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

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

We included 4677 individuals aged 20–100 years 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 nonspecific airflow limitation (n=456).

Compared to individuals with F_{ENO} <25 ppb and blood eosinophils $<0.3\times10^9$ cells·L^{-1}, age- and sex-adjusted odds ratios (95% CI) for wheezing were 1.54 (1.29–1.84) for individuals with F_{ENO} ⩾25 ppb or blood eosinophils $\geqslant0.3\times10^9$ cells·L^{-1} and 2.14 (1.47–3.10) for individuals with F_{ENO} ⩾25 ppb and blood eosinophils $\geqslant0.3\times10^9$ cells·L^{-1}. Corresponding odds ratios 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 F_{ENO} 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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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 (F_{ENO}) level and blood eosinophil count have been suggested as biomarkers to determine and quantify the degree of eosinophilic airway inflammation [3]. Although F_{ENO} 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 T-helper cell cytokine-driven inflammation [4–9]. Therefore, it has been suggested that they be used 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 F_{ENO} 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 were 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, ~25% of those 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 (Herlev, Denmark) 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

F_{ENO} levels in the expiratory volume were 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 F_{ENO} 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 F_{ENO} levels in the airways [14], measurement of F_{ENO} was always performed before spirometry and reversibility testing. Healthcare professionals were trained on proper use of standard operating procedures in the measurement of F_{ENO} and certified on three occasions by more experienced healthcare professionals. Maintenance of the apparatus was undertaken regularly, as recommended by the manufacturer. A F_{ENO} 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 using the ADVIA 120 Hematology System (Siemens Healthcare, Munich, Germany). Analyses were subjected to daily precision testing using internal quality control material and monthly accuracy testing using an external control quality programme [12]. Blood eosinophil counts were reported in $\times 10^9$ cells·L^{-1} together with other leukocyte subpopulations, and percentage of total white blood cell count was calculated. A blood eosinophil count $<0.3\times10^9$ cells·L^{-1} was considered normal and $\geqslant 0.3\times10^9$ cells·L^{-1} increased, as the 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 tobacco consumed, we calculated smoking history (cumulative tobacco consumption) in pack-years for former and current smokers; 1 pack-year corresponded to 20 cigarettes or equivalent (*e.g.* cheroots, cigars, pipe), smoked daily for 1 year.

Spirometry was performed using an EasyOne Spirometer (ndd Medical Technologies, Zurich, Switzerland) in a standing position without the use of a nose-clip. Prebronchodilator forced expiratory volume in 1 s (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 <5% and a correct visual inspection of the spirometry curves. Spirometry use in the CGPS has undergone a rigorous validation process [19]. Individuals with presence of airflow limitation, defined as a prebronchodilator FEV_1/FVC ratio <0.70 were additionally asked to undergo reversibility testing: postbronchodilator FEV_1 and FVC were measured using the same procedures 15 min after inhalation of 400 µg salbutamol, a β_2-agonist, from a dry powder inhaler (Ventoline Diskus; GlaxoSmithKline, Brentford, UK). Percentage 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

