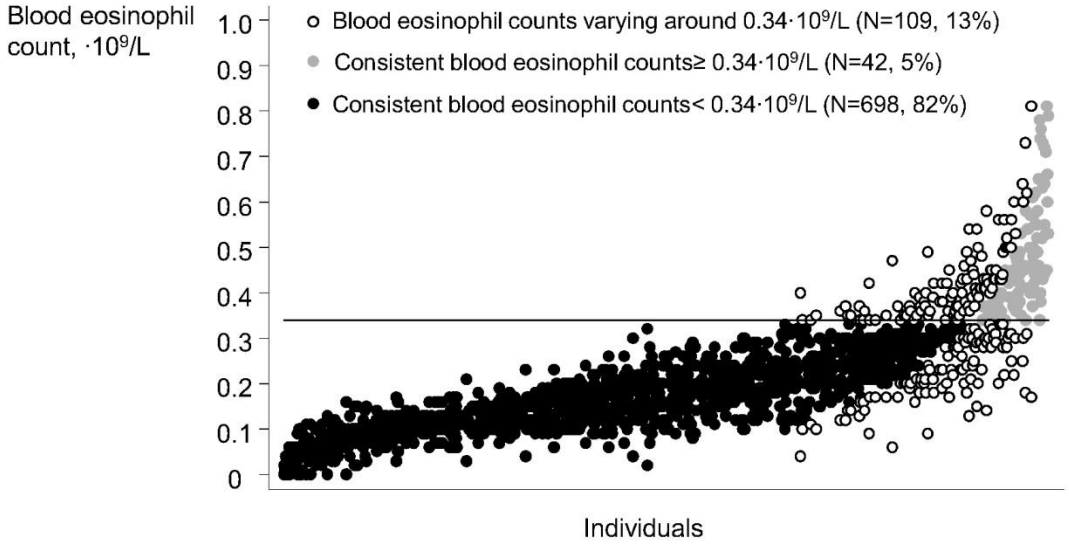
IRR (95%CIs)


N/Pneumonias
Multivariable adjusted model incl. inflammatory biomarkers
IRR (95%CIs)
P for interaction
COPD, FEV1 <50%, N=643
No use of inhaled corticosteroids (ref) 462/105
1.00 (reference)
Use of inhaled corticosteroids 181/70
1.62 (1.03-2.57)
Blood eosinophils < 0.34· 10^9/L 148/46
1.53 (0.90-2.59)
Blood eosinophils ≥ 0.34 · 10^9/L 33/24
2.25 (0.76-6.69)
0.78
Individuals with clinical COPD, N=202
No use of inhaled corticosteroids (ref) 107/19
1.00 (reference)
Use of inhaled corticosteroids 95/39
1.72 (0.81-3.67)
Blood eosinophils < 0.34 · 10^9/L 75/19
1.09 (0.48-2.47)
Blood eosinophils ≥ 0.34· 10^9/L 20/20
6.73 (1.89-23.93)
0.34
0.5 1 2 4 6 8 10
IRR (95%CIs)

COPD, FEV_1<50% of predicted, N=41



COPD, FEV_1≥50% of predicted, N=849



Online Data Supplement

Methods

The Copenhagen General Population Study (CGPS) was conducted according to the declaration of Helsinki and approved by the Regional ethics committee (H-KF 01-144/01). Participants aged 20-100 years old were randomly invited through the national Danish Civil Registration System. The response rate was 42%. Participants filled out an extensive questionnaire concerning health and lifestyle. The questionnaires were validated at the study site at Herlev Hospital, together with an investigator. Physical examinations and spirometry were then preformed, and blood was drawn for a range of blood sample measurements. Recruitment began in 2003 and more than 100,000 individuals have been enrolled.

Information on chronic obstructive pulmonary disease (COPD) exacerbations in the year before baseline used to define clinical COPD was obtained by linking the CGPS to the national Danish Registry of Medicinal Products Statistics and to the national Danish Patient Registry using the World Health Organization International Classification of Diseases code J41-44 and J45-46, as done previously [1]. Likewise, information on the frequency of COPD exacerbations during follow-up was found as previously described [1].

