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Important non-disease related determinants of exhaled NO levels in mild asthma – results from the Swedish GA²LEN study

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Abbreviations:

ATS – American Thoracic Society

CRS – chronic rhinosinusitis

ERS – European Respiratory Society

EP3OS - European Position Paper on Rhinosinusitis and Nasal Polyps

FeNO – fraction of exhaled nitric oxide

FEV₁ – forced expiratory volume in 1 second

FVC – forced vital capacity

GA²LEN – Global Asthma and Allergy Excellence Network

IgE – immunoglobulin E

IL – interleukin

ppb – parts per billion

Th2 – T helper 2

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Abstract (253 words)

Background: Exhaled nitric oxide (FeNO) has a potential clinical role in asthma management. Constitutive factors such as age, height and male gender, as well as individual characteristics, as IgE sensitisation and smoking, affect levels of FeNO in population-based studies. However their effect on FeNO in subjects with asthma has been scarcely studied.

Objective: To study the effects on FeNO of these commonly regarded determinants, as demonstrated in healthy subjects, as well as menarche age and parental smoking, in a population of asthmatics.

Material and Methods: FeNO was measured in 557 subjects with asthma from the Swedish GA2LEN study. Allergic sensitisation was assessed by skin prick tests to most common aeroallergens. Upper airway comorbidities, smoking habits, smoking exposure during childhood, hormonal status (for women) were questionnaire-assessed.

Results: Male gender ($p<0.001$), greater height ($p<0.001$) and sensitisation to both perennial allergens and pollen ($p<0.001$) related to higher FeNO levels. Current smoking ($p<0.001$) and having both parents smoking during childhood, vs having neither ($p<0.001$) or only one parent smoking ($p=0.002$), related to lower FeNO. Women with menarche between 9-11 years of age had lower FeNO than those with menarche between 12-14 years of age ($p = 0.03$) or 15-17 years of age ($p=0.003$).

Conclusions and Clinical relevance: Interpreting FeNO levels in clinical practice is complex and constitutional determinants, as well as smoking and IgE sensitization, are of importance in asthmatic subjects and should be accounted for when interpreting FeNO levels. Furthermore, menarche age and parental smoking during childhood and their effects on lowering FeNO deserve further studies.

Introduction

Fractional exhaled nitric oxide (FeNO) is a non-invasive marker of T helper 2 (Th2)-driven airway inflammation mediated through interleukin (IL)-4/IL-13 mechanisms (1, 2). FeNO levels decrease after introduction of corticosteroids and this effect is dose-dependent (3). Therefore, FeNO measurement has a potential clinical role in asthma management in guiding anti-inflammatory treatment, as a recent meta-analysis suggests benefits in the form of decreasing numbers of exacerbations (4). The current guidelines (5) recommend certain cut-offs of FeNO – 25 and 50 ppb – to be used in asthma management, but these cut-offs have recently been challenged (6, 7). The studies using FeNO to tailor inhaled corticosteroid therapy have chosen other, and to some extent arbitrary, cut-offs to define target FeNO (8-10). However, in order to better understand which FeNO levels we should target with our treatments, we need a better understanding of the non-disease related determinants of exhaled NO levels in subjects with asthma.

In population-based studies, constitutive factors such as age, height and male gender have been found to be significantly related to high FeNO (11). Immunoglobulin E (IgE) sensitisation is an important determinant of exhaled NO (12) and there is a relation between the degree of IgE sensitisation and FeNO (13, 14). However, most of the studies investigating constitutional factors related to FeNO levels are based on non-asthmatic populations and thus there is a need to explore whether these determinants are significant in an asthmatic population as well.

Current smoking is consistently reported to be associated with lower FeNO, both in healthy individuals (15) and asthma subjects (16). Passive smoking has been reported in some studies to decrease FeNO (17, 18), but this effect is not consistent in other studies (19). Exposure to parental smoking has been studied only in children and has been reported to be associated with either increased FeNO (20, 21) or decreased FeNO (22). The timing of exposure appears to play a role. However, the effects of maternal smoking during pregnancy and/or exposure to parental smoking in childhood on exhaled NO levels in adulthood have not been studied yet.

