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Article type : Original Article

Cross-sectional study on exhaled nitric oxide in relation to upper airway inflammatory disorders with regard to asthma and perennial sensitisation

FeNO in relation to upper airway disorders, asthma and sensitisation

Word count: Abstract: 293, Article: 4535, Figures: 2, Tables: 5. On-line supplement: 164, On-line Tables: 4

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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.14019

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Abstract

Background: Fractional exhaled nitric oxide (FeNO) is a well-known marker of type-2 inflammation. FeNO is elevated in asthma and allergic rhinitis, with IgE sensitisation as a major determinant.

Objective: We aimed to see if there was an independent association between upper airway inflammatory disorders (UAID) and FeNO, after adjustment for asthma and sensitisation, in a multi-centre population-based study.

Methods: A total of 741 subjects with current asthma and 4,155 non-asthmatic subjects participating in the second follow-up of the European Community Respiratory Health Survey (ECRHS III) underwent FeNO measurements. Sensitisation status was based on measurement of IgE against airborne allergens; information on asthma, UAID and medication was collected through interview-led questionnaires. Independent associations between UAID and FeNO was assessed in adjusted multivariate regression models and test for interaction with perennial sensitisation and asthma on the relation between UAID and FeNO were made.

Results: UAID were associated with higher FeNO after adjusting for perennial sensitisation, asthma and other confounders: with 4.4 (0.9–7.9) % higher FeNO in relation to current rhinitis and 4.8 (0.7–9.2)% higher FeNO in relation to rhinoconjunctivitis. A significant interaction with perennial sensitisation was found in the relationship between current rhinitis and FeNO (p=0.03) and between rhinoconjunctivitis and FeNO (p=0.03). After stratification by asthma and perennial sensitisation, the association between current rhinitis and FeNO remained in non-asthmatic subjects with perennial sensitisation, with 12.1 (0.2–25.5) % higher FeNO in subjects with current rhinitis than in those without.

Conclusions & Clinical Relevance: Current rhinitis and rhinoconjunctivitis was associated with higher FeNO, with an interaction with perennial sensitisation. This further highlights the concept of united airway disease, with correlations between symptoms and inflammation in the upper and lower airways and that sensitisation needs to be accounted for in the relation between FeNO and rhinitis.

Keywords: FeNO, exhaled nitric oxide, asthma, rhinitis, nasal polyposis, population-based

Key messages:

- Current rhinitis and rhinoconjunctivitis are associated with higher FeNO at a population level.
- There is an interaction with perennial IgE sensitisation on these associations.

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- In non-asthmatic subjects with perennial sensitisation, current rhinitis and rhinoconjunctivitis are related to higher FeNO.

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Introduction

Asthma is a common respiratory disease with a prevalence in adults of over 4% worldwide and a large variation of prevalence between countries [1,2]. Comorbidity with allergic rhinitis (AR) is common in asthma and AR has an impact on asthma control and severity [3,4]. There is also evidence that AR to a certain extent is part of the same entity as asthma, with bronchial hyperreactivity, signs of small airway impairment [5,6], and lower airway inflammation (with increased sputum eosinophils) seen in patients with AR [7,8]. There is ample evidence of a united airway disease linking upper airway symptoms in asthma to structural and inflammatory changes in the lower airways of patients with AR [9]. In epidemiological analyses of more than 15,000 participants, both hypothesis-driven and hypothesis-free approaches showed that multimorbidity of asthma, rhinitis and eczema occurred more frequently than expected by chance and that sensitisation as a mechanism explained only a small part of this multimorbidity [10,11].

Atopic asthma is the most common subgroup of asthma and is associated with type-2 inflammation [12]. Type-2 inflammation is also seen in non-atopic eosinophil asthma, where group 2 innate lymphoid cell (ILC2) play an important role in the inflammation [13]. Exhaled nitric oxide (NO) is a well-established marker of type-2 inflammation leading to activation of inducible NO synthase in the airway epithelium [14]. Fractional exhaled NO (FeNO) is a non-invasive method of measuring NO production in the lower airways, which is elevated in subjects with asthma [15], with higher levels in subjects with allergic asthma than in subjects with non-allergic asthma [16]. In subjects with allergic asthma, both FeNO and inducible NO synthase expression are higher than in healthy controls and FeNO increases further after allergen provocation [17]. FeNO levels can be reduced by treatment with inhaled corticosteroids (ICS) [18] and FeNO has been proposed as a complementary tool for diagnosis and treatment management in asthma [19].

FeNO levels are influenced by constitutional factors like age, gender and height [20]. Current smoking reduces FeNO levels [20], while Immunoglobulin E (IgE) sensitisation is related to increased levels of FeNO in both subjects with asthma [21] and healthy controls [16]. Moreover, the type and degree of sensitisation are important, with perennial sensitisation [22] and multiple sensitisation [23] related to larger increases in FeNO. Within the framework of united airway disease, FeNO has been reported to be increased in subjects with AR without asthma in relation to healthy controls [24,25] and higher FeNO levels have been seen in asthmatic subjects with concomitant AR than in asthmatic subjects without AR [25,26].

In subjects with asthma, chronic rhinosinusitis (CRS) with nasal polyps has been shown to have an impact on health-related quality of life [27], structural lung changes and asthma exacerbations [28]. FeNO is higher in patients with asthma and concomitant nasal polyps than in those without nasal polyps [29,30] and has been

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shown to be reduced upon surgical treatment of the nasal polyps [31]. Furthermore, presence of nasal polyps in subjects with CRS is related to higher FeNO levels [32].

Most of the studies on FeNO in relation to allergic rhinitis or nasal polyps, with or without asthma, have been done on selected samples, without adjustment for the type of allergic sensitisation. The aim of this study was to assess whether there was an independent association between upper airway inflammatory disorders (UAID) (i.e., rhinitis, rhinoconjunctivitis, CRS or nasal polyposis) and FeNO in subjects from a large multi-centre, cross-sectional population-based study, with regard to interaction with IgE sensitisation and asthma. Our hypothesis was that there was an independent association between UAID and FeNO in both subjects with asthma and non-asthmatic subjects, with an interaction with sensitisation.

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Methods

Study sample

We carried out a cross-sectional analysis of the data, collected between the years 2010 and 2013 at 25 centres across 11 countries in Europe and in Australia, as part of the second follow-up of the European Community Respiratory Health Survey (ECRHS III).

The ECRHS is a population-based multi-centre study of asthma and allergy performed mainly in European countries and following a standardised protocol. The first postal survey was sent to randomly selected young adults (age 20–44 years) in the early 1990s. Among the responders, both a random sample and a sample of subjects reporting respiratory symptoms were invited to participate in additional examinations at their respective study centres. These groups have later been invited to follow-up studies (ECRHS II and ECRHS III) in 2000–2002 and 2010–2013, respectively. Further information about the ECRHS can be found on the homepage of the study (www.ecrhs.org), as well as in previously published articles [1,33,34].

