

ORIGINAL ARTICLE

Asthma and Rhinitis

IgE sensitization to food allergens and airborne allergens in relation to biomarkers of type 2 inflammation in asthma

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Summary

Background: We have recently reported that sensitization to food allergens and sensitization to airborne allergens had independent associations with increased fraction of exhaled nitric oxide (FeNO) and blood eosinophils in middle-aged adults and in young subjects with asthma.

Objective: To investigate the relation between IgE sensitization and several type 2 inflammation biomarkers in adult asthmatics.

Methods: FeNO, urinary eosinophil-derived neurotoxin (U-EDN), serum eosinophil cationic protein (S-ECP) and periostin were measured in 396 asthmatics, aged 17–76 years, from the Swedish GA2LEN study. Sensitization to airborne allergens was examined with skin prick tests (≥ 3 mm wheal) and sensitization to food allergens with measurement of specific IgE (≥ 0.35 kU/L).

Results: Asthmatics sensitized to food allergens had higher FeNO, 22.3 ppb (18.6, 26.7) vs 16.1 ppb (14.2, 18.2) ($P = .005$), S-ECP, 17.7 mg/L (14.8, 21.1) vs 12.8 mg/L (10.9, 14.9) ($P = .01$), and periostin, 73.7 (67.5, 80.3) ng/mL vs 59.9 (55.8, 64.2) ng/mL ($P = .003$), than non-sensitized subjects. Periostin levels in this group were also significantly higher than in the group sensitized only to airborne allergens ($P = .01$). Sensitization to food allergens related independently to FeNO ($P = .02$), S-ECP ($P = .006$) and periostin ($P = .004$), whereas sensitization only to airborne allergens related only to FeNO ($P = .02$) after adjustments for age, sex, height, weight and smoking history. FeNO correlated weakly with S-ECP ($r = .17$, $P < .001$), periostin ($r = .19$, $P < .001$) and U-EDN (0.16, $P < .001$). S-ECP also correlated weakly

with U-EDN ($r = .12$, $P = .02$). None of the correlations between the remaining pairs of markers of type 2 inflammation were significant.

Conclusions & Clinical Relevance: Sensitization to food allergens related to several local and systemic type 2 inflammation markers, such as FeNO, S-ECP and periostin. Assessing the profile of allergic sensitization, including to food allergens, might improve the understanding and interpretation of inflammatory markers and potentially improve asthma management.

KEYWORDS

airborne allergens, asthma, food allergens, IgE sensitization, type 2 inflammation

1 | INTRODUCTION

Chronic airway inflammation is a key feature of asthma and to a large extent accounts for the symptoms that an asthmatic experiences.^{1,2} The immunological responses and pathways involved in asthma appear to be more complex than previously thought and, with the introduction of biological drugs, there is a need for better phenotyping and understanding of the disease. Type 2 inflammatory response to inhaled airborne allergens is undoubtedly important in asthma pathogenesis.^{3,4}

Most progress with regard to identifying biomarkers linked to asthma phenotypes has been made regarding type 2 inflammation-related asthma, but the markers used to define type 2 inflammation-high asthma are not yet fully mapped. Fraction of exhaled nitric oxide (FeNO) is used to assess type 2-airway inflammation.⁵ NO is produced in the bronchial epithelium, where it is mainly linked to interleukin (IL)-4/IL-13 pathways.⁶ Another biomarker in asthma is periostin, which is induced by IL-13, and is thought to reflect and define type 2 inflammatory phenotypes.⁷ Serum eosinophil cationic protein (S-ECP) is also a marker of systemic eosinophilic inflammation in asthma and allergic diseases.⁸ Moreover, eosinophil-derived neurotoxin (EDN) has been shown to be a useful biomarker of eosinophilic inflammation in a wide variety of clinical samples.⁹ The 2 eosinophil activation markers mentioned above are more closely linked to IL-5 production,¹⁰ but might reflect different aspects, as EDN relates mainly to lung function,¹¹ while S-ECP relates to symptoms and asthma attacks.¹²

Most, but not all, forms of asthma are attributable to atopy,¹³ and IgE sensitization to airborne allergens has been linked to increased airway and systemic eosinophilic inflammation.¹⁴ We have previously reported that having IgE antibodies against perennial airborne allergens was the type of IgE sensitization that had the strongest relation to asthma and FeNO.^{15,16} S-ECP is also related to sensitization to cat and dog allergens.¹⁷ Sensitization to perennial allergens is associated with higher urinary eosinophil-derived neurotoxin (U-EDN) levels than sensitization to seasonal allergens.¹⁸ We have recently reported that periostin was not related to sensitization to airborne allergens or type of airborne allergen in this study.¹⁹