Hormonal status in women has been reported to be associated with exhaled nitric oxide; an increase in oestrogen levels during the ovarian cycle was related to decreased FeNO, while the opposite was seen for progesterone levels (23). However, these effects were not consistent in cell culture studies, where an increase of NO production in bronchial epithelial cells was found after oestrogen stimulation (24). Early menarche age is related to lower lung function and higher asthma risk in adulthood (25), but the effects of early menarche age or menopause on exhaled nitric oxide have not been studied.

The heterogeneity in the degree of Th2-driven airways inflammation in different subjects with asthma, due to interindividual variations in inflammation patterns and antiinflammatory treatment may diminish the effect of other (non-disease) determinants of FeNO. The aim of this study is to understand the effects of commonly regarded determinants of FeNO, as demonstrated in healthy subjects, in a population of asthmatics. Furthermore, we wanted to study the effects of hormonal status and smoking exposure in childhood on exhaled NO in adults with asthma, effects only scarcely studied by others previously.

Material and methods

Study population

The Global Asthma and Allergy Excellence Network (GA²LEN) postal survey was conducted in 19 European centres, targeting symptoms of asthma, rhinitis, and chronic rhinosinusitis (CRS), and distributed to a random population-based sample (26). Among those who responded, randomly selected subgroups, consisting of individuals with asthma, CRS, both asthma and CRS, or neither asthma nor CRS, were invited for a clinical follow-up. In Sweden, four centres participated: Gothenburg, Stockholm, Uppsala, and Umeå. A total of 27 866 persons responded to the postal survey in 2008 (27), of whom 1 329 persons participated in the follow-up during 2009 and 2010. The follow-up consisted of a structured oral interview (symptoms of asthma and atopic disease, smoking, medication use, and medical history) and a clinical examination.

In the present study, only subjects with asthma from the Swedish centres were included and the definition of asthma was based on the questionnaire at follow-up. Asthma was defined as self-reported diagnosis of asthma and either asthma symptoms (wheezing, attacks of shortness of breath, and/or awakening at night with breathlessness in the previous 12 months) or asthma treatment (taking any asthma medication during the previous 12 months) (n = 605). A control group consisting of 209 individuals with available FeNO measurements was also included in the frame of the study.

Fraction of exhaled NO (FeNO)

FeNO measurements were done in 557 subjects with asthma that had available anthropometric data, information on smoking habits and performed skin prick test. FeNO was measured using NIOX MINO (NIOX MINO[®]; Aerocrine, Stockholm, Sweden), in accordance with the American Thoracic Society (ATS) and European Respiratory Society (ERS) recommendations (28).

Upper airway disease

Rhinitis was defined as having problems with sneezing or runny or blocked nose during the previous 12 months, without having a cold or the flu. Chronic rhinosinusitis (CRS) was defined according to the European Position Paper on Rhinosinusitis and Nasal Polyps (EP₃OS) criteria (29), i.e., presence of at least two of the following symptoms for at least 12 weeks in the past year: (i) nasal blockage, (ii) nasal discharge, (iii) facial pain or pressure, and (iv) reduction in the sense of smell, with at least one of the symptoms being nasal blockage or nasal discharge.

Smoking history

Ever smoking was defined as having a total smoking history of at least 20 packs of cigarettes (30). The division of ever smokers in current and ex-smokers was based on the subjects' report of smoking in the month preceding the study. Pack-years consumption was calculated based on daily smoking consumption in cigarettes (consumption of tobacco pipe, cigar and cigarillos was translated in cigarettes, according to Janson *et al.* (31)), divided by 20, and number of years a subject smoked. Parental smoking during childhood was assessed for each parent separately. For the subject's father, the question covered regular smoking during the subject's childhood, while for the mother, the question covered both regular smoking during the subject's childhood and before the subject's birth.

