

## Original article

# Both allergic and nonallergic asthma are associated with increased FE<sub>NO</sub> levels, but only in never-smokers

**Background:** Allergic asthma is consistently associated with increased FE<sub>NO</sub> levels whereas divergence exists regarding the use of exhaled nitric oxide (NO) as marker of inflammation in nonallergic asthma and in asthmatic smokers. The aim of this study is to analyze the effect of having allergic or nonallergic asthma on exhaled nitric oxide levels, with special regard to smoking history.

**Methods:** Exhaled NO measurements were performed in 695 subjects from Turin (Italy), Gothenburg and Uppsala (both Sweden). Current asthma was defined as self-reported physician-diagnosed asthma with at least one asthma symptom or attack recorded during the last year. Allergic status was defined by using measurements of specific immunoglobulin E (IgE). Smoking history was questionnaire-assessed.

**Results:** Allergic asthma was associated with 91 (60, 128) % [mean (95% CI)] increase of FE<sub>NO</sub> while no significant association was found for nonallergic asthma [6 (–17, 35) %] in univariate analysis, when compared to nonatopic healthy subjects. In a multivariate analysis for never-smokers, subjects with allergic asthma had 77 (27, 145) % higher FE<sub>NO</sub> levels than atopic healthy subjects while subjects with nonallergic asthma had 97 (46, 166) % higher FE<sub>NO</sub> levels than nonatopic healthy subjects. No significant asthma-related FE<sub>NO</sub> increases were noted for ex- and current smokers in multivariate analysis.

**Conclusions:** Both allergic and nonallergic asthma are related to increased FE<sub>NO</sub> levels, but only in never-smoking subjects. The limited value of FE<sub>NO</sub> to detect subjects with asthma among ex- and current smokers suggests the predominance of a noneosinophilic inflammatory phenotype of asthma among ever-smokers.

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There has been a continually increased interest for the use of exhaled breath as noninvasive assessment of airways inflammation in asthma. Exhaled nitric oxide (NO) is the most promising exhaled breath marker and has gone a long way from the observational study reporting increased exhaled NO levels in asthmatics (1) to current investigations regarding a potential role in titrating steroids in asthma management, predicting asthma exacerbations and outcome after steroid withdrawal in stable asthma (2).

Allergic asthma is associated with increased exhaled NO levels (3–5) whereas the effect of nonallergic asthma on the levels of fraction of NO in the exhaled air (FE<sub>NO</sub>) is unclear with small-sized studies (4, 6) reporting similar FE<sub>NO</sub> levels in nonallergic asthma and healthy nonatopic

controls while larger studies are contradictory with two studies reporting higher levels (7, 8) and one study reporting similar FE<sub>NO</sub> levels in nonallergic asthmatics and healthy controls (9).

Although a certain caution is advocated regarding the use of FE<sub>NO</sub> measurements in smokers (2) due to FE<sub>NO</sub> levels decreased with up to 60% in current smokers, the current ATS/ERS recommendations state that the value of FE<sub>NO</sub> as asthma marker is not affected by smoking (10). However this appears to be contradicted by newer studies, where similar FE<sub>NO</sub> levels have been noted in smokers with and without asthma (11, 12).

The aim of the present study was to analyse the effect of having allergic or nonallergic asthma on exhaled nitric oxide levels, with special regard to smoking history.

## Material and methods

### Study participants

The European Community Respiratory Health Survey (ECRHS) is an international multicenter study of asthma and allergy. The first part, ECRHS I, was conducted in 1990–1994 and the follow-up study, ECRHS II, in 1999–2001. The design of ECRHS I and II has been published in detail. (13, 14) Each participant was sent a brief questionnaire (stage 1) and from those who responded, a random sample was selected to undergo a more detailed clinical examination (stage 2). In addition, a 'symptomatic sample', reporting symptoms of waking with shortness of breath, asthma attacks or using asthma medication in stage 1, was also studied. In ECRHS II, subjects who had participated in stage 2 of ECRHS I were invited to participate in the follow-up.

The present study includes data from three of the 29 centers in the ECRHS II – Uppsala and Gothenburg in Sweden and Turin in Italy where 1548 subjects from random sample and 496 from 'symptomatic sample' participated in stage 2 of ECRHS I. Of these subjects 1484 were included in the ECRHS II. Criteria for inclusion in the present study were to have answered the main questionnaire, to perform  $FE_{NO}$  and specific IgE measurements. The number of subjects who corresponded to these inclusion criteria was 728, of which 102 were from Turin, 341 from Gothenburg and 285 from Uppsala. Thirty-three subjects on daily inhaled steroids or oral antileukotrienes were excluded leading to a total of 695 subjects analyzed.