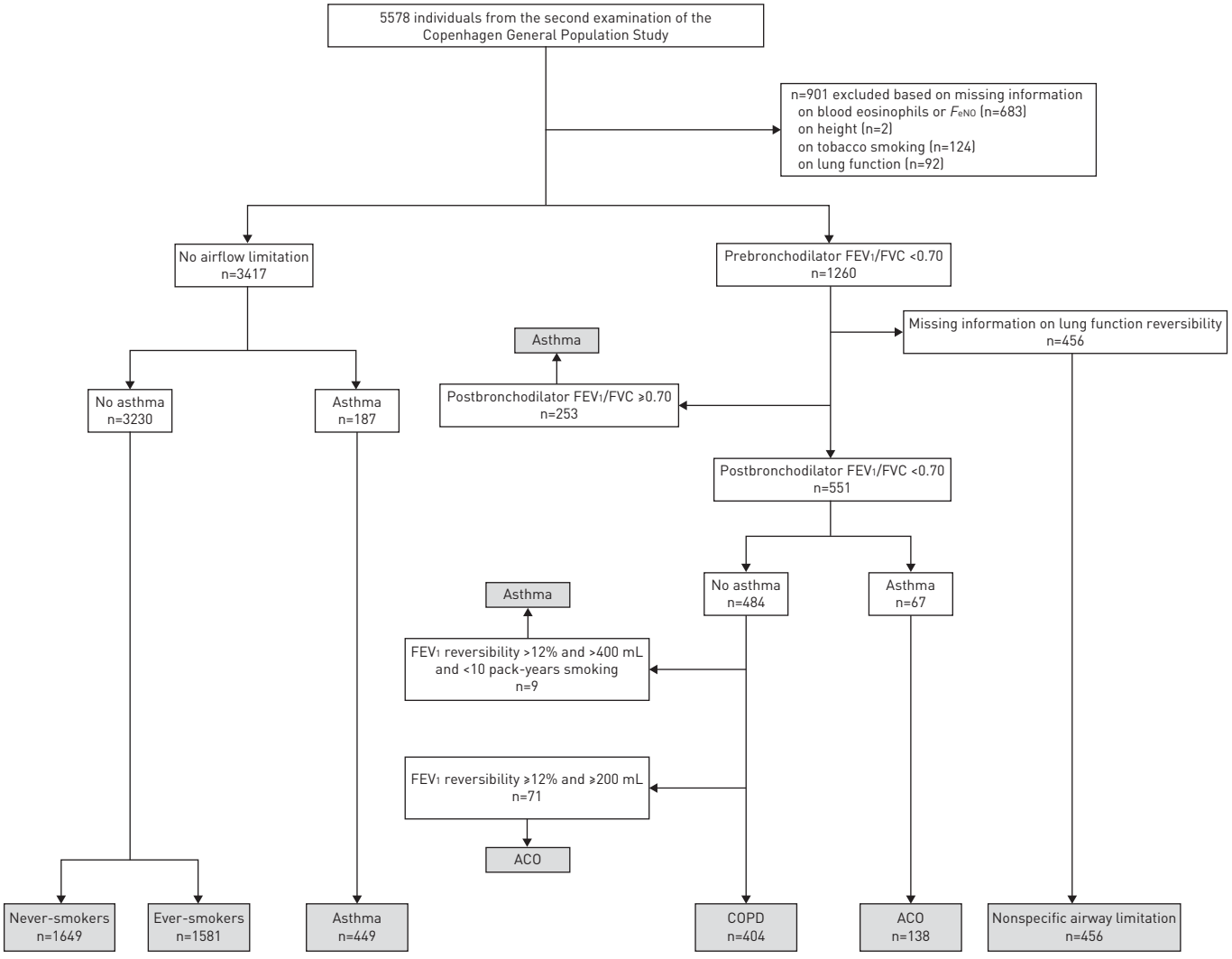

FIGURE 1 Flow chart of the study population. F_{ENO}: exhaled nitric oxide fraction; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; ACO: asthma–chronic obstructive pulmonary disease (COPD) overlap.

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

	Never-smokers	Ever-smokers	Asthma	COPD	ACO	Nonspecific airflow limitation
Subjects	1649	1581	449	404	138	456
Age years	58 (49–68)	60 (51–68)	60 (51–70)	68 (60–75)	68 (59–75)	67 (59–74)
Male	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$_1$ % pred	102 (93–110)	99 (90–108)	94 (85–104)	81 (69–94)	68 (55–79)	85 (71–97)
FVC % pred	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	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 to COPD[¶]	250 (15)	265 (17)	74 (16)	87 (22)	46 (33)	88 (19)
Familial predisposition to asthma[¶]	208 (13)	201 (13)	106 (24)	53 (13)	32 (23)	71 (16)
Familial predisposition to allergy[¶]	315 (19)	246 (16)	99 (22)	41 (10)	17 (12)	61 (13)
Childhood asthma or allergy	315 (19)	253 (16)	137 (31)	68 (17)	31 (22)	92 (20)
Allergy	593 (36)	485 (31)	239 (53)	110 (27)	63 (46)	153 (34)
Use of airway medication	16 (1)	18 (1)	138 (31)	50 (12)	61 (44)	74 (16)
Respiratory symptoms						
Wheezing	138 (8)	247 (16)	148 (33)	104 (26)	66 (48)	105 (23)
Sputum production	87 (5)	177 (11)	78 (17)	77 (19)	36 (26)	73 (16)
Chronic cough	76 (5)	138 (9)	60 (13)	64 (16)	26 (19)	54 (12)
Dyspnoea	397 (24)	525 (33)	202 (45)	217 (54)	79 (57)	198 (43)
Daytime symptoms	142 (9)	219 (14)	135 (30)	120 (30)	54 (39)	106 (23)
Night-time symptoms	100 (6)	133 (8)	74 (16)	46 (11)	29 (21)	54 (12)
Degree of airflow limitation						
FEV$_1$ ⩾80% pred	1561 (95)	1441 (91)	379 (84)	217 (54)	32 (23)	265 (58)
FEV$_1$ 50–79% pred	85 (5)	137 (9)	68 (15)	159 (39)	81 (59)	162 (36)
FEV$_1$ 30–49% pred	2 (<1)	2 (<1)	2 (<1)	26 (6)	24 (17)	23 (5)
FEV$_1$ <30% pred	1 (<1)	1 (<1)	0 (0)	2 (<1)	1 (<1)	6 (1)
Levels of inflammatory biomarkers						
Blood eosinophils ×10^9 cells·L^{-1}	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)
F$_{ENO}$ ppb	13 (10–19)	12 (8–17)	14 (9–21)	12 (8–18)	12 (6–21)	13 (8–19)

Data are presented as n, median (25th and 75th percentile) or n (%), unless otherwise stated. COPD: chronic obstructive pulmonary disease; ACO: asthma–COPD overlap; BMI: body mass index; FEV$_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; *F*$_{ENO}$: exhaled nitric oxide fraction; NA: not applicable. [#]: only for former and current smokers; [¶]: included the answer: "do not know".

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 SD.

In total, 4677 individuals were available with sufficient information on relevant measurements, of whom 1260 had prebronchodilator airflow limitation with FEV$_1$/FVC <0.70. Among these individuals, 456 declined to perform a reversibility test and, therefore, lacked information on postbronchodilator values. We subdivided participants into the following six groups (figure 1). 1) Healthy never-smokers: never-smokers with prebronchodilator FEV$_1$/FVC ⩾0.70 and no self-reported asthma; 2) healthy ever-smokers: former and current smokers with prebronchodilator FEV$_1$/FVC ⩾0.70 and no self-reported asthma; 3) asthma: individuals with prebronchodilator FEV$_1$/FVC ⩾0.70 and self-reported asthma, or with prebronchodilator FEV$_1$/FVC <0.70 and postbronchodilator FEV$_1$/FVC ⩾0.70, or with pre- and postbronchodilator FEV$_1$/FVC <0.70 and who, despite no self-reported asthma have FEV$_1$ reversibility of >12% and >400 mL and <10 pack-years of smoking history; 4) COPD: individuals with pre- and postbronchodilator 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