Of the 5,824 participants in ECRHS III, 126 were excluded due to self-reported emphysema or chronic obstructive pulmonary disease (COPD), and 526 were excluded due to a lack of FeNO measurements (Figure 1). The group that lacked FeNO measurements did not differ in prevalence of self-reported asthma, current rhinitis or rhinosinusitis from subjects with FeNO measurements, but they were older and had a higher percentage of females, as well as more prevalence of severe rhinitis, symptoms of CRS and nasal steroid treatment (On-line Table 1). The remaining participants were divided into subjects with self-reported asthma (with a positive answer to the question “Have you ever had asthma”; n=945) or those without (with a negative answer to the same question; n=4,227). In the self-reported asthma group, 54 individuals were omitted due to no doctor’s diagnosis of asthma and 148 subjects due to absence of asthma symptoms or treatment in the year preceding the study. In the non-asthmatic group, 72 individuals were omitted due to the use of inhaled bronchial corticosteroids during the preceding year. This yielded 741 subjects with current asthma and 4,155 non-asthmatic subjects (Figure 1). The 148 subjects with self-reported doctor’s diagnosis of asthma, but without treatment or symptoms the preceding year (Figure 1). did not differ in gender, age, height or perennial sensitisation from subjects with current asthma, but had lower weight, BMI and reported less UAID and nasal steroid treatment than those with current asthma(On-line Table 2) They also differed significantly in FeNO from both current-asthma and non-asthmatic subjects (On-line supplement).

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Questionnaire and definitions of asthma, rhinitis, smoking habits and medication

All participants answered a detailed, interview-led questionnaire containing questions on respiratory symptoms from the upper and lower airways, self-reported diagnosis of asthma, COPD, emphysema, CRS or nasal polyposis, use of oral, inhaled or nasal medication during the preceding year, allergies and smoking habits. For a few subjects, there are missing data regarding smoking and upper airway disorders; these are reported in the results section.

Current asthma was defined as self-reported doctor-diagnosed asthma and at least one respiratory symptom (self-reported asthma attack, wheezing, nocturnal chest tightness, attacks of shortness of breath at night, after strenuous exercise or at rest) during the preceding 12 months and/or use of asthma medication during the same period.

Current rhinitis was defined as sneezing, or blocked or runny nose without concomitant cold or influenza during the preceding year. Rhinoconjunctivitis was defined as current rhinitis with concomitant itchy or runny eyes. We further divided rhinitis based on persistency and severity. Persistent rhinitis was defined as nasal problems more than 4 days in one week and more than 4 weeks in a row in the preceding 12 months. Intermittent rhinitis was defined as having current rhinitis but not fulfilling the criteria for persistent rhinitis. Severity of rhinitis was grouped based on the extent to which each nasal symptom (runny nose, blocked nose, itchy nose and attacks of sneezing) had affected daily activities and sleep (1. not a problem, 2. a slight problem, but not bothersome, 3. a bothersome problem, but without effect on daily activities or sleep, 4. a problem that affects some activities or sleep). Moderate to severe rhinitis was defined as having at least one of the nasal symptoms affect some activities or sleep. Mild rhinitis was defined as current rhinitis not fulfilling the criterion for moderate to severe rhinitis [3].

CRS and nasal polyposis were defined as self-reported doctor's diagnosis of the respective conditions. Symptoms of CRS were defined as blocked nose combined with at least one other symptom (discoloured secretion from the nose, pain or pressure in the forehead, nose or eyes, or loss of smell) for more than 12 weeks during the preceding 12 months [3].

A smoker was defined as someone who had smoked at least one cigarette per day or one cigar a week for at least one year, or at least 20 packs of cigarettes or 360 g of tobacco in their lifetime. Subjects were defined as never smokers if they had never smoked or had smoked less than the amount stated above. Smokers were further divided into current or ex-smokers based on their smoking habits during the month preceding the study.

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Nasal steroid use was defined as any self-reported use of nasal spray containing corticosteroids during the preceding 12 months. Regular use of leukotriene receptor antagonist (LTRA) was defined as continuous use of LTRA during the preceding 3 months. Use of ICS was divided into regular, intermittent or no use based on reported use during the preceding year: regular if the participant had used ICS continuously during the three months preceding the study, intermittent if ICS had been used, but not continuously, and none if the participant reported no use of ICS in the 12 months preceding the study.

Anthropometry

Weight and height of all participants were measured by trained health technicians and body mass index (BMI) was calculated.

Exhaled nitric oxide

FeNO was measured using an electrochemical analyser (NIOX MINO; Aerocrine AB) at a flow rate of 50 mL/s. The participants were instructed to refrain from eating, drinking, smoking or strenuous exercise during the hour before the measurement. Measurements were performed in accordance with ATS guidelines [35] with the exception that only single measurements were made. NIOX MINO detects FeNO values from 5 to 300 ppb. No value above 207 ppb was measured. A total of 15 subjects (10 subjects without asthma and 5 with current asthma) had levels "below 5 ppb" (no actual value) and those subjects were assigned an arbitrary FeNO value of 3.5 ppb (5 divided by √2).

IgE sensitisation

Blood samples for IgE analyses were drawn at all centres. Blood samples were not obtained in 788 study participants (322 individuals with current asthma and 466 non-asthmatic subjects). The IgE analyses were performed at a single central laboratory (AMC Amsterdam) using the ImmunoCAP system (Thermo Fisher Scientific). Measurements were made of specific IgE against *Dermatophagoides pteronyssinus* (house dust mite), timothy grass and cat. These three sensitisations are the most prevalent in adults within the countries included in the study, even though the variance between countries are high [36,37]. Participants were defined as sensitised toward an individual allergen if the concentration of IgE against that specific allergen was ≥ 0.35 kU_A/L. As the number of participants with only grass sensitisation was low (n = 35

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(8%), in subjects with current asthma and n = 253 (7%) in non-asthmatic subjects), we grouped sensitisation into two groups – perennial sensitisation (cat- and/or house dust mite-sensitised) or no perennial sensitisation (no sensitisation or sensitisation only toward timothy grass) – for stratified and multivariate analyses.

Statistical methods

STATA 15.1 (StataCorp) was used for all statistical analyses. The results are presented as numbers and percentages for categorical variables, means with standard deviations for continuous variables with normal distribution, and geometric means with 95% confidence intervals for variables with a right-skewed distribution (i.e., FeNO). For variables with a right-skewed distribution, logarithmic transformation was performed before statistical testing. Differences in log FeNO levels in relation to categorical variables, such as gender, smoking habits, UAID and perennial sensitisation, were tested separately for participants with current asthma and non-asthmatic subjects, using t-tests for dichotomous variables or one-way ANOVA (adjusted for multiple testing) in multiple strata (i.e., severity and persistency of rhinitis, ICS-use and smoking habits), and tests for interactions with asthma were made. To assess whether there was an independent relationship between UAID and FeNO, both subjects with current asthma and non-asthmatic subjects were further stratified by perennial sensitisation and the same tests were used to evaluate differences in FeNO levels in relation to UAID in the stratified groups. Interaction analysis between sensitisation and UAID with FeNO as outcome was also performed.

To further evaluate the independent association of upper airway disorders with FeNO, multiple linear regression analyses were performed on the groups stratified by current asthma and perennial sensitisation, as well as on the entire study population, with adjustments for gender, age, height, weight, smoking status, centre and treatment for rhinitis and asthma, with log-transformed FeNO as outcome. The UAID that had a relationship to FeNO in the univariate analysis (i.e. current rhinitis, rhinoconjunctivitis and nasal polyps) were tested individually in these multiple linear regression models, as they are related to each other. The results from the analyses, e.g., β coefficient with 95% confidence interval, were than back-transformed and presented as % difference in FeNO (($(10^{\beta}-1)X100$) with 95% confidence interval (($(10^{CI}-1)X100$)) in relation to the reference group. A test for interactions with perennial sensitisation was added individually to all models; the model including all study subjects also had a test for interactions with asthma added.