### Smoking history, atopy and asthma diagnosis

*Smoking history* was questionnaire-assessed. A subject was considered as being a smoker if he/she had been smoking at least one cigarette a day for 1 year or at least 20 packs of cigarettes in a lifetime. Subjects recorded as smokers were further classified as current or ex-smokers, based on the smoking status during the month before the study.

*Specific IgE* was measured against *Dermatophagoides pteronyssinus*, cat, timothy grass and *Cladosporium herbarum*, using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). A person was defined as *atopic* if the titers against at least one of the tested allergens were  $\geq 0.35$  kU/l.

*Current asthma* was defined as self-reported physician-diagnosis of asthma and at least one asthma symptom or one asthma attack during the 12 months preceding the study.

*Allergic and nonallergic asthma* was defined as current asthma with or without a positive atopic status (15).

### Exhaled NO

Exhaled NO measurements were done according to ATS/ERS recommendations (10). Different techniques and flow rates of measuring  $FE_{NO}$  were used in different centers – offline measurements at 350 ml/s in Turin and online measurements at 50 ml/s in Uppsala and Gothenburg. Different NO analyzers were used in the three centers: Sievers NOA 270B, Sievers, Boulder, CA, USA in Uppsala and Turin and Eco Physics NO Analyzer CLD 700 AL, Dürnten, Switzerland in Gothenburg. The methodology of exhaled NO measurements for each center is described in previous published papers from Turin (16), Gothenburg (17) and Uppsala (18).

### Lung function

Forced expiratory volume in 1 s ( $FEV_1$ ) was measured with a standardized method with Biomedin Spirometer (Biomedin, Padova, Italy) in Turin and Spiro Medics Spirometer 2130 (Sensor Medics,

Anaheim, CA, USA) in Uppsala and Gothenburg.  $FEV_1$  was expressed as % of the predicted value (19).

### Statistics

Statistical analyses were performed using STATA 8.0 software (Stata Corp., College Station, TX, USA, 2001). Different  $FE_{NO}$  measurement techniques (20), NO analysers (21) and exhalation flow rates were used and we therefore used percentual increase of  $FE_{NO}$  as measure of effect. Percentual increase of  $FE_{NO}$  was obtained from linear regression models, in the pooled data from the three study centers, where log-transformed  $FE_{NO}$  was the outcome variable. The regression coefficient for the predictor variable of interest (e.g. current asthma) was back-transformed, as described by Franklin et al. (22), in order to obtain the fold increase/difference of  $FE_{NO}$  (e.g. asthmatics vs nonasthmatics), and this was in its turn converted to percentual increase/difference by multiplying with 100 and subtracting 100%.

These regression models were used to study the effect of allergic and nonallergic asthma, respective atopy in the whole population or in different subgroups, obtained after dividing the study population upon smoking history and/or atopy status. Consistency of the found effects was also studied after adjustments have been made for gender, age, height, lung function, study sample (random vs 'symptomatic'), study center and smoking history.

Heterogeneity between centers was tested by means of a meta-analysis of the three centers with regard to the effect of having allergic and nonallergic asthma in the whole population and of having asthma in each smoking strata of our population.

### Ethics

Written informed consent was obtained from each subject before inclusion in the study. The protocol was approved by the local Ethics Committees in each center.

## Results

Effect of known determinants of exhaled NO levels in different study centers

The effect of different known determinants of the levels of  $FE_{NO}$  was investigated per each center and presented as  $FE_{NO}$  percentual change (Table 1). Moreover, heterogeneity was assessed regarding differences between centers in  $FE_{NO}$  percentual change when assessing the effect of a particular variable.

Current smoking and female gender were associated with lower  $FE_{NO}$  values while increased height, presence of atopy and presence of current asthma were associated with increased  $FE_{NO}$  values.

There was heterogeneity regarding the effect of age on  $FE_{NO}$  levels with positive association in Gothenburg and negative in Uppsala and Turin. A trend for heterogeneity was noted regarding the effects of previous-smoking with no effect in Gothenburg and a decrease of  $FE_{NO}$  in Uppsala and Turin.

