

Received Date: 19-Jul-2015

Revised Date: 02-Sep-2015

Accepted Date: 28-Sep-2015

Article Type: Original Article-Asthma and Rhinitis

Title page

Both exhaled nitric oxide and blood eosinophil count were associated with mild allergic asthma only in non-smokers

Condensed title: FENO, blood eosinophil count and smoking status

Authors

Jonathan Giovannelli ^{1,2,3 *}
jonathan.giovannelli@gmail.com

Nathalie Chérot-Kornobis ^{1,2}
nathalie.cherot@univ-lille2.fr

Sébastien Hulo ^{1,2}
sebastien.hulo@chru-lille.fr

Alina Ciuchete ³
alina.ciuchete@gmail.com

Guillaume Clément ^{1,3}
gja.clement@gmail.com

Philippe Amouyel ^{1,2,3}
philippe.amouyel@pasteur-lille.fr

Régis Matran ^{1,2}
regis.matran@univ-lille2.fr

Luc Dauchet ^{1,2,3}
luc.dauchet@pasteur-lille.fr

¹ University of Lille, Faculté de Médecine Henri Warembourg F-59045 Lille Cedex, France

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.12669

This article is protected by copyright. All rights reserved.

² University Hospital of Lille, 2 Avenue Oscar Lambret, F-59000 Lille, France

³ Pasteur Institute of Lille, INSERM U1167 RID-AGE, 1 Rue du Professeur Calmette, F-59800 Lille, France

* Corresponding author: Dr. Jonathan Giovannelli, University Hospital of Lille, Epidemiology Unit, 2 rue du Pr. Laguesse, MRRC, F-59037 Lille Cedex, tel: +33 320 445 518, fax: +33 320 446 945

Abstract

Background. The fractional exhaled nitric oxide (FENO) and the blood eosinophil count (B-eos) are markers of eosinophilic inflammation used in the diagnosis and management of asthma. The relationships between smoking cigarette and both FENO and B-eos are complex and raise questions about the association between these markers and asthma in smokers.

Objective. To determine the relationships between both FENO and B-eos on one hand and asthma and atopy on the other, according to smoking status.

Methods. FENO and B-eos were measured in respectively 1579 and 1496 of the 1607 middle-aged adults randomly selected from the general population in the cross-sectional ELISABET survey. Allergic asthma was defined as asthma (a self-report of physician-diagnosed asthma, and wheezing in the previous 12 months or the use of asthma medications) with atopy (allergic rhinitis or hay fever in the previous 12 months, or a previous positive prick test or allergen desensitization therapy). Nonallergic asthma was defined as asthma without atopy.

Results. The analysis included 812 (51.4%) never-, 473 (30%) former- and 294 (18.6%) current smokers. A total of 490 (32%) participants were atopic, 80 (5.1%) had allergic asthma, and 31 (2%) had nonallergic asthma. Only 16.2% (18/111) of asthmatics were

Accepted Article

treated with glucocorticoid inhalants, suggesting that among them a majority of participants had mild asthma. A positive interaction between smoking status and allergic asthma was observed in multivariate models explaining FENO ($p=0.003$) and B-eos ($p=0.001$). Thus, compared to those without allergic asthma, participants with allergic asthma had higher FENO values (+63.4%, 95%CI=[39; 92]) and higher B-eos (+63.2% [38.2; 92.7]) in never and former smokers, but not in current smokers. Lastly, an analysis of receiver operating characteristic curves showed that each of the two markers was able to discriminate moderately allergic asthma but only in non-smokers.

Conclusions & Clinical Relevance. FENO and B-eos were associated with the presence of mild allergic asthma only in non-smokers, not in current smokers. These findings raise questions about the clinical value of FENO and B-eos in smokers.

Keywords: fractional exhaled nitric oxide; blood eosinophil count; tobacco; smoking status; interaction; airway inflammation

Introduction

The fractional exhaled nitric oxide (FENO) is generally considered as a non-invasive, surrogate marker of eosinophilic inflammation [1]. More recently, the blood eosinophil count (B-eos) has also been studied as another potentially interesting marker; it is associated with bronchial hyperreactivity and late-phase asthmatic reactions [2], asthma attacks [3–5], asthma-related emergency department visits [3], excessive short-acting β_2 -agonist use [4], a short-term risk of deterioration and the need for corticosteroid treatment

in children with mild or moderate allergic asthma [6], severe eosinophilic asthma [7] and a response to omalizumab treatment [8].

It is well known that smoking decreases FENO values [9] and thus complicates their interpretation. The clinical value of FENO measurements in smokers is subject to debate. Although a few studies have evaluated the relationship between FENO and asthma in smokers, the results have been inconsistent [10–15]. In the general population, smoking is associated with an elevated B-eos [16–21]. However, this association appears to be inversed in patients with asthma. Indeed, asthmatic smokers have lower B-eos values than asthmatic non-smokers [16,22,23]. Thus, these findings raise questions about the relationship between B-eos and asthma in smokers.

Analysis of markers of eosinophilic inflammation used in clinical practice in smokers is important because 20% to 35% of asthmatics smoke (a similar proportion to that seen in the general population) [24,25]. Hence, the objective of the present study was to determine the relationships between FENO and B-eos on one hand and asthma and atopy on the other, as a function of smoking status.

Methods

Participants

The participants were men and women (aged from 40 to 64) enrolled in the 2011-2013 *Enquête Littoral Souffle Air Biologie Environnement* (ELISABET) cross-sectional survey of the prevalence of airflow obstruction. All the participants had been resident in the city of Dunkirk (northern France) or one of the 16 other communes in the Dunkirk urban area for at

least the previous 5 years. The non-inclusion criteria were: pregnant women, persons deprived of liberty, unable to consent or not benefiting from social insurance. The participants were selected from electoral rolls by random sampling (stratified for gender and age). All participants were all recruited between January 2011 and November 2013. Each selected participant received a letter asking him/her to contact the coordinating team and make an appointment for data collection. In the absence of a reply, participants were contacted by telephone. Data was collected at home (rarely during a consultation in a healthcare establishment). A detailed questionnaire was administered by a trained, registered nurse. Lung function tests were performed, and a blood sample was also collected in a single visit (or few days after or before for some participants).

The study protocol was approved by the local investigational review board (CPP Nord Ouest IV, reference number: 2010-A00065-34), in compliance with the French legislation on biomedical research. All participants provided their informed, written consent to participation in the study.

Measurements

FENO was measured with the validated NIOX MINO portable analyzer (Aerocrine AB, Solna, Sweden) [26–29] at a constant expiratory flow rate of 50 ml/s, in accordance with the 2005 American Thoracic Society (ATS) and European Respiratory Society (ERS) guideline [30]. To avoid bias, FENO was measured in fasting participants (31,32) prior to any spirometric maneuvers [33,34]. The NIOX MINO device can detect FENO values from 5 to 300 ppb. Values <5 ppb were considered to be below the lower limit of detection (LOD), and were therefore imputed with a value of 3.5 ppb (LOD/v2) [35,36]. FENO was measured twice for each participant and the mean value was subsequently analyzed. The NO level in ambient

air was also measured with the NIOX MINO device. B-eos was measured with an LH780 hematology analyzer (Beckman Coulter, Fullerton, California). Leukocyte populations were analyzed by multidimensional flow cytometry.