Women's hormonal status

Questions were included on menstruations during the 12 months prior to the study and the regularity of menstruations if menstruations during the last 12 months were reported. Hormonal contraceptive use and age of menarche was also recorded.

Skin prick test

Skin prick test (SPT) was performed with a standard set of allergens including timothy grass, mixed grass, *Dermatophagoides pteronyssinus*, cat, birch, *Blattella*, olive, *Alternaria*, dog, *Artemisia*, *Parietaria*, *Dermatophagoides farinae*, histamine (positive control), and diluent (negative control), as previously described (32). A positive SPT was defined as a wheal at least 3 mm at the widest diameter. Atopy was defined as the presence of at least one positive SPT finding.

Spirometry

Lung function measurement was performed using the EasyOne™ Spirometer (nidd Medizintechnik AG, Zurich, Switzerland) in accordance with the ATS spirometry standards (33). Forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) were registered and the European Community for Steel and Coal equations were used as reference values (34). Lower limit of normal for FEV₁/FVC ratio was used from Hankinson *et al.* (35).

Statistical analyses

All analyses were performed using STATA 12 (STATA Corp, College Station, TX, USA). The results are given as prevalence, mean or geometric mean values or 95 % confidence intervals (CI). FeNO was log-transformed. The differences between groups were tested using either a chi-square test or Student's two-sample t-test. Trend analysis was performed as linear regression across different categories (for example menarche age groups). Percentual

increases or changes in FeNO with regard to a certain patient characteristic, as previously defined (36), were compared from simple and multiple linear regression models. A p-value < 0.05 was considered significant.

Ethics

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden (Dnr 2008/1100-31/4) for all participating centres, and the collected personal data were treated in accordance with the Swedish personal data act.

Results

The study population consisted of 557 individuals with asthma. The characteristics of the study subjects are given in Table 1. FeNO levels in subjects with asthma were higher than in subjects without asthma (n=209): 19.3 (18.2, 20.4) vs 15.6 (14.3, 17.0) ppb, $p < 0.001$.

Exhaled NO in relation to anthropometric characteristics

Exhaled NO levels were higher in men than in women: 24.0 (22.1, 26.2) vs 16.4 (15.3, 17.5) ppb, $p < 0.001$ (Figure 1A). Exhaled NO was not significantly related to age ($r = 0.08$, $p = 0.07$). Height was significantly related to FeNO in all subjects ($r = 0.26$, $p < 0.001$) and a trend toward higher FeNO with greater height was found (Figure 1B). Weight was related to FeNO ($r = 0.10$, $p = 0.02$). However, levels of FeNO were similar between subgroups of study participants, defined with regard to BMI: 19.6 ppb (14.8, 26.0) if BMI < 20, 19.5 (17.8, 21.4) if BMI 20-25, 20.0 (18.3, 21.9) if BMI 25-30 and 17.7 (15.7, 20.0) if BMI > 30 ($p = 0.64$).

Exhaled NO in relation to lung function

No relation between FeNO and FEV₁ (% of predicted) ($p = 0.88$) or between FeNO and FVC (% of predicted) was found ($p = 0.88$). Similar levels of FeNO were found for subjects with FEV₁/FVC $\geq$ LLN and < LLN: 18.7 (17.5, 20.0) vs. 19.0 (16.9, 21.4), $p = 0.81$.

Exhaled NO in relation to smoking exposure

Current smokers had lower levels of FeNO than both never- and ex-smokers (both $p < 0.001$), while ex-smokers and non-smokers had similar levels of FeNO ($p = 0.75$) (Figure 2A). No relation with pack-years was found in ever smokers ($p = 0.26$), or in the subgroup of current ($p = 0.11$) or ex-smokers ($p = 0.65$). Passive smokers had lower FeNO than those not exposed to passive smoking ($p < 0.001$) (Figure 2B).

Subjects with both parents smoking during childhood had lower levels than if neither ($p < 0.001$) or only one parent was smoking ($p = 0.002$) (Figure 2C).