Differences regarding population characteristics among centers

We investigated the 695 participants from three study centers regarding their characteristics, which were super-

Table 1. Univariate results per each study center of percentual change of FE<sub>NO</sub> [mean (95% confidence interval (CI))] associated with different variables known to affect FE<sub>NO</sub> levels

| Variable                             | FE <sub>NO</sub> percentual change per center |                              |                           | <i>P</i> -value heterogeneity |
|--------------------------------------|-----------------------------------------------|------------------------------|---------------------------|-------------------------------|
|                                      | Turin ( <i>n</i> = 99)                        | Gothenburg ( <i>n</i> = 328) | Uppsala ( <i>n</i> = 268) |                               |
| Female gender                        | −21% (−37%, −2%)                              | −32% (−41%, −20%)            | −14% (−26%, −1%)          | 0.11                          |
| Age (per 10 years)                   | −11% (−24%, 4%)                               | 11% (−1%, 24%)               | −12% (−20%, −2%)          | 0.005                         |
| Height (per 10 cm)                   | 19% (7%, 32%)                                 | 19% (9%, 29%)                | 13% (5%, 21%)             | 0.58                          |
| FEV <sub>1</sub> (per 10% predicted) | 2% (−5%, 9%)                                  | 3% (−3%, 9%)                 | −5% (−10%, 1%)            | 0.11                          |
| Atopy                                | 22% (−3%, 55%)                                | 35% (14%, 60%)               | 45% (25%, 67%)            | 0.49                          |
| Ex-smoking                           | −16% (−35%, 10%)                              | 4% (−13%, 23%)               | −21% (−32%, −8%)          | 0.07                          |
| Current smoking                      | −19% (−29%, −8%)                              | −23% (−30%, −16%)            | −24% (−31%, −15%)         | 0.79                          |
| Current asthma                       | 57% (12%, 121%)                               | 39% (10%, 74%)               | 46% (20%, 78%)            | 0.83                          |

imposing the variables listed in Table 1. The only noted difference was regarding height with shorter subjects in Turin than Uppsala and Gothenburg (both *P*-values < 0.001).

#### Participation in the study

The study participants were more likely to be taller [1.73 (1.72, 1.74) m *vs* 1.71 (1.70, 1.72) m, *P* = 0.03] and had a slightly higher mean age [42.7 (42.1, 43.2) years *vs* 41.2 (40.4, 42.1) years, *P* = 0.007] than participants who did not undergo FE<sub>NO</sub> measurements. No significant differences were found concerning FEV<sub>1</sub>, smoking history, atopy and current asthma.

Levels of FE<sub>NO</sub> in the study population according to atopy and asthma status

The pooled study population was divided into four groups: nonatopic nonasthmatics (*n* = 432), nonallergic asthmatics (*n* = 33), atopic nonasthmatics (*n* = 165), and allergic asthmatics (*n* = 65). Allergic asthma was associated with 91 (60, 128) % [mean (95% CI)] increase of FE<sub>NO</sub> while no significant FE<sub>NO</sub> increase was found

for nonallergic asthma [6 (−17, 35) %] in univariate analysis, when compared to nonatopic healthy subjects (Fig. 1A). After adjustments for gender, height, age, lung function, smoking history, study sample and center, nonallergic asthmatics and atopic nonasthmatic subjects had similar FE<sub>NO</sub> increases when compared to nonatopic nonasthmatic subjects while allergic asthma subjects had the highest levels (*P* < 0.01 for comparisons to nonallergic asthmatics and atopic nonasthmatics) (Fig. 1B).

The reported results were consistent for both allergic and nonallergic asthmatics in a meta-analysis performed for the three centers with a similar size of the effect as for the pooled data analyses with no heterogeneity between the centers (Table 2).

Atopy and degree of IgE sensitization in subjects grouped upon smoking history

Atopy was associated with increased FE<sub>NO</sub> levels in all three smoking history groups while current asthma was only associated with increased FE<sub>NO</sub> in never and ex-smokers (Table 3).