Spirometry

For the first 14,624 participants in the CGPS a Vitalograph™ (Pulmonary Function Printer, Maids Moreton, Buckinghamshire, UK) was used. For the remainder an EasyOne™ Diagnostics Spirometer (nnd Medizintechnok, Zürich, Switzerland) was used. Three sets of values of FEV_1 and FVC were obtained for each participant; two of the measurements had to differ less than 5% in order to be registered as correct. The highest obtained values of FEV_1 and FVC were used for each participant. Only pre-bronchodilator measurements were available.

Blood eosinophil counts

Daily precision control was performed using internal quality control material and once a month using an external control quality program.

Covariates

We included several potentially confounders in our analyses. Smoking was self-reported and categorized as current smoker, former smoker, or never smoker according to the questions "Do you smoke?" and "Have you previously smoked?". Cumulative smoking was measured in terms of pack-years based on the duration of smoking and the amount consumed; 1 pack-year was 20 cigarettes or equivalent smoked per day for one year. Body mass index was calculated as measured weight divided by measured height (kg/m^2). Low level of education was 3 or fewer years of education following the mandatory Danish public school of 7-9 years. Ischemic heart disease prior to baseline was defined as hospitalization due to ischemic heart disease (World Health Organization International Classification of

Diseases: ICD8 410-414; ICD10 I20-I25) prior to examination date. Frequency of exacerbations (per individual per year) was recorded and calculated as previously described (1).

Furthermore, we included three systemic inflammatory biomarkers as confounders in the analyses; in a previous study from the CGPS these were found to be associated with high risk of exacerbations [2] and comorbidities [3] among individuals with COPD. The systemic inflammatory biomarkers were grouped according to the previous studies; fibrinogen was grouped as high according to the cut-point ≥14 μmol/L, total blood leukocyte count was grouped as high according to the cut point of $\geq 9 \cdot 10^9$/L, and high sensitivity C-reactive protein was grouped as high according to the cut point ≥3 mg/L. Plasma levels of fibrinogen and high sensitivity C-reactive protein were analyzed using standard hospital assays. Total blood leukocyte count was measured together with blood eosinophil counts using the ADVIA™ 120 Hematology system.

Statistics

We determined the lower limit of normal for men and women separately for each spirometer, in a subsample of healthy, asymptomatic, never-smokers using linear regression with age and height as covariates. Individuals were considered asymptomatic if they did not report any respiratory symptoms. Individuals with self-reported asthma were excluded from the COPD definition. FEV_1 as % of the predicted value was also calculated separately for each spirometer and separately for men and women using internally derived reference values based on the same subsample of healthy, asymptomatic, never-smokers without self-reported asthma in a linear regression with age and height as covariates.

In the main analyses sex, smoking status, level of education, body mass index, use of inhaled corticosteroids, and inflammatory biomarkers were used as categorical covariates while age, cumulative smoking, and FEV_1 % predicted were continuous covariates.

Imputation of missing characteristics was done by multivariable regression. Information on age, sex, height, use of inhaled corticosteroids in the year prior to baseline, inflammatory biomarkers, FVC, FEV_1, and FEV_1 in % of predicted was 100% complete, whereas information on other characteristics were > 98% complete.

In sensitivity analyses, we used a Poisson regression model only taking pneumonia during follow-up (yes/no) per individual into account. Furthermore we used a Cox proportional hazard regression model, with age as time scale, to estimate hazard ratios (HR) with 95% confidence intervals of time to first pneumonia. Individuals with a hospitalization due to pneumonia prior to baseline (N=486) were excluded from Cox proportional hazard regression analyses and follow-up for each individual started at baseline and ended at death (n=583), emigration (n=8), or end of follow-up which was 31st December 2011. Multivariable adjustments were done using the same confounders as in our main analyses.

Also, in a sensitivity analysis, we used the Cox proportional hazard regression model, with time since blood eosinophil count measurement as time scale and adjusted for the same confounders as in the main analyses, to estimate hazard ratios (HR) with 95% confidence intervals of all-cause mortality after hospitalization due to pneumonia. Only individuals with a pneumonia event after baseline were included in these analyses. Follow-up for each individual started at the date of hospital admission and ended at death (n=212) or end of follow-up which was 31st December 2011. No emigrations were registered for the individuals in these analyses.