It has been shown in both children²⁰ and adults¹⁶ that not only sensitization to airborne allergens, but also IgE sensitization to food allergens, is linked to increased FeNO and increased risk of asthma. Sensitization to food allergens is highly prevalent in adults²¹ as well as children²² with asthma and is associated with increased use of health care and asthma medication.²² Asthmatic children sensitized to both airborne allergens and food allergens have higher S-ECP levels than those sensitized only to airborne allergens.²³ However, the importance of food allergen sensitization in relation to periostin is not known and no studies have looked at the relation between food allergen sensitization and different markers of eosinophil activity in the same study.

The aim of this study was to investigate the relation between sensitization, especially to food allergens, and type 2 inflammation markers in adult asthmatics. Our hypothesis was that sensitization to food allergens would relate to both local and systemic type 2 inflammation markers.

2 | METHODS

2.1 | Study population

The Global Allergy and Asthma Excellence Network (GA2LEN) survey was a cross-sectional study conducted in 19 European centres in 2008, targeting symptoms of asthma, rhinitis and chronic rhinosinusitis.²⁴ Details about the study have been described elsewhere.²⁵

In this study, only subjects with asthma, aged 17–76 years, from 3 Swedish centres (Stockholm, Uppsala and Umeå) were included and the definition of asthma was based on the questionnaires filled out at clinical examination.²⁵ The questionnaires also provided data on smoking and medication. 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).

Data on allergic sensitization (skin prick tests for aeroallergens and blood tests for food allergens) were available in 396 subjects (Figure 1).

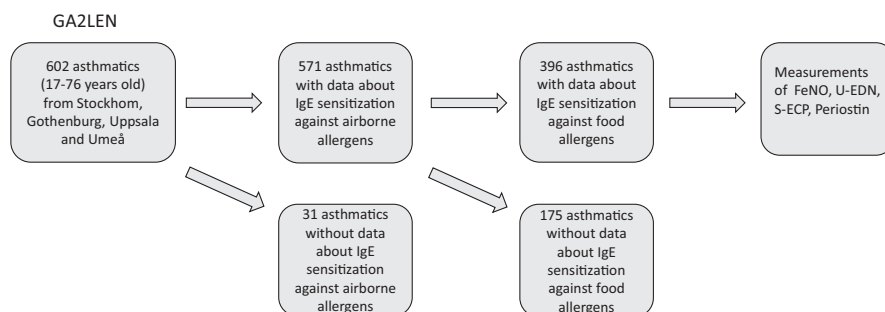


FIGURE 1 Flow chart for the selection of patients

2.2 | Fraction of exhaled NO (FeNO)

FeNO measurements were made using NIOX MINO (NIOX MINO®; Aerocrine, Stockholm, Sweden), in accordance with the American Thoracic Society (ATS) and European Respiratory Society (ERS) recommendations.²⁶

2.3 | Spirometry

Forced vital capacity (FVC) and forced expiratory volume in one-second (FEV₁) were registered before and at least 15 minutes after bronchodilatation (inhalation of 200 micrograms salbutamol). Lung function was measured with EasyOne™ Spirometer (ndd Medizintechnik AG, Zurich, Switzerland) in accordance with the ATS spirometry standards,²⁷ with reference values from the European Community for Steel and Coal.²⁸ Height and weight were also measured before spirometry.

2.4 | Skin prick test (SPT)

A single batch of each SPT allergen solution was purchased centrally from a range of manufacturers. SPT was performed with a standard set of allergens including timothy grass (ALK-Abello, Hamburg, Germany), mixed grass (ALK-Abello), cat (ALK-Abello), birch (Allergopharma, Reinbek, Germany), *Blattella* (Leti, Witten, Germany), olive (Stallergens, Antony, France), *Alternaria* (ALK-Abello), dog (ALK-Abello), *Artemisia* (Stallergens), *Parietaria* (ALK-Abello), *Dermatophagoides farinae* (Stallergens), *Dermatophagoides pteronyssinus* (Stallergens), histamine (positive control; ALK-Abello) and diluent (negative control; ALK-Abello), as previously described.²⁵ A positive SPT was defined as a weal of at least 3 mm at the widest diameter. The protocol of the European GA2LEN study included only SPT towards aeroallergens.

