ORIGINAL ARTICLE
ASTHMA AND ALLERGY

Contributions of asthma, rhinitis and IgE to exhaled nitric oxide in adolescents

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ABSTRACT Exhaled nitric oxide fraction (F_{eNO}) is an indicator of allergic airway inflammation. However, it is unknown how asthma, allergic rhinitis (AR) and allergic sensitisation relate to F_{eNO}, particularly among adolescents and in overlapping conditions. We sought to determine the associations between asthma, AR, and aeroallergen immunoglobulin (Ig)E and F_{eNO} in adolescents.

We measured F_{eNO} among 929 adolescents (aged 11–16 years) in Project Viva, an unselected prebirth cohort in Massachusetts, USA. We defined asthma as ever asthma physician diagnosis plus wheezing in the past year or taking asthma medications in the past month, AR as a physician diagnosis of hay fever or AR, and aeroallergen IgE as any IgE >0.35 IU·mL^{-1} among 592 participants who provided blood samples. We examined associations of asthma, AR and IgE with percent difference in F_{eNO} in linear regression models adjusted for sex, race/ethnicity, age and height, maternal education and smoking during pregnancy, and household/neighbourhood demographics.

Asthma (14%) was associated with 97% higher F_{eNO} (95% CI 70–128%), AR (21%) with 45% higher F_{eNO} (95% CI 28–65%), and aeroallergen IgE (58%) with 102% higher F_{eNO} (95% CI 80–126%) compared to those without each condition, respectively. In the absence of asthma or AR, aeroallergen IgE was associated with 75% higher F_{eNO} (95% CI 52–101), while asthma and AR were not associated with F_{eNO} in the absence of IgE.

The link between asthma and AR with F_{eNO} is limited to those with IgE-mediated phenotypes. F_{eNO} may be elevated in those with allergic sensitisation alone, even in the absence of asthma or AR.

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While asthma, allergic rhinitis (AR) and allergic sensitisation are associated with higher F_{ENO}, asthma and AR in the absence of aeroallergen IgE are not associated with F_{ENO}. When elevated in asthma or AR, F_{ENO} suggests allergic sensitisation. https://bit.ly/3bGgr0r

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Introduction

Exhaled nitric oxide fraction (F_{eNO}) is an indicator of allergic airway inflammation and can aid in the diagnosis of allergic asthma [1]. The influence of concurrent allergic rhinitis (AR) and allergic sensitisation to environmental allergens on F_{eNO} measurements is not well-known in adolescents. How each of these (asthma, AR and aeroallergen immunoglobulin (Ig)E) relate to F_{eNO} is important not only for interpretation of F_{eNO} in clinical use but also for a greater understanding of the factors that lead to elevated F_{eNO}. We sought to understand the associations of asthma, AR, and aeroallergen IgE with F_{eNO} in an adolescent population.

Asthma and AR are common and contribute to significant morbidity, with poorer quality of life, missed days of school, healthcare costs and emergency room visits for asthma exacerbations, resulting from difficulty sleeping, fatigue, and changes in mood and cognition [2]. AR and asthma share epidemiological overlap. AR occurs in >75% of patients with asthma, and asthma is seen in up to 40% of those with AR [3]. In those with both AR and asthma, AR may present first and there is a risk factor for subsequent development of asthma [4–7].

Asthma and AR are each known to be associated with elevated F_{eNO} in both adults and children. Nitric oxide (NO) is an intercellular messenger known to mediate a variety of processes, including immune function and inflammation [8]. In the lung, NO acts as a signalling molecule in bronchial and vascular dilatation, ciliary kinesis, and neurotransmission in the non-adrenergic and non-cholinergic systems [9]. In allergic inflammation, T-helper-2 cells, type 2 innate lymphoid cells, mast cells and eosinophils produce cytokines, like interleukin (IL)-4 and IL-13, with downstream effects including the activation of inducible NO synthase (iNOS) and thus elevated NO levels. Therefore, F_{eNO} is considered a marker of type 2 airway inflammation, which occurs with AR in the upper airway or allergic asthma in the lower airway [9–13]. Elevation of F_{eNO} may indicate not only allergic asthma or AR but also elevations in circulating IgE against environmental allergens. In previous work in this same Project Viva cohort, an unselected pre-birth cohort in Massachusetts, USA, we have found differences in the nasal epigenome in association with asthma, AR, aeroallergen IgE and F_{eNO} that are annotated with genes implicated in type 2 inflammatory responses [14]. Currently, the American Thoracic Society recommends F_{eNO} in the diagnosis of eosinophilic airway inflammation, and suggests that it may be used to support the diagnosis of asthma where the diagnosis is less clear [1]. F_{eNO}, however, may also be elevated in AR or allergic sensitisation to environmental allergens [15, 16]. Therefore, we sought to elucidate the contributors to F_{eNO} and supplement our understanding of its diagnostic utility. In this study, we examined the relationships of asthma, AR, and aeroallergen IgE with F_{eNO} in an adolescent population in Project Viva, a cohort not selected on the basis of asthma or allergy.

