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Early View

Original article

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Combined value of exhaled nitric oxide and blood eosinophils in chronic airway disease: the Copenhagen General Population Study

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Key Words: asthma, chronic obstructive pulmonary disease, airway obstruction, allergy, overlap syndrome.

Take home message: Combination of exhaled nitric oxide and blood eosinophils may have an additive value in chronic airway disease.

Abstract

We investigated whether the combination of increased fraction of exhaled nitric oxide (FeNO) level and blood eosinophil count had an additive value in chronic airway disease in the general population.

We included 4677 individuals aged 20-100years from the Copenhagen General Population Study. Based on pre- and post-bronchodilator spirometry, self-reported asthma, and smoking history, participants were subdivided into:healthy never-smokers (n=1649), healthy ever-smokers (n=1581),

asthma (n=449), chronic obstructive pulmonary disease (COPD) (n=404), asthma-COPD overlap (ACO) (n=138), and non-specific airflow limitation (n=456).

Compared to individuals with FeNO<25ppb and blood eosinophils<$0.3x10^{-9}$/L, age- and sex adjusted odds ratios (ORs) for wheezing were 1.54(95% CI:1.29-1.84) for individuals with FeNO≥25ppb or blood eosinophils≥$0.3x10^{-9}$/L and 2.14(1.47-3.10) for individuals with FeNO≥25ppb and blood eosinophils≥$0.3x10^{-9}$/L. Corresponding ORs were 1.13(0.91-1.41) and 1.83(1.20-2.79) for sputum production, 1.54(1.22-1.94) and 3.26(2.16-4.94) for asthma, 1.03(0.80-1.32) and 0.67(0.36-1.27) for COPD, and 1.32(0.88-1.96) and 2.14(1.05-4.36) for ACO. Among individuals reporting respiratory symptoms, predicting the type of chronic airway disease did not differ between the two biomarkers and did not improve by combining them.

Combination of FeNO and blood eosinophils may have an additive value in characterising chronic airway disease in the general population but still needs to be investigated further with regard to clinical application.

Introduction

Eosinophilic airway inflammation is increasingly recognised as an important feature of patients that are highly responsive to treatment with corticosteroids[1,2]. Both fraction of exhaled nitric oxide (FeNO) level and blood eosinophil count have been suggested as biomarkers to determine and quantify the degree of eosinophilic airway inflammation[3]. Although FeNO and blood eosinophils may be a measure of the same inflammatory component, the two biomarkers seem to be regulated by different inflammatory pathways, which is supported by the weak correlation between them and

by the results from large clinical trials of treatments targeting type 2 helper T-cell (T_H2) cytokine–driven inflammation[4-9]. Therefore, it has been suggested to use them as complementary biomarkers of a clinically important pattern of inflammation[5,10,11]. However, studies on the clinical importance of combining these two biomarkers are still limited.

In the present study, we investigated whether the combination of increased FeNO level and blood eosinophil count had an additive value in chronic airway disease in the general population.

Methods

Study design and participants

We included 5578 individuals aged 20-100 years from the second examination of the Copenhagen General Population Study (CGPS), a population-based prospective cohort study initiated in April 2014 with ongoing enrolment. In the second examination, individuals are invited from the same areas as the first examination (2003-2014), meaning that some are newly invited and some have been examined in the first examination[12,13]. Individuals living in the Capital Region of Denmark were randomly selected from the National Danish Civil Registration System to reflect the adult Danish population by using the unique identification number provided to everyone at birth or immigration. Among individuals aged 20-39 years approximately 25% of the eligible were randomly selected and invited, whereas all eligible individuals aged ≥40 years were randomly selected and invited. All participants completed a comprehensive questionnaire, underwent a physical examination, and provided blood for biochemical analyses. Questionnaires were reviewed in detail at the day of attendance by a healthcare professional together with the participant. The study was approved by Herlev and Gentofte Hospital and a Danish ethical committee, and was

conducted according to the Declaration of Helsinki. All participants provided written informed consent.

Exhaled nitric oxide and blood eosinophils

FeNO levels in the expiratory volume was obtained using an online measurement technique with the portable hand-held device NIOX VERO (Aerocrine, Solna, Sweden), in accordance with the recommendations from the European Respiratory Society (ERS) and American Thoracic Society (ATS)[12,14]. The apparatus has a lowest detection limit of 5 ppb and a measurement range of 5-300 ppb. Measurements were performed with individuals in a sitting position without the use of a nose-clip, as this may lead to accumulation of nitric oxide in the nasal region and promote leakage via the posterior nasopharynx[14]. During the inspiration phase, individuals were required to inhale to their total lung capacity through the mouthpiece, which possesses a protective filter, in order to avoid environmental containment. During the exhalation phase, individuals were guided via an animated interface on the apparatus to maintain a correct constant expiratory flow rate. The apparatus did not analyse the expiratory volume for a FeNO level if individuals failed to sustain a correct constant expiratory flow rate and automatically required the measurement to be repeated. Since spirometry and reversibility testing may affect FeNO levels in the airways[14], measurement of FeNO was always performed before spirometry and reversibility testing. Healthcare professionals were trained properly using standard operating procedures in measurement of FeNO and certified on three occasions by more experienced healthcare professionals. Maintenance of the apparatus was done regularly, as recommended by the manufacturer. A FeNO level <25 ppb was considered as normal and ≥25 ppb as increased, in accordance with the recommendations from the ATS[15].

White blood cell counts were measured on fresh samples by using the ADVIA 120 Hematology System (Siemens Healthcare, Munich, Germany). Analyses were subjected to daily precision testing by using internal quality control material and monthly accuracy testing by using an external control quality programme[12]. Blood eosinophil counts were reported in x 10^9 cells/L together with other leukocyte sub-populations, and percent of total white blood cell count was calculated. A blood eosinophil count <0.3 x 10^9/L was considered as normal and ≥0.3 x 10^9/L as increased, as the following cut off has been associated with significant disease severity and increased risk of exacerbations in chronic airway disease[5,10,12,16].

Definition of chronic airway disease

The clinical groups of chronic airway disease were defined based on information obtained from the questionnaire and spirometry with the highest likelihood principle in accordance with the agreed recommendations from the Global Initiative for Chronic Obstructive Lung Disease (GOLD) and Global Initiative for Asthma (GINA)[17,18].

Information on asthma and tobacco smoking was obtained through self-report. Asthma was defined as an affirmative response to the question: "Do you have asthma?" Smoking status was defined as never, former, and current smokers. Based on information on age at smoking onset, duration of tobacco smoking, and amount of consumed tobacco, we calculated smoking history (cumulative tobacco consumption) in pack-years for former and current smokers; one pack-year corresponded to 20 cigarettes or equivalent, e.g. cheroots, cigars, pipe, smoked daily for one year.

Spirometry was performed with an EasyOne Spirometer (ndd Medical Technologies, Zurich, Switzerland) in a standing position without the use of a nose-clip. Pre-bronchodilator forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were measured with at least

three sets of values, and a validated spirometry performance was based on at least two measurements differing by less than 5% and a correct visual inspection of the spirometry curves. Spirometry use in the CGPS has previously undergone a rigorous validation process[19]. Individuals with presence of airflow limitation, defined as a pre-bronchodilator $FEV_1/FVC<0.70$, were additionally asked to perform a reversibility testing: post-bronchodilator FEV_1 and FVC were measured with the same procedures 15 min after inhalation of 400 µg salbutamol, a beta-2-agonist, from a dry powder inhaler (Ventoline Diskus, GlaxoSmithKline, Brentford Middlesex, UK). Percent of predicted values were calculated separately for men and women using internally derived reference values based on a subsample of healthy asymptomatic never-smokers with age and height as covariates[19]. The lower limit of normal (LLN), defined as the lower 5th percentile of the predicted value for FEV_1 and FEV_1/FVC, was calculated as the mean value minus 1.645 standard deviations.

In total, 4677 individuals were available with sufficient information on relevant measurements, of whom 1260 had pre-bronchodilator airflow limitation with $FEV_1/FVC<0.70$. Among these individuals, 456 wished not to perform a reversibility test and, therefore, lacked information on post-bronchodilator values. We subdivided participants into one of the following six groups (Figure 1):

1) **Healthy never-smokers:** never-smokers with pre-bronchodilator $FEV_1/FVC\geq0.70$ and no self-reported asthma.

2) **Healthy ever-smokers:** former and current smokers with pre-bronchodilator $FEV_1/FVC\geq0.70$ and no self-reported asthma.

3) **Asthma:** individuals with pre-bronchodilator $FEV_1/FVC\geq0.70$ and self-reported asthma, or with pre-bronchodilator $FEV_1/FVC<0.70$ and post-bronchodilator $FEV_1/FVC\geq0.70$, or with

pre-and post-bronchodilator $FEV_1/FVC<0.70$ and despite of no self-reported asthma have a FEV_1 reversibility of >12% and >400 mL and <10 pack-years of smoking history.

4) **Chronic obstructive pulmonary disease (COPD):** individuals with pre- and post-bronchodilator $FEV_1/FVC<0.70$ and no self-reported asthma or FEV_1 reversibility (FEV_1 reversibility of <12% and <200 mL).

5) **ACO:** individuals with pre- and post-bronchodilator $FEV_1/FVC<0.70$ and self-reported asthma or with pre-and post-bronchodilator $FEV_1/FVC<0.70$ and despite of no self-reported asthma have a FEV_1 reversibility of ≥12% and ≥200 mL.

6) **Non-specific airflow limitation:** individuals with pre-bronchodilator $FEV_1/FVC<0.70$ and no reversibility testing.

Other information

Body mass index (BMI) was calculated as measured weight divided by measured height squared (kg/m^2). Familial pre-disposition was defined as at least one first degree relative (father, mother, and/or sibling) with the respective condition. Allergy was defined if the participants reported to have allergy for different allergens (i.e. mold fungus, pollens from trees, grasses, or weeds, dust mites, pets, or other allergens) or asthma, hay fever, or eczema as a reaction to food, medication, grass, flower, animal hair, or other allergens. Information on childhood asthma or allergy was also self-reported. Use of airway medication was defined as taking any kind of medication for asthma/bronchitis (including sprays/dry powders) daily or almost daily. Wheezing was whistling or wheezing while breathing. Sputum production was phlegm from the lungs in the morning and/or during the day as long as three consecutive months each year. Chronic cough was cough lasting more than 8 weeks. Dyspnoea was shortness of breath during different levels of activity, at night-

time, and/or while seated/at rest. Individuals were also asked whether they had respiratory symptoms during the day or at night.

Statistical analyses

Statistical analyses were performed by using STATA/SE 13.1 for Windows (StataCorp, College Station, Texas, US). Logistic regression models were used. Area under the curve (AUC) for the receiver operating characteristics and classification statistics were determined; the positive outcome thresholds were estimated by plotting the sensitivity and specificity versus probability cut-off. First, associations of clinical attributes with an increased FeNO level and blood eosinophil count were investigated. Second, associations of an increased FeNO level and blood eosinophil count with symptoms and type of chronic airway disease were investigated. Third, predictive capabilities of the two biomarkers were investigated in a clinical population reporting at least one respiratory symptom (i.e. wheezing, sputum production, chronic cough, dyspnoea, and respiratory symptoms during the day or at night). All prediction analyses were adjusted for age and sex. All analyses for the two biomarkers were performed separately and combined.

Results

Among 4677 individuals, 1649 were healthy never-smokers, 1581 were healthy ever-smokers, 449 had asthma, 404 had COPD, 138 had ACO, and 456 had non-specific airflow limitation (Figure 1). Individuals with COPD, ACO, and non-specific airflow limitation were older compared to the other groups (Table 1). Individuals with asthma, COPD, and in particular those with ACO had lower lung function and reported more symptoms and greater use of airway medication. Generally, healthy

individuals with atopy compared to those without atopy irrespective of smoking status seemed to have higher FeNO levels and blood eosinophil counts, while healthy current smokers compared to healthy never and former smokers irrespective of presence of atopy seemed to have lower FeNO levels and higher blood eosinophil counts (Figures 2 and S1-S4).

Clinical attributes

A higher age, male sex, pre-bronchodilator $FEV_1/FVC<0.70$, positive reversible test, self-reported atopy, and use of airway medication were all associated with an increased risk of having a FeNO level ≥25 ppb and a blood eosinophil count $\geq 0.3 \times 10^{-9}$/L, both when analysed separately and combined (Figure 2). In contrast, only a higher BMI, a pre-bronchodilator FEV_1/FVC<LLN and a FEV_1 % predicted <LLN and <80 were associated with an increased risk of having a blood eosinophil count $\geq 0.3 \times 10^{-9}$/L, whereas current smoking and smoking history were associated with a reduced risk of having a FeNO level ≥25 ppb.