References

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3 Thomsen M, Dahl M, Lange P, Vestbo J, Nordestgaard BG. Inflammatory biomarkers and comorbidities in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012; 186: 982-8.

Supplementary table S1. Distribution of ICS according to blood eosinophil count in the COPD subpopulation with FEV_1<50% of the predicted value using ICS (n=181).

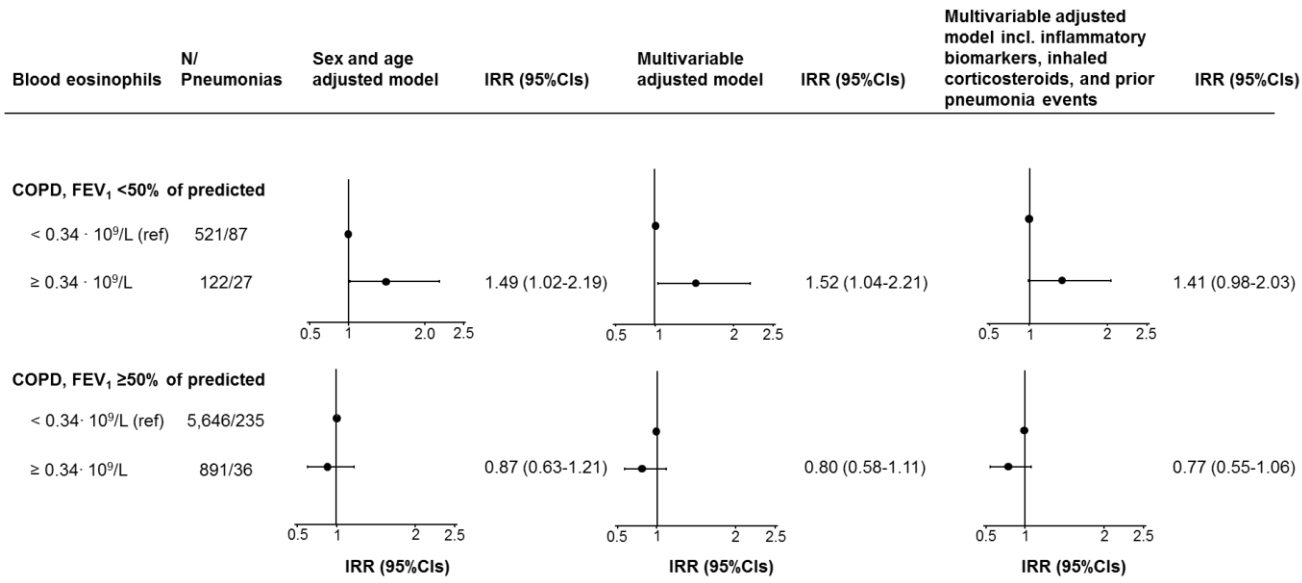
	Blood eosinophils <0.34· 10^9/L, n=148	**Blood eosinophils ≥0.34· 10^9/L, n=33**	**P-value**
Fluticasone	26 (18%)	9 (27%)	0.20
Budesonide	122 (82%)	24 (73%)	

Supplementary table S2. Significant covariates in a stepwise regression model considering all covariates from the full model in Figure 2.

COPD, FEV_1 <50% of predicted N = 643		
Covariate	**IRR (95% confidence interval)**	**P-value**
Prior pneumonia event	4.04 (2.57-6.34)	$1 \cdot 10^{-9}$
Number of high inflammatory biomarkers		
1	2.73 (1.56-4.75)	$4 \cdot 10^{-4}$
2	2.58 (1.49-4.48)	$7 \cdot 10^{-4}$
3	3.22 (1.61-6.45)	$1 \cdot 10^{-3}$
Blood eosinophil count $\geq 0.34 \cdot 10^9$/L	1.66 (1.07-2.60)	0.03

Significant covariates in a stepwise regression model on the risk of pneumonias according to blood eosinophil count in individuals with COPD and FEV_1<50% of predicted (N=643). Significance level was set to 0.05. Stepwise regression was run in the full model including sex, age, smoking status, pack-years of smoking, body mass index, education, FEV_1% predicted, inflammatory biomarkers (C-reactive protein, leukocyte count, and fibrinogen), use of inhaled corticosteroids, and prior pneumonia events. Incidence rate ratios (IRR) with 95% confidence intervals and P-values are from the final model including only the listed significant covariates. Results were similar using both forward and backward stepwise regression.