Exhaled NO in relation to current medication

Subjects who were medicating with inhaled corticosteroids during the week preceding the measurement (n = 192) had lower levels of FeNO than subjects without inhaled corticosteroids (n = 322): 16.8 (15.3, 18.5) vs. 20.4 (19.0, 21.9), p = 0.002. There were few users of leukotriene receptor users (n = 27) and their FeNO levels were similar to those of non-users (n = 518): 19.3 (18.3, 20.5) vs. 16.3 (12.2, 21.6), p = 0.19.

Exhaled NO in relation to upper airway comorbidities

Similar levels of FeNO were found in subjects without rhinitis (n = 116) and subjects with rhinitis (n = 438): 19.0 (16.6, 21.7) vs. 19.3 (18.1, 20.5), p = 0.84. Subjects with CRS (n = 121) had FeNO levels similar to those of subjects without CRS (n = 433): 18.7 (16.5, 21.2) vs. 19.4 (18.3, 20.7), p = 0.58.

Exhaled NO in relation to IgE sensitisation and pollen exposure

Subjects sensitised to both perennial allergens and pollen had higher levels of FeNO than non-sensitised subjects (p < 0.001) and subjects sensitised only to pollen (p=0.003). However, compared with non-sensitised subjects, no significantly higher levels of FeNO were found if subjects were sensitised to only perennial allergens (p = 0.16) or only pollen (p = 0.66) (Figure 3). No significant differences were found when looking at FeNO levels in subjects who performed measurements during pollen season (n=313) vs outside pollen season (n=231) (p=0.14). This was also consistent when looking specifically at subjects only sensitised to pollen (p=0.28) or sensitised to both pollen and perennial allergens (p=0.75).

Exhaled NO in relation to women's hormonal status

A total of 304 women reported age of menarche and the median age was 13 (range 9-17). Women with menarche between 9-11 years of age (n = 52) had lower FeNO levels than those with menarche between 12-14 years of age (n = 219) (p = 0.03) or between 15-17 years of age (n = 33) (p = 0.003) (Figure 4). A positive trend of increasing FeNO among these three groups of menarche age was found (p=0.004).

Similar levels of FeNO were found for women reporting no menstruations in the last 12 months (n = 110) and those reporting menstruations in the last 12 months (n = 183): 17.1 (15.1, 19.3) vs. 15.5 (14.3, 16.9), p = 0.20.

No differences in FeNO between women with regular menstruation (n = 117) vs. irregular menstruation (n = 68) were found: 15.6 (14.0, 17.3) vs. 15.5 (13.4, 18.0), p = 0.99. No significant differences were found between women always having irregular menses (n = 20) and women having irregular menses only in the latest months (n = 48): 12.8 (10.1, 16.2) vs. 16.9 (14.1, 20.2), p = 0.08 or between women always having irregular menses and women always having regular menses (n=117): 12.8 (10.1, 16.2) vs. 15.6 (14.0, 17.3), p = 0.16.

Hormonal contraceptive use (n=83) was not related to differences in FeNO: 15.6 (13.4, 18.1) vs 16.2 (14.9, 17.5) in women not using hormonal contraceptives (n=183), p=0.68.

Multivariate model of determinants of FeNO

In the multivariate analysis, gender ($p = 0.01$), height ($p = 0.02$), age ($p = 0.001$), current smoking ($p < 0.001$), sensitisation to both perennial and pollen allergens ($p < 0.001$), both parents smoking ($p = 0.04$) and ICS use ($p = 0.02$) were all independent predictors of FeNO (Figure 5B). The adjusted effects of these patient characteristics on FeNO are depicted in Figure 5B, alongside a comparison with the univariate effects (Figure 5A). Weight ($p = 0.42$), passive smoking ($p = 0.96$), only perennial ($p = 0.12$) and only pollen sensitisation ($p = 0.19$) were not related to FeNO.

No interaction between gender and any of the characteristics related to FeNO was found in the multiple linear regression model described above.