Respiratory symptoms

An increased FeNO level was associated with an increased risk of wheezing and respiratory symptoms during the day and at night, whereas an increased blood eosinophil count was associated with an increased risk of all types of respiratory symptoms (Figure 3). These associations were attenuated after additional adjustment for potential confounders. Compared to individuals with

FeNO level <25 ppb and blood eosinophil count <0.3 x 10^{-9}/L, age- and sex adjusted odds ratios (ORs) for wheezing were 1.54 (95% confidence interval [CI]:1.29-1.84) for individuals with FeNO level ≥25 ppb or blood eosinophil count ≥0.3 x 10^{-9}/L and 2.14 (1.47-3.10) for individuals with FeNO level ≥25 ppb and blood eosinophil count ≥0.3 x 10^{-9}/L (Figure 4). Corresponding ORs were 1.13 (0.91-1.41) and 1.83 (1.20-2.79) for sputum production, 1.22 (0.96-1.55) and 1.51 (0.92-2.48) for chronic cough, 1.21 (1.04-1.40) and 1.03 (0.73-1.46) for dyspnoea, 1.34 (1.12-1.61) and 1.30 (0.86-1.98) for day-time respiratory symptoms, and 1.33 (1.06-1.68) and 1.56 (0.95-2.56) for night-time respiratory symptoms. Results were attenuated but similar after additional adjustment for potential confounders.

Chronic airway disease

When the two biomarkers were analysed separately, an increased FeNO level and blood eosinophil count were associated with an increased risk of asthma but not COPD or ACO (Figure 5). Compared to individuals with FeNO level <25 ppb and blood eosinophil count <0.3 x 10^{-9}/L, age- and sex adjusted ORs for asthma were 1.54 (95% CI:1.22-1.94) for individuals with FeNO level ≥25 ppb or blood eosinophil count ≥0.3 x 10^{-9}/L and 3.26 (2.16-4.94) for individuals with FeNO level ≥25 ppb and blood eosinophil count ≥0.3 x 10^{-9}/L. Corresponding ORs were 1.03 (0.80-1.32) and 0.67 (0.36-1.27) for COPD and 1.32 (0.88-1.96) and 2.14 (1.05-4.36) for ACO. Adjustment for additional potential confounders gave attenuated but similar results. Results were also similar when defining the clinical groups of obstructive lung disease according to FEV_1/FVC <LLN (Figure S5).

In additional analyses, we restricted to different subgroups of individuals with chronic airway disease. In the subgroup of individuals with asthma and COPD, an increased FeNO level and blood eosinophil count was associated with an increased risk of asthma compared to COPD, especially

when the two biomarkers were combined (Figure 6). Similarly, when individuals with COPD and ACO were analysed separately, an increased FeNO level and blood eosinophil count was associated with an increased risk of ACO compared to COPD. No clear associations were observed in the ACO and asthma subgroup.

Predictive capabilities

Among individuals reporting respiratory symptoms, FeNO and blood eosinophils had a poor sensitivity and specificity with regard to predicting asthma, COPD, or ACO (Table 2). Furthermore, the negative predictive value was high (≥90%) and the positive predictive value was low (≤18%). No differences were observed between the two biomarkers, and the combination of them did not seem to improve the predictive capability. AUC values for predicting asthma were 0.62 (0.58-0.65) for FeNO ≥25 ppb, 0.60 (0.57-0.64) for blood eosinophils ≥0.3 x 10^{-9}/L, and 0.61 (0.58-0.64) for FeNO ≥25 ppb and/or blood eosinophils ≥0.3 x 10^{-9}/L. Corresponding AUC values were 0.68 (0.65-0.72), 0.68 (0.64-0.71), and 0.69 (0.65-0.72) for COPD and 0.63 (0.57-0.68), 0.64 (0.59-0.69), and 0.64 (0.59-0.69) for ACO.

After restricting the analyses to subgroups with chronic airway disease, no differences could be observed between the two biomarkers and the combination of them with regard to the predictive capability of differentiating between asthma, COPD, or ACO. Although the overall predictive capabilities were poor, the two biomarkers seemed to have an acceptable performance with regard to differentiating between asthma and COPD.

Discussion

In this large random sample from the general population, we found that compared to individuals with normal FeNO level and blood eosinophil count, individuals with both increased biomarkers had an increased risk of respiratory symptoms and asthma and ACO with higher risk estimates than to those with only one increased biomarker. Among individuals reporting respiratory symptoms, predicting the type of chronic airway disease did not differ between the two biomarkers and did not improve by combining them; however, use of the two biomarkers seemed to rule out chronic airway disease with a negative predictive value of ≥90%. Thus, our findings suggest that although the combination of these two biomarkers may have an additive value in characterising chronic airway disease, it still needs to be investigated further with regard to potential clinical application.

Exhaled nitric oxide is believed to arise due to local inflammation in the airways related to the activation of interleukin (IL)-4 and IL-13, whereas blood eosinophils are believed to reflect systemic inflammation with the activation of interleukin IL-5[2]. In large clinical trials, treatment with mepolizumab (monoclonal anti-IL-5) reduced blood eosinophil counts significantly without any noteworthy effect on FeNO levels[7-9], while treatment with lebrikizumab (anti-IL-13) and dupilumab (anti-IL-4 and anti-IL-13) reduced FeNO levels significantly with no or a very modest increase in blood eosinophil counts[4,6]. This suggests that these two biomarkers should not be used interchangeably but instead be combined in order to determine different aspects of an eosinophilic airway inflammation. In the present study, we observed not only an additive value of combining these two biomarkers, but also that these biomarkers were differently associated with some of the clinical attributes when analysed separately. A greater airflow limitation, a well-known clinical attribute of severe obstructive lung disease[20,21], was associated with an increased risk of having a blood eosinophil count ≥0.3 x 10^{-9}/L but not with FeNO level ≥25 ppb. Furthermore, measures of atopy and smoking were associated with FeNO rather than with blood eosinophils.

Another interesting finding was that both an increased FeNO level and blood eosinophil count were associated with asthma and ACO but not with COPD. We do not necessarily believe that FeNO and blood eosinophils are able to differentiate asthma and ACO from COPD, but rather identify a pathophysiological trait more common in asthma and ACO rather than in COPD[22]. However, the use of FeNO and blood eosinophils had low sensitivity and specificity among symptomatic individuals for diagnosing the type of chronic airway disease, suggesting that the two biomarkers have their limitations and should be more thoroughly investigated in clinical studies before implementation in routine practice. Yet, the two biomarkers had a negative predictive value of ≥90% and therefore seem to be useful for excluding presence of chronic airway disease among symptomatic individuals in a general population setting. Since the positive and negative predictive values are also dependent on the prevalence of the disease irrespective of the sensitivity and specificity, a potential explanation for observing a high negative predictive value in combination with a low sensitivity and specificity in the present study may be the low prevalence of chronic airway disease in the present sample of the general population.

Previous studies have shown an increased FeNO level and blood eosinophil count to be independently associated with increased disease severity and acute attacks among patients with asthma and COPD[12,16,23-26]. On the other hand, the additive value by combining these two biomarkers has not been investigated extensively. In the National Health and Nutrition Examination Survey, combining FeNO and blood eosinophils gave an additive value with regard to determining risk of current asthma, wheezing, asthma attack, and asthma-related emergency department visits[5,11]. Furthermore, the same investigators could also observe an additive value by combining the two biomarkers among asthmatic individuals with regard to determining severity of airflow limitation, degree of bronchial responsiveness, and having uncontrolled asthma and frequent asthma attacks[10]. Lastly, since the majority of individuals with asthma in the present study had mild

disease, the median values of FeNO and blood eosinophils were lower compared to those with a more severe disease, which is often seen in secondary care[27].

An important limitation of the present study includes the definitions of the clinical groups of chronic airway disease. Despite of being able to differentiate between reversible- and irreversible airflow limitation, both asthma and COPD are very complex diseases with regard to clinical presentation and natural history and may be difficult to separate, and in particular the definition of ACO is still controversial. Although we had a highest likelihood principle to define the clinical groups by taking the agreed recommendations from GOLD and GINA into account[18], our findings warrant replication in clinical settings, where more detailed characterisation of type of chronic airway disease is possible. Another limitation was that we were unable to determine the type of used airway medication. Lastly, although spirometry use in the CGPS has previously undergone a rigorous validation process, some of the procedures were not in accordance with the recommendations from the ERS and ATS[28].

In conclusion, the combination of FeNO and blood eosinophils may have an additive value in characterising chronic airway disease in the general population but still needs to be investigated further with regard to clinical application.

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BGN, JLM, and PL. Acquisition, analyses, or interpretation of data: YÇ, SA, BGN, JLM, and PL. Drafting of the manuscript: YÇ. Critical revision of the manuscript for important intellectual content: YÇ, SA, BGN, JLM, and PL. Statistical analyses: YÇ and SA. Obtained funding: BGN and PL. Administrative, technical, or material support: BGN. Study supervision: PL.

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References

(1) George L, Brightling CE. Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease. Ther Adv Chronic Dis 2016;7:34-51.

(2) Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, et al. After asthma: redefining airways diseases. Lancet 2018;391:350-400.

(3) Pavord ID, Afzalnia S, Menzies-Gow A, Heaney LG. The current and future role of biomarkers in type 2 cytokine-mediated asthma management. Clin Exp Allergy 2017;47:148-60.

(4) Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011;365:1088-98.

(5) Malinovschi A, Fonseca JA, Jacinto T, Alving K, Janson C. Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subjects. J Allergy Clin Immunol 2013;132:821-7.

(6) Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, et al. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med 2013;368:2455-66.

(7) Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med 2009;360:973-84.

(8) Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med 2014;371:1198-207.

(9) Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.

(10) Malinovschi A, Janson C, Borres M, Alving K. Simultaneously increased fraction of exhaled nitric oxide levels and blood eosinophil counts relate to increased asthma morbidity. J Allergy Clin Immunol 2016;138:1301-8.

(11) Pavord ID, Bafadhel M. Exhaled nitric oxide and blood eosinophilia: independent markers of preventable risk. J Allergy Clin Immunol 2013;132:828-9.

(12) Vedel-Krogh S, Nielsen SF, Lange P, Vestbo J, Nordestgaard BG. Blood Eosinophils and Exacerbations in Chronic Obstructive Pulmonary Disease. The Copenhagen General Population Study. Am J Respir Crit Care Med 2016;193:965-74.

(13) Çolak Y, Afzal S, Nordestgaard BG, Vestbo J, Lange P. Prognosis of asymptomatic and symptomatic, undiagnosed COPD in the general population in Denmark: a prospective cohort study. Lancet Respir Med 2017;5:426-34.

(14) ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912-30.

(15) Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med 2011;184:602-15.

(16) Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. Lancet Respir Med 2015;3:849-58.

(17) Lange P, Çolak Y, Ingebrigtsen TS, Vestbo J, Marott JL. Long-term prognosis of asthma, chronic obstructive pulmonary disease, and asthma-chronic obstructive pulmonary disease overlap in the Copenhagen City Heart study: a prospective population-based analysis. Lancet Respir Med 2016;4:454-62.

(18) Diagnosis of Diseases of Chronic Airflow Limitation: Asthma, COPD, and Asthma-COPD Overlap Syndrome (ACOS). Global Initiative for Asthma (GINA) and Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Initiative for Asthma (GINA) and Global Initiative for Chronic Obstructive Lung Disease (GOLD). Available at: www.goldcopd.org/asthma-copd-asthma-copd-overlap-syndrome (accessed March 13 2018).

(19) Løkke A, Marott JL, Mortensen J, Nordestgaard BG, Dahl M, Lange P. New Danish reference values for spirometry. Clin Respir J 2013;7:153-67.

(20) Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Available at: www.goldcopd.org/gold-reports (accessed March 13 2018).

(21) Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA). Available at: www.ginasthma.org/2018-gina-report-global-strategy-for-asthma-management-and-prevention/ (accessed March 13 2018).

(22) Agusti A, Bel E, Thomas M, Vogelmeier C, Brusselle G, Holgate S, et al. Treatable traits: toward precision medicine of chronic airway diseases. Eur Respir J 2016;47:410-9.