2.5 | Serum IgE measurements

Venous blood samples were drawn from all participants during the clinical visits and frozen at −20°C. In the Swedish GA2LEN study, measurements of serum IgE antibodies against a mix of food allergens (fx5), shrimp and timothy were performed. All measurements of IgE were performed with the ImmunoCAP system

(Immunodiagnosics, Thermo Fisher Scientific, Uppsala, Sweden). The results are presented in kU/L and values of IgE ≥ 0.35 kU/L for an individual allergen defined a subject as being sensitized to that allergen.²⁹ Subjects with titres of ≥ 0.35 kU/L against food mix (fx5) were further characterized regarding IgE antibody levels against each individual allergen in this food panel (fx5): egg white, milk, cod fish, wheat, peanut and soya bean. Those with titres < 0.35 kU/L were not further examined for each individual allergen in the food panel.

2.6 | Allergic sensitization

Allergic sensitization to aeroallergens was defined as the presence of at least one positive skin prick test finding (for the allergens listed above) or/and values of IgE ≥ 0.35 kU/L against timothy. Allergic sensitization to food allergens was defined as values of ≥ 0.35 kU/L against the investigated mix of food allergens mix (fx5) or/and values of ≥ 0.35 kU/L against shrimp allergen.

The subjects were divided into 3 groups based on their allergic sensitization profile: non-allergic asthmatics, asthmatics sensitized only to airborne allergens and asthmatics sensitized to food allergens with or without sensitization to airborne allergens.

2.7 | S-ECP

Blood samples collected for the measurement of serum eosinophil cationic protein (S-ECP) were allowed to coagulate for 60 minutes at 24°C before they were centrifuged. Sera were stored at −20°C. The concentration of S-ECP was assayed using the ImmunoCAP system (Thermo Fisher Scientific).

2.8 | Total IgE

Total IgE was measured using the ImmunoCAP system (Thermo Fisher Scientific).

2.9 | U-EDN

U-EDN, previously named eosinophil protein X (EPX), was measured using a sandwich ELISA (Diagnostics Development, Uppsala, Sweden), in accordance with the manufacturer's instructions. The values of U-EDN were adjusted for creatinine.

TABLE 1 Characteristics of the studied asthma population in relation to IgE sensitization to various allergens

	Non-allergic	Sensitized only to airborne allergens	P-value *	Sensitized to food allergens with/without concomitant sensitization to airborne allergens	P-value *	P-value #
N	105	217		74		
Female (%)	67.6	52.1	.008	50.0	.02	.76
Age (y)	48.8 ± 15.7	41.9 ± 13.4	<.001	42.7 ± 15.7	.02	>.99
Height (cm)	169 ± 9.8	172 ± 9.7	.02	171 ± 9.9	.26	>.99
Weight (kg)	80.7 ± 19.3	78.1 ± 16.4	>.99	75.1 ± 14.3	.27	>.99
Smoking			.003		.06	.65
Never smokers (%)	44.8	60.8		62.2		
Ex-smokers (%)	40.0	33.6		29.7		
Current smokers (%)	15.2	5.5		8.1		
FEV ₁ (L)	2.9 ± 0.9	3.3 ± 0.9	.002	3.3 ± 0.8	.1	>.99
FEV ₁ /FVC	75.6 ± 10.2	77.4 ± 8.7	.89	77.1 ± 9.6	>.99	>.99
Wheeze (%)	76.2	86.6	.02	85.1	.14	.75
Nocturnal cough (%)	60.0	49.8	.09	44.6	.042	.44
Breathlessness following effort (%)	43.8	35.5	.15	41.9	.79	.32
Asthma attack last 12 mo (%)	43.8	42.7	.85	45.9	.77	.63
Leukotriene receptor antagonists (%)	6.7	3.7	.20	8.1	.7	.10
Oral corticosteroids (%)	5.7	5.2	.80	1.4	.14	.16
Inhaled corticosteroids use (%)	36.1	31.8	.47	50.0	.08	.009
Total IgE	25.8 (19.7,33.9)	60.8 (52.1,70.9)	<.001	259 (191,351)	<.001	<.001

*vs non-allergic subjects; #vs subjects only sensitized to airborne allergens.