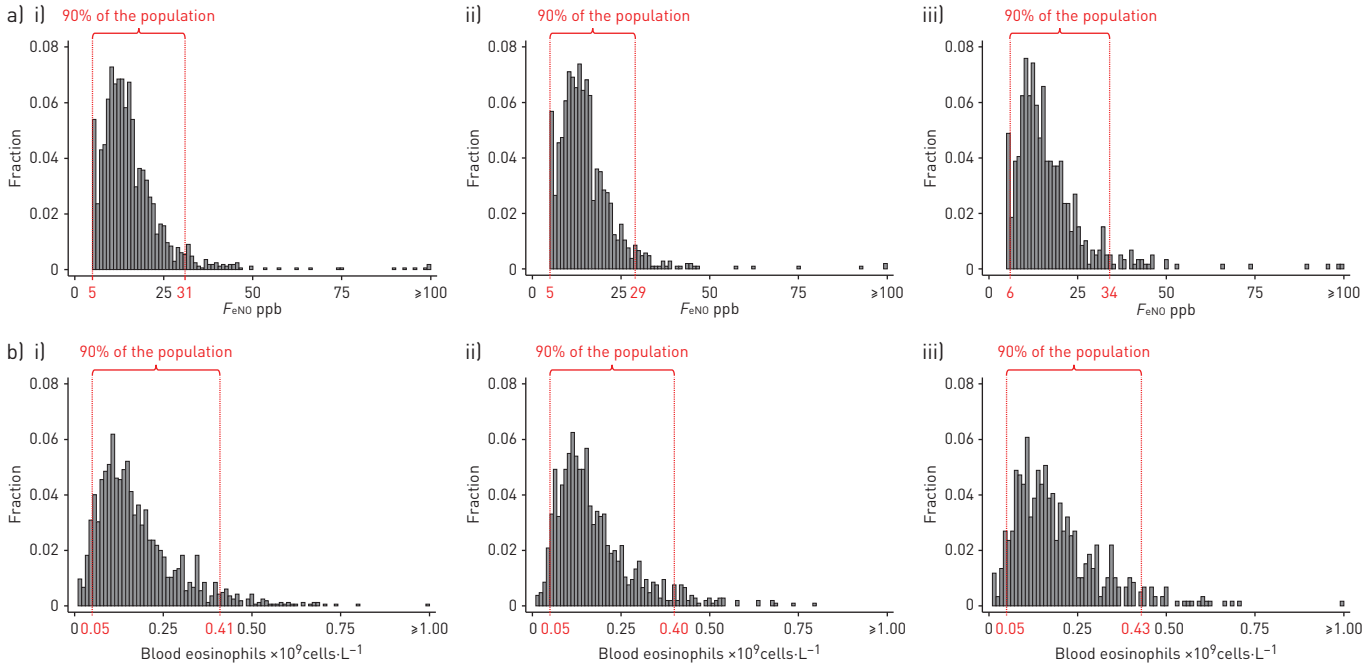

FIGURE 2 Distribution of a) exhaled nitric oxide fraction (F_{ENO}) levels and b) blood eosinophil counts in i) healthy never-smokers ii) without and iii) with atopy.

self-reported asthma or with pre- and postbronchodilator FEV1/FVC <0.70 and who, despite no self-reported asthma have FEV1 reversibility of ⩾12% and ⩾200 mL; 6) nonspecific airflow limitation: individuals with prebronchodilator FEV1/FVC <0.70 and no reversibility testing.

Other information

Body mass index (BMI) was calculated as measured weight divided by measured height squared ($kg \cdot m^{-2}$). Familial predisposition was defined as at least one first-degree relative (father, mother and/or sibling) with the condition in question. Allergy was defined if the participants reported to have allergy for different allergens (*i.e.* mould fungus, pollens from trees, grasses or weeds, dust mites, pets or other allergens) or asthma, hay fever or eczema as a reaction to food, medications, grass, flowers, animal hair or other allergens. In addition, information on childhood asthma or allergy was 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 for three consecutive months each year. Chronic cough was cough lasting >8 weeks. Dyspnoea was shortness of breath during different levels of activity, at night-time and/or while seated/at rest. Individuals were asked whether they had respiratory symptoms during the day or at night.

Statistical analyses

Statistical analyses were performed using STATA/SE 13.1 for Windows (StataCorp, College Station, TX, USA). 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 F_{ENO} level and blood eosinophil count were investigated. Second, associations of an increased F_{ENO} 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 nonspecific airflow limitation (figure 1). Individuals with COPD, ACO and nonspecific airflow limitation were older compared to the other groups (table 1).