Spirometric examinations were performed using weekly calibrated Micro 6000 devices (Medisoft, Belgium), accordingly to the 2005 ATS/ERS guideline [37]. No reversibility test (with administration of bronchodilator) was performed prior to spirometric examinations. The latter were validated by a trained specialist.

Current asthma was defined by a "yes" answer in response to the question "have you ever been diagnosed with asthma by a physician?", and self-reported wheezing in the previous 12 months or asthma medications (including adrenergic, glucocorticoid or anticholinergic drugs). Atopy was defined as self-reported symptoms of allergic rhinitis or hay fever in the previous 12 months, or a previous positive skin prick test or allergen desensitization therapy. Allergic asthma and nonallergic asthma were then defined as asthma with and without atopy, respectively. Airflow obstruction was defined as a ratio between the forced expiratory volume in 1 second and the forced vital capacity <0.7 . Smoking status was qualified as either current (at least one cigarette per day for a year), former or never. Lastly, usual intakes of fruits and vegetables were assessed using a validated food frequency questionnaire [38].

Statistical analyses

The agreement between the two sets of FENO measurements was evaluated by calculating the intraclass correlation coefficient (ICC) and generating a Bland-Altman plot [39]. The Pearson product-moment correlation coefficients for the relationship between FENO and B-eos were computed as a function of smoking status.

In order to study the relationships between FENO and asthma/atopy as a function of smoking status, three different analyses were performed. Similar analyses were performed for B-eos and asthma/atopy. Firstly, the characteristics (including asthma and atopy) of groups of participants below and above a FENO or B-eos threshold were compared as a function of smoking status (using Student's tests for means and Fisher's exact tests for percentages). For FENO, thresholds were obtained by calculating the corresponding 95% upper reference value (RV) as a function of smoking status. The latter was estimated from data on healthy participants (i.e. participants with no self-reported respiratory disease, lung symptoms, asthma medication use, atopy or airflow obstruction) by back-transformation of the mean $+1.64 \times$ the standard deviation of logarithm-transformed FENO values ($\ln(\text{FENO})$) [40–42]. The normality of $\ln(\text{FENO})$ was evaluated in a Shapiro-Wilk test. A sensitivity analysis was performed with a fixed threshold of 25 ppb. For B-eos, a threshold of 300 cells/mm³ was used. A sensitivity analysis was performed with a threshold of 500 cells/mm³. Secondly, two multiple linear regression models were built with $\ln(\text{FENO})$ and $\ln(\text{B-eos})$ as the dependent variables, respectively. The explanatory variables were as follows: age, gender, height, body mass index (BMI), smoking status (current vs. former or never), current allergic and nonallergic asthma, inhaled glucocorticoid use, atopy, airflow obstruction, a respiratory infection in the previous 4 weeks, and for the model with $\ln(\text{FENO})$ as the dependent variable: usual dietary intakes of vegetables and fruits, and NO in the ambient air. Interaction terms between smoking status and the other explanatory variables were tested one at a time in both models. Lastly, models stratified for smoking status were performed. The regression coefficients were expressed as the percentage increase in FENO or B-eos value [95%CI] per unit change in the explanatory variable. For this, they were back-

transformed and converted to percentage increase by multiplying with 100 and subtracting 100% (10,43). Regression diagnostics were performed.

Thirdly, to assess the ability of FENO and B-eos to discriminate between participants with current allergic asthma (and with participants with current asthma (allergic or nonallergic) in a sensibility analysis) and other participants, receiver operating characteristic (ROC) curves were plotted and the areas under the curve (AUC) were calculated as a function of smoking status. Optimal thresholds that maximized sensitivity and specificity were also calculated.

All statistical analyses were performed using R software, version 3.1.2 (R: A Language and Environment for Statistical Computing, R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria); the threshold for statistical significance was set to $p < 0.05$.

Results

A total of 4670 people were eligible for inclusion. Of these, 1485 did not reply and 1578 refused to participate. A total of 1607 were included, giving a participation rate of 34.4%.

FENO was measured and analyzed in 1579 participants. The degree of agreement between the two sets of FENO measurements was evaluated by calculating the ICC and by drawing up a Bland-Altman plot. The ICC [95%CI] was 0.97 [0.96; 0.98], and the 95% lower and upper limits of agreement were -7.2 and 6.2 ppb, respectively.

B-eos was measured in 1496 participants. For the population as a whole, a significant correlation between FENO and B-eos was observed (0.26 [0.21; 0.31], $p < 10^{-9}$). Significant

correlations were also noted in never smokers (0.35 [0.29; 0.41], $p < 10^{-9}$) and former smokers (0.32 [0.23; 0.40], $p < 10^{-9}$) but not in current smokers (0.10 [-0.02; 0.21], $p=0.1$).

The participants' characteristics as a function of smoking status are summarized in Table 1. The median (interquartile range) value was 14.5 (11) ppb for FENO and 165 (139) cells/mm³ for B-eos. There were 812 (51.4%) never smokers, 473 (30%) former smokers and 294 (18.6%) current smokers. A total of 490 (32%) participants had atopy, 80 (5.1%) had current allergic asthma, 31 (2%) had current nonallergic asthma and 234 (15.2%) had airflow obstruction. Among the participants with asthma, 45.2%, 38.3% and 16.5% were never, former and current smokers, respectively. Current smokers had, compared with never smokers: lower FENO values (geometric mean [95%CI] =10.4 [9.7; 11.3] ppb vs. 16.3 [15.6; 16.9] ppb, respectively), a higher B-eos (200 [187; 214] cells/mm³ vs. 149 [143; 155]) and greater proportions of wheezing in the past 12 months (28.9% vs. 10.6%), male gender (57.5% vs. 34.1%) and airflow obstruction (20.9% vs. 11.3%).

The characteristics of participants with FENO values below and above the 95%RV are presented as a function of smoking status in Table 2. The 95%RVs for never, former and current smokers were 34, 32 and 25 ppb, respectively. For both never and former smokers, participants with FENO values above the 95%RV had (compared to those with FENO values below the 95%RV) greater proportions of current asthma, current allergic asthma, inhaled adrenergic bronchodilator use, atopy, allergic rhinitis or hay fever in the past 12 months, airflow obstruction and respiratory infection in the past 4 weeks, and a higher B-eos. In contrast, there were no differences for current smokers with FENO values below or above the 95%RV. Use of a fixed threshold of 25 ppb gave similar results (Supplementary Table 1).

The characteristics of participants with B-eos below and above the threshold of 300 cells/mm³ are presented as a function of smoking status in Table 3. In both never smokers and former smokers, participants with B-eos above the threshold had greater proportions of current asthma, current allergic asthma, wheezing in the past 12 months and inhaled adrenergic bronchodilator use and higher FENO values than those below the threshold. No differences were observed for current smokers. Use of a threshold of 500 cells/mm³ gave similar results (data not shown).