In a sub-analysis among women where menarche age group was added to the list of above determinants, higher FeNO levels were found in females with menarche at age 12-14 years vs. age 9-11 ($p = 0.04$), while the difference in FeNO between women reporting menarche at 15-17 years vs. 9-11 years was not significant ($p = 0.07$).

Discussion

We could confirm that constitutional characteristics, such as male gender and greater height, related to higher exhaled NO. Current smoking and current use of ICS related to lower levels of exhaled NO, while allergic sensitisation related to higher levels of exhaled NO. This is to our knowledge the first report in a large population of asthmatics to show that these determinants are relevant and have similar effect magnitude as previously reported in healthy individuals. We can report two novel findings: parental smoking during childhood and lower menarche age are related to lower FeNO levels in adulthood.

Gender, height, allergic sensitisation and current smoking are important patient characteristics to be accounted for in interpreting the levels of FeNO in patients with asthma. These results are in line with results from population-based studies (12). For example, the effect of gender is in line with results from Taylor *et al.* in an epidemiological study of healthy adults (12). Only part of the effect could be explained by differences in height, as the gender differences in FeNO were consistent after adjusting for height, probably signalling a difference in NO production between genders (37). The relation with height is thought to reflect a larger airway size, leading to a larger airway surface as a source of NO; in line with this, a clear relation with height can be found in children (38). The association with weight is in line with results from univariate analysis from healthy adults, reported by Jo *et al.* (39). This could in theory be explained by Th1-polarization (40) and lower levels of Th2-cytokines seen in obese patients with asthma (41) that could lead to lower FeNO levels. However, this relation became statistically non-significant after adjustments in the present study and it was mainly explained by gender differences in the report of Jo *et al.* (39). Allergic sensitisation was related to higher levels of FeNO, especially when patients were sensitised to both perennial and pollen allergens. This probably signals a higher level of degree of IgE sensitisation, as FeNO is known to relate to the degree of IgE sensitisation (13, 14) and this is probably due to further activation of epithelial iNOS (2). Current smoking is a known, important factor related to lower FeNO levels and the size of this decrease is similar to the one reported in healthy populations (12). This is also in line with our recent report that FeNO

can be used to diagnose asthma in a population with respiratory symptoms, even in ex- or current smokers, if the cut-off is lowered accordingly (42). The reasons why current smokers have decreased FeNO are probably reduced substrate bioavailability for NO production, increased catabolism of NO and effects in the oropharyngeal cavity (2).

Current use of inhaled corticosteroids is expected to lower FeNO levels, as inhaled corticosteroids reduce FeNO in a dose-dependent way (3) and this is probably due to decreased IL-4/IL-13 production (2). We could report similar results in a cohort of patients with asthma from primary and specialist care in Sweden (43). No relation to airway obstruction could be found in the present material and this is in line with the literature where weak or no relations to FeNO are found (2).

Parental smoking during childhood was related to lower FeNO levels in the present material. This is to our knowledge the first study reporting such findings. This relation is particularly clear if both parents were smoking during childhood. It is known that parental smoking is related to increased incidence of wheeze and asthma (44) and it could be speculated that this might be due to down-regulation of endogenous NO production, which regulates the bronchial tonus and is bronchoprotective (45). However, in children exposed to parental smoking, increased rather than decreased FeNO has been described (20, 21) and the authors speculated that this might be due to acute irritant effects of ETS or that these subjects to a larger extent are IgE sensitised, as early-life exposure to ETS increases the risk of atopy. Hollams *et al.* (46) reported that maternal smoking during pregnancy was related to poorer lung function and increased risk for current asthma in adolescents.

Lower menarche age was related to lower FeNO levels in the present material and this is a novel finding. Previously, it was reported that women with early menarche were characterized by a lower FEV₁ (25). It could be speculated that our finding might be due to the lower average height achieved by women with early menarche (47), as lower FeNO may be explained by lower height. This could partly explain our findings as the associations were weaker in the multiple logistic regression model after adjustments for height, among other variables.