(23) Alcazar-Navarrete B, Ruiz RO, Conde BP, Romero Palacios PJ, Agusti A. Persistently elevated exhaled nitric oxide fraction is associated with increased risk of exacerbation in COPD. Eur Respir J 2018;51.

(24) Bafadhel M, McKenna S, Terry S, Mistry V, Reid C, Haldar P, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. Am J Respir Crit Care Med 2011;184:662-71.

(25) Bjerregaard A, Laing IA, Backer V, Sverrild A, Khoo SK, Chidlow G, et al. High fractional exhaled nitric oxide and sputum eosinophils are associated with an increased risk of future

virus-induced exacerbations: A prospective cohort study. Clin Exp Allergy 2017;47:1007-13.

(26) Coumou H, Westerhof GA, de Nijs SB, Zwinderman AH, Bel EH. Predictors of accelerated decline in lung function in adult-onset asthma. Eur Respir J 2018;51.

(27) Schleich FN, Chevremont A, Paulus V, Henket M, Manise M, Seidel L, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. Eur Respir J 2014;44:97-108.

(28) Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26:319-38.

TABLE 1 Characteristics of individuals from the Copenhagen General Population Study according to clinical groups of chronic airway disease

	Never-smokers (n=1649)	Ever-smokers (n=1581)	Asthma (n=449)	COPD (n=404)	ACO (n=138)	Non-specific AL (n=456)
Age – years	58 (49-68)	60 (51-68)	60 (51-70)	68 (60-75)	68 (59-75)	67 (59-74)
Men – no. (%)	700 (42)	745 (47)	142 (32)	220 (54)	58 (42)	218 (48)
BMI – kg/m^2	26 (23-29)	27 (24-30)	25 (23-29)	25 (23-28)	26 (23-29)	25 (23-28)
FEV_1 predicted – %	102 (93-110)	99 (90-108)	94 (85-104)	81 (69-94)	68 (55-79)	85 (71-97)
FVC predicted – %	104 (95-112)	102 (92-111)	105 (94-116)	104 (89-117)	90 (78-103)	102 (88-116)
FEV_1/FVC	0.78 (0.75-0.81)	0.77 (0.74-0.80)	0.69 (0.67-0.75)	0.62 (0.57-0.66)	0.58 (0.53-0.63)	0.67 (0.60-0.69)
Current smokers – no. (%)	NA	335 (21)	55 (12)	100 (25)	40 (29)	87 (19)
Smoking history – pack-years*	NA	14 (5-25)	15 (5-27)	31 (18-45)	35 (18-50)	24 (10-38)
Familial predisposition for COPD – no. (%)†	250 (15)	265 (17)	74 (16)	87 (22)	46 (33)	88 (19)
Familial predisposition for asthma – no. (%)†	208 (13)	201 (13)	106 (24)	53 (13)	32 (23)	71 (16)
Familial predisposition for allergy – no. (%)†	315 (19)	246 (16)	99 (22)	41 (10)	17 (12)	61 (13)
Childhood asthma or allergy – no. (%)	315 (19)	253 (16)	137 (31)	68 (17)	31 (22)	92 (20)
Allergy – no. (%)	593 (36)	485 (31)	239 (53)	110 (27)	63 (46)	153 (34)
Use of airway medication – no. (%)	16 (1)	18 (1)	138 (31)	50 (12)	61 (44)	74 (16)
Respiratory symptoms						
Wheezing – no. (%)	138 (8)	247 (16)	148 (33)	104 (26)	66 (48)	105 (23)
Sputum production – no. (%)	87 (5)	177 (11)	78 (17)	77 (19)	36 (26)	73 (16)
Chronic cough – no. (%)	76 (5)	138 (9)	60 (13)	64 (16)	26 (19)	54 (12)
Dyspnoea – no. (%)	397 (24)	525 (33)	202 (45)	217 (54)	79 (57)	198 (43)
Day-time symptoms – no. (%)	142 (9)	219 (14)	135 (30)	120 (30)	54 (39)	106 (23)
Night-time symptoms – no. (%)	100 (6)	133 (8)	74 (16)	46 (11)	29 (21)	54 (12)
Degree of airflow limitation‡						
FEV_1 % predicted ≥80 – no. (%)	1561 (95)	1441 (91)	379 (84)	217 (54)	32 (23)	265 (58)
FEV_1 % predicted 50-79 – no. (%)	85 (5)	137 (9)	68 (15)	159 (39)	81 (59)	162 (36)
FEV_1 % predicted 30-49 – no. (%)	2 (<1)	2 (<1)	2 (<1)	26 (6)	24 (17)	23 (5)
FEV_1 % predicted <30 – no. (%)	1 (<1)	1 (<1)	0 (0)	2 (<1)	1 (<1)	6 (1)
Levels of inflammatory biomarkers						
Blood eosinophils – x 10^9/L	0.15 (0.10-0.22)	0.16 (0.11-0.24)	0.16 (0.11-0.27)	0.18 (0.12-0.26)	0.19 (0.13-0.28)	0.18 (0.12-0.27)
FeNO – ppb	13 (10-19)	12 (8-17)	14 (9-21)	12 (8-18)	12 (6-21)	13 (8-19)

Data presented as median and 25th and 75th percentiles, or number (percent), unless otherwise stated. ACO: asthma-COPD overlap; AL: airflow limitation; BMI: body mass index; COPD: chronic obstructive pulmonary disease; FeNO: fraction of exhaled nitric oxide; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; NA: not applicable. *Only for former and current smokers. †Also included the answer: "Do not know".

TABLE 2 Predictive capabilities of increased exhaled nitric oxide level and blood eosinophil count with regard to chronic airway disease in symptomatic individuals

	Sensitivity	Specificity	PPV	NPV	AUC (95% CI)	P-value versus FeNO	P-value versus blood eosinophils	P-value versus FeNO and blood eosinophils
Asthma								
FeNO ≥25 ppb	57%	60%	18%	90%	0.62 (0.58-0.65)	NA	0.10	0.36
Blood eosinophils ≥0.3 x 10^{-9}/L	61%	55%	17%	90%	0.60 (0.57-0.64)	0.10	NA	0.16
FeNO ≥25 ppb **and/or** blood eosinophils ≥0.3 x 10^{-9}/L	58%	59%	18%	90%	0.61 (0.58-0.64)	0.36	0.16	NA
COPD								
FeNO ≥25 ppb	61%	64%	18%	93%	0.68 (0.65-0.72)	NA	0.26	0.37
Blood eosinophils ≥0.3 x 10^{-9}/L	60%	64%	18%	92%	0.68 (0.64-0.71)	0.26	NA	0.12
FeNO ≥25 ppb **and/or** blood eosinophils ≥0.3 x 10^{-9}/L	60%	64%	18%	93%	0.69 (0.65-0.72)	0.37	0.12	NA
ACO								
FeNO ≥25 ppb	54%	64%	7%	97%	0.63 (0.57-0.68)	NA	0.34	0.10
Blood eosinophils ≥0.3 x 10^{-9}/L	57%	66%	7%	97%	0.64 (0.59-0.69)	0.34	NA	0.89
FeNO ≥25 ppb **and/or** blood eosinophils ≥0.3 x 10^{-9}/L	51%	66%	7%	97%	0.64 (0.59-0.69)	0.10	0.89	NA
Asthma versus COPD								
FeNO ≥25 ppb	67%	66%	69%	64%	0.74 (0.70-0.78)	NA	0.32	0.27
Blood eosinophils ≥0.3 x 10^{-9}/L	67%	65%	69%	63%	0.73 (0.69-0.77)	0.32	NA	0.08
FeNO ≥25 ppb **and/or** blood eosinophils ≥0.3 x 10^{-9}/L	67%	67%	70%	64%	0.74 (0.70-0.78)	0.27	0.08	NA
COPD versus ACO								
FeNO ≥25 ppb	58%	57%	77%	35%	0.57 (0.50-0.64)	NA	0.51	0.26
Blood eosinophils ≥0.3 x 10^{-9}/L	50%	66%	79%	34%	0.59 (0.52-0.65)	0.51	NA	0.94
FeNO ≥25 ppb **and/or** blood eosinophils ≥0.3 x 10^{-9}/L	59%	58%	78%	36%	0.58 (0.52-0.65)	0.26	0.94	NA
ACO versus asthma								
FeNO ≥25 ppb	59%	63%	35%	82%	0.68 (0.62-0.74)	NA	0.94	0.69
Blood eosinophils ≥0.3 x 10^{-9}/L	63%	60%	35%	83%	0.68 (0.62-0.74)	0.94	NA	0.86
FeNO ≥25 ppb **and/or** blood eosinophils ≥0.3 x 10^{-9}/L	62%	62%	36%	83%	0.68 (0.63-0.74)	0.69	0.86	NA

Data presented as percent, unless otherwise stated. Individuals reported to have at least one respiratory symptom (i.e. wheezing, sputum production, chronic cough, dyspnoea, and respiratory symptoms during the day or at night). Logistic regression models were used to calculate the statistics and included age and sex as covariates. P-values were from testing the equality of two AUCs for the receiver operating characteristics. ACO: asthma-COPD overlap; AUC: area under the curve; CI: confidence interval; COPD: chronic obstructive pulmonary disease; FeNO: fraction of exhaled nitric oxide; NA: not applicable; NPV: negative predictive value; PPV: positive predictive value.

Figure legends

Figure 1. Flow chart of the study population.

ACO: asthma-COPD overlap; AL: airflow limitation; COPD: chronic obstructive pulmonary disease; FeNO: fraction of exhaled nitric oxide; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity.

Figure 2. Distribution of FeNO levels and blood eosinophil counts in healthy never-smokers with and without atopy.

FeNO: fraction of exhaled nitric oxide.

Figure 3. Clinical attributes associated with increased exhaled nitric oxide level and blood eosinophil count.

Reversibility was defined as FEV_1 reversibility of ≥12% and ≥200 mL. Logistic regression models were used. Estimates are unadjusted. P-values were from Wald's test. BMI: body mass index; CI: confidence interval; COPD: chronic obstructive pulmonary disease; FeNO: fraction of exhaled nitric oxide; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; LLN: the lower limit of normal; OR: odds ratio.

Figure 4. Separate association of increased exhaled nitric oxide level and blood eosinophil count with respiratory symptoms.

Logistic regression models were used. Multivariable adjustment included age, sex, body mass index, smoking status, smoking history, familial pre-disposition for chronic obstructive pulmonary

disease and asthma, atopy, and use of airway medication. P-values were from Wald's test. CI: confidence interval; FeNO: fraction of exhaled nitric oxide; OR: odds ratio.

Figure 5. Combined association of increased exhaled nitric oxide level and blood eosinophil count with respiratory symptoms.

Logistic regression models were used. Multivariable adjustment included age, sex, body mass index, smoking status, smoking history, familial pre-disposition for chronic obstructive pulmonary disease and asthma, atopy, and use of airway medication. P-values were from Wald's test. CI: confidence interval; FeNO: fraction of exhaled nitric oxide; OR: odds ratio.

Figure 6. Association of increased exhaled nitric oxide level and blood eosinophil count with asthma, COPD, and ACO.

Panel A: Illustrates the separate association analyses. *Panel B:* Illustrates the combined association analyses. Logistic regression models were used. Multivariable adjustment included age, sex, body mass index, smoking status, smoking history, familial pre-disposition for COPD and asthma, atopy, and use of airway medication. P-values were from Wald's test. ACO: asthma-COPD overlap; CI: confidence interval; COPD: chronic obstructive pulmonary disease; FeNO: fraction of exhaled nitric oxide; OR: odds ratio.

Figure 7. Increased exhaled nitric oxide level and blood eosinophil count favouring more asthma, COPD, or ACO.

Panel A: Illustrates the separate association analyses. *Panel B:* Illustrates the combined association analyses. Logistic regression models were used. Multivariable adjustment included age, sex, body

mass index, smoking status, smoking history, familial pre-disposition for COPD and asthma, atopy, and use of airway medication. P-values were from Wald's test. ACO: asthma-COPD overlap; CI: confidence interval; COPD: chronic obstructive pulmonary disease; FeNO: fraction of exhaled nitric oxide; OR: odds ratio.