In multivariate analyses with models explaining ln(FENO) and ln(B-eos), positive interaction terms were noted between smoking status on one hand and (i) current allergic asthma ($p < 0.003$ and $p < 0.001$, respectively), (ii) atopy ($p = 0.01$ and $p = 0.02$, respectively), and (iii) BMI ($p = 0.04$ and $p = 0.01$, respectively) on the other (Tables 4 and 5). Thus, for these variables, results must to be interpreted from models stratified for smoking status. Compared to those without allergic asthma, participants with allergic asthma had higher FENO values (+63.4% [39; 92]) and higher B-eos (+63.2% [38.2; 92.7]) in never and former smokers, but not in current smokers. Similar results were observed for atopy: in never and former smokers, the increase in FENO values was statistically significant (+18% [9.9; 26.7]) but the increase in B-eos did not reach significance (+7.2% [-0.7; 15.7], $p = 0.07$). No association was noted for current smokers (Tables 4 and 5). Similar results were observed for the BMI. Furthermore, FENO was associated with: current smoking, female gender, height, respiratory infection in the past 4 weeks, usual dietary intake of fruit and NO in ambient air, with no interaction with smoking status. B-eos was also associated with current smoking, female gender and respiratory infection in the past 4 weeks, with no interaction with smoking status (Tables 4 and 5).

In terms of FENO's ability to discriminate between the presence and absence of current allergic asthma, the AUC was: 0.75 [0.64; 0.82], 0.71 [0.59; 0.81] and 0.41 [0.26; 0.60] for never, former and current smokers, respectively. For B-eos, it was: 0.76 [0.67; 0.84], 0.73 [0.62; 0.80] and 0.44 [0.29; 0.60], respectively (Table 6). (The 2x2 contingency tables are presented in supplementary tables 2-9.) A sensibility analysis was conducted when considering all asthmatics (allergic and nonallergic asthma). For FENO, the AUC was: 0.69 [0.60; 0.77], 0.58 [0.48; 0.68] and 0.47 [0.33; 0.62] for never, former and current smokers, respectively. For B-eos, it was: 0.71 [0.62; 0.79], 0.65 [0.55; 0.73] and 0.41 [0.29; 0.55], respectively.

Discussion

In the present study, we performed several analyses of the relationships between two widely used minimally invasive markers of eosinophilic inflammation (FENO and B-eos) on one hand and asthma and atopy on the other, as a function of smoking status. Our main finding was that allergic asthma was associated or could discriminated moderately by FENO and B-eos only in never and former smokers, not in current smokers.

The present study had strengths and limitations. One of the strengths was the use of a large enough general population sample size to reveal significant interactions in multivariate analyses. The study's main limitation was the absence of skin prick tests or specific immunoglobulin E assays for more accurate phenotyping of participants with atopy. The latter was defined by the presence of symptoms of allergic rhinitis or hay fever in the

previous 12 months, or previous positive prick test or allergen desensitization therapy. However, the prevalence of atopy in the present study (32%) was in the range of others conducted in the general population [42,44]. Moreover, for technical reasons, two reproducible measurements (as recommended by the ATS; delta $\leq 10\%$ for FENO values ≥ 30 ppb, and delta ≤ 2 ppb for values < 30 ppb) could not be obtained in all participants [30]. We carried out two sets of FENO measurements per participant and retrospectively evaluated the agreement between them. The ICC was high (0.97, [0.96-0.98]). However, the 95% limits of agreement (-7.2; 6.2 ppb) slightly exceeded the manufacturer's specifications (± 5 ppb) but remained clinically acceptable. Lastly, only 16.2% (18/111) of participants with asthma were treated with glucocorticoid inhalants, suggesting that among asthmatics a majority of participants had mild asthma. So, our findings should be applied to people with mild asthma, but they might be not applied to patients with more severe asthma.

It is well known that smoking decreases FENO values in both asthmatic and non-asthmatic people [9]. This effect is reversible (over 4 to 8 weeks) [45,46] and can be explained by several factors: (i) the high concentration of NO in cigarette smoke partially inhibits the activity of NO synthase (NOS) -2 [47], (ii) the activity of NOS-2 and -3 does not increase during exposure to cigarette smoke, despite an increase in the expression of their genes [46], (iii) exposure to cigarette smoke increases the production of endogenous NOS inhibitors [48], and (iv) exposure to cigarette smoke may decrease local production of NO above the upper trachea [49]. In the present study, FENO values and thus the 95% RVs were lower for current smokers than for non-smokers. The observed 95% RVs for never, former and current smokers (34, 32 and 25 pbb, respectively) were in agreement with the literature values [36,40–42,50].

Given that smoking reduces and complicates the interpretation of FENO values, the clinical use of FENO measurements in smokers is subject to debate. While it is well known that FENO can differentiate between asthmatics and non-asthmatics in never smokers [1], contradictory results have been observed in smokers. Malinovschi et al. reported that both allergic and nonallergic asthma are associated with increased FENO levels, but only in never-smokers [10]. In addition, Spears et al. showed that FENO was significantly lower in smokers with asthma and did not change significantly in response to dexamethasone [11]. However, Malinovschi et al.'s study of participants with asthma-like symptoms found that FENO could differentiate between asthmatics and non-asthmatics equally well in both never smokers and current smokers [12]. Furthermore, Michils et al.'s longitudinal study found that sequential changes in FENO are related to asthma control in both smokers and non-smokers [13].

Our results showed that FENO was associated with allergic asthma and atopy in never and former smokers, but not in current smokers. Indeed, in contrast to the situation for non-smokers, the proportion of allergic asthma was similar in current smokers with FENO values above and below the 95% RV of FENO (4.4% vs. 4.8%, respectively; $p=1$). These results were confirmed in multivariate analysis. We observed an interaction between smoking status and allergic asthma ($p=0.003$). Thus, the association between FENO and allergic asthma was noted only in non-smokers. The same results were observed for atopy. Lastly, ROC curves showed that allergic asthma was not discriminated by FENO in current smokers (AUC=0.41 [0.26; 0.60]). In non-smokers, allergic asthma was discriminated moderately by FENO (AUC=0.75 [0.64; 0.82] in never smokers, =0.71 [0.59; 0.81] in former smokers). However, considering that optimal values of sensitivity and specificity were only between 0.59 and 0.75, the use of FENO to discriminate allergic asthma in non-smokers would lead to large

number of false positives and false negatives. Although the results of this cross-sectional study do not allow conclusions on the usefulness or not of FENO in asthma management, they confirmed that FENO cannot discriminate allergic asthmatic in smokers.

Previous studies of the general population have reported that smoking increase B-eos [16–21]. The fact that smoking also increases IgE suggests that smoking has a profound effect on the immunological system [16,51,52]. However, B-eos seems to be lower in asthmatic smokers than in asthmatic non-smokers [16,22,23]. Sunyer et al. specifically studied the relationships between asthma, smoking and B-eos and concluded that smoking probably has different immunological role in asthmatics vs. non-asthmatics [16]. Our results are in agreement with Sunyer et al.'s study.