A p value of < 0.05 was considered statistically significant.

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Ethics

Approval for the study was obtained at each participating centre from the regional committee for medical research ethics, in accordance with national legislation (**Australia:** Human Research Ethics Committee of Alfred Hospital – Nr - CF11/1818 – 2011001012, **Belgium:**-Adviescommissie Medische Ethiek UZA-UA (CME). Nr: 11/41/288 – UA, **Denmark:** Regionshuset Viborg, De Videnskabsetiske Komiteer – Nr: 20110106, **Estonia:** - Research Ethics Committee of the University of Tartu, Estland. Nr: 209T-17, **France:** – Comite de protection des personnes, Sud V Est Nr: 2011-A00013-38, **Germany:** – Ethik-Kommission der Bayerischen Landesarztekammer. Nr: 10015, **Iceland:** - National Biotecs Committe of Iceland (NBCI) Nr: VSNb2011090016/03.11, **Italy: – Verona** - Comitato Etico per la sperimentazione, Azienda Ospedaliera Universitaria Integrata Verona. Nr: N. Prog 1393, **Turin** - Ethics Committee of the Local Health Authority TO2 of Turin. Nr: 569/09/08, Meeting of June 10th 2008, **Norway:** - Universitetet i Bergen, Regional komité for medisinsk og helsefaglig forskningsetikk, Vest-Norge (REK Vest) Nr: 2010/759, **Sweden:** - Ethics Committee at the Medical Faculty, Uppsala University Nr: 1999/313 and 2010/068, **Spain: - Barcelona** - Comité Ético de Investigación Clínica del Instituto Municipal de Asistencia Sanitaria, Barcelona, Spain), Nr PS09/00716 and 2009/3500/I, **Galdakao** - Comité Éticode Investigacióndel Hospital de Galdakao, Spain Nr: 20101104, **Huelva** - Comisión de Investigación del Hospital Juan Ramón Jiménez de Huelva Nr: 20090417, **Oviedo** - Comité Ético de Investigación Clínica Regional, Hospital Universitario Central de Asturias, Nr: 20110415, **Albacete** - Comité de Ética e Investigación de Complejo Hospitalario de Albacete, Nr: 04/09, **England** : NRES Committee London – Stanmore REC Reference 11/LO/0965, IRAS number 70769.). All participants gave informed consent prior to inclusion in the study.

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Results

A total of 741 participants with current asthma and 4,155 non-asthmatic subjects were included in the study. The baseline characteristics of both groups are given in Table 1. There was a higher proportion of females in the group with asthma than in the group without asthma. Age and weight were similar between groups, but the group with asthma had higher BMI and lesser height than the non-asthmatic group. The proportion of current smokers was lower among subjects with current asthma than those without asthma. The proportion of participants sensitised towards perennial allergens and the proportion with UAID were higher in the group with current asthma than in the non-asthmatic group. There was also a higher proportion of participants who used nasal steroids among those with current asthma (Table 1). Subject characteristics and FeNO levels in groups stratified by current rhinitis are included in on-line supplement. Subjects with current rhinitis were younger, encompassed more females and had a higher prevalence of current asthma, sensitisation to perennial allergens, and treatment for asthma and rhinitis (On-line Table 3).

FeNO levels in relation to sensitisation and upper airway inflammatory symptoms or diagnosis

When looking the whole study population FeNO differed significantly between perennial (20.9 (19.9 – 21.9) ppb, n = 763), seasonal (18.2 (17.1 – 19.5) ppb, n = 337) and no sensitisation (16.2 (15.8 – 16.3) ppb, n = 3,446) ($p<0.01$ for all). As the number of subjects sensitised to only seasonal allergen was low and the relative difference in FeNO was smaller between seasonal and non-sensitised we chose to group sensitisation into perennial sensitisation (± grass sensitisation) or no perennial sensitisation (either no sensitisation or only grass sensitisation) for stratified and multivariate analyses. Subjects with perennial sensitisation had higher FeNO levels than those without, among both subjects with current asthma and non-asthmatic subjects (Table 2).

Current rhinitis was associated with higher FeNO in both subjects with current asthma and non-asthmatic subjects, whereas rhinoconjunctivitis only was associated with higher FeNO in non-asthmatic subjects (Table 2). No interaction with asthma status on the relation between FeNO and current rhinitis or rhinoconjunctivitis was found. When grouping rhinitis based on persistency and severity, no differences in FeNO levels were seen between those with persistent rhinitis and those with intermittent rhinitis, or between those with moderate to severe rhinitis and those with mild rhinitis, neither among subjects with current asthma nor among non-asthmatic subjects (data not shown). Nasal polyposis was associated with increased FeNO in asthmatics only. No association with CRS was observed (Table 2).

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FeNO levels in relation to upper airway inflammatory symptoms or diagnosis – stratified analyses for asthma and IgE sensitisation for perennial allergens

In non-asthmatic subjects with perennial sensitisation, both current rhinitis and rhinoconjunctivitis related to higher FeNO. No such effects were seen in the other three groups of subjects (Table 3). In subjects with current asthma without perennial sensitisation, those with nasal polyps had higher levels of FeNO than those without, while no such differences were seen for the other three groups (Table 3).

In non-asthmatic subjects, a significant interaction with perennial sensitisation was found on the relationship between current rhinitis and FeNO (p interaction = 0.04), as well as on the relationship between rhinoconjunctivitis and FeNO (p interaction = 0.005).

FeNO levels in relation to anthropometric variables, gender and smoking

There was a positive association between FeNO and height in patients with current asthma, as well as non-asthmatic subjects, whereas a positive association between FeNO and age and weight only was seen in non-asthmatic subjects (On-line supplement). FeNO was higher in males than females among both subjects with current asthma and non-asthmatic subjects (On-line Table 4). Current smokers had lower levels of FeNO than previous smokers and never smokers among both subjects with current asthma and non-asthmatic subjects (On-line Table 4).

Multivariate models of independent associations of upper airway inflammatory disorders and FeNO

In a multivariate regression model stratified by perennial sensitisation and asthma, and adjusted for variables known to be associated with FeNO (smoking, gender, height and asthma medication), as well as for weight, study centre and use of nasal steroids, we found an independent association between FeNO and current rhinitis, as well as rhinoconjunctivitis, only in non-asthmatic subjects with perennial sensitisation. No association between FeNO and nasal polyps was found in either subjects with current asthma or controls (Table 4). In subjects with current asthma and perennial sensitisation, use of nasal steroids was associated with lower FeNO and continuous as well as intermittent use of ICS was associated with higher FeNO. No relation between FeNO and medication was seen in subjects with current asthma without perennial sensitisation (Table 4).

In the same multivariate model including all study participants (adjusted for smoking, gender, height, weight, study centre, asthma medication, use on nasal steroids, as well as current asthma and perennial sensitisation), current rhinitis as well as rhinoconjunctivitis were associated with higher FeNO. There was

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a significant interaction with perennial sensitisation on the relationships between current rhinitis and rhinoconjunctivitis and FeNO, and a significant interaction with current asthma on the relationship between nasal polyposis and FeNO (Table 5). There was also an independent association between FeNO and asthma, as well as FeNO and perennial sensitisation (Table 5).