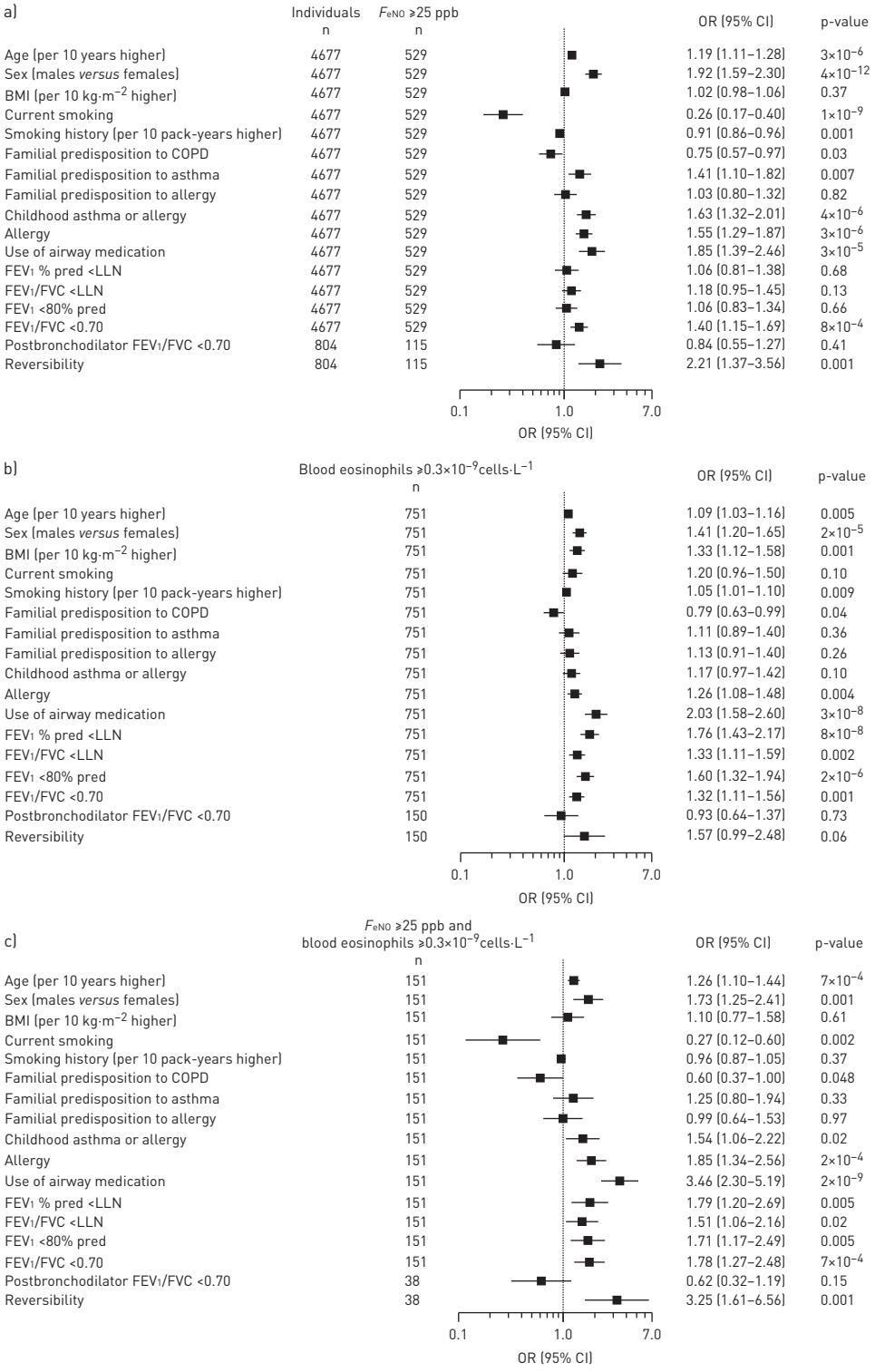

FIGURE 3 Clinical attributes associated with a) increased exhaled nitric oxide fraction (F_{ENO}; ⩾25 ppb); b) increased blood eosinophil count (⩾0.3×10^9 cells·L^{-1}); and c) combined value. Reversibility was defined as forced expiratory volume in 1 s (FEV_1) reversibility of ⩾12% and ⩾200 mL. Logistic regression models were used. Estimates are unadjusted. p-values were from Wald's test. BMI: body mass index; COPD: chronic obstructive pulmonary disease; LLN: lower limit of normal; FVC: forced vital capacity.