2.10 | Serum periostin

Serum periostin levels were measured using an “in-house” developed ELISA with 2 rat anti-human periostin mAbs (clones SS18A and SS17B), as previously described.³⁰

2.11 | 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. Percentage differences in FeNO, S-ECP, U-EDN and periostin were obtained using linear regression models, where the log-transformed value was the outcome variable. The regression coefficient for the predictor variable of interest (eg, sensitization only to airborne allergens) was back-transformed, as described in previous studies,³¹ in order to obtain fold differences in FeNO, S-ECP, U-EDN and periostin (eg, sensitized only to airborne allergens vs non-allergic), and these were in turn converted to percentage differences by multiplying the obtained fold differences by 100 and subtracting 100%. These models were adjusted for either age, sex, height, weight and smoking history, or age, sex, height, weight, smoking history, use of inhaled

corticosteroids (ICS), leukotriene receptor antagonists and oral steroids. Correlation between different markers of type 2 inflammation was examined with Pearson's correlation coefficients for log-transformed biomarker values. The percentage contribution of levels of IgE against food allergens or IgE against timothy to levels of total IgE was defined as the ratio of IgE against respective allergen to levels of total IgE, multiplied by 100. A *P* value <.05 was considered significant.

2.12 | 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 personal data gathered were processed in accordance with the Swedish Personal Data Act. All subjects provided informed written consent.

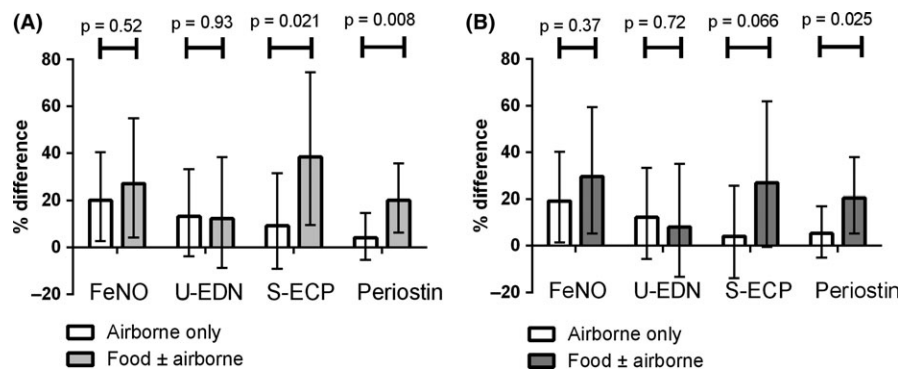
3 | RESULTS

A total of 54.8% of the subjects were sensitized only to airborne allergens, 18.7% were sensitized to food allergens, with or without sensitization to airborne allergens, and 26.5% were not sensitized to any of the measured allergens. Of those sensitized to food allergens,

TABLE 2 Markers of type 2 inflammation (FeNO, U-EDN, S-ECP, Periostin; geometric mean [95% CI]) in relation to allergic sensitization profile

	Non-allergic	Sensitized only to airborne	P-value *	Sensitized to food allergen with/without concomitant sensitization to airborne allergens	P-value *	P-value #
N	105	217		74		
FeNO (ppb)	16.1 (14.2, 18.2)	20.7 (18.9, 22.6)	.005	22.3 (18.6, 26.7)	.005	>.99
U-EDN (μg/mmol creatinine)	46.9 (41.7, 52.7)	50.2 (45.7, 55.0)	>.99	50.3 (41.8, 60.6)	>.99	>.99
S-ECP (mg/L)	12.8 (10.9, 14.9)	14.2 (12.9, 15.7)	.72	17.7 (14.8, 21.1)	.015	.10
Periostin (ng/mL)	59.9 (55.8, 64.2)	62.6 (59.1, 66.4)	>.99	73.6 (67.5, 80.3)	.003	.01

*vs non-allergic subjects; #vs subjects only sensitized to airborne allergens.

**FIGURE 2** Percentage difference of FeNO, U-EDN, S-ECP and periostin depending on the type of IgE sensitization in a model adjusted for age, sex, height, weight and smoking history (A) and in a model adjusted for age, sex, height, weight, smoking history, use of inhaled corticosteroids, leukotriene receptor antagonists and oral corticosteroids (B)

89.8% were also sensitized to at least one airborne allergen. The most common sensitization to food allergens was shrimp, followed by peanut and wheat (Table S1). The characteristics of the studied population are described in Table 1. Non-allergic asthmatics were older, more often female and less often never smokers.