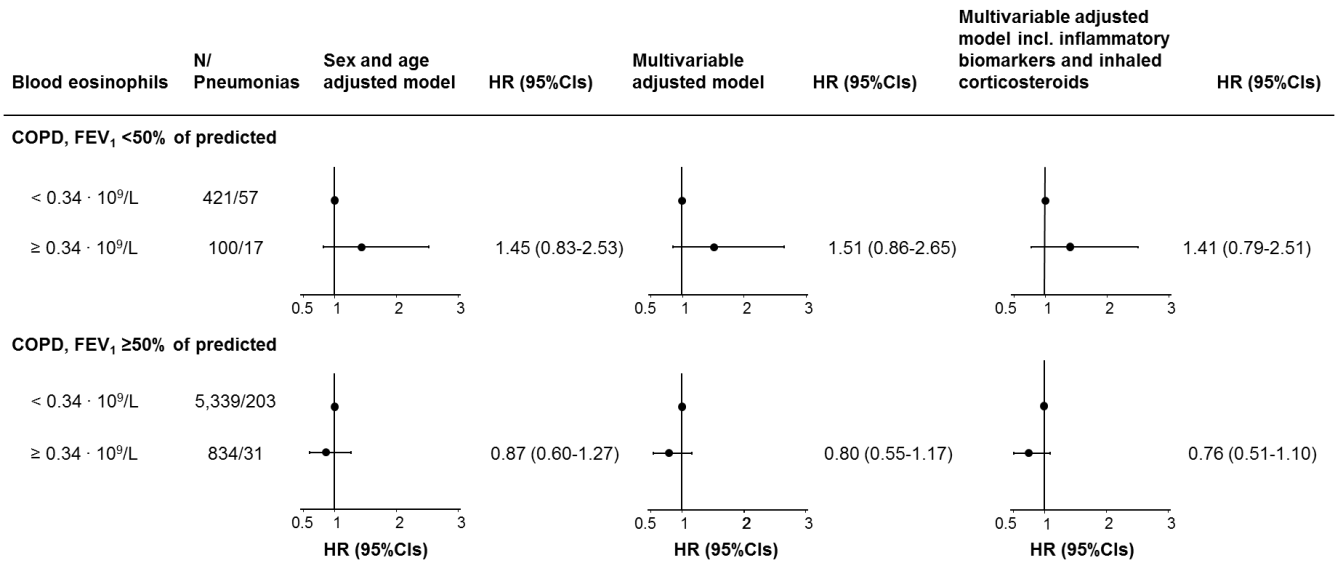
Supplementary figure S1. Risk of pneumonia according to blood eosinophil count and stratified according to COPD severity in a Poisson regression model only taking pneumonia status (yes/no) per individual during follow-up into account.



Multivariable adjustment was for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% predicted. Inflammatory biomarkers were C-reactive protein, leukocytes, and fibrinogen.

COPD: Chronic Obstructive Pulmonary Disease; IRR: Incidence Rate Ratio; CI: Confidence Interval.