Whereas B-eos has recently been highlighted as a marker of systemic eosinophilic inflammation in asthma [3–8], this study is the first, to the best of our knowledge, to evaluate the association between B-eos and asthma or atopy as a function of smoking status. In this respect, our results for B-eos are similar to those for FENO. Thus, the proportion of allergic asthma in smokers did not vary significantly according to whether B-eos was above or below a fixed threshold of 300 cells/mm³ (3% vs. 5.6%, respectively; $p=0.53$) – unlike non-smokers. Furthermore, we observed in multivariate analysis an interaction between smoking status and allergic asthma ($p=0.001$), and the association between B-eos and allergic asthma was noted only in non-smokers. Similar results were observed for atopy. Lastly, ROC curves confirmed that B-eos could not discriminate allergic asthma in current smokers (AUC= 0.44 [0.29; 0.60]). In non-smokers, allergic asthma was discriminated moderately by B-eos (AUC=0.76 [0.67; 0.84] in never smokers, =0.73 [0.62; 0.80] in former smokers). However, in the same way as for FENO, considering that optimal

values of sensitivity and specificity were only between 0.68 and 0.79, the use of B-eos to discriminate allergic asthma in non-smokers would lead to an elevated misclassification rate. Thus, the study results (i) confirmed that the elevation in B-eos caused by smoking in the general population is reversed in asthmatics, and (ii) showed that B-eos was significantly associated with allergic asthma only in non-smokers.

FENO and B-eos are markers of local and systemic eosinophilic inflammation, respectively. According to the literature, biomarkers of eosinophilic inflammation (FENO, B-eos and others, such as the sputum eosinophil count) are well correlated with each other [53–55]. However, our results indicated that FENO and B-eos were significantly correlated in never smokers (0.35 [0.29; 0.41]) and former smokers (0.32 [0.23; 0.40]) but not in current smokers (0.10 [-0.02; 0.21]).

The absence of an association between FENO and B-eos and allergic asthma in smokers may be due to the changes in inflammation caused by cigarette smoking in patients with asthma. Indeed, asthmatic smokers have higher neutrophil counts and lower eosinophil counts in sputum than non-smokers [56,57]. The lower proportion of eosinophilic inflammation in smokers with asthma was confirmed by Broekema et al.'s study of bronchial biopsies; a lower bronchial eosinophil count and a higher mast cell count were observed in current smokers, relative to never smokers [58]. Furthermore, Broekema et al. suggested that smoking-related changes are reversible because the epithelium did not appear to be modified in former smokers [58]. This was consistent with our study, with similar results in never smokers and former smokers. Furthermore, smoking in asthma is associated with a corticosteroid-insensitive increase in multiple airway cytokines [59]. It has been also

observed that cigarette smoke exposure can impair the bioavailability of L-arginine (the substrate for NOS) in asthmatic patients [60,61].

Conclusion

FENO and B-eos (both alone and when combined) were associated with the presence of mild allergic asthma only in never and former smokers, not in current smokers. Our results suggest that local and systemic eosinophilic inflammation is modified in asthmatic smokers and raise questions about the clinical value of FENO and B-eos in this population.

Acknowledgements

The authors thank the CHRU de Lille, the University of Lille, the Institut Pasteur de Lille, and the Centre Hospitalier Général de Dunkerque. They thank the nurses, physicians and secretarial staff of the University of Lille and the Institut Pasteur de Lille. They thank the Service de Médecine du Travail, the Service de Biologie Spécialisé, and the Laboratoire d'Analyses Génomiques at the Institut Pasteur de Lille, the Department of Biology and the Department of Pulmonology at the Centre Hospitalier Général de Dunkerque, the Institut de Biologie et de Pathologie at the CHRU de Lille and the Centre Universitaire de Mesures et d'Analyses (CUMA) at the University of Lille.

Funding

The ELISABET survey was sponsored by the CHRU de Lille. The present study was funded by the Nord Pas-de-Calais Region and the European Regional Development Fund (ERDF-FEDER

Presage N°36034) in the frame of CPER Institut de Recherche en ENvironnement Industriel (IRENI).

Conflicts of interest

None of the authors state potential conflicts of interest.

References

1. Taylor DR, Pijnenburg MW, Smith AD. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax*. 2006 Sep;61(9):817–27.
2. Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin Allergy*. 1985 Sep;15(5):411–8.
3. 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 Oct;132(4):821–827.e1–5.
4. Zeiger RS, Schatz M, Li Q, Chen W, Khatry DB, Gossage D, et al. High blood eosinophil count is a risk factor for future asthma exacerbations in adult persistent asthma. *J Allergy Clin Immunol Pract*. 2014 Dec;2(6):741–50.
5. Tran TN, Khatry DB, Ke X, Ward CK, Gossage D. High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2014 Jul;113(1):19–24.

- Accepted Article
6. Lönnkvist K, Hellman C, Lundahl J, Halldén G, Hedlin G. Eosinophil markers in blood, serum, and urine for monitoring the clinical course in childhood asthma: impact of budesonide treatment and withdrawal. *J Allergy Clin Immunol*. 2001 May;107(5):812–7.
 7. Katz LE, Gleich GJ, Hartley BF, Yancey SW, Ortega HG. Blood eosinophil count is a useful biomarker to identify patients with severe eosinophilic asthma. *Ann Am Thorac Soc*. 2014 May;11(4):531–6.
 8. Busse W, Spector S, Rosén K, Wang Y, Alpan O. High eosinophil count: a potential biomarker for assessing successful omalizumab treatment effects. *J Allergy Clin Immunol*. 2013 Aug;132(2):485–486.e11.
 9. Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med*. 2014 Jun;108(6):830–41.
 10. Malinovschi A, Janson C, Högman M, Rolla G, Torén K, Norbäck D, et al. Both allergic and nonallergic asthma are associated with increased FE(NO) levels, but only in never-smokers. *Allergy*. 2009 Jan;64(1):55–61.
 11. Spears M, Weir CJ, Smith AD, McSharry C, Chaudhuri R, Johnson M, et al. Bronchial nitric oxide flux (J'aw) is sensitive to oral corticosteroids in smokers with asthma. *Respir Med*. 2011 Dec;105(12):1823–30.
 12. Malinovschi A, Backer V, Harving H, Porsbjerg C. The value of exhaled nitric oxide to identify asthma in smoking patients with asthma-like symptoms. *Respir Med*. 2012 Jun;106(6):794–801.

- Accepted Article
13. Michils A, Louis R, Peché R, Baldassarre S, Van Muylem A. Exhaled nitric oxide as a marker of asthma control in smoking patients. *Eur Respir J*. 2009 Jun;33(6):1295–301.
 14. Karrasch S, Ernst K, Behr J, Heinrich J, Huber RM, Nowak D, et al. Exhaled nitric oxide and influencing factors in a random population sample. *Respir Med*. 2011 May;105(5):713–8.
 15. McSharry CP, McKay IC, Chaudhuri R, Livingston E, Fraser I, Thomson NC. Short and long-term effects of cigarette smoking independently influence exhaled nitric oxide concentration in asthma. *J Allergy Clin Immunol*. 2005 Jul;116(1):88–93.
 16. Sunyer J, Springer G, Jamieson B, Conover C, Detels R, Rinaldo C, et al. Effects of asthma on cell components in peripheral blood among smokers and non-smokers. *Clin Exp Allergy J Br Soc Allergy Clin Immunol*. 2003 Nov;33(11):1500–5.
 17. Sørensen LT, Toft BG, Rygaard J, Ladelund S, Paddon M, James T, et al. Effect of smoking, smoking cessation, and nicotine patch on wound dimension, vitamin C, and systemic markers of collagen metabolism. *Surgery*. 2010 Nov;148(5):982–90.
 18. Freedman DS, Flanders WD, Barboriak JJ, Malarcher AM, Gates L. Cigarette smoking and leukocyte subpopulations in men. *Ann Epidemiol*. 1996 Jul;6(4):299–306.
 19. Schwartz J, Weiss ST. Cigarette smoking and peripheral blood leukocyte differentials. *Ann Epidemiol*. 1994 May;4(3):236–42.
 20. Campesi I, Carru C, Zinellu A, Occhioni S, Sanna M, Palermo M, et al. Regular cigarette smoking influences the transsulfuration pathway, endothelial function, and

Accepted Article

inflammation biomarkers in a sex-gender specific manner in healthy young humans. *Am J Transl Res.* 2013;5(5):497–509.