Asthmatics with sensitization to food allergens, with or without sensitization to airborne allergens, had significantly higher levels of FeNO, S-ECP and periostin than non-sensitized asthmatics (Table 2). Periostin levels were higher also compared with those of asthmatics sensitized only to airborne allergens ($P < .001$). U-EDN did not differ significantly between groups (Table 2). Asthmatics sensitized to food allergens, with or without sensitization to airborne allergens, had less nocturnal coughing than non-allergic subjects ($P = .04$), but did not differ in other symptoms (Table 1). Asthmatics sensitized only to airborne allergens had higher FeNO ($P = .005$; Table 2) and reported wheeze more often than non-allergic subjects ($P = .02$; Table 1). Subjects sensitized to food allergens, with or without sensitization to airborne allergens, had higher levels of total IgE than non-sensitized subjects or subjects sensitized only to aeroallergens (both P -values $< .001$; Table 1).

IgE sensitization to food allergens, with or without concomitant sensitization to airborne allergens, was related independently to FeNO ($P = .02$), S-ECP ($P = .006$) and periostin ($P = .004$) in regression models adjusted for age, sex, height, weight and smoking

history. Sensitization only to airborne allergens was related only to FeNO ($P = .02$) in these models. IgE sensitization to food allergens with or without sensitization to airborne allergens was related to higher percentage increase in S-ECP and periostin (Figure 2A) than IgE sensitization only to airborne allergens.

We also examined the independent association of the presence of IgE against food allergens with different markers of type 2 inflammation. Sensitization to shrimp, egg and milk was independently associated with S-ECP ($P = .01$, $P < .001$ and $P = .005$) and sensitization to shrimp, milk and fish with periostin ($P < .001$, $P = .01$ and $P = .04$), respectively (Table 3). Sensitization to wheat and peanut was independently associated with FeNO ($P = .02$ and $P = .03$), and sensitization to shrimp was independently associated with U-EDN ($P = .0498$). No other significant associations between presence of IgE towards food allergens and the markers of type 2 inflammation were found.

When the regression models were adjusted also for use of ICS, leukotriene receptor antagonists and oral corticosteroids, IgE sensitization to food allergens with or without sensitization to airborne allergens was related independently to FeNO ($P = .01$) and periostin ($P = .006$; Figure 2B). Association of IgE sensitization to food allergens, with or without sensitization to airborne allergens, with S-ECP was weaker in the last model ($P = .054$). Sensitization only to airborne allergens was related only to FeNO ($P = .03$) in the above

TABLE 3 Percentage difference (95% CI) of FeNO, U-EDN, S-ECP and periostin depending on the type of IgE sensitization in a model adjusted for age, sex, height, weight and smoking history

	FeNO percentage difference	U-EDN percentage difference	S-ECP percentage difference	Periostin percentage difference
Egg sensitization	19.2 (−18.3, 74.2)	10.4 (−27.9, 69.0)	115 (40.5, 231)	18.9 (−5.5, 49.5)
Milk sensitization	13.8 (−19.2, 60.2)	−4.8 (−35.5, 40.5)	74.4 (17.9, 157)	30.9 (6.5, 60.9)
Fish sensitization	19.5 (−28.5, 99.9)	−11.1 (−49.8, 57.7)	31.0 (−29.2, 142)	39.3 (0.8, 92.4)
Wheat sensitization	43.0 (6.6, 91.9)	17.8 (−15.1, 63.3)	58.2 (12.3, 122)	13.1 (−5.6, 35.6)
Peanut sensitization	32.7 (2.6, 71.4)	−16.6 (−37.2, 10.9)	23.7 (−8.6, 67.6)	10.1 (−6.1, 29.1)
Soy sensitization	−16.5 (−42.1, 20.5)	27.9 (−9.9, 81.8)	25.7 (−16.2, 88.6)	14.6 (−7.3, 41.8)
Shrimp sensitization	6.9 (−12.7, 30.6)	24.4 (0.02, 54.6)	36.7 (7.8, 73.3)	25.6 (10.9, 42.2)

TABLE 4 Correlation between different markers of type 2 inflammation. Results are presented as Pearson's *r*-value (*P*-value)

	FeNO	S-ECP	Periostin	U-EDN
FeNO	1	0.17 (<.001)	0.193 (<.001)	0.16 (<.001)
S-ECP	0.17 (<.001)	1	0.06 (.21)	0.12 (.02)
Periostin	0.193 (<.001)	0.06 (0.21)	1	0.07 (.12)
U-EDN	0.16 (<.001)	0.12 (0.02)	0.07 (.12)	1

models (Figure 2 B). IgE sensitization to food allergens, with or without sensitization to airborne allergens, was related to a higher percentage increase in periostin (Figure 2B) than sensitization only to airborne allergens.

