Online Data Supplement

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

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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^{TM} (Pulmonary Function Printer, Maids Moreton, Buckinghamshire, UK) was used. For the remainder an EasyOne^{TM} 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^{TM} 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

1 Vedel-Krogh S, Nielsen SF, Lange P, Vestbo J, Nordestgaard BG. Blood Eosinophils and Exacerbations in COPD: the Copenhagen General Population Study. Am J Respir Crit Care Med 2015.

2 Thomsen M, Ingebrigtsen TS, Marott JL, Dahl M, Lange P, Vestbo J, Nordestgaard BG. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. JAMA 2013; 309: 2353-61.

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).

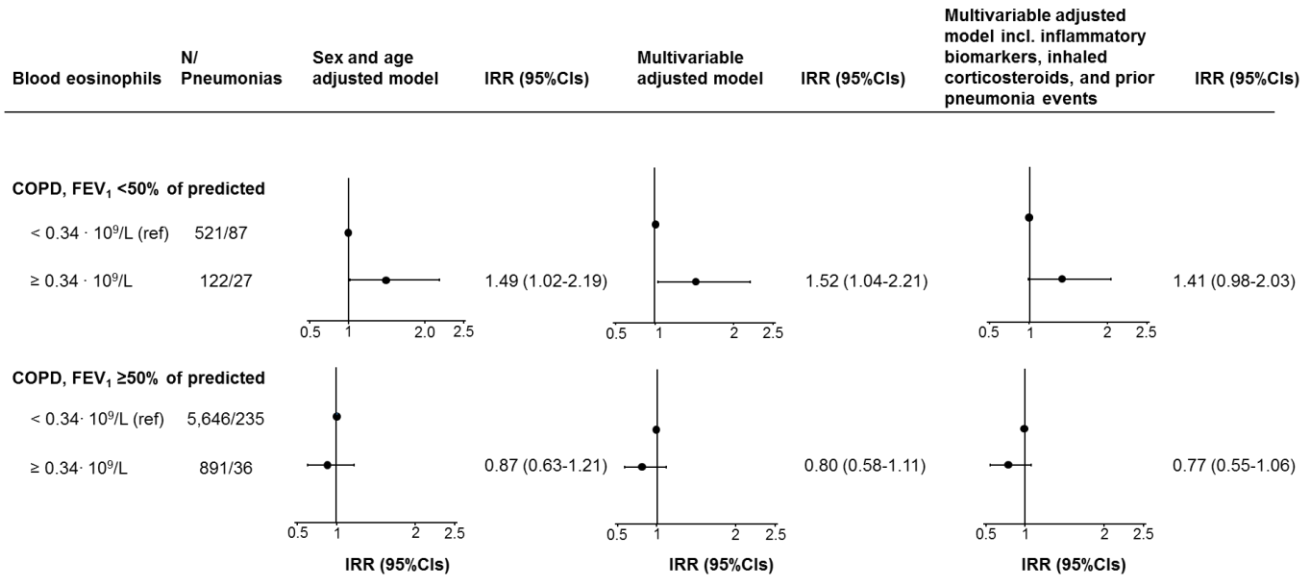
	Blood eosinophils <0.34· 10^9/L, n=148	**Blood eosinophils ≥0.34· 10^9/L, n=33**	**P-value**
Fluticasone	26 (18%)	9 (27%)	0.20
Budesonide	122 (82%)	24 (73%)	

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.