21. Hou L, Lloyd-Jones DM, Ning H, Huffman MD, Fornage M, He K, et al. White blood cell count in young adulthood and coronary artery calcification in early middle age: coronary artery risk development in young adults (CARDIA) study. *Eur J Epidemiol.* 2013 Sep;28(9):735–42.
22. Telenga ED, Kerstjens HAM, Ten Hacken NHT, Postma DS, van den Berge M. Inflammation and corticosteroid responsiveness in ex-, current- and never-smoking asthmatics. *BMC Pulm Med.* 2013;13:58.
23. Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, et al. Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe asthma. *J Allergy Clin Immunol.* 2013 Apr;131(4):1008–16.
24. James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, et al. Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. *Am J Respir Crit Care Med.* 2005 Jan 15;171(2):109–14.
25. Siroux V, Pin I, Oryszczyn MP, Le Moual N, Kauffmann F. Relationships of active smoking to asthma and asthma severity in the EGEA study. *Epidemiological study on the Genetics and Environment of Asthma. Eur Respir J.* 2000 Mar;15(3):470–7.
26. Alving K, Janson C, Nordvall L. Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respir Res.* 2006;7:67.

- Accepted Article
27. Khalili B, Boggs PB, Bahna SL. Reliability of a new hand-held device for the measurement of exhaled nitric oxide. *Allergy*. 2007 Oct;62(10):1171–4.
 28. McGill C, Malik G, Turner SW. Validation of a hand-held exhaled nitric oxide analyzer for use in children. *Pediatr Pulmonol*. 2006 Nov;41(11):1053–7.
 29. Menzies D, Nair A, Lipworth BJ. Portable exhaled nitric oxide measurement: Comparison with the “gold standard” technique. *Chest*. 2007 Feb;131(2):410–4.
 30. American Thoracic Society, European Respiratory Society. 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 Apr 15;171(8):912–30.
 31. Olin AC, Aldenbratt A, Ekman A, Ljungkvist G, Jungersten L, Alving K, et al. Increased nitric oxide in exhaled air after intake of a nitrate-rich meal. *Respir Med*. 2001 Feb;95(2):153–8.
 32. Vints A-M, Oostveen E, Eeckhaut G, Smolders M, De Backer WA. Time-dependent effect of nitrate-rich meals on exhaled nitric oxide in healthy subjects. *Chest*. 2005 Oct 1;128(4):2465–70.
 33. Silkoff PE, Wakita S, Chatkin J, Ansarin K, Gutierrez C, Caramori M, et al. Exhaled nitric oxide after beta2-agonist inhalation and spirometry in asthma. *Am J Respir Crit Care Med*. 1999 Mar;159(3):940–4.

- Accepted Article
34. Deykin A, Halpern O, Massaro AF, Drazen JM, Israel E. Expired nitric oxide after bronchoprovocation and repeated spirometry in patients with asthma. *Am J Respir Crit Care Med*. 1998 Mar;157(3 Pt 1):769–75.
 35. Brody DJ, Zhang X, Kit BK, Dillon CF. Reference values and factors associated with exhaled nitric oxide: U.S. youth and adults. *Respir Med*. 2013 Nov;107(11):1682–91.
 36. See KC, Christiani DC. Normal values and thresholds for the clinical interpretation of exhaled nitric oxide levels in the US general population: results from the National Health and Nutrition Examination Survey 2007-2010. *Chest*. 2013 Jan;143(1):107–16.
 37. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J*. 2005 Aug;26(2):319–38.
 38. Giovannelli J, Dallongeville J, Wagner A, Bongard V, Laillet B, Marecaux N, et al. Validation of a short, qualitative food frequency questionnaire in French adults participating in the MONA LISA-NUT study 2005-2007. *J Acad Nutr Diet*. 2014 Apr;114(4):552–61.
 39. Bland JM, Altman DG. Measurement error. *BMJ*. 1996 Jun 29;312(7047):1654.
 40. Olin A-C, Bake B, Torén K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. *Chest*. 2007 Jun;131(6):1852–6.
 41. Matsunaga K, Hirano T, Kawayama T, Tsuburai T, Nagase H, Aizawa H, et al. Reference ranges for exhaled nitric oxide fraction in healthy Japanese adult population. *Allergol Int Off J Jpn Soc Allergol*. 2010 Dec;59(4):363–7.

- Accepted Article
42. Travers J, Marsh S, Aldington S, Williams M, Shirtcliffe P, Pritchard A, et al. Reference ranges for exhaled nitric oxide derived from a random community survey of adults. *Am J Respir Crit Care Med*. 2007 Aug 1;176(3):238–42.
 43. Franklin PJ, Turner SW, Le Souëf PN, Stick SM. Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. *Thorax*. 2003 Dec;58(12):1048–52.
 44. Olin A-C, Rosengren A, Thelle DS, Lissner L, Bake B, Torén K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. *Chest*. 2006 Nov;130(5):1319–25.
 45. Robbins RA, Millatmal T, Lassi K, Rennard S, Daughton D. Smoking cessation is associated with an increase in exhaled nitric oxide. *Chest*. 1997 Aug;112(2):313–8.
 46. Högman M, Holmkvist T, Wålinder R, Meriläinen P, Lúdvíksdóttir D, Håkansson L, et al. Increased nitric oxide elimination from the airways after smoking cessation. *Clin Sci Lond Engl* 1979. 2002 Jul;103(1):15–9.
 47. Buga GM, Griscavage JM, Rogers NE, Ignarro LJ. Negative feedback regulation of endothelial cell function by nitric oxide. *Circ Res*. 1993 Nov;73(5):808–12.
 48. Schilling J, Holzer P, Guggenbach M, Gyurech D, Marathia K, Geroulanos S. Reduced endogenous nitric oxide in the exhaled air of smokers and hypertensives. *Eur Respir J*. 1994 Mar;7(3):467–71.