Discussion

Current rhinitis and rhinoconjunctivitis were associated with higher FeNO with a significant interaction with perennial sensitisation on the relationships between current rhinitis and rhinoconjunctivitis and FeNO. When subjects were stratified by asthma and perennial sensitisation and adjustment was made for further confounders, the associations with higher FeNO in current rhinitis and in rhinoconjunctivitis were consistent in non-asthmatic controls sensitised to perennial allergens. Moreover, treatment with nasal steroids related to lower FeNO levels in asthmatic subjects sensitised to perennial allergens.

The association of allergic rhinitis with higher FeNO levels has previously been found in both subjects with asthma [26,38] and non-asthmatic subjects [24], as has a positive association between quality of life and FeNO during allergen exposure in children with rhinoconjunctivitis [39]. IgE sensitisation is a major determinant of FeNO, leading to increased FeNO values in both healthy subjects [16,40-42] and subjects with rhinitis and asthma [21,23,43]. Hedman *et al.* recently reported, in a study of mono- and dizygotic twins, that the relation between FeNO and asthma is mostly governed by genetic factors influencing IgE levels [44]. However, Olin *et al.* suggested that increased FeNO was found in subjects with sensitisation toward airborne allergens only if they had symptoms of airway inflammation (i.e., asthma or rhinitis) [45]. Our study confirmed the association between FeNO and perennial sensitisation in both subjects with current asthma and non-asthmatic controls. Most studies on the relationship between AR and FeNO have not been adjusted for sensitisation or type of sensitisation, although a study by Jouaville *et al.* on 1,156 children showed higher FeNO in non-asthmatic subjects with sensitisation and rhinitis than in non-asthmatic subjects with sensitisation, but without rhinitis [23]. Our study results are in line with this evidence and suggest that there is a relationship between FeNO and rhinitis, but that this relation is influenced by perennial sensitisation. When the study population was stratified by asthma and perennial sensitisation, no relation between FeNO and rhinitis was seen in non-asthmatic or asthmatic subjects without sensitisation toward perennial allergens. The effect size of rhinitis and rhinoconjunctivitis on FeNO was around 5% when looking at the whole population, this effect was about one third of the effect of asthma and perennial sensitisation on FeNO. The size effect of 5% might be questioned regarding its clinical relevance. However, we think that

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the main finding is that upper airway inflammatory symptoms interacts with perennial sensitisation in the association with FeNO as a marker of lower airway inflammation. Moreover, in non-asthmatic subjects with perennial sensitisation current rhinitis is associated with12% higher FeNO, which could be clinically relevant. Most likely, the increased FeNO in rhinitis in our analysis can be explained by an allergic inflammation in the lower airways triggered by an allergen response in the upper airways, or a simultaneous exposure to allergens in both the upper and lower airways, a schematic depiction of this relation is given in Figure 2. Previous studies have shown higher eosinophilic counts in induced sputum in subjects with AR than in healthy controls [7,8]. The lack of a significant relationship between FeNO and current rhinitis in subjects with current asthma might be explained by either the fact that the number of subjects with current asthma was relatively small, or that allergic sensitisation was driving both higher FeNO and symptoms of rhinitis in asthmatic subjects, or that the additive effect of rhinitis on FeNO in subjects with asthma was diminished in the presence of asthma-related inflammation in the lower airways, and the fact that this relationship could be confounded by asthma treatment. Counterintuitively, asthma treatment related to higher FeNO levels in our population, which might be explained by subjects with more severe disease and higher degree of inflammation being prescribed ICS. These subjects may have higher FeNO levels even though they are clinically responding to ICS treatment [46]. The mean levels of FeNO in subjects with current asthma was within normal range in our study. As this is a population-based study this may indicate that most of the subjects with current asthma had a mild or well-controlled asthma. However the relation between ICS treatment and higher FeNO may reflect that the subpopulation with ICS treatment had a more severe asthma, reflected in increased lower airway inflammation and higher FeNO.

The elevated FeNO levels in subjects with rhinitis without asthma might be a biomarker for future onset of asthma. Several studies have shown that non-asthmatic subjects with AR and high FeNO at baseline have a higher prevalence of asthma at follow-up than those with lower FeNO, suggesting that FeNO may be a predictor of asthma development in subjects with AR [47-49]. However, it is unclear whether the presence of AR triggers both the march towards asthma and higher FeNO, or an underlying inflammation in the united airways clinically manifests as upper airway symptoms first. We have shown, in a cohort of adolescents, that subjects without rhinitis with high FeNO levels at baseline had a higher risk of developing new onset of rhinitis and persistent rhinitis than those with lower FeNO levels at baseline, in line with the united airway disease concept [50].

Nasal corticosteroids related to lower FeNO levels in asthmatic subjects sensitised to perennial allergens. This finding is in line with the evidence of Oka *et al.* showing a reduction of FeNO and improvement in

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asthma quality of life scores after add-on therapy with nasal corticosteroids in adults [51]. However, the evidence on the effect on FeNO by treatment of upper airway disease with nasal corticosteroids in subjects with asthma is inconclusive, with longitudinal studies on nasal steroid treatment and FeNO in children not showing a significant decrease in FeNO upon treatment with nasal steroids [52,53]. It is not possible to deduce from our cross-sectional study if the relationship between nasal corticosteroids and FeNO is due to a lower level of inflammation in the upper airways resulting in lower inflammation in the lower airways.

In our study we found no relation between persistency or severity of rhinitis and FeNO. This is somewhat surprising, as previous studies on subjects with AR have reported higher FeNO in persistent than intermediate rhinitis [24,51,54], as well as in moderate to severe than mild rhinitis [51].

A relationship between nasal polyposis and higher FeNO was seen only in univariate analysis in subjects with current asthma and not in non-asthmatic controls. CRS with nasal polyps has an impact on asthma severity, health-related quality of life and asthma exacerbations [27,28] and is related to higher FeNO [30-32]. Treatment of type-2 inflammation with dupilumab reduces the size of nasal polyps as well as the severity of symptoms [55]. In our multivariate analysis of all subjects we found a significant interaction with asthma on the relation between FeNO and nasal polyposis although no significant relation between FeNO and nasal polyps was found. Nor was any significant association between FeNO and nasal polyps found in the multivariate model stratified by asthma and perennial sensitisation. This might be explained by the lower number of individuals in each stratum after stratification or due to the fact that the definition of nasal polyposis in our study only was based on self-reported doctor's diagnose of polyposis and no objective examinations.

The main strength of our analysis is that the ECRHS is a large multi-centre survey including many subjects with current asthma and non-asthmatic controls. The questionnaires were administered through interviews, with clear instructions to the participating centres, the methods for measurements of FeNO were standardised, and sensitisation was measured at a single central laboratory. The main limitation of our study is that we carried out a cross-sectional analysis of the relationship between FeNO and UAID with no information on the development of respiratory symptoms and diagnosis over time. Other major limitations are the high amount of missing data on IgE sensitisation in subjects with asthma and, to a lesser degree, in non-asthmatic subjects, and that IgE sensitisation only have been assessed against three allergens, due to limited resources. This may result in misclassification of sensitisation, with an underestimation of both perennial and seasonal sensitisations. However cat and house dust mite are the most prevalent perennial sensitisation and timothy grass the most prevalent seasonal sensitisation in

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Europe even though there is a wide difference in pattern of sensitisations between countries in Europe [37,56]. A further limitation is the large heterogeneity in asthma treatment and levels of FeNO between the participating centres. Information on doctor's diagnosis of CRS or nasal polyposis, as well as asthma, was self-reported by the study participants and no objective confirmation of the diagnoses was included in the study.