We also studied the correlation between different markers of type 2 inflammation (Table 4). FeNO correlated weakly with S-ECP ($r = .17$, $P < .001$), periostin ($r = .19$, $P < .001$) and U-EDN ($r = 0.16$, $P < .001$). S-ECP also correlated weakly with U-EDN ($r = .12$, $P = .02$). None of the correlations between the remaining pairs of markers of type 2 inflammation were significant.

4 | DISCUSSION

Sensitization to food allergens, with or without sensitization to airborne allergens, is related to several local and systemic type 2 inflammation markers, such as FeNO, S-ECP and periostin, in the present population of subjects with asthma from the Swedish GA2LEN study. Sensitization only to airborne allergens was related to increased FeNO, but not to S-ECP, periostin or urinary EDN.

The relationship of sensitization to food allergens and FeNO and blood eosinophil count (B-Eos) is in line with previous studies.^{14,20,32} According to previous studies,^{20,32} children sensitized to food allergens had high levels of FeNO that could not be accounted for solely by comorbidities such as allergic rhinitis or sensitization to airborne allergens (house dust mite). Sensitization to food allergens was independently related to increased FeNO levels in both middle-aged adults³³ and in young asthmatics¹⁶ in our previous studies. In the current study, those sensitized to food allergens and those sensitized only to airborne allergens had higher levels of FeNO than non-atopic asthmatics, but their FeNO levels did not differ, one from the other. The effect of sensitization to food allergens is probably weaker in this population with age range between 17 and 76 years; we know that the

effect of sensitization to food allergens is stronger in children than in adults at least regarding markers of systemic type 2 inflammation (B-Eos).¹⁴ Furthermore, this study examined a smaller number of food allergens compared with the previous studies.^{14,16} In the present study, S-ECP levels were higher in asthmatics sensitized to food allergens, but this increase was not consistent after adjustments for potential confounders. In a previous study,¹⁷ adults sensitized only to food allergens did not differ in S-ECP levels compared with non-allergic asthmatics. According to Koh et al.,³⁴ serum S-ECP correlates directly with activated eosinophils in bronchial mucosa in adult asthmatics³⁵ and is a more sensitive marker of asthma severity than peripheral blood eosinophil counts in acute exacerbations in children.³⁶ IgE sensitization to food allergens, with or without sensitization to airborne allergens, was associated with a higher percentage increase in S-ECP and periostin than IgE sensitization only to airborne allergens. This study also shows that the type of sensitization to individual food allergens was important for the magnitude of the type 2 inflammation. For example, sensitization to shrimp was associated with elevated levels of 3 markers of type 2 inflammation, S-ECP, U-EDN and periostin. This might indicate that subclinical exposure to certain kind of foods in sensitized subjects could promote type 2 inflammation.

The different investigated markers of type 2 inflammation were weakly correlated in the present study. Nitric oxide (NO) has been found to be correlated with sputum eosinophil count.³⁷ Serum periostin was also found to correlate with sputum eosinophils.⁷ In a study of asthmatic children, FeNO correlated positively to ECP in both groups with and without inhaled steroids.³⁸

The present study is the first to report an association of sensitization to food allergens and periostin. The higher periostin levels of asthmatics sensitized to food allergens compared with asthmatics sensitized only to airborne allergens imply that IgE sensitization to food allergens has more systemic effect than type 2 inflammation. In a previous study, based on the GA2LEN-population, no relation was

found to the presence of IgE sensitization to aeroallergens or type of aeroallergen.¹⁹ However, the relation to food allergen sensitization was not studied in this previous analysis.

No relation of sensitization to food allergens or airborne allergens was found with urinary EDN in the present study. In previous studies, serum EDN had significant correlation with changes in lung function and severity of asthma.³⁹ According to Gore et al.,⁴⁰ urinary EDN levels reflect atopy and symptoms of atopic disease at 3 years of age, as sensitization to airborne allergens is related to higher U-EDN levels. In the above study by Gore et al., positive SPT results to milk and egg had the second and third highest mean U-EDN levels, while positive results to mould had the highest. However, there was a weak correlation between degree of allergic sensitization and levels of U-EDN in this study.