5578 individuals from the second examination of the Copenhagen General Population Study

n=901 excluded based on

- Missing information on blood eosinophils or FeNO (*n=683*)
- Missing information on height (*n=2*)
- Missing information on tobacco smoking (*n=124*)
- Missing information on lung function (*n=92*)

No airflow limitation

n=3417

No asthma

n=3230

Asthma

n=187

Never-smokers

n=1649

Ever-smokers

n=1581

Asthma

n=449

Pre-bronchodilator FEV_1/FVC<0.70

n=1260

Asthma

Post-bronchodilator FEV_1/FVC ≥0.70

n=253

Missing information on lung function reversibility

n=456

Post-bronchodilator FEV_1/FVC<0.70

n=551

Asthma

FEV_1 reversibility >12% and >400 mL and <10 pack-years

n=9

FEV_1 reversibility ≥12% and ≥200 mL

n=71

ACO

No asthma

n=484

Asthma

n=67

COPD

n=404

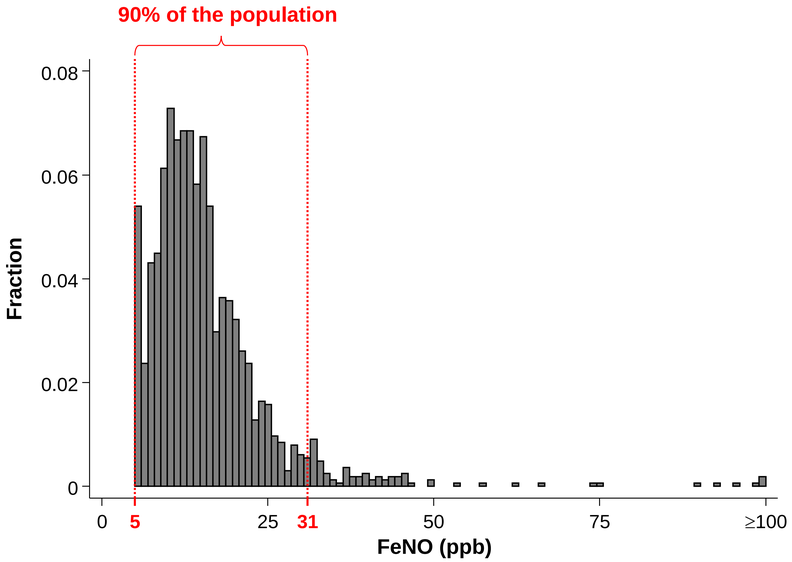
ACO

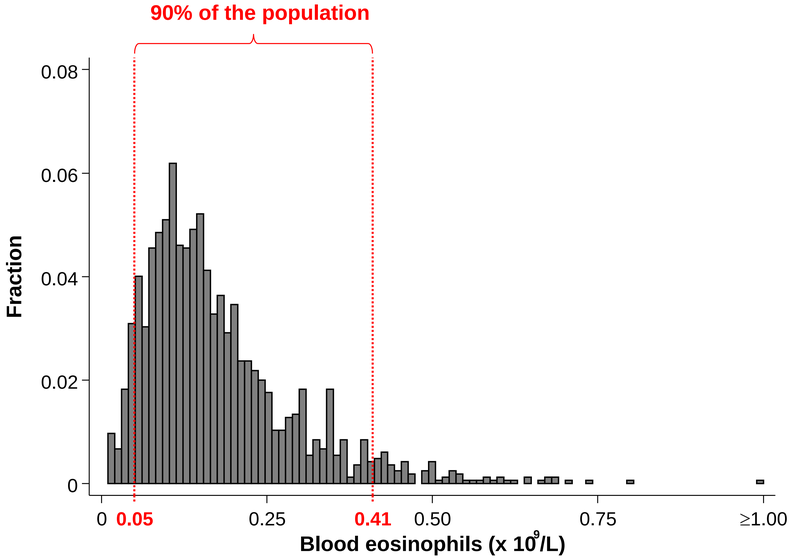
n=138

Non-specific AL

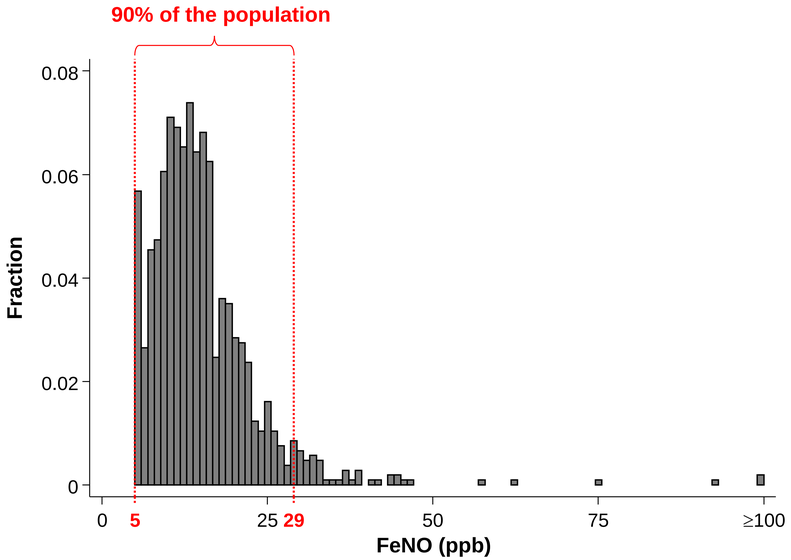
n=456

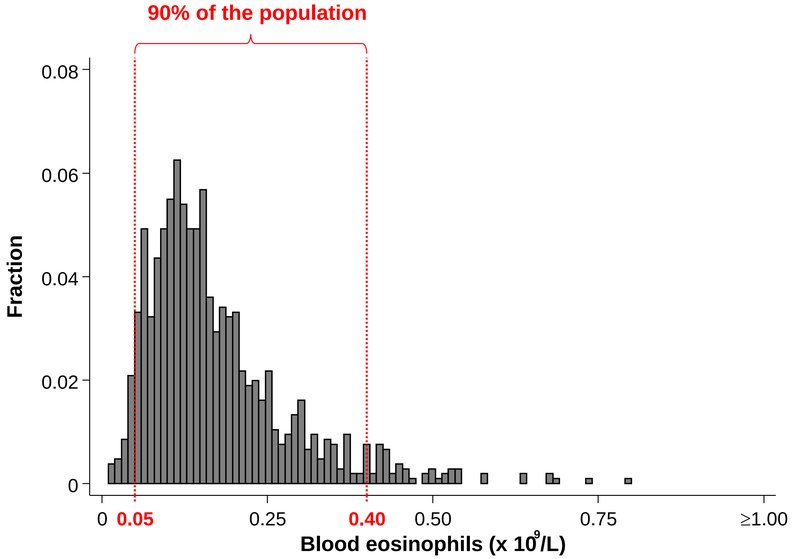
Never-smokers



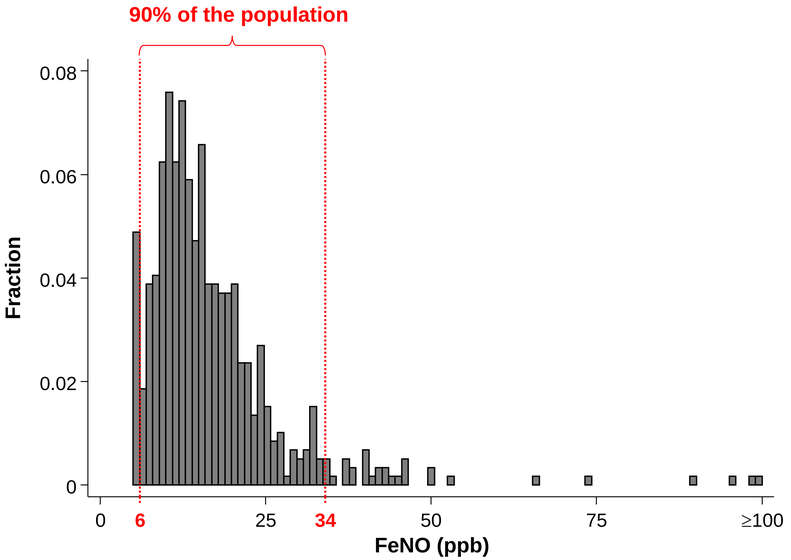


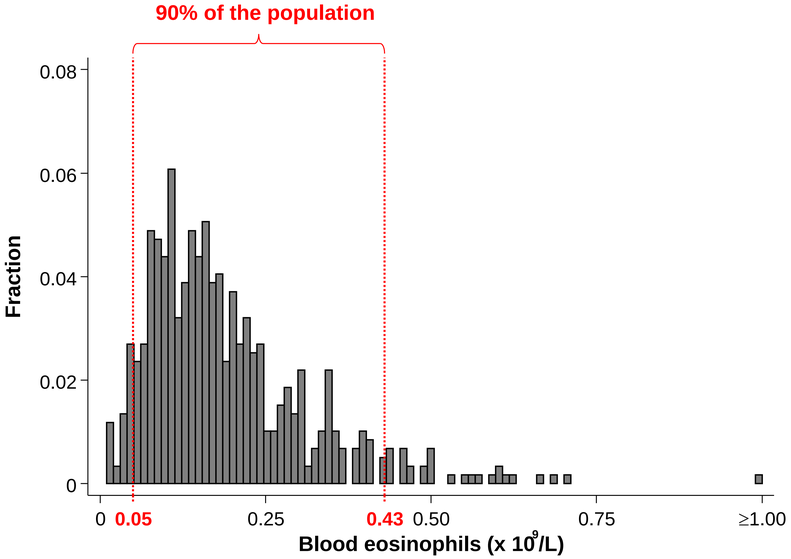
Never-smokers without atopy

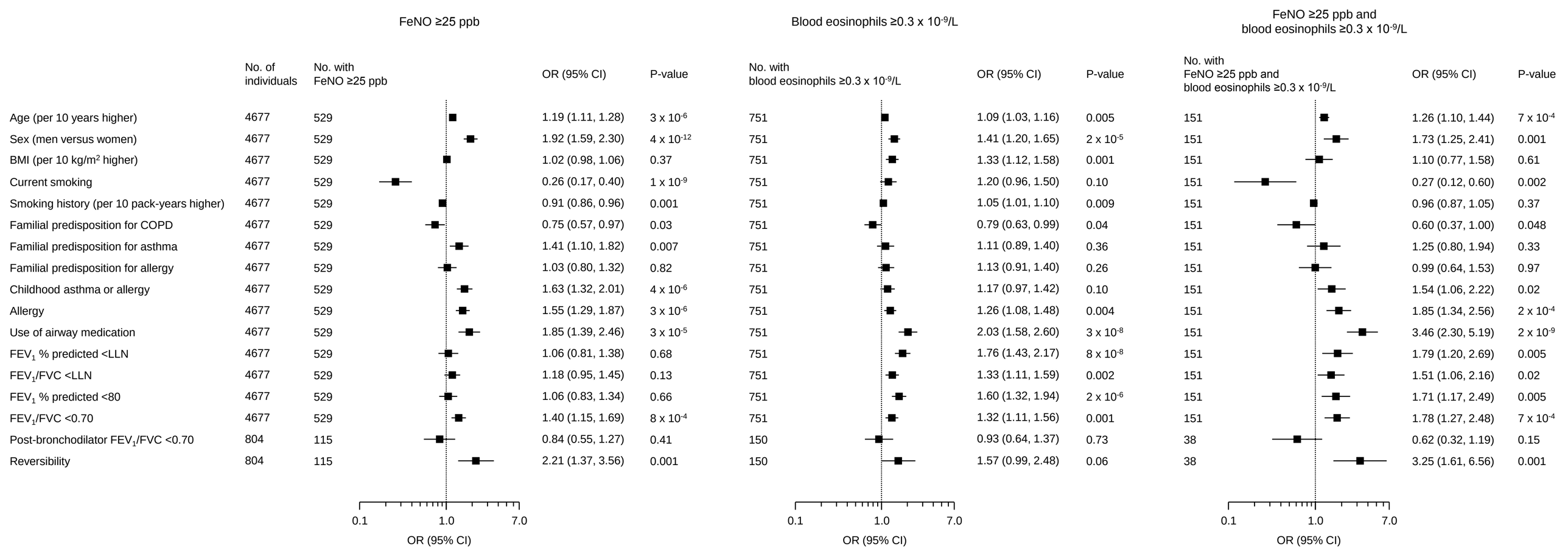




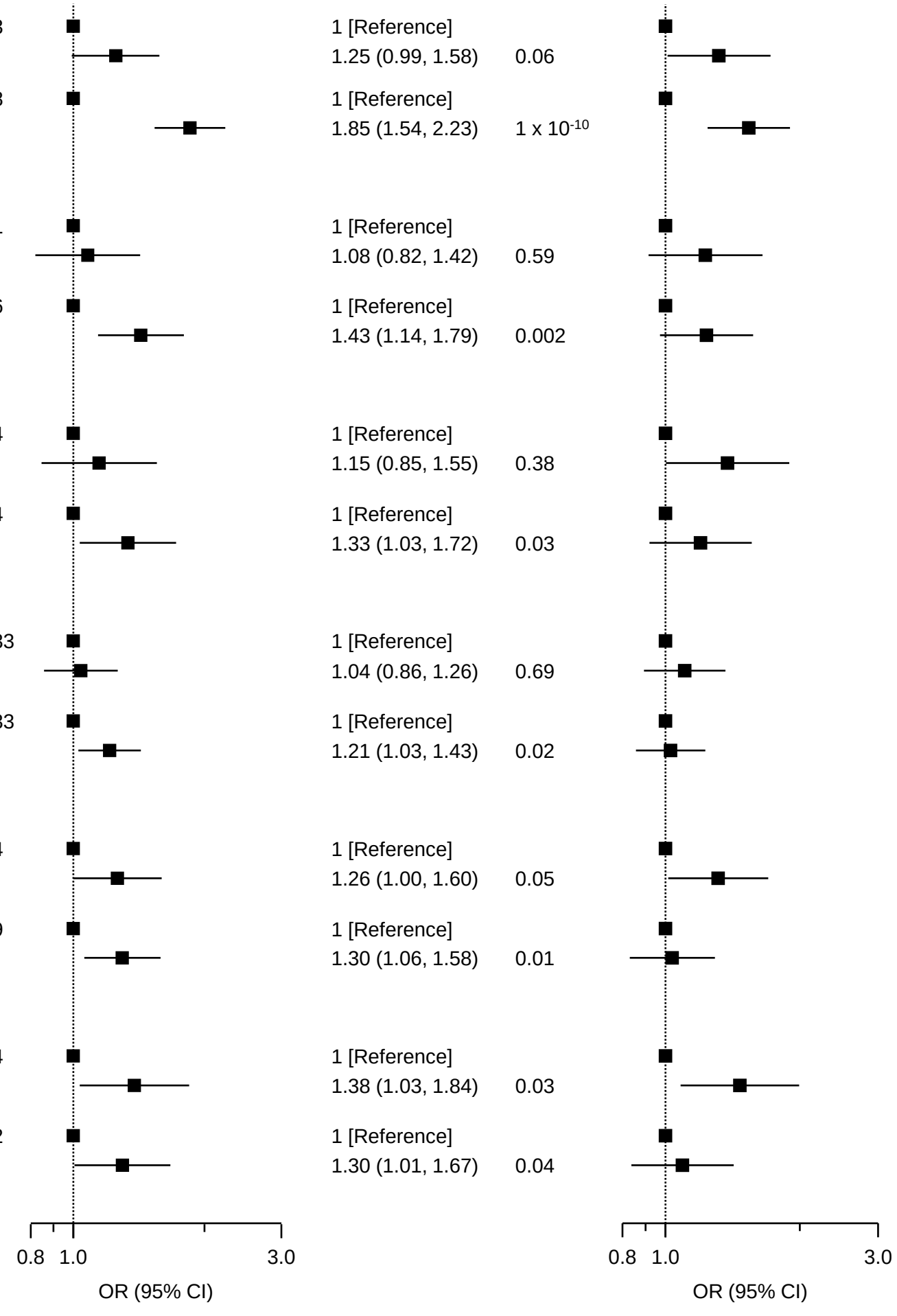
Never-smokers with atopy



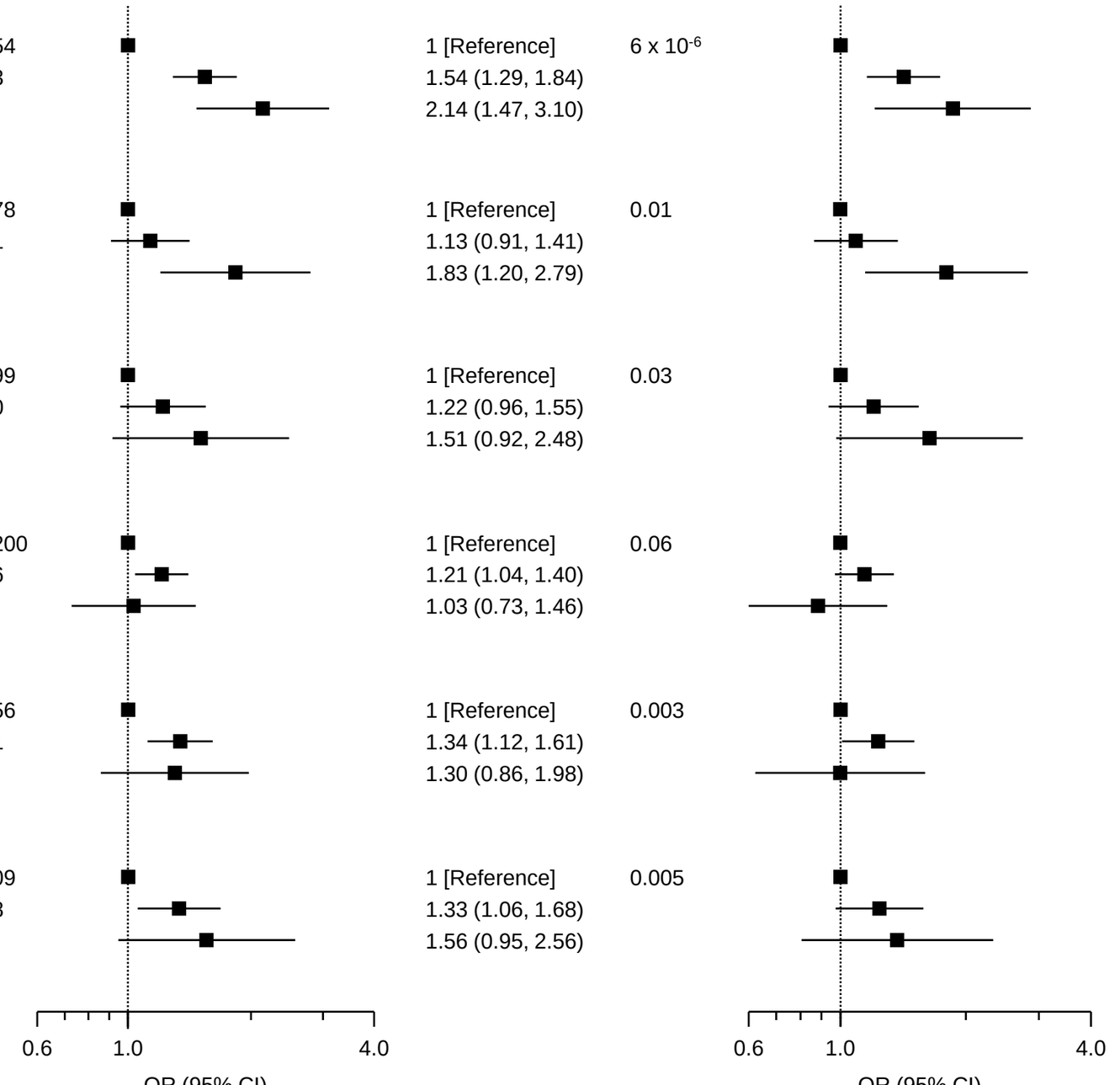



FeNO ≥25 ppb
Blood eosinophils ≥0.3 x 10-9/L
FeNO ≥25 ppb and
blood eosinophils ≥0.3 x 10-9/L
No. of individuals
No. with FeNO ≥25 ppb
OR (95% CI)
P-value
No. with blood eosinophils ≥0.3 x 10-9/L
OR (95% CI)
P-value
No. with FeNO ≥25 ppb and blood eosinophils ≥0.3 x 10-9/L
OR (95% CI)
P-value
Age (per 10 years higher) 4677 529 1.19 (1.11, 1.28) 3 x 10-6 751 1.09 (1.03, 1.16) 0.005 151 1.26 (1.10, 1.44) 7 x 10-4
Sex (men versus women) 4677 529 1.92 (1.59, 2.30) 4 x 10-12 751 1.41 (1.20, 1.65) 2 x 10-5 151 1.73 (1.25, 2.41) 0.001