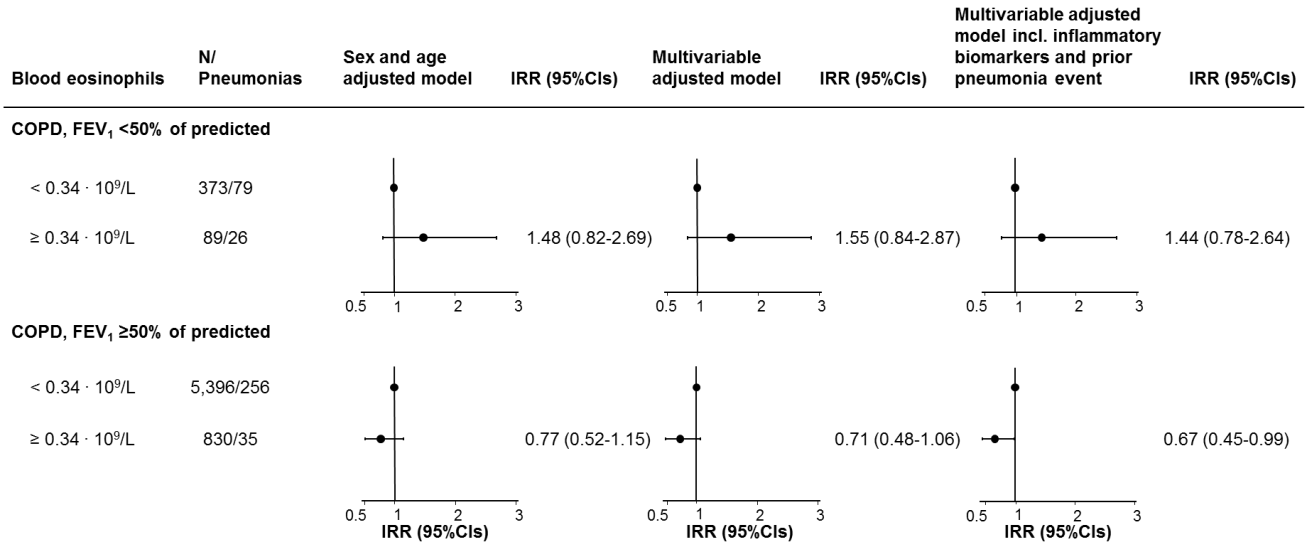
Supplementary figure S2. Risk of pneumonias according to blood eosinophil count excluding individuals with a hospitalization due to pneumonia before baseline, and stratified according to COPD severity in a Cox proportional hazards regression model.



Individuals with pneumonia (N=486) events prior to baseline measurement of blood eosinophil counts are excluded from the analyses. Multivariable adjustment was for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% predicted. The use of inhaled corticosteroids was assessed at baseline. Inflammatory biomarkers were C-reactive protein, leukocytes, and fibrinogen.

COPD: Chronic Obstructive Pulmonary Disease; HR: Hazard Ratio; CI: Confidence Interval.