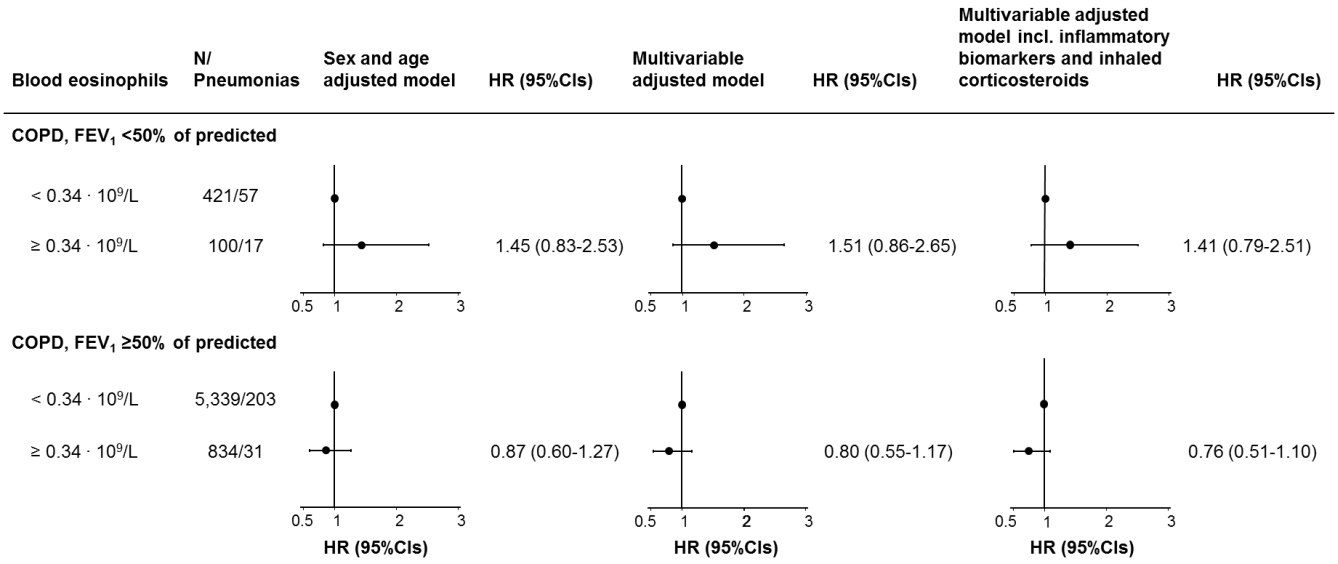
Supplementary figure S1. Risk of pneumonia according to blood eosinophil count and stratified according to COPD severity in a Poisson regression model only taking pneumonia status (yes/no) per individual during follow-up into account.



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.

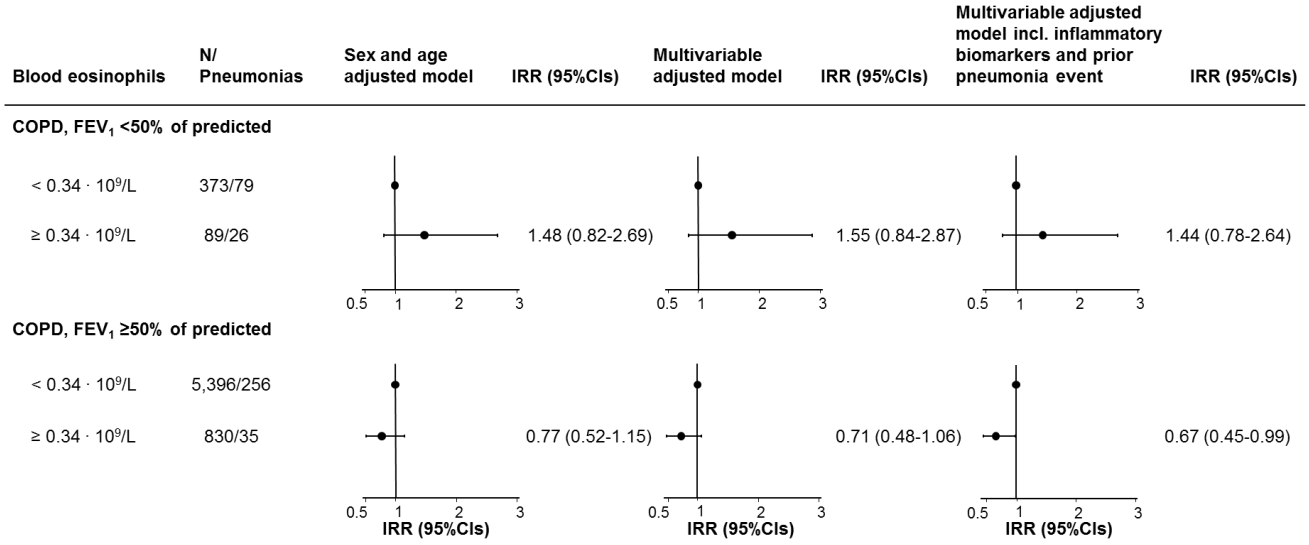
Supplementary figure S2. Risk of pneumonias according to blood eosinophil count excluding individuals with a hospitalization due to pneumonia before baseline, and stratified according to COPD severity in a Cox proportional hazards regression model.