Materials and methods

Study design and participants

Between 1999 and 2002 we recruited women in early pregnancy into Project Viva from eight obstetric offices of Atrius Harvard Vanguard Medical Associates, a multi-specialty group practice in eastern Massachusetts. Exclusion criteria included multiple gestation, inability to answer questions in English, and gestational age ⩾22 weeks at recruitment. Details of recruitment and retention are available elsewhere [17]. Of the 2128 infants, we included in this analysis those participants with F_{eNO} measurements at an in-person visit in early adolescence, which totalled 929. Compared with the 929 included participants, the 1199 excluded participants were less likely to have college-educated mothers (59% *versus* 71%) and more likely to have mothers who smoked during pregnancy (15% *versus* 9%). However, maternal age at enrolment, gestational age at delivery, and sex and race/ethnicity were similar. Those 1199 were excluded due to either missing F_{eNO} or no early adolescent visit. The median adolescent age was 12.9 years with a range of 11.9–16.6 years; there were no participants older than this due to cohort inception dates.

Asthma and AR

We defined current asthma as a maternal report of ever asthma diagnosis plus wheeze symptoms in the past 12 months or use of asthma medications in the past month, reported on an early teen questionnaire in keeping with the Study of Asthma and Allergies in Childhood questionnaire [18]. We used as a comparison group with no asthma diagnosis, no wheezing and no use of asthma medications or wheezing in the past 12 months. Participants were defined as having AR if they reported ever receiving a physician diagnosis of hay fever or AR. Both current asthma and AR were reported by parental questionnaire at the early teen follow-up visit.

Aeroallergen IgE

Trained research phlebotomists collected blood from participants at the early teen visit, which we centrifuged and stored at −80C. We measured plasma IgE against *Dermatophagoides farina* (dust mite), cat or dog dander, *Aspergillus fumigatus* (mould), *Alternaria alternata* (plant fungi), common ragweed, oak, ryegrass, or silver birch. Allergen extract-specific IgE antibodies were measured by ImmunoCap (Thermo Fisher Scientific/Phadia, Kalamazoo, Michigan); a widely used *in vitro* sandwich immunoassay, as described previously [19, 20]. We defined aeroallergen IgE as having IgE against any of these outdoor or indoor allergens >0.35 $IU \cdot mL^{-1}$. We defined "perennial IgE" as having IgE >0.35 $IU \cdot mL^{-1}$ against any of the following: *D. farina* (dust mite), cat or dog dander, *A. fumigatus* (mould) or *A. alternata* (plant fungi). We defined "seasonal IgE" as having any IgE >0.35 $IU \cdot mL^{-1}$ against any of the following: common ragweed, oak, ryegrass or silver birch.

Measurement of F_{eNO}

Exhaled NO levels were measured twice for each participant with a portable electrochemical device (NIOX MINO; Aerocrine AB); this has been validated by chemiluminescence technology, with an accuracy of ±5 ppb [21]. Prior to each measurement, participants breathed in through an NO scrubbing filter and exhaled into room air twice. This was done in keeping with prior studies using F_{eNO}; the ambient air NO was not measured [22–24]. On the third breath, participants inhaled through the filter and exhaled into the F_{eNO} analyser. The last 3 s of the exhalation were utilised for F_{eNO} measurement; this ensures lower rather than upper airway measurement. Nose clips were not used.

Statistical analysis

Potential confounders were selected *a priori*, based on known or suspected associations with asthma or allergy. Model 1 adjusted for sex and age and height at the early teen visit. Model 2 additionally adjusted for race/ethnicity, maternal education and smoking during pregnancy, median value of owner-occupied housing and education (% with a bachelor degree) based on census tract of home residence at time of mid-childhood visit from 2000, and household income and any smokers at home at the early teen visit. We additionally adjusted for body mass index in model 3.