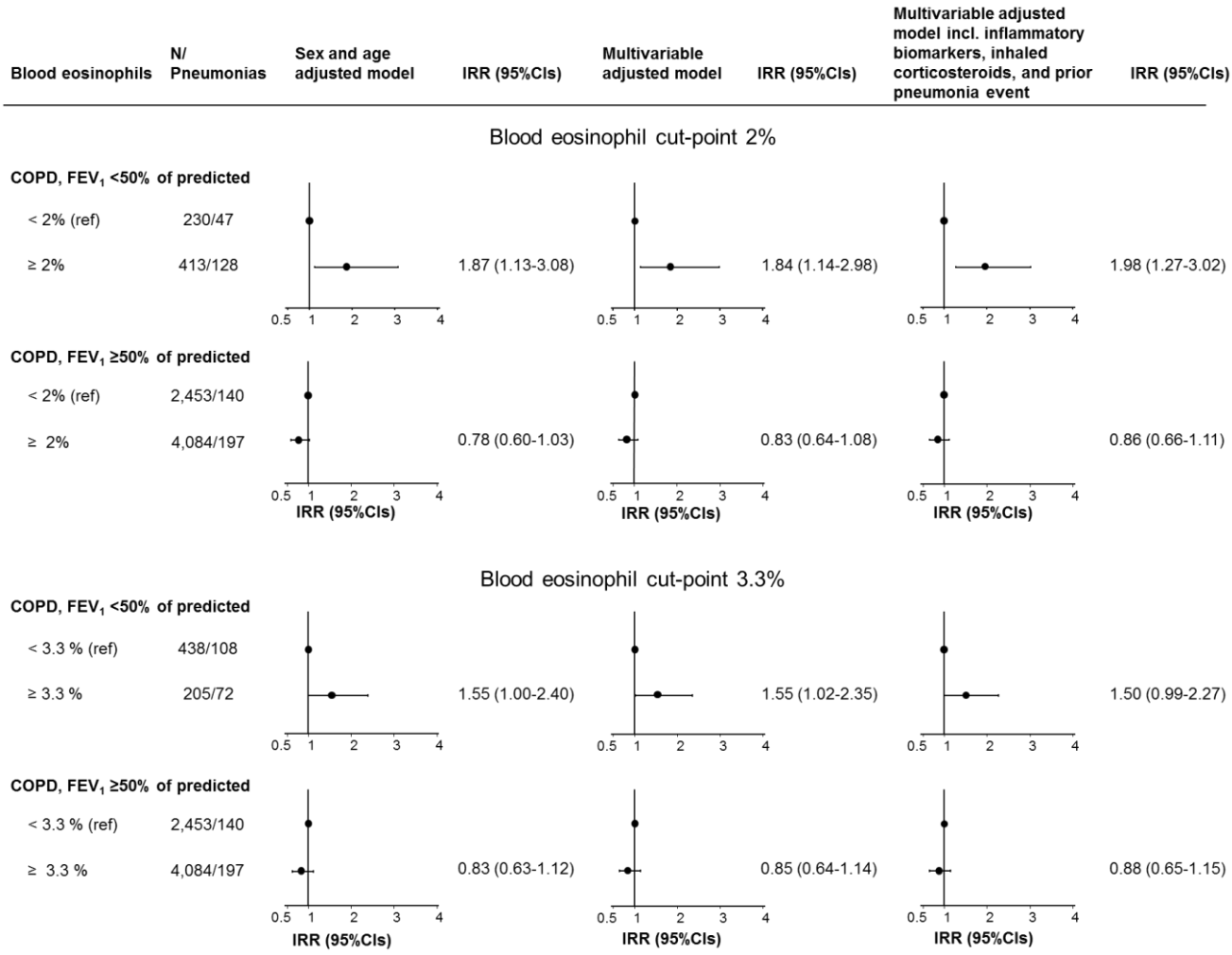
Supplementary figure S3. Risk of pneumonias according to blood eosinophil count excluding individuals with use of inhaled corticosteroids prior to baseline, and stratified according to COPD severity in individuals with COPD.



Multivariable adjustment was for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% predicted. Inflammatory biomarkers were C-reactive protein, leukocytes, and fibrinogen.

COPD: Chronic Obstructive Pulmonary Disease; IRR: Incidence Rate Ratio; CI: Confidence Interval.

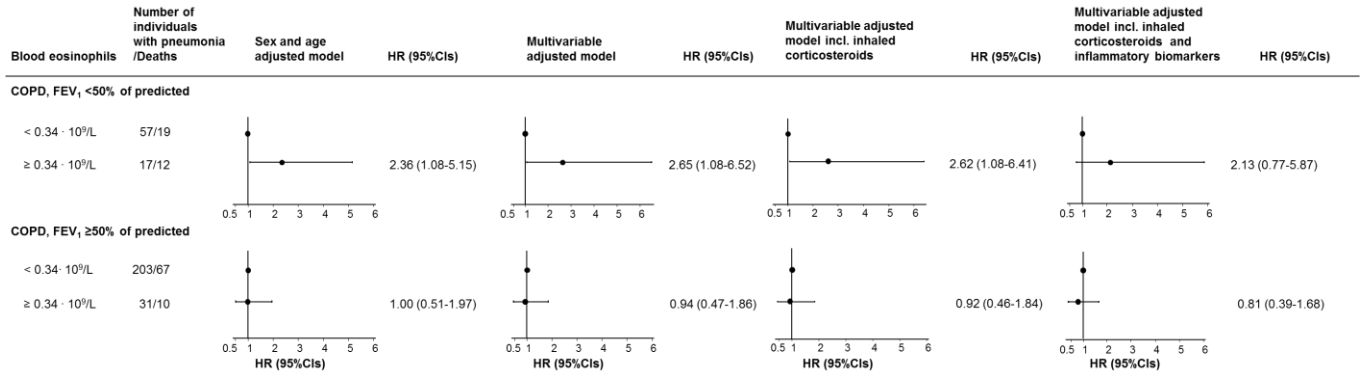
Supplementary figure S4. Risk of pneumonias according to blood eosinophil cut-points 2% and 3.3%.