The FE<sub>NO</sub> increase associated with current asthma persisted in meta-analysis for never and ex-smokers and

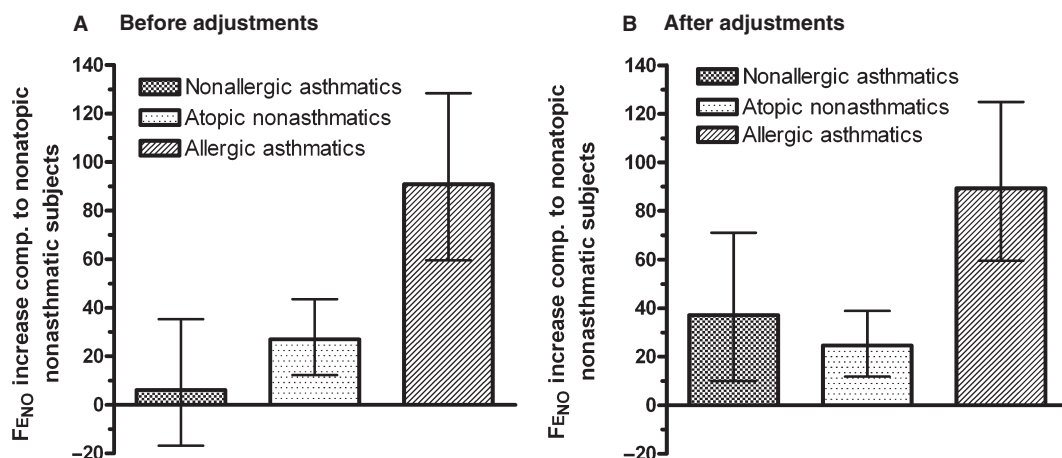


Figure 1. The percentual increase of FE<sub>NO</sub> [mean (95% CI)] in subjects with nonallergic asthma, atopic nonasthmatics or allergic asthmatics compared to nonatopic nonasthmatics, before (Panel A) and after adjustments for gender, height, age, lung function, smoking history, study sample and center (Panel B).

Table 2. Percentual increase of  $FE_{NO}$  [mean (95% CI)] associated with allergic and nonallergic asthma respectively, compared to nonatopic nonasthmatic subjects, in pooled data analysis and meta-analysis

|                    | Percentual increase of $FE_{NO}$ * in pooled data | Percentual increase of $FE_{NO}$ * in meta-analysis | Heterogeneity between centers |
|--------------------|---------------------------------------------------|-----------------------------------------------------|-------------------------------|
| Allergic asthma    | 89 (60, 125) %                                    | 88 (59, 123) %                                      | 0.53                          |
| Nonallergic asthma | 37 (10, 71) %                                     | 39 (13, 70) %                                       | 0.75                          |

\*After adjustments for gender, height, age, lung function, smoking history, study sample and center.

Table 3. Percentual increase of  $FE_{NO}$  [mean (95% CI)] associated with presence of atopy or current asthma in the three smoking strata of our study population

|                 | Never smokers  | Ex-smokers    | Current smokers |
|-----------------|----------------|---------------|-----------------|
| Atopy*          | 41 (21, 65) %  | 37 (15, 64) % | 42 (12, 80) %   |
| Current asthma† | 70 (39, 108) % | 46 (14, 88) % | -12 (-38, 24) % |

\*Compared to nonatopic individuals from the same strata of smoking history.

†Compared to nonasthmatic individuals from the same strata of smoking history.

Table 4. Percentual increase of  $FE_{NO}$  [mean (95% CI)] in asthmatic subjects, compared to nonasthmatic subjects, for each of the six groups obtained after dividing our population by allergic status and smoking history

|                 | Nonatopic subjects                | Atopic subjects                  |
|-----------------|-----------------------------------|----------------------------------|
| Never-smokers   | 62.1% (16.7, 125.2)<br>(n = 199)  | 49.3% (11.1, 100.7)<br>(n = 119) |
| Ex-smokers      | 4.9% (-31.7, 61.1)<br>(n = 160)   | 44.2% (1.8, 104.4)<br>(n = 64)   |
| Current smokers | -25.4% (-51.5, 14.6)<br>(n = 100) | 5.4% (-39.9, 84.9)<br>(n = 46)   |

no heterogeneity was noted with exception for current smokers group ( $P = 0.02$ ), where trends for positive association of asthma with  $FE_{NO}$  were noted for Uppsala and Turin and a negative association for Gothenburg.