We averaged the two F_{eNO} measurements and included the log-transformed value as a continuous outcome in linear regression models. We present effect estimates as % change (95% CI) in F_{eNO}, calculated as (exponentiated (β)−1)×100. In secondary analyses, we examined F_{eNO} in categories (<20, 20–⩽35 and >35 ppb) based on American Thoracic Society guidelines [1] using multinomial logistic regression models. We examined the associations of asthma, AR, and aeroallergen IgE with F_{eNO} using separate linear and logistic regression models. We repeated linear regression models for the associations of seasonal and perennial IgE with F_{eNO}.

To examine associations of overlapping conditions of asthma, AR and aeroallergen IgE, we derived an eight-category exposure based on the combination of these three exposures. We ran linear regression models with this eight-category exposure and used no aeroallergen IgE, no AR, no asthma as the reference category. We performed all analyses using SAS 9.4 (SAS Institute).

Results

Study population

The characteristics of the 929 study participants included in any analysis and their mothers are shown in table 1. Reported smoking in the home was rare (12%) and mean reported annual household income was \$109 000. Children were predominantly white (64%) and their average age was 13 years. Mean±SD F_{eNO} was 26±27 ppb and 19% of study participants had exhaled NO levels above the upper limit of normal for this age group (35 ppb) [1].

Overall, 115 (14%) out of 797 participants reported asthma while 179 (21%) out of 869 reported AR. Aeroallergen IgE >0.35 $IU \cdot mL^{-1}$ was detected in 345 (58%) out of 592 of those providing blood samples with IgE results. Mean±SD F_{eNO} in those with asthma was 48.4±43.0 ppb compared to 21.8±20.4 ppb in those without asthma. Mean±SD F_{eNO} in those with AR was 35.7±32.8 ppb compared to 23.5±24.9 ppb without AR. Participants with aeroallergen IgE had mean±SD F_{eNO} 36.2±34.2 ppb compared to 14.8±9.5 ppb in those without IgE.

Associations of each condition with F_{eNO} are shown in table 2, where model 1 is parsimoniously adjusted and model 2 is fully adjusted, with similar results. Asthma was common in those with F_{eNO} >35 ppb; 35% of teens with F_{eNO} >35 ppb had asthma *versus* 7% among participants with F_{eNO} <20 ppb and 18% among participants with F_{eNO} 20–⩽35 ppb. Of the three conditions, the presence of aeroallergen IgE and asthma diagnosis each had a similar association with F_{eNO}, while the magnitude of the association was half as great for AR. Specifically, those with asthma had a 97% higher F_{eNO} (95% CI 70–128%) compared to those without asthma. Those with AR had a 45% higher F_{eNO} (95% CI 28–65%) compared to those without AR, and those with aeroallergen IgE had 102% higher F_{eNO} (95% CI 80–126%) compared to those without aeroallergen IgE. Perennial IgE was associated with 119% higher F_{eNO} (95% CI 97–144) while seasonal IgE

TABLE 1 Participant characteristics (N=929)

Subjects n	929
Child	
Sex	
Male	466 (50)
Female	463 (50)
Race/ethnicity	
Black	154 (17)
Hispanic	40 (4)
Asian	28 (3)
White	593 (64)
Other or >1 race/ethnicity	113 (12)
Early teen visit	
Age years	13±1
Age at visit years	
11.9 to <13	505 (54.4)
13.0 to <15.0	374 (40.3)
15.0 to 16.6	50 (5.4)
Height cm	160±9
BMI percentile category %	
<5th	30 (3)
5–<85th	634 (68)
>85th	262 (28)
Exhaled nitric oxide ppb	26±27
Exhaled nitric oxide	
<20 ppb	553 (60)
20–⩽35 ppb	196 (21)
>35 ppb	180 (19)
Current asthma	
No	682 (86)
Yes	115 (14)
Ever allergic rhinitis	
No	690 (79)
Yes	179 (21)
Any aeroallergen IgE >0.35 $kU \cdot L^{-1}$	
No	247 (42)
Yes	345 (58)
Annual household income at early teen visit $1000	109±44
Any smokers at home at early teen visit	114 (12)
Mother/family	
Pregnancy smoking status	
Never	653 (71)
Former	188 (20)
During pregnancy	85 (9)
College graduate	
No	264 (29)
Yes	662 (71)
Median value owner-occupied housing $1000[#]	263±149
Percent with education higher than a bachelor's degree[#]	42±20

Data are presented as n (%) or mean±SD, unless otherwise stated. BMI: body mass index; Ig: immunoglobulin. #: based on census tract, mid-childhood.

was associated with 71% higher F_{eNO} (95% CI 52–93). Similar results were seen in logistic regression analyses for the clinical thresholds of F_{eNO}. Asthma was associated with eight times higher odds (95% CI 5–14) of F_{eNO} >35 ppb, AR was associated with three times higher odds (95% CI 2–5) of F_{eNO} >35 ppb and aeroallergen IgE was associated with 28 times higher odds (95% CI 12–63) of F_{eNO} >35 ppb compared to those without each condition (table 2).