- Accepted Article
49. Marteus H, Mavropoulos A, Palm JP, Ulfgren A-K, Bergström J, Alving K. Nitric oxide formation in the oropharyngeal tract: possible influence of cigarette smoking. *Nitric Oxide Biol Chem Off J Nitric Oxide Soc.* 2004 Nov;11(3):247–55.
 50. Olivieri M, Talamini G, Corradi M, Perbellini L, Mutti A, Tantucci C, et al. Reference values for exhaled nitric oxide (reveno) study. *Respir Res.* 2006;7:94.
 51. Taylor RG, Gross E, Joyce H, Holland F, Pride NB. Smoking, allergy, and the differential white blood cell count. *Thorax.* 1985 Jan;40(1):17–22.
 52. Burrows B, Halonen M, Lebowitz MD, Knudson RJ, Barbee RA. The relationship of serum immunoglobulin E, allergy skin tests, and smoking to respiratory disorders. *J Allergy Clin Immunol.* 1982 Sep;70(3):199–204.
 53. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med.* 2001 Oct 15;164(8 Pt 1):1376–81.
 54. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax.* 1998 Feb;53(2):91–5.
 55. Silvestri M, Spallarossa D, Frangova Yourukova V, Battistini E, Fregonese B, Rossi GA. Orally exhaled nitric oxide levels are related to the degree of blood eosinophilia in atopic children with mild-intermittent asthma. *Eur Respir J.* 1999 Feb;13(2):321–6.

56. Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. *Chest*. 2001 Dec;120(6):1917–22.
57. Boulet L-P, Lemièrre C, Archambault F, Carrier G, Descary MC, Deschesnes F. Smoking and asthma: clinical and radiologic features, lung function, and airway inflammation. *Chest*. 2006 Mar;129(3):661–8.
58. Broekema M, ten Hacken NHT, Volbeda F, Lodewijk ME, Hylkema MN, Postma DS, et al. Airway epithelial changes in smokers but not in ex-smokers with asthma. *Am J Respir Crit Care Med*. 2009 Dec 15;180(12):1170–8.
59. Spears M, McSharry C, Chaudhuri R, Weir CJ, de Wet C, Thomson NC. Smoking in asthma is associated with elevated levels of corticosteroid resistant sputum cytokines-an exploratory study. *PloS One*. 2013;8(8):e71460.
60. Bergeron C, Boulet L-P, Page N, Laviolette M, Zimmermann N, Rothenberg ME, et al. Influence of cigarette smoke on the arginine pathway in asthmatic airways: increased expression of arginase I. *J Allergy Clin Immunol*. 2007 Feb;119(2):391–7.
61. Bruce CT, Zhao D, Yates DH, Thomas PS. L-arginine reverses cigarette-induced reduction of fractional exhaled nitric oxide in asthmatic smokers. *Inflammopharmacology*. 2010 Feb;18(1):9–16.

Legends to Figures

None

Tables

Table 1. Characteristics of the study population (overall and as a function of smoking status).

Variable	Overall population (n=1579)	Smoking status			<i>p</i> ^(a)
		Never smokers (n=812)	Former smokers (n=473)	Current smokers (n=294)	
Age (years), mean (SD)	53.3 (7.3)	53.6 (7.3)	54.5 (7.1)	50.6 (6.9)	<10 ⁻⁹
Gender, n (%) males	765 (48.5)	277 (34.1)	319 (67.4)	169 (57.5)	<10 ⁻⁹
Height (cm), mean (SD)	169.2 (9.5)	167.3 (9.4)	171.6 (8.9)	170.6 (9.6)	<10 ⁻⁹
Body mass index (kg/m ²), mean (SD)	27.6 (5.4)	27.2 (5.5)	28.6 (5)	26.8 (5.4)	<10 ⁻³
Healthy participants, n (%)	569 (36.9)	327 (41.6)	160 (34.5)	82 (28.2)	<10 ⁻³
Current asthma, n (%)	111 (7.3)	50 (6.4)	43 (9.4)	18 (6.3)	0.122
Current allergic asthma, n (%)	80 (5.1)	39 (4.8)	27 (5.7)	14 (4.8)	0.754
Current nonallergic asthma, n (%)	31 (2.0)	11 (1.4)	16 (3.4)	4 (1.4)	0.041
Wheezing in the previous 12 months, n (%)	252 (16)	86 (10.6)	81 (17.1)	85 (28.9)	<10 ⁻⁹
Adrenergic inhalants, n (%)	58 (3.7)	21 (2.6)	26 (5.5)	11 (3.7)	0.030
Glucocorticoid inhalants, n (%)	18 (1.1)	11 (1.4)	7 (1.5)	0 (0)	0.074
Atopy, n (%)	490 (32)	259 (32.9)	147 (32.2)	84 (29.4)	0.551
Previous positive prick test, n (%)	262 (17.3)	146 (18.8)	78 (17.2)	38 (13.2)	0.105
Previous allergen desensitization therapy, n (%)	128 (8.1)	66 (8.1)	41 (8.7)	21 (7.1)	0.775
Allergic rhinitis or hay fever in the previous 12 months, n (%)	335 (21.4)	178 (22.1)	100 (21.4)	57 (19.7)	0.705
Airflow obstruction, n (%)	234 (15.2)	90 (11.3)	84 (18.1)	60 (20.9)	<10 ⁻³
FEV1 (% predicted), mean (SD)	1.04 (0.18)	1.06 (0.18)	1.02 (0.19)	0.99 (0.17)	<10 ⁻⁶
FVC (% predicted), mean (SD)	1.13 (0.20)	1.15 (0.22)	1.10 (0.18)	1.10 (0.16)	<10 ⁻³
Respiratory infection in the previous 4 weeks, n (%)	156 (9.9)	69 (8.5)	43 (9.1)	44 (15)	0.007
Usual dietary intake of vegetables (frequency/day), mean (SD)	1.84 (1.07)	1.88 (1.03)	1.92 (1.04)	1.62 (1.19)	<10 ⁻³
Usual dietary intake of fruits (frequency/day), mean (SD)	1.64 (1.17)	1.76 (1.12)	1.61 (1.17)	1.36 (1.26)	<10 ⁻³
FENO (ppb), geometric mean [95%CI]	14.7 [14.3; 15.2]	16.3 [15.6; 16.9]	15.5 [14.7; 16.3]	10.4 [9.7; 11.3]	<10 ⁻⁹
NO in ambient air (ppb), geometric mean [95%CI]	8.2 [7.8; 8.6]	8.3 [7.7; 8.9]	8.2 [7.5; 9.0]	8.0 [7.1; 9.0]	0.891
Blood eosinophil count (cells/mm ³), geometric mean [95%CI]	163 [158; 168]	149 [143; 155]	168 [159; 178]	200 [187; 214]	<10 ⁻⁹

n: number; SD: standard deviation; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; FENO: fractional exhaled nitric oxide; ppb: parts per billion; NO: nitric oxide; CI: confidence interval.

^(a) In a one-way analysis of variance for quantitative variables and Fisher's exact test for qualitative variables.

Table 2. Characteristics of participants with FENO values below and above the 95% reference value, as a function of smoking status.