Conclusions

Current rhinitis and rhinoconjunctivitis were related to increased FeNO and the relationship was modified by presence of IgE sensitisation toward perennial allergens. Specifically, current rhinitis was associated with higher FeNO, with a significant interaction with perennial sensitisation, which suggests a need to take IgE sensitisation toward perennial allergens into account when evaluating the importance of FeNO in subjects with current rhinitis. The finding on increased FeNO in non-asthmatic subjects with current rhinitis, sensitised to perennial allergens, suggested that these subjects had high levels of type-2 inflammation and might therefor be at higher risk of developing asthma, but this, as well as the use of FeNO in monitoring patients with rhinitis need to be studied in future, longitudinal studies.

Acknowledgements

Grants and/or financial support:

The coordination of ECRHS III was supported by the Medical Research Council (Grant Number 92091), and the following grants contributed to funding ECRHS III:

Australia: National Health & Medical Research Council. **Belgium:** Antwerp South, Antwerp City – Research Foundation Flanders (FWO), grant code G.0.410.08.N.10 (both sites). **Estonia:** Tartu – SF0180060s09 from the Estonian Ministry of Education. Estonian Research Council Personal Research Grant no 562. **France:** (All) Ministère de la Santé, Programme Hospitalier de Recherche Clinique (PHRC) national 2010. Bordeaux – INSERM U897 Université Bordeaux Segalen. Grenoble - Comité Scientifique AGIRadom 2011. Paris – Agence Nationale de la Santé, Région Ile de France, domaine d'intérêt majeur (DIM). **Germany:** Erfurt – German Research Foundation HE 3294/10-1. Hamburg – German Research Foundation MA 711/6-1, NO 262/7-1. **Iceland:** Reykjavik – The Landspítali University Hospital Research Fund, University of Iceland

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Research Fund, ResMed Foundation, California, USA, Orkuveita Reykjavíkur (Geothermal plant), Vegagerðin (The Icelandic Road Administration (ICERA). **Italy:** All Italian centres were funded by the Italian Ministry of Health, Chiesi Farmaceutici SpA. Verona received additional funding from the Cariverona foundation, Education Ministry (MIUR). **Norway:** Norwegian Research Council grant no 214123, Western Norway Regional Health Authorities grant no 911631, Bergen Medical Research Foundation. **Spain:** Fondo de Investigación Sanitaria (PS09/02457, PS09/00716 09/01511) PS09/02185 PS09/03190), Servicio Andaluz de Salud, Sociedad Española de Neumología y Cirurgía Torácica (SEPAR 1001/2010), Fondo de Investigación Sanitaria (PS09/02457). Barcelona – Fondo de Investigación Sanitaria (FIS PS09/00716). Galdakao – Fondo de Investigación Sanitaria (FIS 09/01511). Huelva – Fondo de Investigación Sanitaria (FIS PS09/02185) and Servicio Andaluz de Salud. Oviedo – Fondo de Investigación Sanitaria (FIS PS09/03190). "ISGlobal acknowledges support from the Spanish Ministry of Science and Innovation through the 'Centro de Excelencia Severo Ochoa 2019-2023' Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program." **Sweden:** All centres were funded by the Swedish Heart and Lung Foundation, the Swedish Asthma and Allergy Association, the Swedish Association against Lung and Heart Disease, and the Swedish Research Council for Health, Working Life and Welfare (FORTE). Göteborg also received further funding from the Swedish Council for Working Life and Social Research. Umeå also received funding from a Västerbotten County Council ALF grant. **Switzerland:** The Swiss National Science Foundation (grants no 33CSCO-134276/1, 33CSCO-108796, 3247BO-104283, 3247BO-104288, 3247BO-104284, 3247-065896, 3100-059302, 3200-052720, 3200-042532, 4026-028099), The Federal Office for Forest, Environment and Landscape, The Federal Office of Public Health, The Federal Office of Roads and Transport, the canton governments of Aargan, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais, and Zürich, the Swiss Lung League, the Lung Leagues of the cantons Basel Stadt/Basel, Landschaft, Geneva, Ticino, Valais, and Zurich, SUVA, Freiwillige Akademische Gesellschaft, UBS Wealth Foundation, Talecris Biotherapeutics GmbH, Abbott Diagnostics, European Commission 018996 (GABRIEL), Wellcome Trust WT 084703MA. **UK:** Medical Research Council (Grant Number 92091). Support was also provided by the National Institute for Health Research through the Primary Care Research Network.

Conflicts of interest

The authors declare no conflict of interest.

Author contribution

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C Krantz and A Malinovschi conceived, designed or analysed and interpreted the data; drafted the article or revised it; provided intellectual content of critical importance to the work described; and are accountable for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. S Accordini, K Alving, A G Corsico, P Demoly, D S Ferreira, B Forsberg, J Garcia-Aymerich, T Gislason, J Heinrich, C Janson, R Jõgi, A Johannessen, B Leynaert, A Marcon, J Martínez-Moratalla Rovira, E Nerpin, D Nowak, A-C Olin, M Olivieri, A Pereira-Vega, C Raherison-Semjen, F Gómez Real, T Sigsgaard and G Squillacioti conceived, designed or analysed and interpreted the data; drafted the article or revised it; provided intellectual content of critical importance to the work described.

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Tables and Figures

Table 1. Characteristics of subjects with current asthma and non-asthmatic controls. Data presented as means ± standard deviations for continuous variables and as numbers (%) for categorical variables.

	Current asthma (n=741)	Non-asthmatic subjects (n=4155)	p value
Female	439 (59.2%)	2065 (49.7%)	< 0.001
Age (years)	54.0 (± 7.1)	54.2 (± 7.1)	0.4
Height (cm)	169.0 (± 9.8)	170.4 (± 9.6)	< 0.001
Weight (kg)	79.5 (± 16.8)	78.8 (± 16.4)	0.3
BMI (kg/m^2)	27.8 (± 5.5)	27.1 (± 4.8)	< 0.001
Smoking habits: Never smoker Ex-smoker Current smoker	363 (49.1%) 277(37.5%) 99 (13.4%)	1796 (43.4%) 1570 (37.9%) 774 (18.7%)	0.002
Sensitisation to perennial allergens	170 (40.6%)	490 (13.3%)	< 0.001
Current rhinitis	496 (67.3%)	1513 (36.6%)	< 0.001
Rhinoconjunctivitis	390 (52.9%)	757 (18.3%)	< 0.001
Persistent rhinitis	163 (22.1%)	448 (10.9%)	0.2[1]
Severe rhinitis	146 (19.8%)	235 (5.7%)	< 0.001[2]
Symptoms of CRS	69 (9.4%)	117 (2.8%)	< 0.001
CRS	71 (9.6%)	213 (5.2%)	< 0.001
Nasal polyposis	103 (14.0%)	252 (6.2%)	< 0.001
Nasal steroid use	208 (28.2%)	356 (8.6%)	< 0.001
ICS use: Chronic Intermittent	207 (27.9%) 127 (17.1%)	0 (0%)	n.a.