Multivariable adjustment was for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% predicted. The use of inhaled corticosteroids was assessed at baseline. Inflammatory biomarkers were C-reactive protein, leukocytes, and fibrinogen.

COPD: Chronic Obstructive Pulmonary Disease; IRR: Incidence Rate Ratio; CI: Confidence Interval

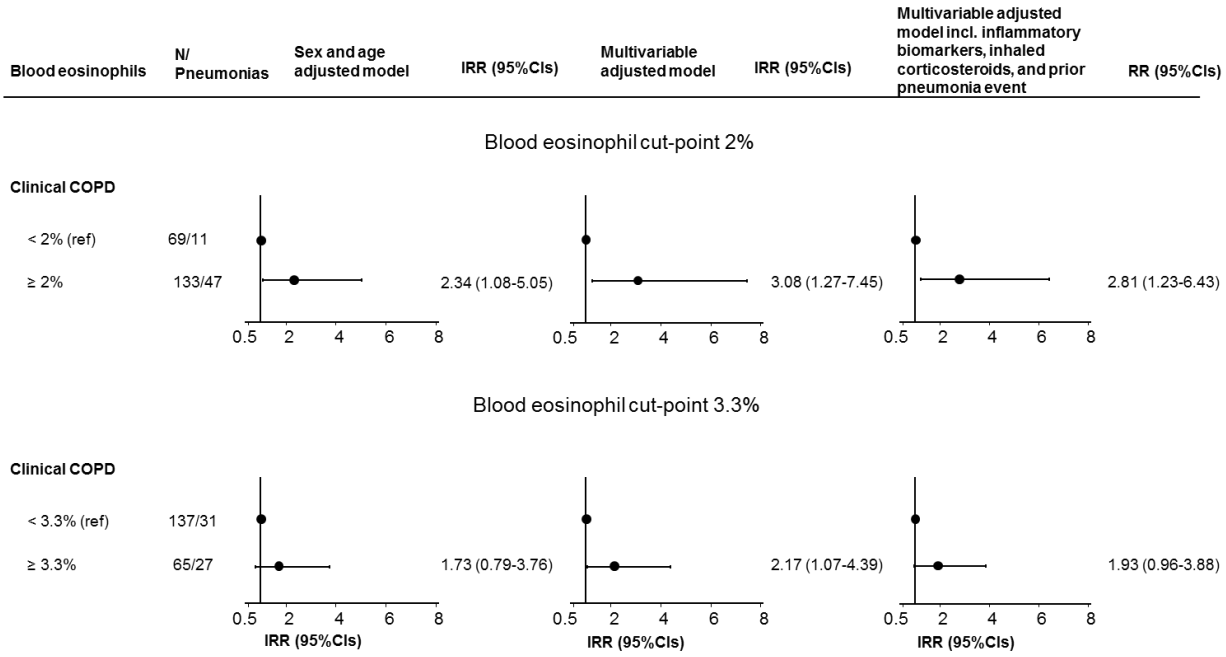
Supplementary figure S5. Risk of all-cause mortality after pneumonia according to blood eosinophil count and stratified according to COPD severity.



Multivariable adjustment was for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% predicted. The use of inhaled corticosteroids was assessed at baseline. Inflammatory biomarkers were C-reactive protein, leukocytes, and fibrinogen.

COPD: Chronic Obstructive Pulmonary Disease; HR: Hazard Ratio; CI: Confidence Interval

Supplementary figure S6. Risk of pneumonias according to blood eosinophil cut-points 2% and 3.3% in the clinical COPD population.



Multivariable adjustment was for sex, age, smoking status, pack-years of smoking, body mass index, education, and FEV_1% predicted. The use of inhaled corticosteroids was assessed at baseline. Inflammatory biomarkers were C-reactive protein, leukocytes, and fibrinogen.

COPD: Chronic Obstructive Pulmonary Disease; IRR: Risk Ratio; CI: Confidence Interval