BMI (per 10 kg/m2 higher) 4677 529 1.02 (0.98, 1.06) 0.37 751 1.33 (1.12, 1.58) 0.001 151 1.10 (0.77, 1.58) 0.61
Current smoking 4677 529 0.26 (0.17, 0.40) 1 x 10-9 751 1.20 (0.96, 1.50) 0.10 151 0.27 (0.12, 0.60) 0.002
Smoking history (per 10 pack-years higher) 4677 529 0.91 (0.86, 0.96) 0.001 751 1.05 (1.01, 1.10) 0.009 151 0.96 (0.87, 1.05) 0.37
Familial predisposition for COPD 4677 529 0.75 (0.57, 0.97) 0.03 751 0.79 (0.63, 0.99) 0.04 151 0.60 (0.37, 1.00) 0.048
Familial predisposition for asthma 4677 529 1.41 (1.10, 1.82) 0.007 751 1.11 (0.89, 1.40) 0.36 151 1.25 (0.80, 1.94) 0.33
Familial predisposition for allergy 4677 529 1.03 (0.80, 1.32) 0.82 751 1.13 (0.91, 1.40) 0.26 151 0.99 (0.64, 1.53) 0.97
Childhood asthma or allergy 4677 529 1.63 (1.32, 2.01) 4 x 10-6 751 1.17 (0.97, 1.42) 0.10 151 1.54 (1.06, 2.22) 0.02
Allergy 4677 529 1.55 (1.29, 1.87) 3 x 10-6 751 1.26 (1.08, 1.48) 0.004 151 1.85 (1.34, 2.56) 2 x 10-4
Use of airway medication 4677 529 1.85 (1.39, 2.46) 3 x 10-5 751 2.03 (1.58, 2.60) 3 x 10-8 151 3.46 (2.30, 5.19) 2 x 10-9
FEV1 % predicted <LLN 4677 529 1.06 (0.81, 1.38) 0.68 751 1.76 (1.43, 2.17) 8 x 10-8 151 1.79 (1.20, 2.69) 0.005
FEV1/FVC <LLN 4677 529 1.18 (0.95, 1.45) 0.13 751 1.33 (1.11, 1.59) 0.002 151 1.51 (1.06, 2.16) 0.02
FEV1 % predicted <80 4677 529 1.06 (0.83, 1.34) 0.66 751 1.60 (1.32, 1.94) 2 x 10-6 151 1.71 (1.17, 2.49) 0.005
FEV1/FVC <0.70 4677 529 1.40 (1.15, 1.69) 8 x 10-4 751 1.32 (1.11, 1.56) 0.001 151 1.78 (1.27, 2.48) 7 x 10-4
Post-bronchodilator FEV1/FVC <0.70 804 115 0.84 (0.55, 1.27) 0.41 150 0.93 (0.64, 1.37) 0.73 38 0.62 (0.32, 1.19) 0.15
Reversibility 804 115 2.21 (1.37, 3.56) 0.001 150 1.57 (0.99, 2.48) 0.06 38 3.25 (1.61, 6.56) 0.001
0.1
1.0
7.0
OR (95% CI)