A major strength of the study is that it is one of the biggest studies including FeNO that has been performed in an asthmatic population. Another strength of the study is the large span in subject age, making the results applicable to all adult asthmatics. The FeNO measurements have been done with identical instruments in different study centres. It can be speculated that the asthmatics included here, because of the population-based study design, might have milder forms of the disease. Furthermore, a subgroup of them could have mostly symptoms during pollen season. However, no differences in FeNO could be found between subjects examined during and outside season and the large majority of subjects sensitised to pollen were also sensitised to perennial allergens so this drawback has probably influenced the results only to a limited extent. The epidemiological nature of the study relied on self-reported diagnosis of asthma with current medication or symptoms. Therefore, these results should be replicated also in a cohort of asthma patients. The parental smoking exposure assessment has limitations, as details on smoking indoors or outdoors are lacking. Furthermore, the mother's smoking habits cannot be separated into antenatal, postnatal or both.

In conclusion, interpreting FeNO levels in clinical practice is complex and we demonstrate that previously proven constitutional determinants, as well as smoking and IgE sensitisation, are of importance in asthmatic subjects. Therefore, gender, height, IgE sensitisation and current smoking should be taken into account when interpreting FeNO levels in asthmatic patients. This has not been previously demonstrated in a large cohort of subjects with asthma. These effects should probably be acknowledged when determining a target FeNO level to be achieved in titrating steroids, although this must be further studied. Parental smoking exposure during childhood might result in lower FeNO levels later in adulthood, but this finding warrants further studies with higher resolution regarding exposure and time of exposure as well as mechanisms. Lower menarche age appear to be related to lower FeNO levels in the present study and this also warrants further studies of whether this signals a different inflammatory phenotype in women with early menarche or if the reduced capacity of NO production is due to early menarche.

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Table 1. Characteristics of the study population. Results presented as mean $\pm$ standard deviation, n (%) or geometric mean (95 % confidence interval) (the lastnamed for FeNO).

	All subjects (n = 557)
Age (years)	44.3 $\pm$ 14.7
Height (m)	1.71 $\pm$ 0.10
Weight (kg)	78.3 $\pm$ 17.1
BMI (kg/m ²)	26.6 $\pm$ 5.0
Atopy	387 (69.7 %)
IgE sensitization (n (%))	None
	167 (30.0 %)
	Only perennial
	58 (10.4 %)
Only pollen	67 (12.0 %)
	Perennial and pollen
	265 (47.6 %)
Smoking habits	Never smokers
	298 (53.5 %)
	Ex-smokers
Current smokers	204 (36.6 %)
	55 (9.9 %)
Smoking history (pack-years)	Ex-smokers
	15.7 $\pm$ 20.1
Current smokers	17.0 $\pm$ 15.3
Parents' smoking habits	None
	195 (36.7 %)
	One
Two	200 (37.6 %)
	137 (25.7 %)
Passive smoking	67 (12.1 %)
Chronic rhinosinusitis	121 (21.8 %)
Rhinitis	438 (79.1 %)

Use of inhaled corticosteroids (ICS) in last week	192 (37.4)
Use of leukotriene receptor antagonist	27 (5.0)
FeNO (ppb)	19.3 (18.2, 20.4)
FEV ₁ (L)	3.0 ± 0.90
FEV ₁ (% predicted)	91.4 ± 17.3
FVC (L)	4.1 ± 1.1
FVC % (% predicted)	103.3 ± 16.1
FEV ₁ /FVC	0.75 ± 0.09

Figure legend

Figure 1. FeNO (GM (95 % CI)) in relation to gender (A) and height (B).

Figure 2. FeNO (GM (95 % CI)) in relation to personal smoking habits (A), parental smoking during childhood (B) and current passive smoking exposure (C).

Figure 3. FeNO (GM (95 % CI)) in relation to IgE sensitisation pattern.

Figure 4. FeNO (GM (95 % CI)) in relation to menarche age.

Figure 5. FeNO percentual change in relation to different participant characteristics in univariate (A) and multivariate models (B).