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None	407 (54.9%)		
Regular LTRA use	18 (2.4%)	0 (0%)	n.a.

CRS = chronic rhinosinusitis, nasal steroid use = any use of intranasal steroid during the preceding 12 months, ICS = inhaled corticosteroids, chronic= continuous use during the preceding 3 months, intermittent = any but not continuous use during the preceding 12 months, none = no use during the preceding 12 months, LTRA = leukotriene receptor antagonist, n.a. = non-applicable. p value[1]= persistent vs intermittent rhinitis, p value[2]= moderate to severe vs mild rhinitis. Sensitisation based on n = 419 subjects with current asthma, and n = 3,689 non-asthmatic controls. In 17 cases (2 individuals with current asthma and 15 non-asthmatic subjects), the question on smoking was not answered.

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Table 2. FeNO levels (ppb) in relation to sensitisation and upper airway inflammatory symptoms in subjects with current asthma and non-asthmatic subjects. Results presented as geometric means (95% confidence intervals).

	Current asthma (n=741)	p value	Non-asthmatic subjects (n=4,155)	p value	p value interaction
Perennial sensitisation:					
No	18.1 (16.7–19.7)	< 0.001	16.0 (15.7–16.3)	< 0.001	0.06
Yes	24.4 (21.9–27.3)		19.2 (18.2–20.4)		
Current rhinitis:					
No	19.5 (17.9–21.2)	0.02	16.1 (15.7–16.4)	< 0.001	0.18
Yes	22.1 (20.7–23.5)		17.0 (16.6–17.5)		
Rhinoconjunctivitis					
No	20.3 (18.9–21.9)	0.1	16.1 (15.8–16.4)	< 0.001	0.6
Yes	22.0 (20.5–23.6)		17.8 (17.1–18.6)		
CRS symptoms					
No	21.2 (20.2–22.4)	0.6	16.4 (16.1–16.7)	0.7	0.5
Yes	20.4 (16.9–24.6)		16.7 (15.1–18.5)		
CRS diagnosis					
No	21.3 (20.2–22.5)	0.4	16.4 (16.1–16.7)	0.4	0.2
Yes	19.8 (16.6–23.5)		16.9 (15.7–18.3)		
Nasal polyposis					
No	20.7 (19.6–21.9)	0.04	16.4 (16.1–16.7)	0.9	0.04
Yes	24.2 (21.2–27.5)		16.6 (15.5–17.7)		

FeNO = Fractional exhaled nitric oxide, CRS = chronic rhinosinusitis, p value interaction relates to the interaction between current asthma and the relation of each variable and log FeNO.

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Table 3. FeNO levels (ppb) in relation to rhinitis and nasal polyposis, in subjects with current asthma and non-asthmatic subjects, stratified by perennial sensitisation. Results presented as geometric means (95% confidence intervals).

	Current rhinitis		p value
	No	Yes	
Current asthma and perennial sensitisation	21.6 (17.5–26.8) n=42	25.6 (22.5–29.2) n=127	0.2
Current asthma, no perennial sensitisation	17.3 (15.3–19.5) n=97	18.6 (16.7–20.9) n=150	0.4
Non-asthmatic subjects and perennial sensitisation	18.1 (16.7–19.7) n=241	20.6 (19.1–22.3) n=243	0.03
Non-asthmatic subjects, no perennial sensitisation	15.9 (15.5–16.2) n=2,117	16.2 (15.8–16.8) n=1,067	0.2
	Rhinoconjunctivitis		
	No	Yes	
Current asthma and perennial sensitisation	25.2 (21.2–29.9) n=68	24.1 (20.8–28.0) n=101	0.7
Current asthma, no perennial sensitisation	18.0 (16.1–20.1) n=135	18.2 (16.0–20.7) n=112	0.9
Non-asthmatic subjects and perennial sensitisation	18.1 (17.0–19.4) n=324	22.0 (19.9–24.3) n=160	0.002
Non-asthmatic subjects, no perennial sensitisation	15.9 (15.6–16.2) n=2,696	16.4 (15.7–17.2) n=488	0.2
	Nasal polyposis		
	No	Yes	
Current asthma and perennial sensitisation	23.8 (21.1–26.8) n=150	28.5 (21.2–38.3) n=19	0.3
Current asthma, no perennial sensitisation	17.4 (15.9–19.0) n=214	23.2 (18.5–29.1) n=32	0.02
Non-asthmatic subjects and perennial sensitisation	19.3 (18.2–20.4) n=454	19.6 (15.6–24.5) n=32	0.9

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Non-asthmatic subjects, no perennial sensitisation	16.0 (15.7–16.3) n=2,592	16.0 (14.9–17.2) n=198	1.0

Missing data on current rhinitis and rhinoconjunctivitis in 4 subjects with current asthma and 25 non-asthmatic controls, as well as on self-reported nasal polyposis in 5 subjects with current asthma and 58 non-asthmatic subjects.

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Table 4. Independent associations of upper airway inflammatory disorders and treatment on FeNO in subjects with current asthma and non-asthmatic subjects, stratified by perennial sensitisation, adjusted for age, weight, height, gender, smoking status and centre.

	Current asthma			Non-asthmatic subjects		
	Perennial atopy (n=168)	No perennial atopy (n=243)	p value inter.	Perennial atopy (n=482)	No perennial atopy (n=3,164)	p value inter.
	% difference (95% CI)	% difference (95% CI)		% difference (95% CI)	% difference (95% CI)	
Current rhinitis	20.7 (-9.5–60.9)	7.8 (-10.0–29.2)	0.7	**12.1 (0.2–25.5)**	1.9 (-1.6–5.6)	**0.02**
Nasal steroids preceding year	**-31.3 (-48.0–(-9.2))**	9.3 (-9.8–32.4)	0.06	-6.5 (-20.6–10.0)	3.9 (-2.3–10.6)	0.9
Regular LTRA use	-31.3 (-67.2–44.1)	10.5 (-31.9–79.2)	0.3	Not applicable		
Use of ICS Intermittent use Regular use	 **40.0 (3.9–88.5)** **67.1 (14.7–143.4)**	 -2.2 (-20.2–19.8) 5.4 (-14.6–30.1)	 0.5 0.12	Not applicable		
Nasal polyposis	15.5 (-21.1–68.9)	18.9 (-8.4–54.3)	0.6	1.3 (-18.2–25.5)	1.6 (-5.0–8.7)	0.9
Rhinoconjunctivitis	6.5 (-16.1–35.0)	5.0 (-12.2–23.5)	0.7	**15.9 (3.4–29.9)**	2.1 (-22.5–6.9)	**0.003**

Perennial = perennial +/- grass sensitisation, non-perennial = no sensitisation or sensitisation only towards grass, LTRA = leukotriene receptor antagonist, ICS = inhaled corticosteroids. CI = confidence interval, results presented as % difference ($(10^{\beta\text{-coefficient}} - 1) \times 100$), with 95% CI, expressed as % difference in relation to reference group (i.e., no rhinitis, no treatment, no nasal polyposis, no rhinoconjunctivitis). 95% CIs that do not include 0 are statistically significant ($p < 0.05$) and are highlighted in bold. p value inter= p value for interaction with perennial sensitisation in relation to each variable and log FeNO.