	No. of individuals/cases	Age- and sex adjusted OR (95% CI)	Age- and sex adjusted P-value	Multivariable adjusted OR (95% CI)	Multivariable adjusted P-value
Wheezing					
FeNO <25 ppb	4148/703	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/105	1.25 (0.99, 1.58)	0.06	1.32 (1.01, 1.72)	0.04
Blood eosinophils <0.3 x 10^{-9}/L	3926/618	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/190	1.85 (1.54, 2.23)	1 x 10^{-10}	1.54 (1.24, 1.90)	8 x 10^{-5}
Sputum production					
FeNO <25 ppb	4148/461	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/67	1.08 (0.82, 1.42)	0.59	1.23 (0.91, 1.65)	0.17
Blood eosinophils <0.3 x 10^{-9}/L	3926/416	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/112	1.43 (1.14, 1.79)	0.002	1.23 (0.97, 1.57)	0.09
Chronic cough					
FeNO <25 ppb	4148/364	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/54	1.15 (0.85, 1.55)	0.38	1.38 (1.00, 1.89)	0.05
Blood eosinophils <0.3 x 10^{-9}/L	3926/334	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/84	1.33 (1.03, 1.72)	0.03	1.20 (0.92, 1.56)	0.18
Dyspnoea					
FeNO <25 ppb	4148/1433	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/185	1.04 (0.86, 1.26)	0.69	1.10 (0.89, 1.36)	0.36
Blood eosinophils <0.3 x 10^{-9}/L	3926/1333	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/285	1.21 (1.03, 1.43)	0.02	1.03 (0.86, 1.23)	0.78
Day-time symptoms					
FeNO <25 ppb	4148/674	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/102	1.26 (1.00, 1.60)	0.05	1.31 (1.01, 1.70)	0.04
Blood eosinophils <0.3 x 10^{-9}/L	3926/629	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/147	1.30 (1.06, 1.58)	0.01	1.03 (0.83, 1.29)	0.77
Night-time symptoms					
FeNO <25 ppb	4148/374	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/62	1.38 (1.03, 1.84)	0.03	1.47 (1.08, 1.99)	0.01
Blood eosinophils <0.3 x 10^{-9}/L	3926/352	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/84	1.30 (1.01, 1.67)	0.04	1.09 (0.84, 1.42)	0.52

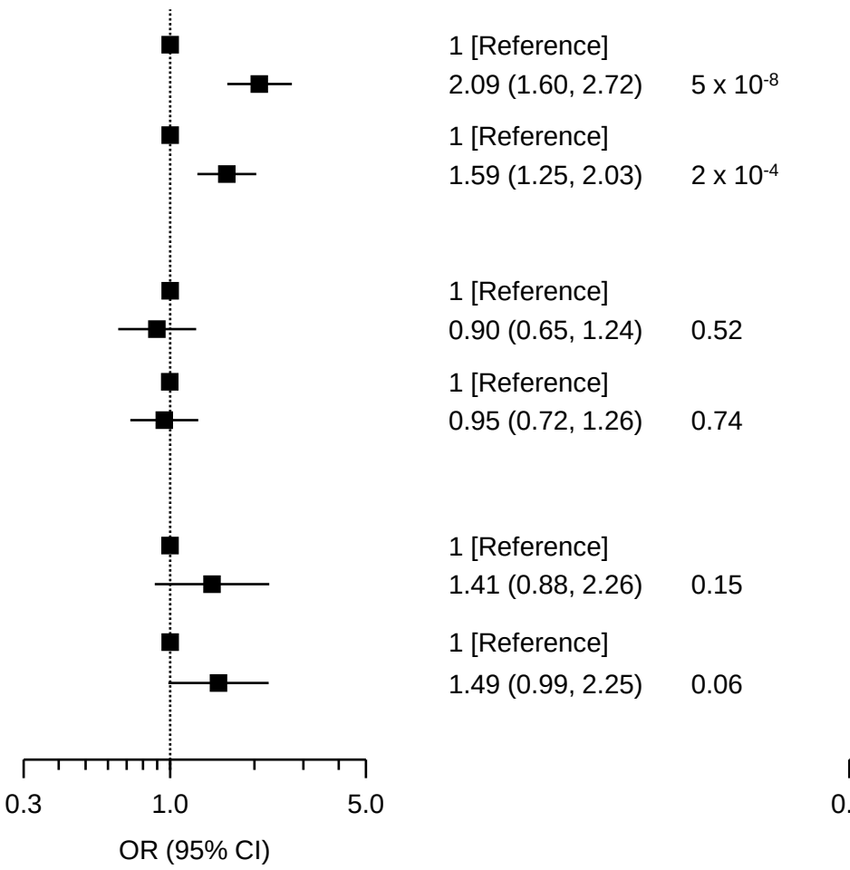


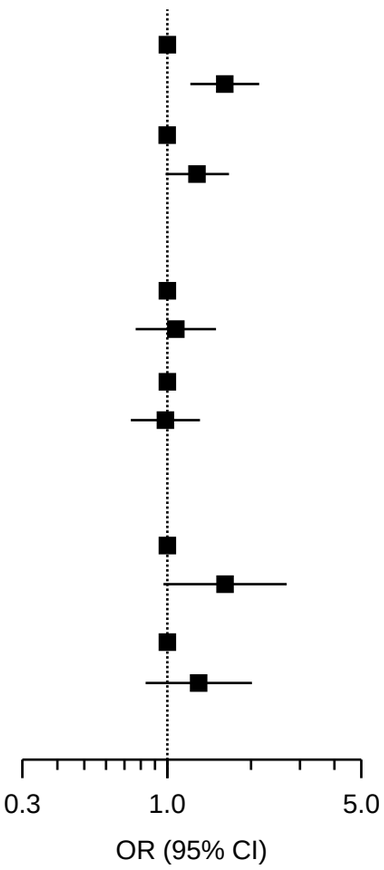
	Age- and sex adjusted			Multivariable adjusted		
	No. of Individuals/cases	OR (95% CI)	P-value for trend	OR (95% CI)	P-value for trend
Wheezing						
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/554	1 [Reference]	6 x 10^{-6}	1 [Reference]	4 x 10^{-5}
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/213	1.54 (1.29, 1.84)		1.42 (1.16, 1.73)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/41	2.14 (1.47, 3.10)		1.86 (1.21, 2.87)	
Sputum production					
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/378	1 [Reference]	0.01	1 [Reference]	0.04
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/121	1.13 (0.91, 1.41)		1.09 (0.86, 1.37)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/29	1.83 (1.20, 2.79)		1.80 (1.14, 2.82)	
Chronic cough					
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/299	1 [Reference]	0.03	1 [Reference]	0.03
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/100	1.22 (0.96, 1.55)		1.20 (0.93, 1.54)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/19	1.51 (0.92, 2.48)		1.64 (0.98, 2.75)	
Dyspnoea					
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/1200	1 [Reference]	0.06	1 [Reference]	0.45
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/366	1.21 (1.04, 1.40)		1.14 (0.97, 1.34)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/52	1.03 (0.73, 1.46)		0.88 (0.60, 1.29)	
Day-time symptoms					
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/556	1 [Reference]	0.003	1 [Reference]	0.15
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/191	1.34 (1.12, 1.61)		1.23 (1.01, 1.50)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/29	1.30 (0.86, 1.98)		1.00 (0.62, 1.59)	
Night-time symptoms					
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/309	1 [Reference]	0.005	1 [Reference]	0.05
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/108	1.33 (1.06, 1.68)		1.24 (0.97, 1.58)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/19	1.56 (0.95, 2.56)		1.37 (0.80, 2.33)	



A

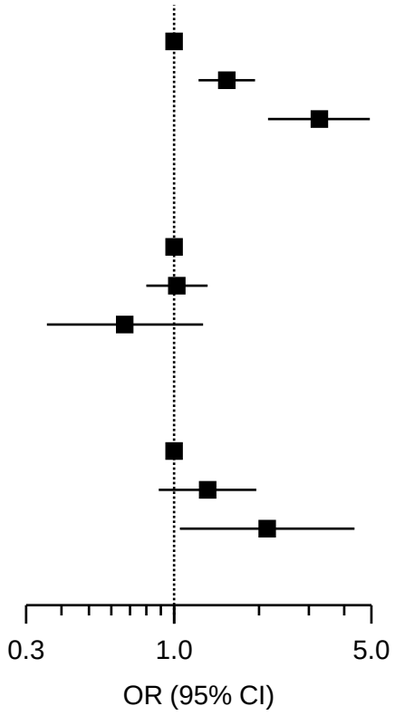
	No. of individuals/cases	Age- and sex adjusted OR (95% CI)	Age- and sex adjusted P-value	Multivariable adjusted OR (95% CI)	Multivariable adjusted P-value
Asthma					
FeNO <25 ppb	4148/368	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/81	2.09 (1.60, 2.72)	5×10^{-8}	1.61 (1.21, 2.15)	0.001
Blood eosinophils <0.3 x 10^{-9}/L	3926/352	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/97	1.59 (1.25, 2.03)	2×10^{-4}	1.28 (0.98, 1.66)	0.66
COPD					
FeNO <25 ppb	4148/355	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/49	0.90 (0.65, 1.24)	0.52	1.07 (0.77, 1.50)	0.69
Blood eosinophils <0.3 x 10^{-9}/L	3926/336	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/68	0.95 (0.72, 1.26)	0.74	0.98 (0.74, 1.31)	0.89
ACO					
FeNO <25 ppb	4148/116	1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	529/22	1.41 (0.88, 2.26)	0.15	1.61 (0.96, 2.69)	0.07
Blood eosinophils <0.3 x 10^{-9}/L	3926/107	1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	751/31	1.49 (0.99, 2.25)	0.06	1.29 (0.83, 2.01)	0.25

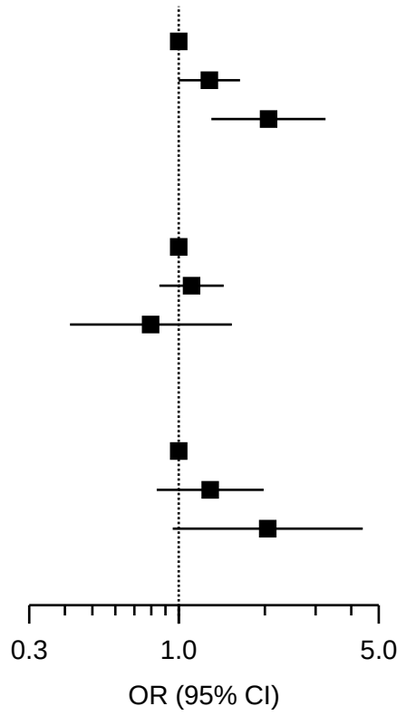




B

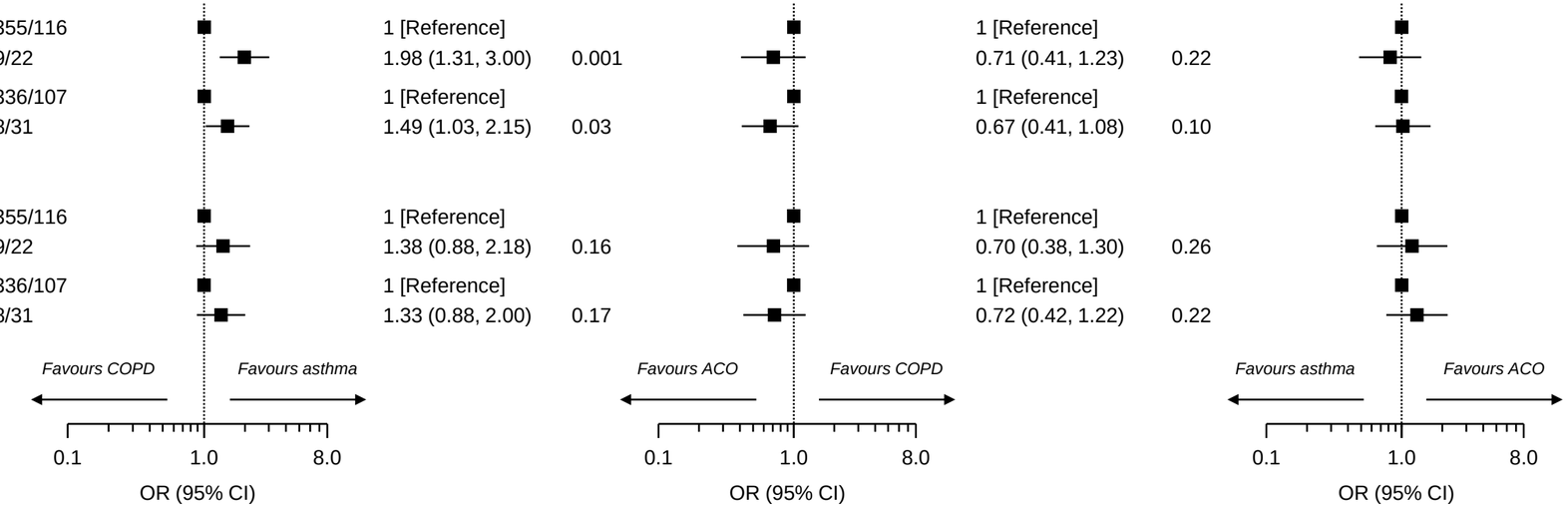
	No. of individuals/cases	Age- and sex adjusted OR (95% CI)	Age- and sex adjusted P-value for trend	Multivariable adjusted OR (95% CI)	Multivariable adjusted P-value for trend
Asthma					
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/303	1 [Reference]	1×10^{-9}	1 [Reference]	0.001
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/114	1.54 (1.22, 1.94)		1.28 (1.00, 1.64)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/32	3.26 (2.16, 4.94)		2.06 (1.30, 3.25)	
COPD					
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/298	1 [Reference]	0.52	1 [Reference]	0.88
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/95	1.03 (0.80, 1.32)		1.11 (0.86, 1.43)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/11	0.67 (0.36, 1.27)		0.80 (0.42, 1.53)	
ACO					
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	3548/94	1 [Reference]	0.02	1 [Reference]	0.05
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	978/35	1.32 (0.88, 1.96)		1.29 (0.84, 1.98)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	151/9	2.14 (1.05, 4.36)		2.04 (0.95, 4.39)	