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.

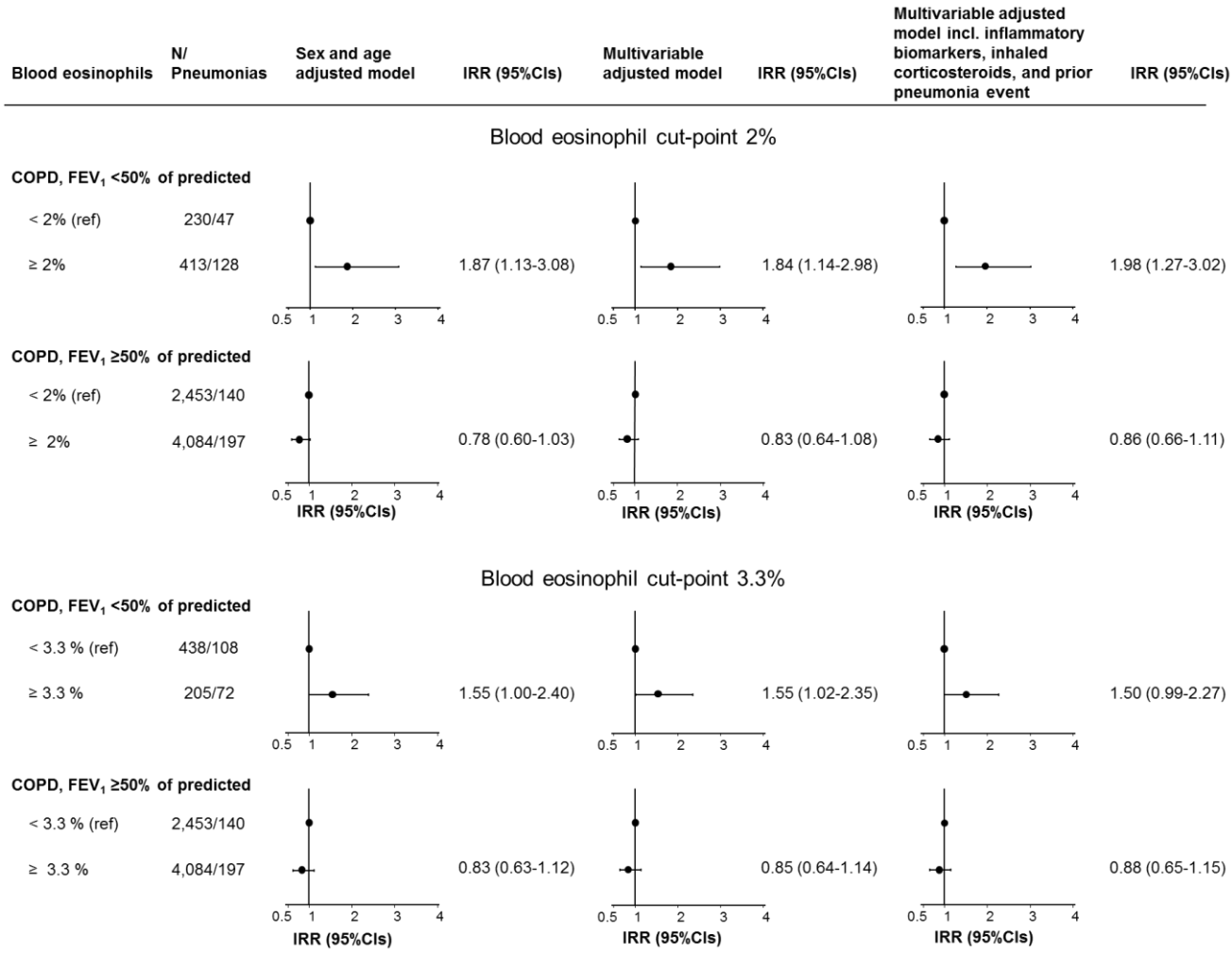
Supplementary figure S3. Risk of pneumonias according to blood eosinophil count excluding individuals with use of inhaled corticosteroids prior to baseline, and stratified according to COPD severity in individuals with COPD.



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.