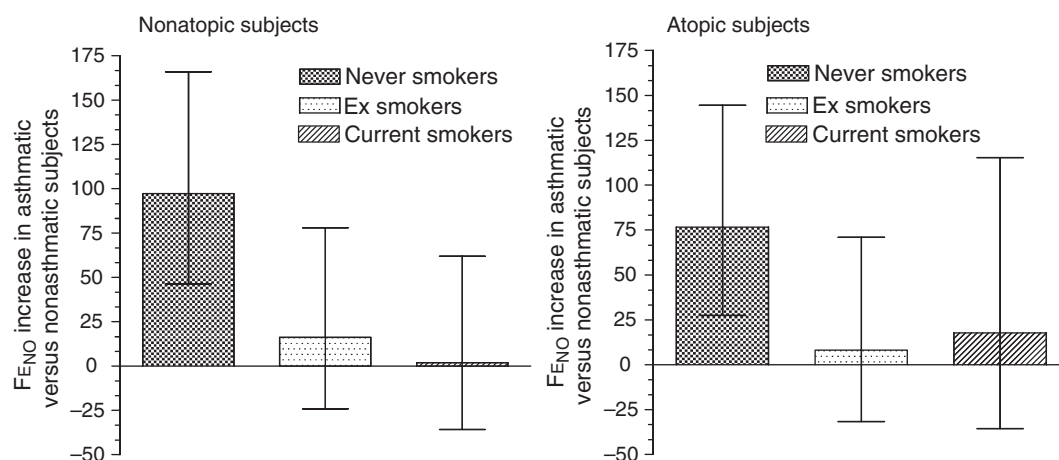


Figure 2. Percentual increase of  $FE_{NO}$  in asthmatics vs nonasthmatics in relation to smoking history, in nonatopic and atopic subjects after adjustment for gender, age, height,  $FEV_1$ (%pred), study sample and center.

Table 4 shows the percentual increase in  $FE_{NO}$  in asthmatic vs nonasthmatics after stratification for both atopic status and smoking history. In never-smokers asthma was associated with increased  $FE_{NO}$  levels both among nonatopics and atopics. In ex-smokers asthma was related to increased  $FE_{NO}$  only in atopics, while no association between asthma and  $FE_{NO}$  was found in current smokers.

When adjusting for gender, height, age, lung function, study sample and center an independent association was found between asthma and  $FE_{NO}$  only in never-smokers, both in the nonatopic and atopic group (Fig. 2). The effects of allergic and nonallergic asthma among never-smokers were consistent in a meta-analysis and no heterogeneity was recorded ( $P = 0.17$  and  $0.51$ ).

The reported results were maintained in a subanalysis done in ex-smokers when the nine subjects who quit smoking during the year previous to the study were excluded. Furthermore, no effect of time since quitting smoking on the  $FE_{NO}$  levels was noted when it was adjusted for in the model described above (data not shown).

## Discussion

The main finding in this study is that both allergic and nonallergic asthma are related to increased  $FE_{NO}$  levels, but only among never-smoking subjects. The use of  $FE_{NO}$  measurements in order to identify asthmatic subjects in a population appears to be of limited value in ex-smokers and of no value in the case of current smokers.

The strength of our study resides in the fact that this is the first population-based multicenter study investigating the value of  $FE_{NO}$  in detecting subjects with asthma when special regard is taken both to the type (allergic vs nonallergic) of asthma and the subjects' smoking history. The use of different techniques and/or equipment for

measuring NO in different study centers represents the main weakness of our study as it lead to different absolute FE<sub>NO</sub> values and therefore the necessity of reporting the results as percentual increases of FE<sub>NO</sub>. However, the extrapolation of our results might prove to be easier when using the percentual change of FE<sub>NO</sub>. Similar effects in all study centers of known determinants of FE<sub>NO</sub> were observed by meta-analysis, suggesting valid data. The heterogeneity regarding the effects of age and previous smoking is in line with results from the two big available population-based studies looking at determinants of FE<sub>NO</sub> levels (12, 23).

A higher exhalation flow rate was used in Turin than in the other centers. A higher exhalation flow rate leads to a more peripheral NO sampling, but the contribution from central airways to measured exhaled NO is still predominant (24). The asthma-related increase of FE<sub>NO</sub> is in a majority of cases related to the increase production of NO in the central airways (24), that will be reflected in exhaled NO measured at flow-rates between 50 and 200 ml/s with online methods and 50–350 ml/s with offline methods (25). Furthermore our main findings remained significant even when excluding Turin from the analyses (data not shown).