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Table 5. Independent associations of upper airway inflammatory disorders and treatment on FeNO in all subjects, with information on interaction with current asthma and perennial sensitisation. Also adjusted for age, weight, height, gender, smoking status and centre.

	% difference (95% CI) All subjects n=4,057	p interaction asthma	p interaction perennial sensitisation
Current asthma	**14.5 (7.1–22.4)**	n.a.	0.1
Perennial sensitisation	**18.4 (13.4–23.7)**	0.1	n.a.
Nasal steroids	0.2 (-5.0–5.6)	0.2	0.12
LTRA	-2.6 (-27.8–31.4)	n.a.	0.17
ICS Intermittent use Regular use	 4.7 (-6.8–17.6) **20.2 (5.2–37.3)**	 n.a.	 0.4 **0.02**
Current rhinitis	**4.4 (0.9–7.9)**	0.2	**0.03**
Rhinoconjunctivitis	**4.8 (0.7–9.2)**	0.6	**0.03**
Nasal polyposis	5.3 (- 1.0–12.0)	**0.03**	0.7

Perennial = perennial +/- grass sensitisation, non-perennial = no sensitisation or sensitisation only towards grass, LTRA = leukotriene receptor antagonist, ICS = inhaled corticosteroids. CI = confidence interval, results presented as % difference ($(10^{\beta\text{-coefficient}} - 1) \times 100$), with 95% CI, expressed as % difference in relation to reference group (i.e., no rhinitis, no treatment, no nasal polyposis, no rhinoconjunctivitis). 95% CIs that do not include 0 are statistically significant ($p < 0.05$) and are highlighted in bold. Upper airway inflammatory disorders (current rhinitis, rhinoconjunctivitis and nasal polyposis), as well as interactions, were added to the model individually. p interaction= p value for interaction with perennial sensitisation or current asthma in relation to each variable and log FeNO.

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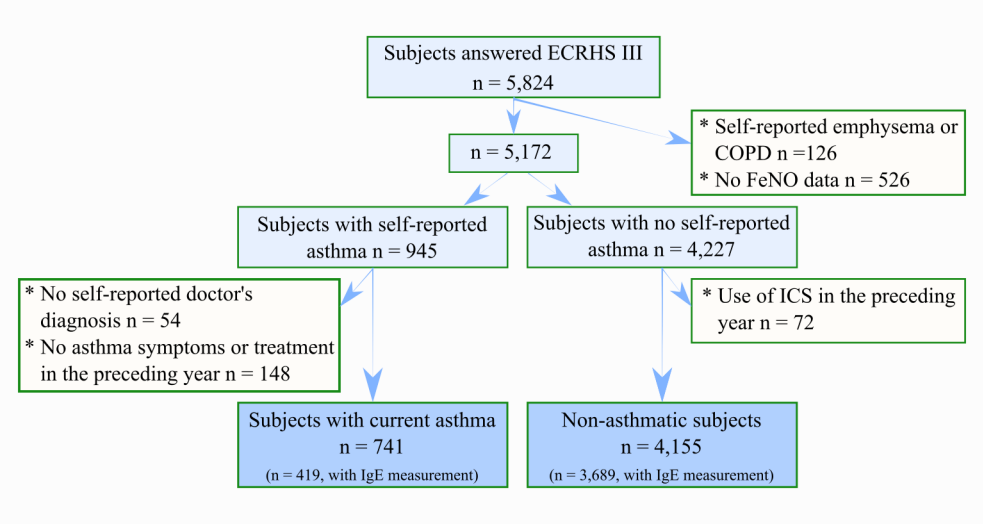
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Figure 1. Flowchart of subject selection for this study.

Figure 2. Schematic illustration of the relation between FeNO and allergic sensitisation, asthma and upper airway inflammatory disorders.

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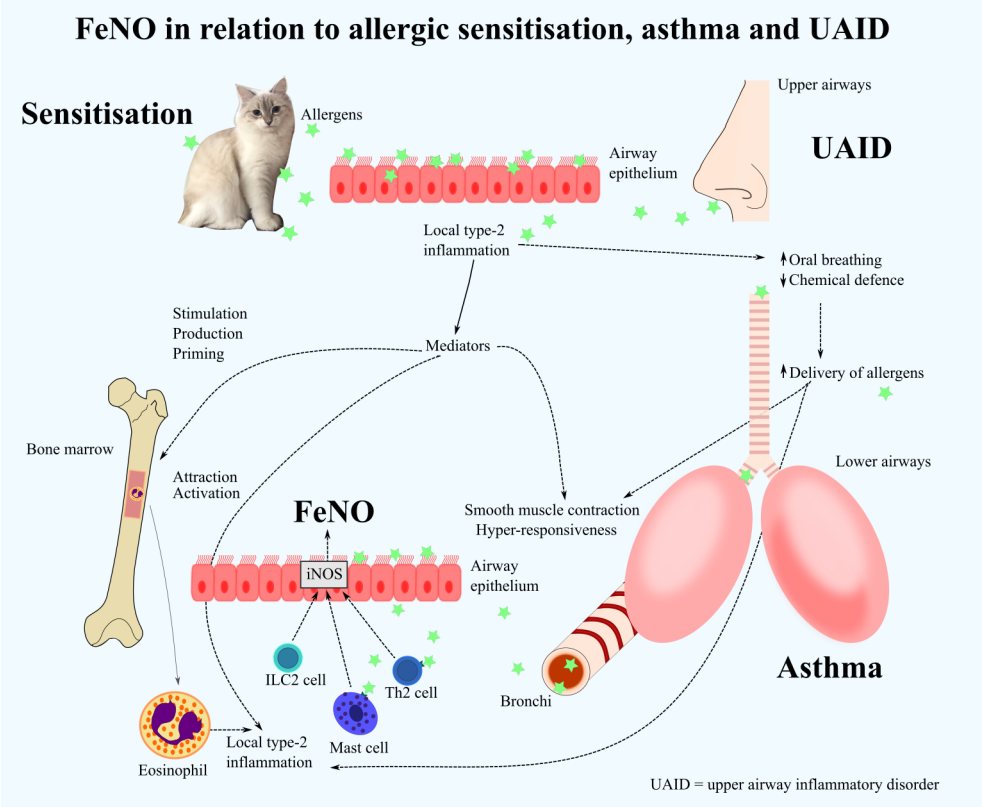
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Cross-sectional study on exhaled nitric oxide in relation to upper airway inflammatory disorders with regard to asthma and perennial sensitisation

On-line supplement:

On-line Table 1: Characteristics of subjects with or without measurements of FeNO. Data presented as number (percentage) for categorical variables and means (± standard deviation) for age.