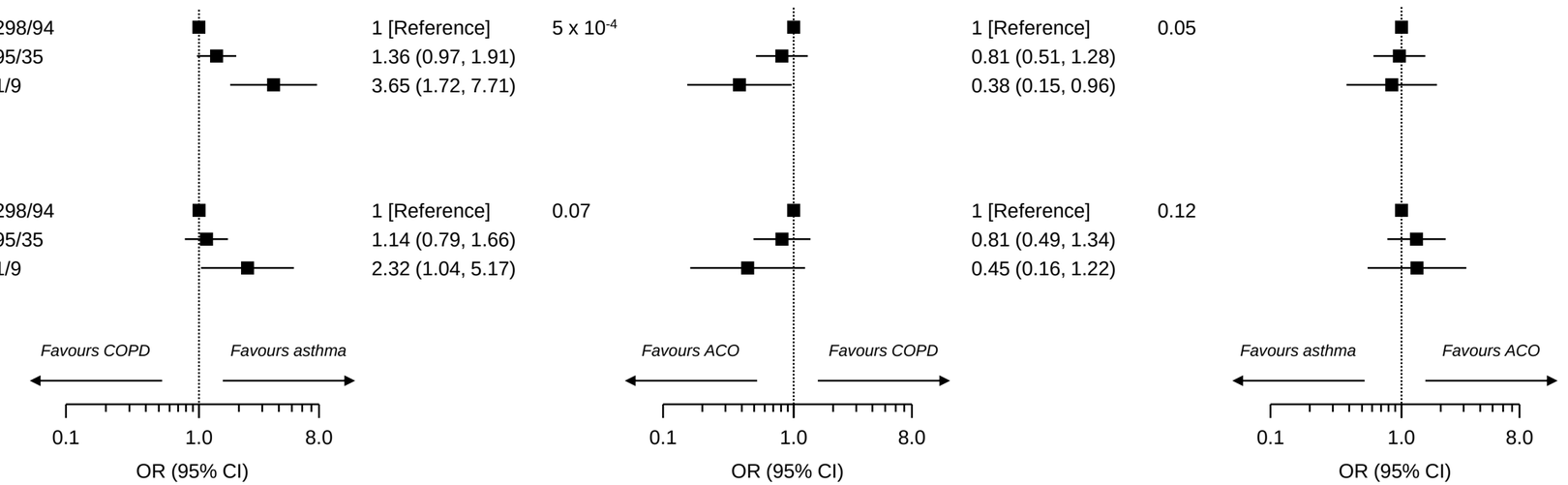
A

	No. of asthma/COPD/ACO	Asthma versus COPD: OR (95% CI)	P-value	COPD versus ACO: OR (95% CI)	P-value	ACO versus asthma: OR (95% CI)	P-value
Age- and sex adjusted							
FeNO <25 ppb	368/355/116	1 [Reference]		1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	81/49/22	1.98 (1.31, 3.00)	0.001	0.71 (0.41, 1.23)	0.22	0.82 (0.48, 1.39)	0.47
Blood eosinophils <0.3 x 10^{-9}/L	352/336/107	1 [Reference]		1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	97/68/31	1.49 (1.03, 2.15)	0.03	0.67 (0.41, 1.08)	0.10	1.02 (0.64, 1.63)	0.93
Multivariable adjusted							
FeNO <25 ppb	368/355/116	1 [Reference]		1 [Reference]		1 [Reference]	
FeNO ≥25 ppb	81/49/22	1.38 (0.88, 2.18)	0.16	0.70 (0.38, 1.30)	0.26	1.20 (0.66, 2.17)	0.56
Blood eosinophils <0.3 x 10^{-9}/L	352/336/107	1 [Reference]		1 [Reference]		1 [Reference]	
Blood eosinophils ≥0.3 x 10^{-9}/L	97/68/31	1.33 (0.88, 2.00)	0.17	0.72 (0.42, 1.22)	0.22	1.30 (0.77, 2.19)	0.32



B

	No. of asthma/COPD/ACO	Asthma versus COPD: OR (95% CI)	P-value for trend	COPD versus ACO: OR (95% CI)	P-value for trend	ACO versus asthma: OR (95% CI)	P-value for trend
Age- and sex adjusted							
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	303/298/94	1 [Reference]	5 x 10^{-4}	1 [Reference]	0.05	1 [Reference]	0.70
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	114/95/35	1.36 (0.97, 1.91)		0.81 (0.51, 1.28)		0.97 (0.61, 1.53)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	32/11/9	3.65 (1.72, 7.71)		0.38 (0.15, 0.96)		0.84 (0.38, 1.87)	
Multivariable adjusted							
FeNO <25 ppb and blood eosinophils <0.3 x 10^{-9}/L	303/298/94	1 [Reference]	0.07	1 [Reference]	0.12	1 [Reference]	0.30
FeNO ≥25 ppb **or** blood eosinophils ≥0.3 x 10^{-9}/L	114/95/35	1.14 (0.79, 1.66)		0.81 (0.49, 1.34)		1.31 (0.78, 2.19)	
FeNO ≥25 ppb **and** blood eosinophils ≥0.3 x 10^{-9}/L	32/11/9	2.32 (1.04, 5.17)		0.45 (0.16, 1.22)		1.32 (0.55, 3.14)	



Supplement

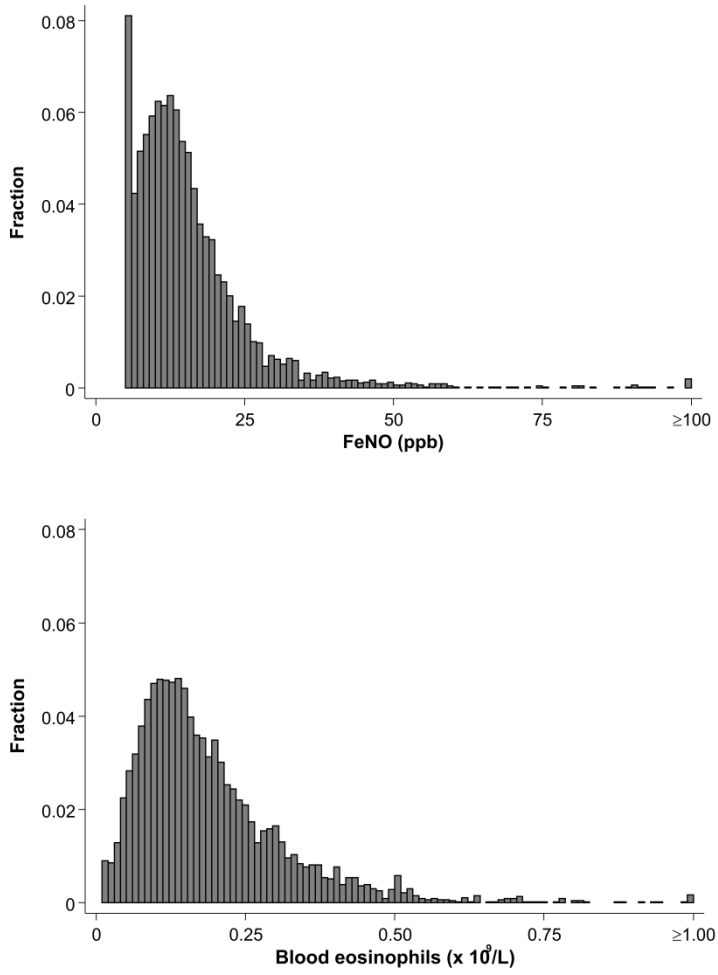


Figure S1. Distribution of FeNO levels and blood eosinophil counts in the Copenhagen General Population Study. FeNO = fraction of exhaled nitric oxide.

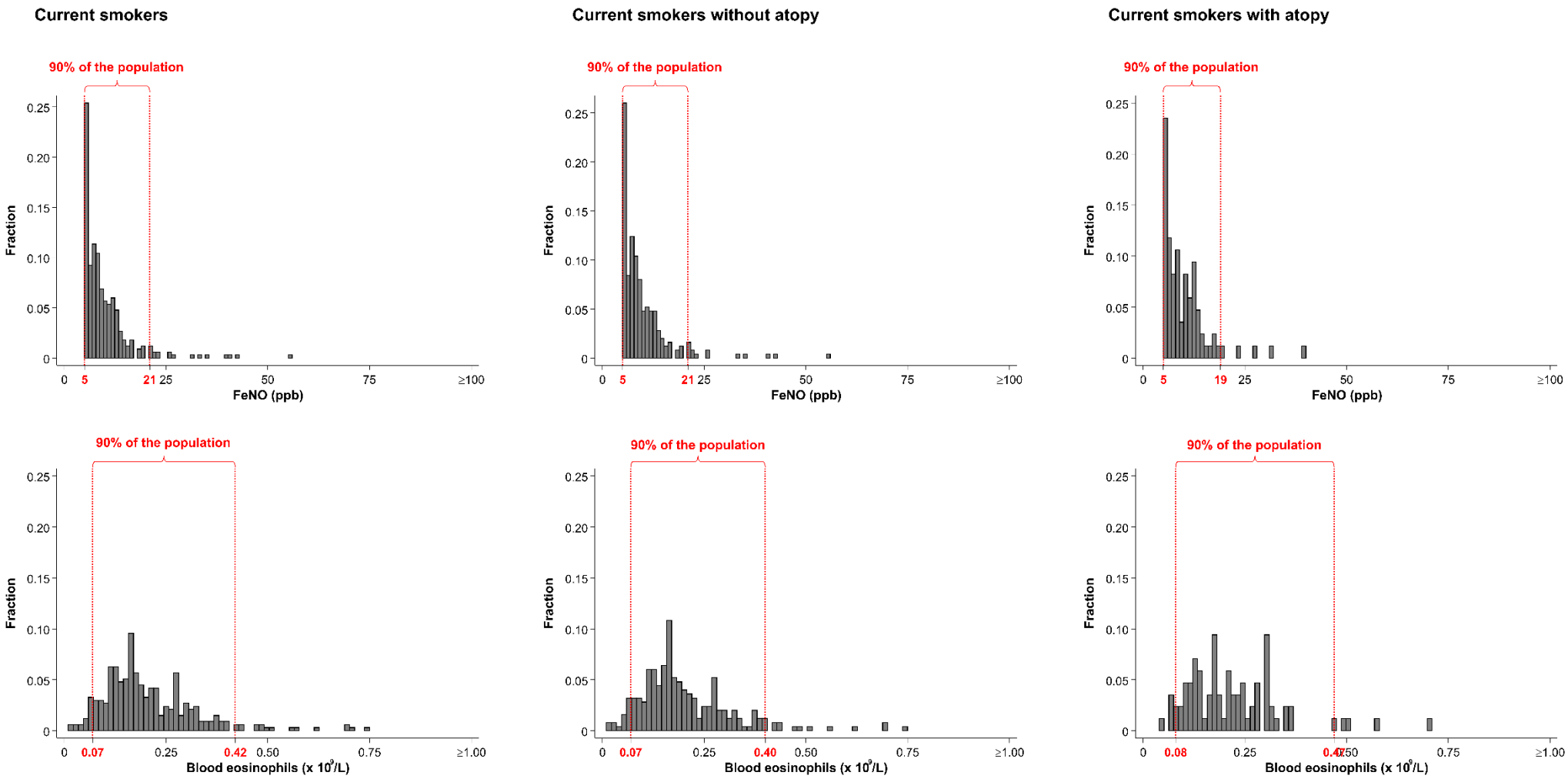


Figure S2. Distribution of FeNO levels and blood eosinophil counts in healthy current smokers with and without atopy. FeNO = fraction of exhaled nitric oxide.