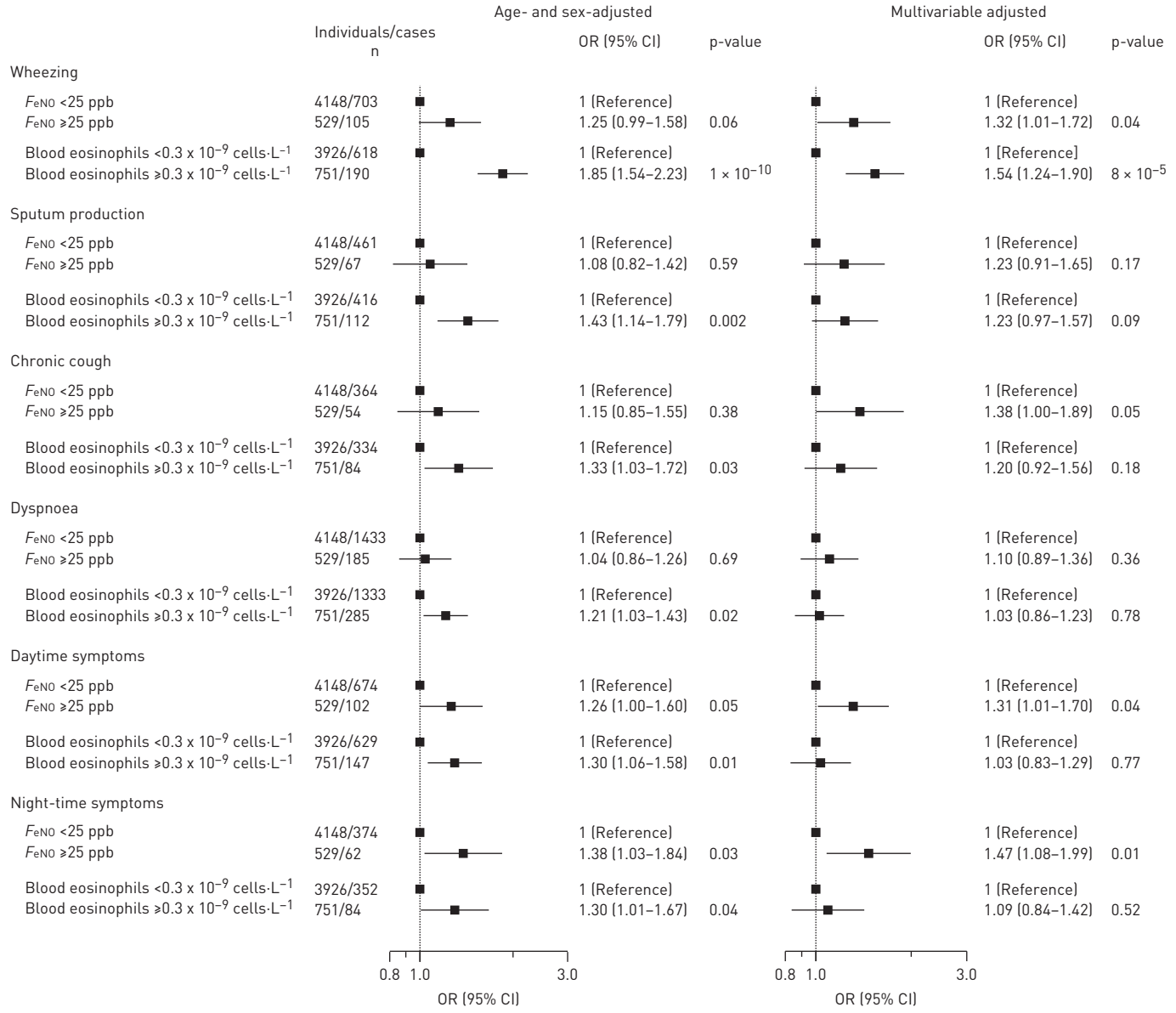

FIGURE 4 Separate association of increased exhaled nitric oxide level and blood eosinophil count with respiratory symptoms. Logistic regression models were used. Multivariable adjustment included age, sex, body mass index, smoking status, smoking history, familial predisposition for chronic obstructive pulmonary disease and asthma, atopy and use of airway medication. p-values were from Wald's test. *F*eNO: exhaled nitric oxide fraction.

Individuals with asthma, COPD and particularly 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 *F*eNO 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 *F*eNO levels and higher blood eosinophil counts (figure 2 and online supplementary figures S1–S4).

Clinical attributes

A higher age, male sex, prebronchodilator FEV1/FVC <0.70, positive reversible test, self-reported atopy and use of airway medication were all associated with an increased risk of having a *F*eNO level ⩾25 ppb and a blood eosinophil count ⩾0.3×10^9 cells·L^{-1}, both when analysed separately and combined (figure 3). In contrast, only higher BMI, prebronchodilator FEV1/FVC <LLN and FEV1 % pred <LLN and <80% were associated with an increased risk of having a blood eosinophil count ⩾0.3×10^9 cells·L^{-1}, whereas current smoking and smoking history were associated with a reduced risk of having a *F*eNO level ⩾25 ppb.