We found similar associations when stratified by sex (table S2). Since steroids can affect F_{eNO}, we ran a sensitivity analysis, excluding participants who used oral or inhaled corticosteroids within 72 h of F_{eNO} testing (n=22) and found similar associations between asthma, AR and aeroallergen IgE with higher F_{eNO}.

TABLE 2 Associations of asthma, allergic rhinitis (AR) and aeroallergen immunoglobulin (Ig)E with exhaled nitric oxide fraction (F_{eNO}) using linear and multinomial logistic regression models

	Model 1#			Model 2¶		
	Linear regression % difference in F_{eNO} (95% CI)	Logistic regression+ OR (95% CI)		Linear regression % difference in F_{eNO} (95% CI)	Logistic regression+ OR (95% CI)	
		20–⩽35 ppb	>35 ppb		20–⩽35 ppb	>35 ppb
Asthma *versus* no asthma	97 (73–124)	3 (2–5)	8 (5–13)	97 (70–128)	3 (1–5)	8 (5–14)
AR *versus* no AR	48 (32–66)	2 (1–3)	3 (2–5)	45 (28–65)	2 (1–3)	3 (2–5)
Aeroallergen IgE *versus* no aeroallergen IgE	99 (79–121)	4 (3–7)	26 (12–59)	102 (80–126)	4 (3–7)	28 (12–63)

Current asthma: ever having an asthma diagnosis plus wheezing in the past year or taking asthma medications in the past month; no asthma: no asthma: no wheezing and no use of asthma medications or wheezing in the past 12 months; AR: ever having a diagnosis of hay fever or AR; no AR: never having a diagnosis of hay fever or AR; aeroallergen IgE; having any aeroallergen IgE >0.35 IU·mL^{-1}; no aeroallergen IgE: IgE⩽0.35 IU·mL^{-1}. #: adjusted for sex and current age and height; ¶: model 1 additionally adjusted for race/ethnicity, maternal education and smoking during pregnancy, median value owner-occupied housing and percent⩾bachelor's degree (census tract, mid-childhood), and household income and any smokers at home at early teen visit; +: reference group is <20 ppb.

Finally, we adjusted for body mass index and found similar associations with asthma, AR and aeroallergen IgE with F_{eNO} in both linear and logistic regression models (table S3).

Overlapping associations of allergy, asthma and aeroallergen IgE with F_{eNO}

To examine associations of overlapping conditions of asthma, AR and aeroallergen IgE, we derived an eight-category exposure based on the combination of these three exposures among 476 participants with non-missing values for the three exposures. The reference category was no asthma, no AR and no aeroallergen IgE (figure 1, table S1). We found that, in the absence of asthma or AR, aeroallergen IgE alone (n=173, 36%) was associated with 75% higher F_{eNO} (95% CI 52–101%). Asthma in the absence of AR and aeroallergen IgE (n=10, 2%), AR in the absence of asthma and aeroallergen IgE (n=14, 3%), and asthma and AR in the absence of aeroallergen IgE (n=6, 1%) were uncommon and not associated with