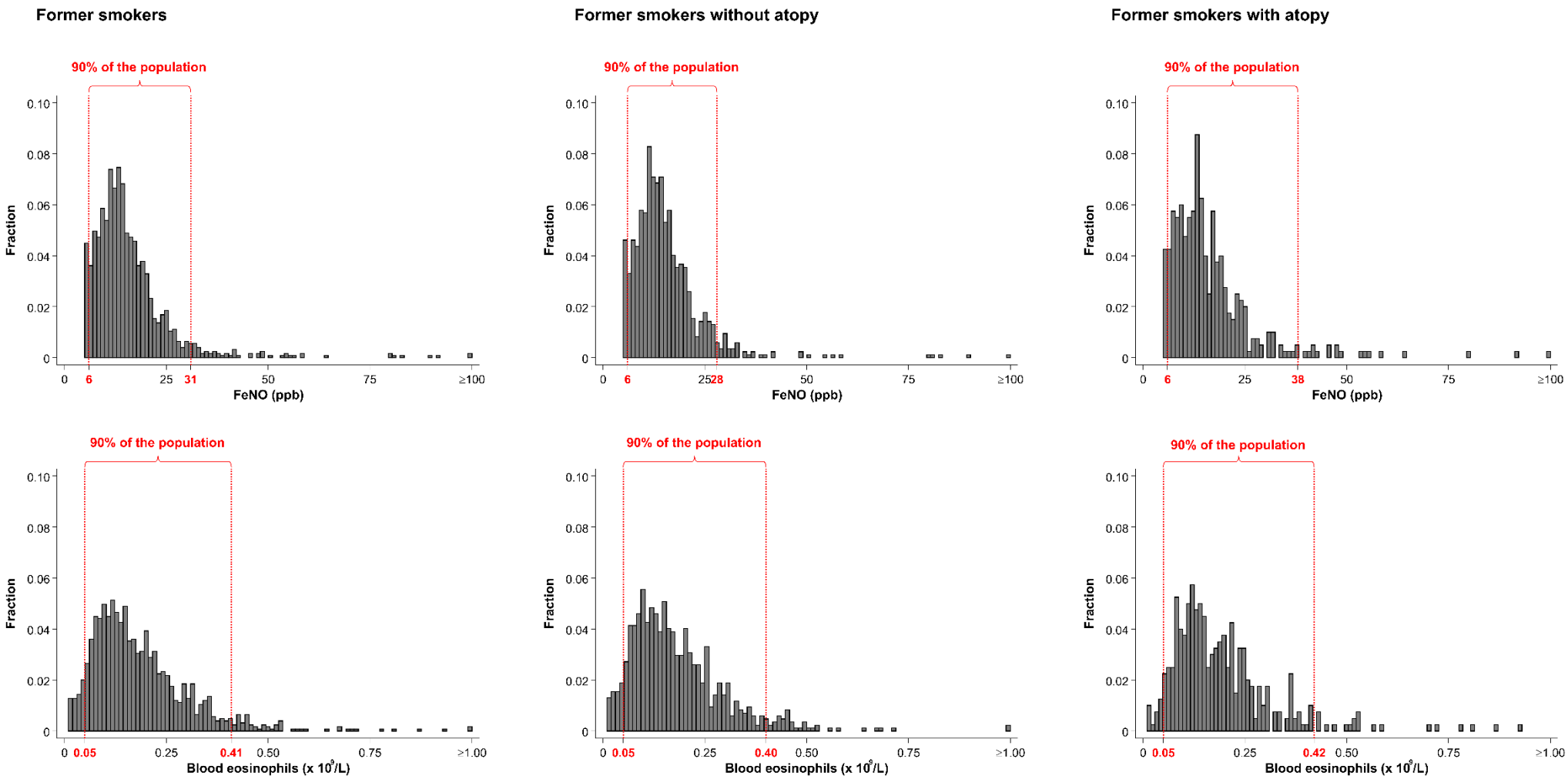


Figure S3. Distribution of FeNO levels and blood eosinophil counts in healthy former smokers with and without atopy. FeNO = fraction of exhaled nitric oxide.

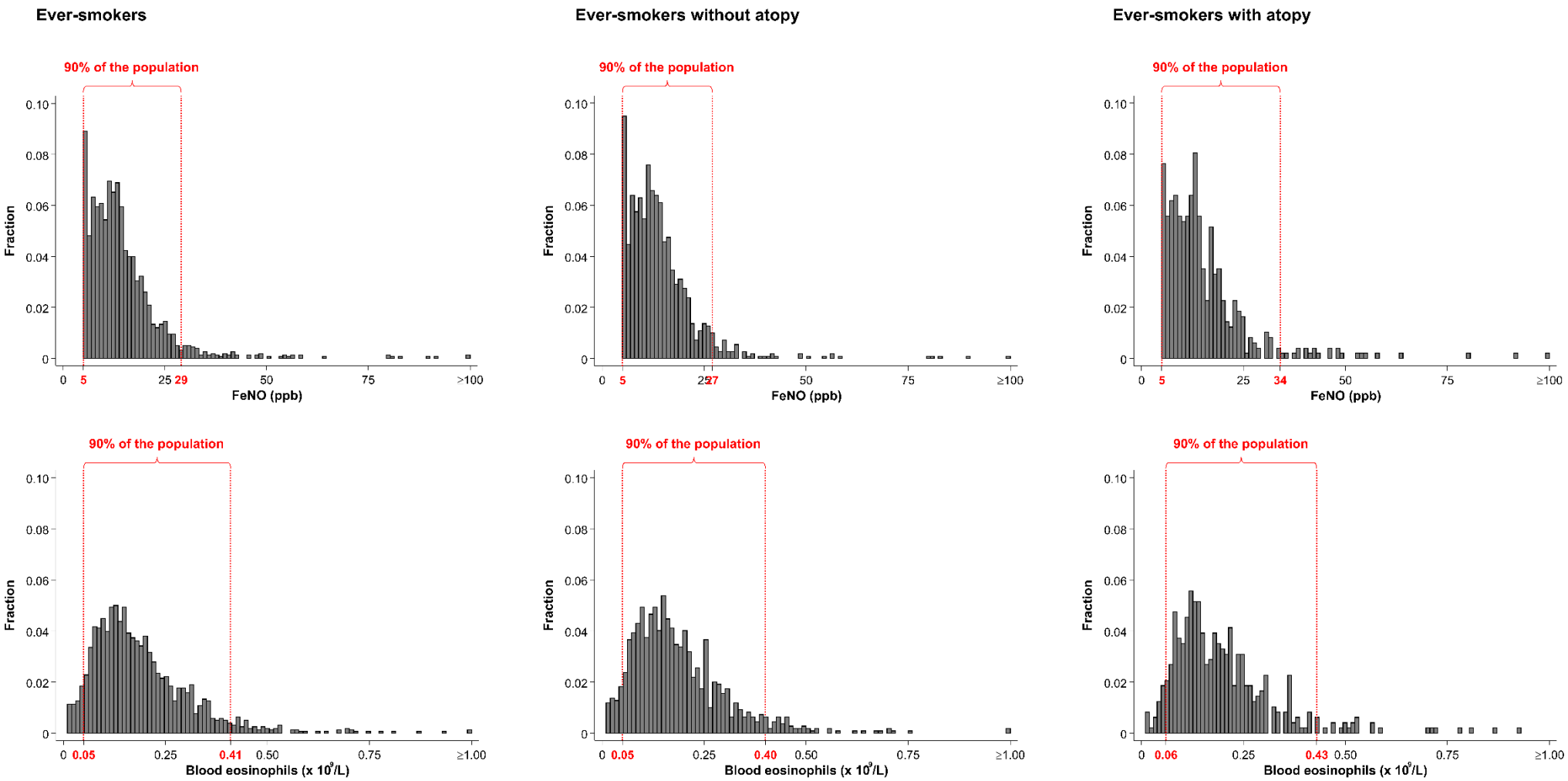


Figure S4. Distribution of FeNO levels and blood eosinophil counts in healthy ever-smokers with and without atopy. FeNO = fraction of exhaled nitric oxide.

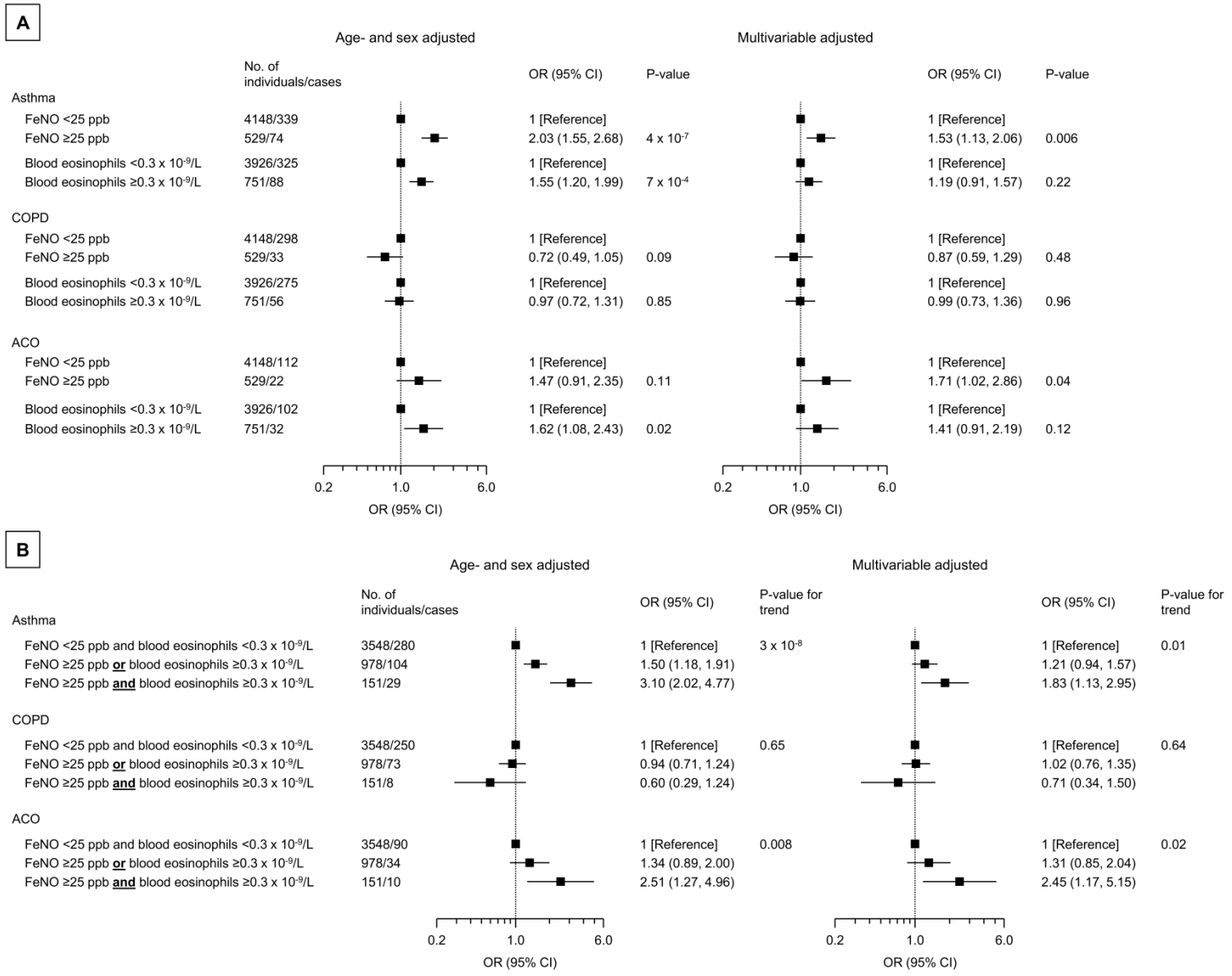


Figure S5. Association of increased exhaled nitric oxide level and blood eosinophil count with asthma, COPD, and ACO. The clinical groups of chronic airway disease were defined according to forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) <lower limit of normal (LLN). *Panel A:* Illustrates the separate association analyses. *Panel B:* Illustrates the combined association analyses. Logistic regression models were used. Multivariable adjustment included age, sex, body mass index, smoking status, smoking history, familial predisposition for COPD and asthma, atopy, and use of airway medication. P-values were from Wald's test. ACO: asthma-COPD overlap; CI: confidence interval; COPD: chronic obstructive pulmonary disease; FeNO: fraction of exhaled nitric oxide; OR: odds ratio.