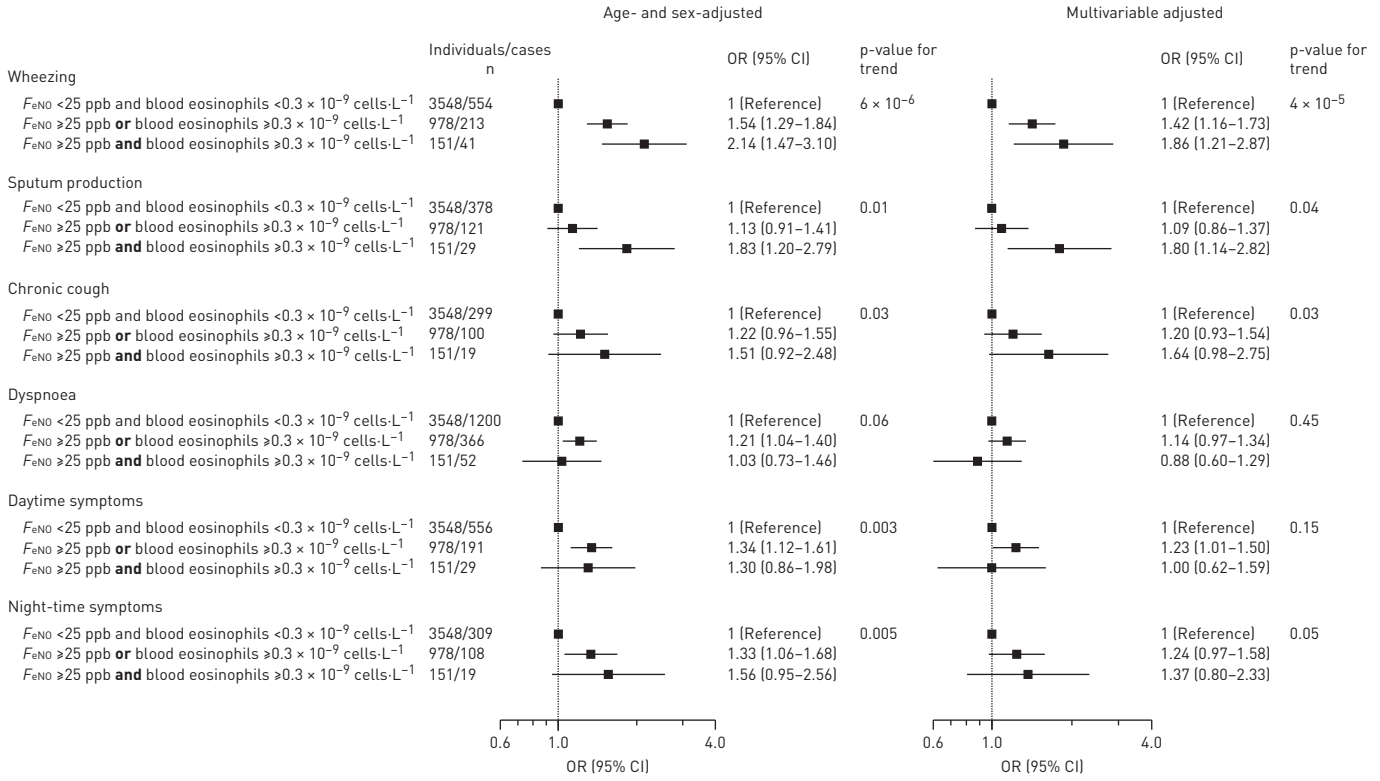

FIGURE 5 Combined association of increased exhaled nitric oxide level and blood eosinophil count with respiratory symptoms. Logistic regression models were used. Multivariable adjustment included age, sex, body mass index, smoking status, smoking history, familial predisposition to chronic obstructive pulmonary disease and asthma, atopy and use of airway medication. p-values were from Wald's test. *F*eNO: fraction of exhaled nitric oxide.

Respiratory symptoms

An increased *F*eNO 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 4). These associations were attenuated after additional adjustment for potential confounders. Compared to individuals with *F*eNO level <25 ppb and blood eosinophil count <0.3×10^9 cells·L^{-1}, age- and sex-adjusted odds ratios (95% CI) for wheezing were 1.54 (1.29–1.84) for individuals with *F*eNO level ⩾25 ppb or blood eosinophil count ⩾0.3×10^9 cells·L^{-1} and 2.14 (1.47–3.10) for individuals with *F*eNO level ⩾25 ppb and blood eosinophil count ⩾0.3×10^9 cells·L^{-1} (figure 5). Corresponding odds ratios (95% CI) 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 daytime 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 *F*eNO level and blood eosinophil count were associated with an increased risk of asthma, but not COPD or ACO (figure 6). Compared to individuals with *F*eNO level <25 ppb and blood eosinophil count <0.3×10^9 cells·L^{-1}, age- and sex-adjusted odds ratios (95% CI) for asthma were 1.54 (1.22–1.94) for individuals with *F*eNO level ⩾25 ppb or blood eosinophil count ⩾0.3×10^9 cells·L^{-1} and 3.26 (2.16–4.94) for individuals with *F*eNO level ⩾25 ppb and blood eosinophil count ⩾0.3×10^9 cells·L^{-1}. Corresponding odds ratios (95% CI) 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 similar when defining the clinical groups of obstructive lung disease according to FEV1/FVC <LLN (online supplementary 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 *F*eNO level and blood eosinophil count was associated with an increased risk of asthma compared to COPD, especially when the two biomarkers were combined (figure 7). Similarly, when individuals with COPD and ACO were analysed separately, an