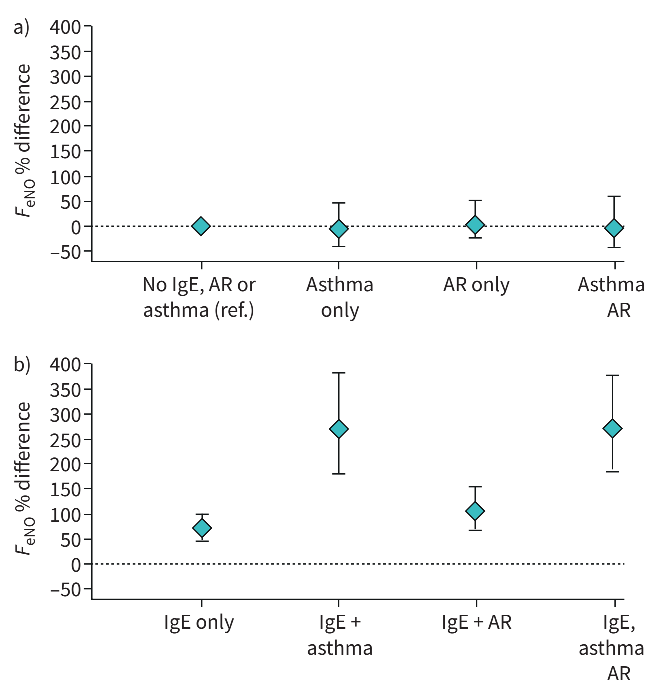

FIGURE 1 Percent difference in exhaled nitric oxide fraction (F_{eNO}) relative to reference group a) without detectable aeroallergen immunoglobulin (Ig)E and b) with aeroallergen IgE. AR: allergic rhinitis.

F_{eNO}. The presence of all three exposures (asthma, AR and aeroallergen IgE (n=32, 7%)) was associated with 272% higher F_{eNO} (95% CI 189–379%) compared to having none of these (figure 1).

Discussion

In this study of adolescents, we found, as expected, that asthma and allergy were each associated with higher F_{eNO}. However, this association was absent among those without detectable aeroallergen IgE. Additionally, aeroallergen IgE was associated with elevated F_{eNO} even in the absence of asthma or AR.

The association between asthma and elevated F_{eNO} among adolescents in this study is consistent with the well-established connection between allergic asthma and airway inflammation as measured by F_{eNO}. Exhaled NO >35 ppb in children strongly supports the diagnosis of asthma and can be used as a diagnostic tool [1]. F_{eNO} is a marker of an allergic asthma phenotype, characterised by elevations in type 2 cytokines IL-4, IL-5 and IL-13 and eosinophils in sputum, serum, and bronchial biopsy [25–27].

However, we found that this association between asthma and increased F_{eNO} was seen only when aeroallergen IgE was detected. In the absence of aeroallergen IgE, the association between asthma and F_{eNO} was lost. Our results suggest that asthma is only associated with F_{eNO} in the presence of allergic sensitisation, and that F_{eNO} is more likely to indicate the presence of aeroallergen IgE. We found that F_{eNO} >35 ppb had a positive predictive value of only 35% for the diagnosis of asthma, compared to a positive predictive value of 94% for the presence of aeroallergen IgE. Our findings contrast with a study of 1156 children in France, which found that non-atopic asthmatics had higher F_{eNO} than non-atopic children without asthma: average F_{eNO} 13.4 ppb in non-atopic children with asthma *versus* 10.6 ppb in non-atopic children without asthma [28]. However, our findings are consistent with other birth cohorts, including one from the Netherlands, which observed higher F_{eNO} only among young adults with atopic asthma, defined as asthma plus allergic sensitisation with positive serum IgE against 10 common aeroallergens, but not non-atopic asthma [29]. The Isle of Wight birth cohort in England, UK, similarly found that atopy (measured by skin-prick testing) was associated with higher F_{eNO}, while the level of F_{eNO} did not differ between non-atopic teens with and without asthma [30]. The authors concluded, as we do, that F_{eNO} is a biomarker for atopy rather than asthma.

Our study also confirmed the association between AR and higher F_{eNO} among adolescents, which has been well established among adults and children [31–35]. Similar to our findings in asthma, we found that this association between AR and F_{eNO} was observed only in the presence of aeroallergen IgE. Other studies have also found that those with atopy and rhinitis have higher F_{eNO} than those with rhinitis but no atopy [28, 36, 37]. This same large cohort of 1156 children in France found that non-asthmatic atopic participants (as determined by skin-prick test to common allergens) with rhinitis had significantly higher F_{eNO} than non-atopic participants with rhinitis (20.7±13 *versus* 12.5±6.4 ppb) [28]. Likewise, in an adult population examining atopic participants identified by skin-prick testing, Gratziou *et al.* [37] found that F_{eNO} was significantly higher in atopic rhinitis than in those with non-atopic rhinitis (13.3±1.3 *versus* 5.8±1.2 ppb).

One explanation of our findings is that asthma in the setting of low aeroallergen IgE is mediated by a non-allergic inflammatory pathway. This distinct non-allergic asthma phenotype represents a subset of asthmatics that may have more severe and more difficult to control asthma [38, 39]. Rather than allergic, type 2-mediated pathways, these non-atopic asthmatics may have neutrophilic airway inflammation [40]. This may be determined, in part, by genetic factors, and those with allergic and non-allergic asthma have distinct HLA haplotypes [41]. Environmental exposures such as particulate air pollution or childhood viral infections may also contribute to non-allergic asthma, in a process mediated through neutrophilic inflammation [42]. A similar neutrophilic process may be occurring in our participants with asthma or AR and no significant serum IgE.