	Subjects with FeNO measurements (n = 5,172)	**Subjects without FeNO measurements** (n = 526)	p-value
Female	2,673 (52 %)	326 (62 %)	< 0.01
Age	54.1 (± 7.1)	54.8 (± 7.4)	0.03
Smoking status: Current Previous	 918 (18 %) 1,936 (38 %)	 106 (20 %) 179 (34 %)	0.3[1]
Current rhinitis	2,157 (42 %)	210 (44 %)	0.4
Rhinoconjunctivitis	1,247 (24 %)	122 (25 %)	0.6
Persistent rhinitis	664 (13 %)	48 (10 %)	0.2
Severe rhinitis	408 (8 %)	51 (11 %)	0.04
CRS (symptoms)	201 (4 %)	30 (6 %)	0.01
CRS diagnos	308 (6 %)	32 (7 %)	0.5
Nasal polyposis	388 (8 %)	16 (3 %)	< 0.01
Nasal steroids last 12 months	620 (12 %)	85 (18 %)	< 0.01
ICS treatment Daily Intermittent	 186 (4 %) 224 (4 %)	 24 (5 %) 21 (4 %)	0.3
Self-reported asthma	945 (18 %)	100 (19 %)	0.7

CRS = chronic rhinosinusitis, ICS = inhaled corticosteroids. [1] difference in current and never smokers. *In 17 cases (2 individuals with current asthma and 15 non-asthmatic subjects), the question on smoking was not answered. Missing data on current rhinitis and rhinoconjunctivitis in 30 subjects with FeNO measurements and in 47 subjects without FeNO measurements.*

On-line Table 2. Characteristics of subjects with self-reported doctor diagnosis of asthma, but without treatment or asthma symptoms the preceding year and subject with current asthma. Data presented as number (percentage) for categorical variables and means (± standard deviations) for continuous variables.

	Current asthma (n=741)	Non current asthma (n=148)	p value
Female	439 (59.2%)	93 (63%)	0.4
Age (years)	54.0 (± 7.1)	53.1 (± 7.3)	0.4
Height (cm)	169.0 (± 9.8)	168.6 (± 9.0)	0.6
Weight (kg)	79.5 (± 16.8)	75.8 (± 13.7)	0.01
BMI (kg/m^2)	27.8 (± 5.5)	26.6 (± 4.1)	0.01
Smoking habits: Never smoker Ex-smoker Current smoker	363 (49.1%) 277(37.5%) 99 (13.4%)	80 (54.4%) 51 (34.7%) 16 (10.9%)	0.3[1]
Sensitisation to perennial allergens	170 (40.6%)	33 (33%)	0.2
Current rhinitis	496 (67.3%)	74 (50%)	< 0.01
Rhinoconjunctivitis	390 (52.9%)	54 (36.5%)	< 0.01
Persistent rhinitis	163 (22.1%)	31 (21.0%)	0.04[2]
Moderate to severe rhinitis	146 (19.8%)	13 (8.8%)	< 0.01[3]
Symptoms of CRS	69 (9.4%)	4 (2.7%)	< 0.01
Self-reported doctor diagnosis of CRS	71 (9.6%)	11 (7.4%)	0.4
Nasal polyposis	103 (14.0%)	21 (14.2%)	1.0
Nasal steroid use	208 (28.2%)	21 (14.3%)	< 0.01
ICS use: Chronic Intermittent None	207 (27.9%) 127 (17.1%) 407 (54.9%)	0 (0%)	n.a.
Regular LTRA use	18 (2.4%)	0 (0%)	n.a.

CRS = chronic rhinosinusitis, ICS = inhaled corticosteroids. LTRA = leukotriene receptor antagonist. [1] difference in current and never smokers, [2] in relation to intermittent rhinitis. [3] in relation to mild *rhinitis. In 3 cases (2 individuals with current asthma and 1 non-current asthma), the question on smoking was not answered. Missing data on current rhinitis and rhinoconjunctivitis in 4 subjects with current asthma.*

Levels of FeNO differed significantly between subjects with non-current asthma (18.7 (17.1–20.5) ppb), current asthma (21.2 (20.1–22.3) ppb) and non-asthmatic subjects (16.4 (16.1–16.7) ppb) (p = 0.046 current vs non-current asthma, p = 0.02 non-current asthma vs non-asthmatic subjects, p < 0.01 current asthma vs non-asthmatic subjects).

On-line Table 3. Characteristics of subjects stratified by current rhinitis. Data presented as number (percentage) for categorical variables and means (± standard deviations) for continuous variables.

	Current rhinitis (n = 2,009)	No current rhinitis (n = 2,858)	p value
Female	1,116 (55.6%)	1,366 (47.8%)	< 0.01
Age (years)	53.6 (± 7.1)	54.5 (± 7.0)	< 0.01
Height (cm)	169.6 (± 9.6)	170.6 (± 9.7)	< 0.01
Weight (kg)	78.3 (± 16.6)	79.3 (± 16.3)	0.046
BMI (kg/m^2)	27.1 (± 5.0)	27.2 (± 4.8)	0.8
Smoking habits:			< 0.01[1]
Never smoker	924 (46.1%)	1,222 (42.9%)	
Ex-smoker	757 (37.8%)	1,079 (37.9%)	
Current smoker	322 (16.1%)	546 (19.2%)	
Sensitisation to perennial allergens	370 (23.3%)	283 (11.3%)	< 0.01
Current asthma	496 (24.7%)	241 (8.4%)	< 0.01
Self-reported doctor diagnosis of CRS	180 (9.0%)	104 (3.7%)	< 0.01
Nasal polyposis	190 (9.5%)	164 (5.8%)	< 0.01
Nasal steroid use	463 (23.1%)	101 (3.5%)	< 0.01
ICS use:			< 0.01
Chronic	86 (4.3%)	41 (1.4%)	
Intermittent	140 (7.0%)	66 (2.3%)	
None	1,783 (88.8%)	2,752 (96.3%)	
Regular LTRA use	14 (0.7%)	4 (0.1%)	< 0.01
FeNO	18.2 (17.7–18.6)	16.3 (16.0–16.6)	< 0.01

CRS = chronic rhinosinusitis, ICS = inhaled corticosteroids. LTRA = leukotriene receptor antagonist. [1] difference in current and never smokers. *In 15 cases (6 individuals with current rhinitis and 9 without current rhinitis), the question on smoking was not answered. Missing data on current rhinitis and rhinoconjunctivitis in 29 subjects included in the study.*

Relations between FeNO and constitutional factors

In subjects with current asthma, there was a positive association between FeNO and height (r=0.11, p<0.01), but no association was seen with either weight (r=0.02, p=0.5) or age (r=--0.005, p=0.9). In non-asthmatic subjects, positive associations between FeNO and height (r=0.14, p<0.01), weight (r=0.08, p<0.01), and age (r=0.11, p<0.01) were seen. A negative interaction was found with asthma and the relationship between age and FeNO (p<0.01), which remained after adjustment for gender (p=0.01), but no interaction with asthma and the relationship between weight and FeNO (p=0.3) was found. No association between FeNO and BMI was seen in either subjects with current asthma or non-asthmatic subjects (data not shown).

On-line Table 4. *FeNO levels (ppb) in relation to gender and smoking habits in subjects with current asthma and non-asthmatic subjects. Results presented as geometric means (95% confidence*

intervals).

	Current asthma (n=741)	p value	Non-asthmatic subjects (n=4,155)	p value	p value interaction
Females	19.6 (18.3–20.9)	< 0.01	14.8 (14.5–15.2)	< 0.01	0.9
Males	23.7 (22.0–25.7)		18.1 (17.7–18.5)		
Never smoker	23.4 (21.7–25.1)	< 0.01*	18.1 (17.7–18.5)	< 0.01*	< 0.01*
Ex-smoker	22.8 (21.2–24.7)	< 0.01*	17.5 (17.1–17.9)	< 0.01*	< 0.01*
Current smoker	12.0 (10.7–13.4)		11.5 (11.1–11.9)		

In 17 cases (2 individuals with current asthma and 15 non-asthmatic subjects), the question on smoking was not answered.