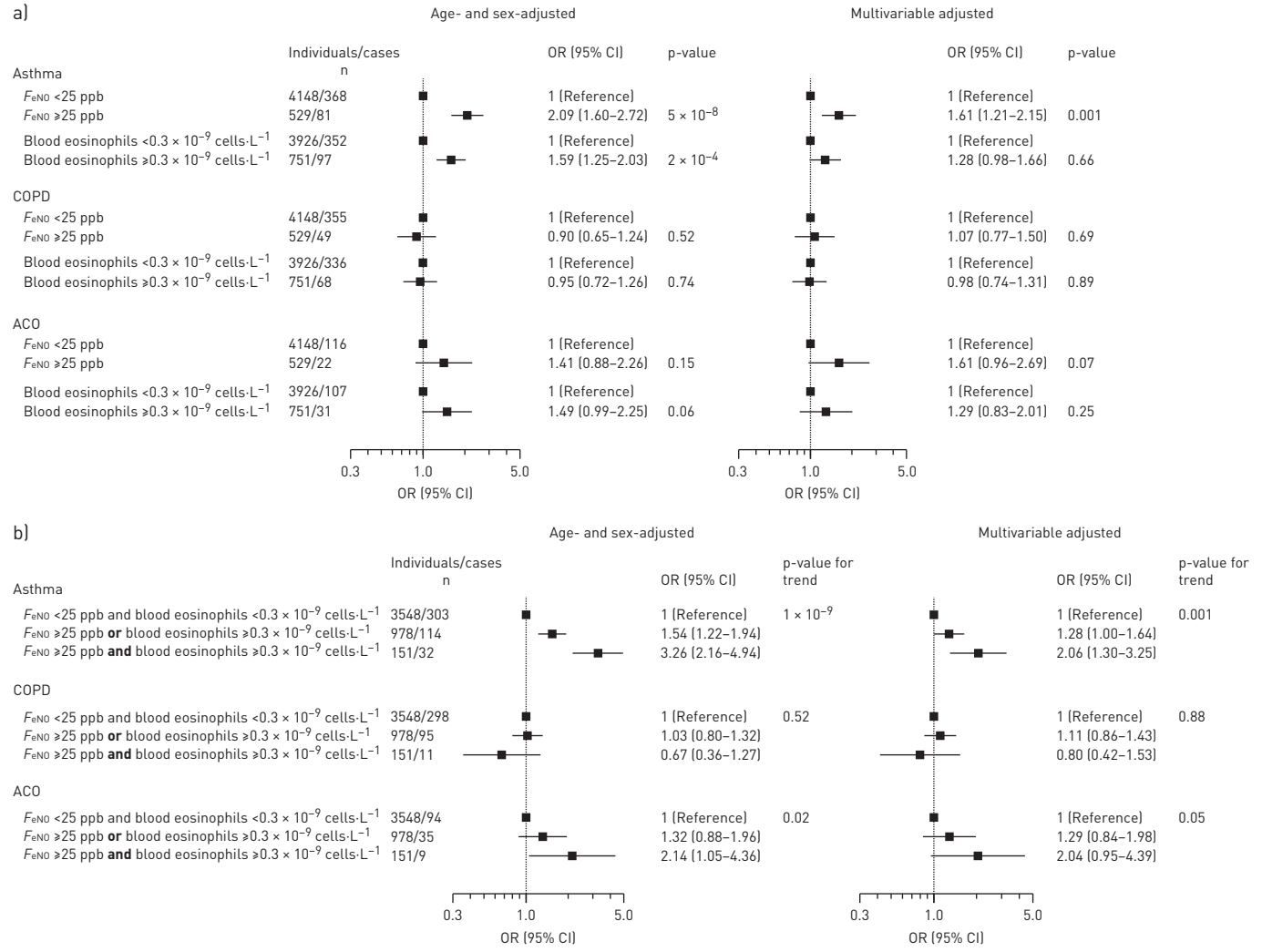

FIGURE 6 Association of increased exhaled nitric oxide level and blood eosinophil count with asthma, chronic obstructive pulmonary disease (COPD) and asthma–COPD overlap (ACO). a) Separate association analyses; b) combined association analyses. Logistic regression models were used. Multivariable adjustment included age, sex, body mass index, smoking status, smoking history, familial predisposition to COPD and asthma, atopy and use of airway medication. p-values were from Wald's test. F_{eNO}: exhaled nitric oxide fraction.

increased F_{eNO} 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, F_{eNO} 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 (95% CI) values for predicting asthma were 0.62 (0.58–0.65) for F_{eNO} ⩾25 ppb, 0.60 (0.57–0.64) for blood eosinophils $\geqslant 0.3\times10^9$ cells·L^{-1} and 0.61 (0.58–0.64) for F_{eNO} ⩾25 ppb and/or blood eosinophils $\geqslant 0.3\times10^9$ cells·L^{-1}. Corresponding AUC (95% CI) 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.