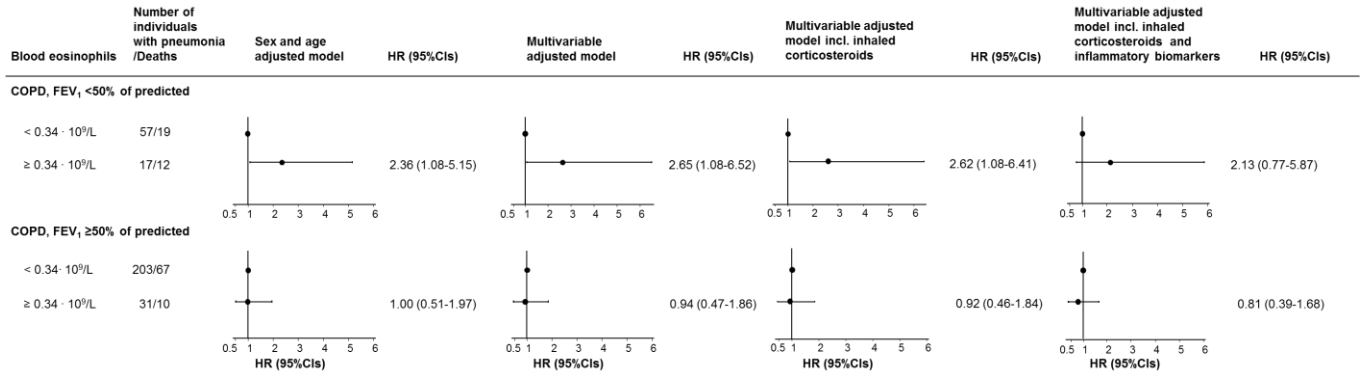
Supplementary figure S4. Risk of pneumonias according to blood eosinophil cut-points 2% and 3.3%.



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

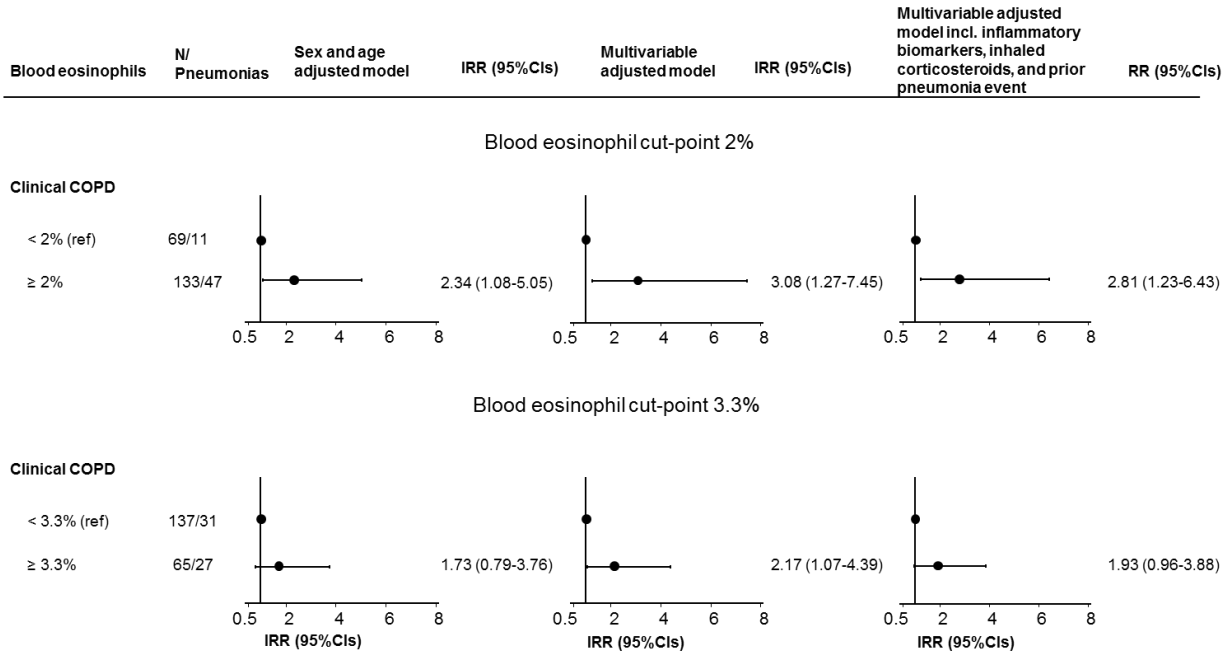
Supplementary figure S5. Risk of all-cause mortality after pneumonia according to blood eosinophil count and stratified according to COPD severity.



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