#### Allergic and nonallergic asthma

In our material, both allergic and nonallergic asthma were associated with increased FE<sub>NO</sub> levels, but only in never-smokers. This is in disagreement with some of the previous studies that report similar levels of FE<sub>NO</sub> in nonallergic asthmatic and nonatopic controls (4–6), but in agreement with two other studies (7, 8). Multiple explanations may lie behind the divergent findings of these studies. The FE<sub>NO</sub> increase reported by one of the studies was small (8) so in order to detect a significant difference between nonallergic asthmatics and nonatopic nonasthmatics it is essential to have adequate power. One important aspect regarding nonallergic asthma is the heterogeneity of the inflammation patterns with divergent reports regarding the extent of the eosinophilic inflammation with no differences (26) or lower eosinophil involvement (27) when compared to allergic asthma. Therefore differences between studies regarding the proportion of subjects with eosinophilic pattern of inflammation among the subjects with nonallergic asthma could to some extent explain the different results.

The association of allergic asthma with increased levels of FE<sub>NO</sub> is a finding in line with the literature (3–5). The mechanism behind this increase appears to be the activation of iNOS in the bronchial epithelial cells (28) as iNOS in lung epithelial cells has been reported be the main determinant of the FE<sub>NO</sub> levels in recent studies (29, 30). The link between the eosinophil activation and epithelial iNOS activation was demonstrated in a murine model of allergic asthma (31) and human studies support this by demonstrating good correlations between exhaled

nitric oxide and blood, lung tissue and sputum eosinophils (9, 32–34).

#### Smoking history

Both current (35, 36) and previous smoking (37) are associated with decreased FE<sub>NO</sub> levels. Although the ERS/ATS guidelines (10) state, based on the study by Horvath et al. (38), that exhaled NO is still reflecting the presence of asthma in current smokers, this appears to be contradicted by two more recent studies where no difference was found between smokers with and without asthma (11, 12). The difference in the results between studies may be partially explained by the lack of adjustment of results for atopy in the study by Horvath et al. where all the subjects with asthma included in the study were polysensitized while the controls were probably mostly nonsensitized as no allergic status is reported for the control group.

The results concerning the limited value of FE<sub>NO</sub> as asthma marker in ex-smokers are in accordance with a previous population-based study (12) where no relation of FE<sub>NO</sub> to physician-diagnosed asthma and asthma symptoms was found among ex-smokers, although ex-smokers had similar FE<sub>NO</sub> levels as never-smokers in the respective study.

The main explanation for the lack of association between exhaled NO and asthma in current smokers is probably the dominance of the neutrophilic phenotype of inflammation in asthmatic smokers as increased neutrophilic inflammation and lower eosinophil number in induced sputum have been documented in smokers with asthma (39, 40). This would limit the use of exhaled NO as a marker of asthmatic inflammation in smokers, since exhaled NO is a marker of eosinophilic inflammation (41–43). It is unclear if there is any shift of the inflammatory phenotype of asthma in ex-smokers as studies with long follow-up time after smoking cessation are lacking, in contrast with chronic obstructive pulmonary disease where such studies are available and the results point consistently towards the persistence of the neutrophilic pattern of inflammation after smoking cessation (44). No relation between time since quitting smoking and exhaled NO could be found in our material, suggesting no shift in the inflammation pattern. The only available smoking cessation studies in asthmatics have shown better response to oral steroids at 1 year (45), but not at 8 weeks (46), after smoking cessation when assessing PEF, but no effect on FEV<sub>1</sub> or asthma control. Similarly a lower number of neutrophils in induced sputum has been reported both at 8 weeks and 1 year (45, 46) after smoking cessation, but no decrease in neutrophil mediators (46).

In the present study, atopy was associated with similar increases of FE<sub>NO</sub> levels both in never- and ever-smokers. Atopy is characterized by subclinical eosinophilic inflammation (47) and increased exhaled NO levels (48). There is also well studied association between exhaled NO levels

and eosinophilic inflammation (2). Taken together this indicates that smoking does not affect the association between atopic eosinophilic inflammation and  $FE_{NO}$ . The lack of association between asthma and  $FE_{NO}$  among ever-smokers is therefore more likely explained by a predominant noneosinophilic asthma phenotype in ever-smokers.

In conclusion, both allergic and nonallergic asthma were associated with increased  $FE_{NO}$  levels in never-smoking subjects, confirming an independent effect of having asthma on increased  $FE_{NO}$  levels. The limited use of  $FE_{NO}$  measurements in ex- and current smokers for

detecting subjects with asthma may be explained by the dominance of noneosinophilic inflammation in ever-smoking asthmatics.

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