Variable	Never smokers (n=812)			Former smokers (n=473)			Current smokers (n=294)		
	FENO <34 ppb (n=734)	FENO ≥34 ppb (n=78)	<i>p</i> ^(a)	FENO <32 ppb (n=427)	FENO ≥32 ppb (n=46)	<i>p</i> ^(a)	FENO <25 ppb (n=271)	FENO ≥25 ppb (n=23)	<i>p</i> ^(a)
Current asthma, n (%)	32 (4.5)	18 (23.1)	<10 ⁻⁶	34 (8.3)	9 (20)	0.026	16 (6.1)	2 (8.7)	0.647
Current allergic asthma, n (%)	22 (3)	17 (21.8)	<10 ⁻⁶	18 (4.2)	9 (19.6)	<10 ⁻³	13 (4.8)	1 (4.4)	1
Current nonallergic asthma, n (%)	10 (1.4)	1 (1.3)	1	16 (3.8)	0 (0)	0.386	3 (1.1)	1 (4.4)	0.282
Wheezing in the previous 12 months, n (%)	61 (8.3)	25 (32.1)	<10 ⁻⁶	69 (16.2)	12 (26.1)	0.1	80 (29.5)	5 (21.7)	0.632
Adrenergic inhalants, n (%)	13 (1.8)	8 (10.3)	<10 ⁻³	19 (4.5)	7 (15.2)	0.008	10 (3.7)	1 (4.4)	0.598
Glucocorticoid inhalants, n (%)	10 (1.4)	1 (1.3)	1	6 (1.4)	1 (2.2)	0.514	0 (0)	0 (0)	1
Atopy, n (%)	213 (30)	46 (59)	<10 ⁻⁶	124 (30.1)	23 (51.1)	0.006	79 (30)	5 (21.7)	0.481
Previous positive prick test, n (%)	114 (16.2)	32 (43.8)	<10 ⁻⁶	58 (14.2)	20 (44.4)	<10 ⁻³	37 (14)	1 (4.4)	0.332
Previous allergen desensitization therapy, n (%)	50 (6.8)	16 (20.5)	<10 ⁻³	31 (7.3)	10 (21.7)	0.003	20 (7.4)	1 (4.4)	1
Allergic rhinitis or hay fever in the previous 12 months, n (%)	144 (19.8)	34 (43.6)	<10 ⁻⁶	86 (20.4)	14 (31.1)	0.124	52 (19.5)	5 (21.7)	0.786
Airflow obstruction, n (%)	76 (10.6)	14 (18.4)	0.055	69 (16.6)	15 (32.6)	0.014	56 (21.2)	4 (17.4)	0.794
FEV1 (% predicted), mean (SD)	1.07 (0.16)	1.02 (0.32)	0.031	1.03 (0.19)	0.99 (0.18)	0.142	0.99 (0.17)	1 (0.14)	0.89
FVC (% predicted), mean (SD)	1.15 (0.17)	1.14 (0.49)	0.611	1.1 (0.18)	1.08 (0.16)	0.316	1.1 (0.17)	1.06 (0.14)	0.205
Respiratory infection in the previous 4 weeks, n (%)	57 (7.8)	12 (15.4)	0.031	37 (8.7)	6 (13)	0.291	37 (13.7)	7 (30.4)	0.06
Eosinophil count (cells/mm ³), geometric mean [95%CI]	143 [137; 149]	218 [187; 255]	<10 ⁻⁶	162 [153; 171]	243 [205; 289]	<10 ⁻³	197 [184; 212]	229 [182; 288]	0.249

FENO: fractional exhaled nitric oxide; n: number; SD: standard deviation; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; CI: confidence interval.

^(a) In Student's test for quantitative variables and Fisher's exact test for qualitative variables.

Table 3. Characteristics of participants with a blood eosinophil count below and above the threshold of 300 cells/mm³, as a function of smoking status.

Variable	Never smokers (n=767)			Former smokers (n=449)			Current smokers (n=280)		
	B-eos <300 cells/mm ³ (n=671)	B-eos ≥300 cells/mm ³ (n=96)	<i>p</i> ^(a)	B-eos <300 cells/mm ³ (n=365)	B-eos ≥300 cells/mm ³ (n=84)	<i>p</i> ^(a)	B-eos <300 cells/mm ³ (n=213)	B-eos ≥300 cells/mm ³ (n=67)	<i>p</i> ^(a)
Current asthma, n (%)	30 (4.6)	17 (18.1)	<10 ⁻³	26 (7.4)	15 (18.1)	0.006	16 (7.7)	2 (3.2)	0.382
Current allergic asthma, n (%)	22 (3.3)	16 (16.7)	<10 ⁻³	16 (4.4)	10 (11.9)	0.016	12 (5.6)	2 (3)	0.531
Current nonallergic asthma, n (%)	8 (1.2)	1 (1.1)	1	10 (2.8)	5 (6)	0.172	4 (1.9)	0 (0)	0.576
Wheezing in the previous 12 months, n (%)	51 (7.6)	24 (25)	<10 ⁻³	52 (14.3)	25 (29.8)	0.001	67 (31.5)	16 (23.9)	0.284
Adrenergic inhalants, n (%)	9 (1.3)	12 (12.5)	<10 ⁻⁶	15 (4.1)	11 (13.1)	0.004	9 (4.2)	2 (3)	1
Glucocorticoid inhalants, n (%)	8 (1.2)	2 (2.1)	0.362	5 (1.4)	2 (2.4)	0.62	0 (0)	0 (0)	1
Atopy, n (%)	203 (31.3)	39 (41.1)	0.062	114 (32.4)	28 (33.7)	0.796	64 (30.6)	18 (28.6)	0.876
Previous positive prick test, n (%)	108 (16.8)	27 (29)	0.006	62 (17.7)	14 (17.3)	1	26 (12.4)	12 (18.5)	0.223
Previous allergen desensitization therapy, n (%)	48 (7.2)	13 (13.5)	0.042	33 (9)	5 (6)	0.514	17 (8)	4 (6)	0.791
Allergic rhinitis or hay fever in the previous 12 months, n (%)	140 (21)	26 (27.1)	0.187	75 (20.8)	22 (26.5)	0.302	44 (20.9)	11 (16.9)	0.595
Airflow obstruction, n (%)	70 (10.7)	18 (19)	0.026	62 (17.2)	20 (25)	0.114	45 (21.6)	13 (20)	0.863
FEV1 (% predicted), mean (SD)	1.07 (0.15)	0.99 (0.19)	<10 ⁻⁶	1.03 (0.19)	1.01 (0.17)	0.253	0.98 (0.16)	1.02 (0.16)	0.105
FVC (% predicted), mean (SD)	1.16 (0.16)	1.1 (0.19)	<10 ⁻³	1.11 (0.18)	1.09 (0.16)	0.342	1.1 (0.16)	1.12 (0.15)	0.321
Respiratory infection in the previous 4 weeks, n (%)	56 (8.4)	12 (12.5)	0.181	31 (8.5)	11 (13.1)	0.114	35 (16.5)	9 (13.4)	0.595
FENO (ppb), geometric mean [95%CI]	15.5 [14.9; 16.1]	23.2 [20.3 26.4]	<10 ⁻⁹	14.7 [13.9; 15.6]	20.0 [17.3; 23.0]	<10 ⁻³	10.3 [9.5; 11.2]	11.2 [9.7; 13.0]	0.319

n: number; SD: standard deviation; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; CI: confidence interval; FENO: fractional exhaled nitric oxide; ppb: parts per billion; B-eos: blood eosinophil count.

^(a) In Student's test for quantitative variables and Fisher's exact test for qualitative variables.

Table 4. Factors associated with FENO, as a function of smoking status.