Levels of total IgE increased from the non-allergic group to the group with sensitization to airborne allergens being highest in the group with sensitization to food allergens, with or without sensitization to airborne allergens. Whether this difference reflects a higher level of production of IgE than specifically a higher production of specific IgE to food allergens, we know from a previous study⁴¹ that the levels of specific IgE for various allergens explain about 1% of total IgE so the contribution of specific IgE to the total IgE is relatively low. The percentage contribution of levels of IgE against food allergens (fx5 mix and shrimp) to levels of total IgE in the present study was about 2% implying that the contribution of specific IgE to total IgE was at about the same level as in previous studies.

Sensitization only to airborne allergens was related to increased FeNO, but not S-ECP or periostin. We have previously reported that subjects sensitized only to airborne allergens (perennial) have increased levels of FeNO and higher prevalence of asthma¹⁶ than non-allergic subjects. The levels of FeNO increase with an increasing degree of sensitization to airborne allergens (both perennial and seasonal) in both adults¹⁵ and asthmatic children.⁴² However, in the present study, we did not find an association with S-ECP, in contrast with previous findings in younger adults¹⁷ and asthmatic children.⁴⁰ The current study had the strength of having examined a large number of asthmatics of a wide age range and several markers of type 2 inflammation, but also had some limitations. We measured a limited number of food allergens compared with our previous study^{16,33} where sensitization to food allergens was independently related to FeNO. We did not have data about clinical food allergy but previous studies report that about 1 of 10 subjects with sensitization to food allergens have clinical food allergy so a similar proportion would have to be expected in the present population.⁴³ A limitation of our study is that we did not have data about allergen components and therefore could not study the role of cross-reactive allergen components in relation to increased type 2 inflammatory markers. The information on allergic sensitization to aeroallergens using skin prick tests and blood tests can also give some discrepant information.^{44,45} No higher burden of disease was found in the subjects sensitized to food allergens, but due to the cross-sectional design, it cannot be excluded that high levels of type 2 inflammation seen in these subjects might have consequences in the future. Generally, the levels of

type 2 inflammation markers can be regarded as relatively low in the whole population, but this probably reflects the fact that the subjects identified from population-based studies are having milder form of disease.

In conclusion, several type 2 markers are elevated to a greater extent in asthmatics subjects sensitized to food allergens. It could be important to examine both airborne and food allergen sensitization in order to understand the type 2 inflammation in asthma and guide treatment accordingly, so as to decrease both local and systemic inflammation. Future studies are needed to confirm present results, and the use of larger panels of food allergens and longitudinal data could shed light on the predictive value of sensitization to food allergens for having persistently high type 2 inflammation and poorer disease outcome.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Online Table S1: Prevalence of sensitisation to airborne and food allergens in asthmatic patients.

	All	Non-allergic	Sensitised only to airborne allergens	Sensitised to food allergens with and without concomitant sensitisation to aeroallergens
N	396	105	217	74
Cat	223 (56.3%)	0	166 (76.5%)	57 (77.0%)
Dog	193 (48.7%)	0	137 (63.1%)	56 (75.7%)
Birch	179 (45.2%)	0	131 (60.4%)	48 (64.9%)
Timothy	186 (46.9%)	0	134 (61.7%)	52 (70.3%)
Mite	65 (16.4%)	0	37 (17.1%)	28 (37.8%)
Mould	23 (13.5%)	0	13 (5.9%)	10 (13.5%)
Shrimp	43 (10.9%)	0	0	43 (58.1%)
Egg	12 (2.9%)	0	0	12 (16.2%)
Milk	15 (3.7%)	0	0	15 (20.3%)
Cod	6 (1.5%)	0	0	6 (8.1%)
Wheat	20 (5.0%)	0	0	20 (27.0%)
Peanut	25 (6.3%)	0	0	25 (33.7%)
Soy	14 (3.5%)	0	0	14 (18.9%)

Sensitisation to all airborne allergens except timothy was examined with skin prick tests (≥ 3 mm wheal considered positive) and sensitisation to food allergens and timothy with measurement of specific IgE (≥ 0.35 kU/L).