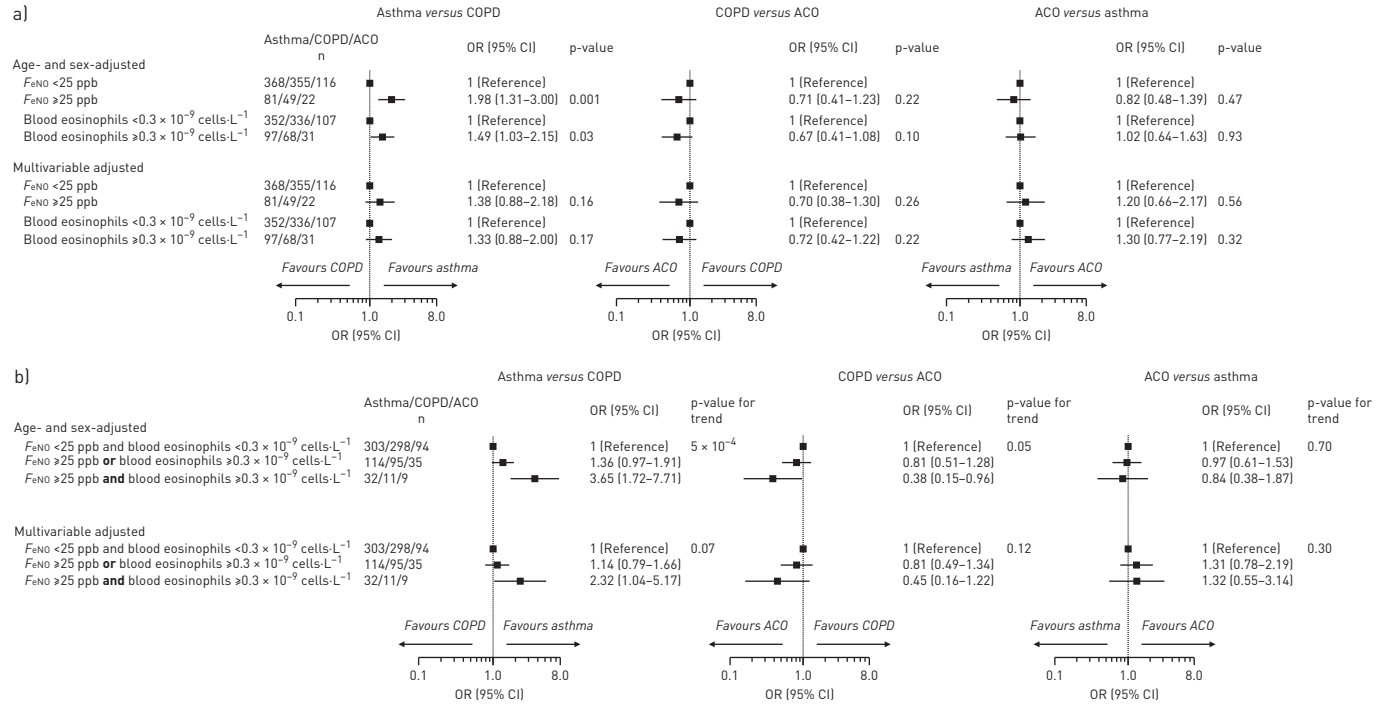

FIGURE 7 Increased exhaled nitric oxide level and blood eosinophil count favouring more asthma, chronic obstructive pulmonary disease (COPD) or asthma–COPD overlap (ACO). a) Separate association analyses; b) combined association analyses. Logistic regression models were used. Multivariable adjustment included age, sex, body mass index, smoking status, smoking history, familial predisposition to COPD and asthma, atopy and use of airway medication. p-values were from Wald's test. *F*eNO: exhaled nitric oxide fraction.

Discussion

In this large random sample from the general population, we found that compared to individuals with normal *F*eNO 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 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 *F*eNO levels [7–9], while treatment with lebrikizumab (anti-IL-13) and dupilumab (anti-IL-4 and -13) reduced *F*eNO 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 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 $\geqslant 0.3\times10^9$ cells·L^{-1} but not with *F*eNO level ⩾25 ppb. Furthermore, measures of atopy and smoking were associated with *F*eNO rather than with blood eosinophils.

Another interesting finding was that both an increased *F*eNO level and blood eosinophil count were associated with asthma and ACO, but not with COPD. We do not necessarily believe that *F*eNO 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 *F*eNO 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

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* F_{ENO}	p-value *versus* blood eosinophils	p-value *versus* F_{ENO} and blood eosinophils
Asthma								
F_{ENO} ⩾25 ppb	57	60	18	90	0.62 (0.58–0.65)	NA	0.10	0.36
Blood eosinophils ⩾0.3×10^9 cells·L^{-1}	61	55	17	90	0.60 (0.57–0.64)	0.10	NA	0.16
F_{ENO} ⩾25 ppb and/or blood eosinophils ⩾0.3×10^9 cells·L^{-1}	58	59	18	90	0.61 (0.58–0.64)	0.36	0.16	NA
COPD								
F_{ENO} ⩾25 ppb	61	64	18	93	0.68 (0.65–0.72)	NA	0.26	0.37
Blood eosinophils ⩾0.3×10^9 cells·L^{-1}	60	64	18	92	0.68 (0.64–0.71)	0.26	NA	0.12
F_{ENO} ⩾25 ppb and/or blood eosinophils ⩾0.3×10^9 cells·L^{-1}	60	64	18	93	0.69 (0.65–0.72)	0.37	0.12	NA
ACO								
F_{ENO} ⩾25 ppb	54	64	7	97	0.63 (0.57–0.68)	NA	0.34	0.10
Blood eosinophils ⩾0.3×10^9 cells·L^{-1}	57	66	7	97	0.64 (0.59–0.69)	0.34	NA	0.89
F_{ENO} ⩾25 ppb and/or blood eosinophils ⩾0.3×10^9 cells·L^{-1}	51	66	7	97	0.64 (0.59–0.69)	0.10	0.89	NA
Asthma *versus* COPD								
F_{ENO} ⩾25 ppb	67	66	69	64	0.74 (0.70–0.78)	NA	0.32	0.27
Blood eosinophils ⩾0.3×10^9 cells·L^{-1}	67	65	69	63	0.73 (0.69–0.77)	0.32	NA	0.08
F_{ENO} ⩾25 ppb and/or blood eosinophils ⩾0.3×10^9 cells·L^{-1}	67	67	70	64	0.74 (0.70–0.78)	0.27	0.08	NA
COPD *versus* ACO								
F_{ENO} ⩾25 ppb	58	57	77	35	0.57 (0.50–0.64)	NA	0.51	0.26
Blood eosinophils ⩾0.3×10^9 cells·L^{-1}	50	66	79	34	0.59 (0.52–0.65)	0.51	NA	0.94
F_{ENO} ⩾25 ppb and/or blood eosinophils ⩾0.3×10^9 cells·L^{-1}	59	58	78	36	0.58 (0.52–0.65)	0.26	0.94	NA
ACO *versus* asthma								
F_{ENO} ⩾25 ppb	59	63	35	82	0.68 (0.62–0.74)	NA	0.94	0.69
Blood eosinophils ⩾0.3×10^9 cells·L^{-1}	63	60	35	83	0.68 (0.62–0.74)	0.94	NA	0.86
F_{ENO} ⩾25 ppb and/or blood eosinophils ⩾0.3×10^9 cells·L^{-1}	62	62	36	83	0.68 (0.63–0.74)	0.69	0.86	NA

Data are presented as %, 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 areas under the curve (AUC) for the receiver operating characteristics. PPV: positive predictive value; NPV: negative predictive value; F_{ENO}: exhaled nitric oxide fraction; COPD: chronic obstructive pulmonary disease; ACO: asthma–COPD overlap; NA: not applicable.

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 *F*eNO 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]. However, the additive value of combining these two biomarkers has not been investigated extensively. In the National Health and Nutrition Examination Survey, combining *F*eNO 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 *F*eNO 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 the differentiation 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 *F*eNO 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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