Variable	Overall population			Never or former smokers		Current smokers	
	Percentage increase in FENO values [95% CI] ^(a)	<i>p</i>	<i>p</i> value for interaction with current smoking ^(b)	Percentage increase in FENO values [95% CI] ^(a)	<i>p</i>	Percentage increase in FENO values [95% CI] ^(a)	<i>p</i>
Current smoking	-35 [-39.7; -29.8]	<10 ⁻⁹	-	-	-	-	-
Age (per 5-year increment)	1.3 [-0.8; 3.5]	0.234	0.322	1.7 [-0.6; 4]	0.145	-2.2 [-7.4; 3.4]	0.432
Female gender	-12.5 [-19.1; -5.2]	0.001	0.360	-10.4 [-17.9; -2.3]	0.013	-20.8 [-34.4; -4.3]	0.016
Height (per 10 cm increment)	7.7 [3.3; 12.3]	<10 ⁻³	0.386	7.9 [3.1; 13]	0.001	6.5 [-3.1; 17.1]	0.190
Body mass index (per 1 kg/m ² increment)	0.5 [0; 1.1]	0.059	0.036	0.8 [0.2; 1.5]	0.008	-1 [-2.3; 0.4]	0.162
Current allergic asthma	51.3 [30.3; 75.5]	<10 ⁻⁶	0.003	63.4 [39; 92]	<10 ⁻⁶	2.4 [-29.7; 49.1]	0.901
Current nonallergic asthma	-3.1 [-22.3; 21]	0.782	0.113	-8.6 [-27.9; 16]	0.461	50.6 [-17.8; 176]	0.184
Glucocorticoid inhalants	-24.3 [-44.3; 3]	0.076	-	-26.3 [-45.8; 0.3]	0.052	-	-
Atopy	15.5 [8.2; 23.3]	<10 ⁻³	0.011	18 [9.9; 26.7]	<10 ⁻³	4 [-11.5; 22.2]	0.635
Airflow obstruction	1.7 [-6.5; 10.5]	0.701	0.392	2.4 [-6.8; 12.6]	0.620	-6.4 [-21.6; 11.7]	0.461
Respiratory infection in the previous 4 weeks	14.9 [4.4; 26.5]	0.004	0.136	8.8 [-2.5; 21.6]	0.132	22.9 [0.5; 50.2]	0.045
Usual dietary intake of vegetable (for a 1 per day increment)	0.9 [-1.9; 3.7]	0.531	0.866	1 [-2.1; 4.2]	0.519	2.3 [-3.8; 8.8]	0.469
Usual dietary intake of fruit (for a 1 per day increment)	3.6 [1; 6.2]	0.006	0.087	2.3 [-0.6; 5.2]	0.115	7.9 [1.9; 14.2]	0.009
NO in ambient air (per 10 ppb increment)	1 [0; 1.9]	0.044	0.282	1.2 [0.2; 2.2]	0.020	-0.5 [-3; 2.1]	0.702

FENO: fractional exhaled nitric oxide; NO: nitric oxide; ppb: parts per billion; CI: confidence interval.

^(a) Performed using a multiple linear regression model. Results are expressed as the percentage increase in FENO values per unit change in the explanatory variable.

^(b) Interaction terms between smoking status (current smoking: yes/no) and the other explanatory variables were tested one at a time in the multiple linear regression model.

Table 5. Factors associated with the blood eosinophil count, as a function of smoking status.

Variable	Overall population			Never or former smokers		Current smokers	
	Percentage increase in B-eos values [95% CI] ^(a)	<i>p</i>	<i>p</i> value for interaction with current smoking ^(b)	Percentage increase in B-eos values [95% CI] ^(a)	<i>p</i>	Percentage increase in B-eos values [95% CI] ^(a)	<i>p</i>
Current smoking	25.3 [15.7; 35.7]	<10 ⁻⁹	-	-	-	-	-
Age (per 5-year increment)	1.1 [-1.2; 3.3]	0.355	0.244	1.5 [-0.9; 3.9]	0.234	-1.8 [-7; 3.7]	0.513
Female gender	-14.1 [-21.1; -6.5]	<10 ⁻³	0.979	-11.7 [-19.5; -3]	0.009	-21.4 [-35.4; -4.3]	0.017
Height (per 10 cm increment)	0.1 [-0.4; 0.6]	0.649	0.576	0.3 [-0.2; 0.8]	0.311	-0.5 [-1.5; 0.6]	0.367
Body mass index (per 1 kg/m ² increment)	1.4 [0.8; 2]	<10 ⁻³	0.010	1.8 [1.1; 2.5]	<10 ⁻⁶	-0.2 [-1.5; 1.2]	0.811
Current allergic asthma	47.7 [27.1; 71.6]	<10 ⁻⁶	0.001	63.2 [38.2; 92.7]	<10 ⁻⁶	-2.9 [-31.1; 36.9]	0.868
Current nonallergic asthma	-9.1 [-28.3; 15.2]	0.429	0.224	-0.6 [-23.2; 28.6]	0.962	-32.2 [-63; 24.2]	0.208
Glucocorticoid inhalants	7.9 [-22.3; 49.9]	0.648	-	-2 [-29.7; 36.6]	0.903	-	-
Atopy	4.9 [-2.1; 12.4]	0.176	0.017	7.2 [-0.7; 15.7]	0.074	-5.8 [-20; 10.9]	0.468
Airflow obstruction	2.7 [-5.9; 12]	0.555	0.124	4.5 [-5.3; 15.4]	0.382	-5.8 [-21.3; 12.7]	0.511
Respiratory infection in the previous 4 weeks	15.3 [4.3; 27.5]	0.005	0.822	13 [0.5; 27]	0.041	22.2 [0.3; 49]	0.047

B-eos: blood eosinophil count; CI: confidence interval.

^(a) Performed using a multiple linear regression model. Results are expressed as the percentage increase in blood eosinophil count values per unit change in the explanatory variable.

^(b) Interactions between smoking status (current smoking: yes/no) and the other explanatory variables were tested one at a time in the multiple linear regression model.

Table 6. The ability of FENO and blood eosinophil count to discriminate between participants with current allergic asthma and other participants.

Population	FENO (ppb)						Blood eosinophil count (cells/mm ³)					
	AUC [95% CI]	Optimal threshold ^(a)	Se	Sp	PPV	NPV	AUC (95% CI)	Optimal threshold ^(a)	Se	Sp	PPV	NPV
Overall population	0.66 [0.59; 0.73]	19	0.59	0.69	0.09	0.97	0.70 [0.63; 0.75]	220	0.64	0.70	0.11	0.97
Never smokers	0.75 [0.64; 0.82]	19.5	0.74	0.67	0.10	0.98	0.76 [0.67; 0.84]	230	0.68	0.79	0.14	0.98
Former smokers	0.71 [0.59; 0.81]	21	0.59	0.75	0.13	0.97	0.73 [0.62; 0.80]	220	0.69	0.68	0.12	0.97
Current smokers	0.41 [0.26; 0.60]	13	0.29	0.66	0.04	0.95	0.44 [0.29; 0.60]	225	0.43	0.57	0.05	0.95

FENO: fraction of exhaled nitric oxide; ppb: parts per billion; AUC: area under the curve; CI: confidence interval; Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value

^(a) The threshold maximizing sensitivity and specificity