We found that aeroallergen IgE is associated with elevated F_{eNO} even in the absence of asthma or AR. A few studies have similarly found that atopy is associated with F_{eNO} regardless of symptoms. For example, both Choi *et al.* [36] and Franklin *et al.* [43] examined children with asthma and atopy and found that atopy increases F_{eNO} regardless of asthma diagnosis. This has been similarly observed among Pacific Islander adults, where positive skin-prick testing to house dust mite was associated with higher exhaled and nasal NO even in the absence of asthma symptoms [44]. In a study comparing asthmatic and healthy children, Barreto *et al.* [45] found that even in those without respiratory symptoms, atopy (defined by skin-prick testing) and peripheral eosinophil count were associated with significantly higher F_{eNO} compared to those without atopy or eosinophilia. In a population of school children, van Amsterdam *et al.* [46] similarly found that allergic sensitisation was associated with higher F_{eNO} even in those without wheeze, although the association was significantly augmented in those with wheeze.

Our findings suggest that IgE is an important factor in the elevated F_{eNO} observed in asthma and AR in adolescents. While the exact mechanism by which IgE may influence F_{eNO} is not entirely clear, the pathophysiology by which each are elevated in asthma and AR has been previously explored. IgE may increase F_{eNO} along with inflammatory cytokines inducing iNOS. In immediate hypersensitivity reactions, IgE cross-links to the FcεR1 receptor on mast cells, resulting in mast cell degranulation, inflammatory cytokine release, and activation of inflammatory cells at sites sensitive to the inciting allergy. Local IgE-mediated mechanisms may explain the localised reactions in skin, nasal turbinates, airways, and gut in eczema, rhinitis, asthma, and food allergy, respectively. In allergic asthma and AR specifically, the inflammatory cytokines that occur as a result of IgE-FcεR1 cross-linking may induce iNOS, resulting in the increased F_{eNO} observed in AR and asthma [47, 48]. The upregulated eosinophils in type 2 inflammation of asthma and AR may also exert direct oxidative damage and promote the continued release of inflammatory cytokines that activate iNOS, increasing NO in exhaled air [28, 45, 49].

Our study is one of the largest studies conducted in a well-characterised adolescent age group that examines the associations of asthma, allergy and IgE with airway inflammation as measured by F_{eNO}. However, we acknowledge limitations to this study. Specifically, this is a cross sectional study evaluating F_{eNO}, asthma and AR at a single time-point in adolescence. Asthma and AR vary widely in symptoms, severity and control, and so associations may change depending on different time-points in the disease trajectory. Additionally, steroid use may reduce F_{eNO} although we had similar findings when excluding participants with any steroid use within 72 h of testing. In addition, there were very few participants who had asthma or AR but no aeroallergen IgE. This may represent the fact that the majority of those with asthma or AR in this study had an allergic asthma phenotype. There were, however, a large portion of our participants (36%) who did not have asthma or AR but who did have aeroallergen IgE. Finally, we did not include those with previous or non-active asthma for those without wheeze in the last 12 months. Similarly, we did not include IgE positivity to non-tested allergens such as food-specific IgE. Future work may include these other allergens. We would also explore the comparison of F_{eNO} with blood eosinophils as a more widely available biomarker than F_{eNO}.

Our study confirms the expected pattern of F_{eNO} elevation among adolescents with asthma and AR and implicates IgE-mediated pathways in the association between asthma, AR and F_{eNO}. Our findings suggest that the presence of aeroallergen IgE was critical to the F_{eNO} elevations observed in those with asthma or AR. Without aeroallergen IgE, that relationship was lost. Aeroallergen IgE can be considered a marker for elevated F_{eNO}, since F_{eNO} was detected whenever aeroallergen IgE was present, regardless of the presence of clinical disease. Furthermore, even if F_{eNO} is elevated, it does not necessarily indicate asthma or AR, and patients with asthma and no aeroallergen IgE may not have an elevated F_{eNO}. Therefore, this study places F_{eNO} into perspective as a marker predominantly for sensitisation rather than for clinical disease, and further raises the need to identify alternative markers for airway inflammation in those children and adolescents with non-IgE-mediated asthma or AR.

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