

Relationship between exhaled nitric oxide and IgE sensitisation in patients with asthma: influence of steroid treatment

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Abstract

Introduction: The influence of the degree of immunoglobulin E (IgE) sensitisation on the fraction of expired nitric oxide (FE_{NO}) in asthma patients being treated with inhaled corticosteroids (ICS) is not well known.

Objectives: To investigate the relationship between IgE sensitisation and FE_{NO}, and the effect of a step-up in ICS treatment on this relationship, in patients with allergic asthma.

Methods: A primary health care centre recruited 20 non-smoking patients with perennial allergic asthma (18 years–50 years, six male) outside the pollen season. At every visit (0, 2, 4, 8 weeks), FE_{NO} was measured and an exposure questionnaire was completed. ICS dose was adjusted according to FE_{NO} (≥ 22 ppb prescribed increase in ICS). Quantitative analyses of serum IgE (eight common aeroallergens) confirmed allergy.

Results: At baseline, FE_{NO} and the sum of IgE antibody titres for perennial allergens correlated significantly ($r = 0.47$, $P = 0.04$). After a step-up in ICS treatment, this correlation had disappeared. Nine patients had persistently elevated FE_{NO} at last visit (mean 35 ppb vs 16 ppb). This group was more frequently exposed to relevant allergens or colds (89% vs 27% of patients, $P < 0.05$) and had higher IgE antibody titres (perennial allergens) compared with the normalised group (mean 28.9 kU/L vs 10.7 kU/L, $P < 0.05$).

Conclusion: Serum IgE against perennial allergens and FE_{NO} correlate in patients with allergic asthma. However, this relationship disappears after a high-dose ICS regimen, suggesting that FE_{NO} relates to bronchial inflammation and not IgE levels *per se*. High degree of IgE sensitisation together with allergen exposure may lead to ICS-resistant airways inflammation.

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Key words

allergen – asthma – breath test – IgE – nitric oxide

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Authorship

Jörgen Syk performed and designed the study, analysed the data and wrote the manuscript. Anna-Lena Undén and Kjell Alving analyzed the data and wrote the manuscript, and designed the study.

Ethics

The study has been approved by the Regional Ethics Board of Stockholm. All subjects gave their informed consent prior to inclusion in the study.

Conflicts of interest

Kjell Alving is an associate of Aerocrine AB. Jörgen Syk has received research support from Aerocrine AB. Anna-Lena Undén declares no conflict of interest.

Introduction

Steroid-naïve patients with allergy and asthma develop a chronic eosinophilic inflammation in the airways (1). Present guidelines for asthma management advocate lung function and symptom scores as the preferred method to adjust the anti-inflammatory therapy. In many patients whose asthma is considered to be clinically controlled or in remission, airway

hyperresponsiveness (AHR) and airway inflammation may persist (2).

The fraction of expired nitric oxide (FE_{NO}) is increased in asthma (3, 4), particularly in allergic asthma (5, 6), and seems to be a reliable marker for eosinophilic inflammation (2, 7, 8). A relationship between the degree of allergic sensitisation, measured as total serum immunoglobulin E (IgE) or number of positive skin prick tests, and FE_{NO} has previously been

Table 1. Patient characteristics at baseline

Patient no.	Daily asthma medication before visit 1 (μ g)	Baseline FEV ₁ (% predicted)	No. of IgE positivities (perennial)	No. of IgE positivities (seasonal)	FE _{NO} at baseline (ppb)
1	Budesonide 800	85	3	2	18
2	0	104	2	0	28
3	Budesonide 200 + formoterol 4.5	108	4	2	55
4	Budesonide 200	84	3	2	42
5	0	95	1	0	35
6	0	57	4	2	45
7	Budesonide 800 + formoterol 18	91	3	1	24
8	Budesonide 400	97	2	1	14
9	Budesonide 400	81	3	1	140
10	0	95	3	1	39
11	Fluticasone 500 + salmeterol 100	125	2	2	27
12	Beclometasone 800	101	2	1	48
13	0	103	2	1	25
14	Budesonide 800 + formoterol 18	111	2	3	9
15	0	97	3	2	26
16	0	100	1	2	93
17	Budesonide 400 + formoterol 9	94	3	2	27
18	0	102	4	1	62
19	Budesonide 400 + formoterol 9	101	2	1	23
20	Budesonide 800	97	3	1	24

In total, eight common airborne allergens were tested (five perennial and three seasonal). FEV₁, forced expiratory volume in 1 s; IgE, immunoglobulin E; FE_{NO}, expired nitric oxide.

reported (9, 10), and some authors have suggested that an elevated FE_{NO} level is more a marker of atopy than a marker of airway inflammation (11, 12).

Based on the fact that treatment with inhaled corticosteroids (ICS) lower exhaled NO (13, 14) and that an increase in FE_{NO} seems to predict forthcoming asthma exacerbations (15), some studies have recently been performed where possible improvement of asthma treatment by using FE_{NO} as a guide to adjust the ICS therapy has been compared with traditional adjustment based primarily on symptoms. One study showed a significant reduction of the mean daily dose of ICS in the group with ICS adjustments according to FE_{NO} (16). Another study, in children, showed that titration of ICS on FE_{NO} and symptoms for 1 year reduced AHR (17).

However, a recent study looking at children with moderate to severe allergic asthma showed that some patients have persistently high FE_{NO} levels despite high ICS doses and FE_{NO} could not be reduced by improving the inhalation technique or increasing ICS doses, suggesting that FE_{NO} measurements might not be useful in all patients with asthma (18).

The aims of the present study were to investigate the relation between FE_{NO} and the degree of IgE sensitisation in adult patients with allergic asthma, and to see if

the relation would change after a period of intensified ICS treatment. Further, we wanted to investigate if FE_{NO} could be normalised by ICS treatment, by following a stepwise algorithm based on the FE_{NO} value, and if not, investigate if persistently elevated FE_{NO} depends on exposure to allergens.

Materials and methods

This study was carried out at Runby primary health care centre (PHC) in Upplands Väsby, a northern part of the county of Stockholm, in a district with about 7000 inhabitants. All patients were followed by one general practitioner (JS) during all visits. All patients gave their written informed consent and the study was approved by the regional ethics committee.

Study group

Twenty patients with physician-diagnosed allergic asthma, with perennial asthma symptoms, between 18 years and 50 years of age (mean 33.4 years; six male), were consecutively recruited from Runby PHC district (Table 1). All patients were non-smokers since at least 6 months and had not smoked more than 5 years.

Patients using long acting β_2 -agonists and/or anti-leukotrienes had to change therapy or were excluded. Six patients using combined inhalation therapy with corticosteroids and long acting β_2 -agonists, changed therapy to corticosteroids (budesonide or fluticasone) and short-acting β_2 -agonists (salbutamol or terbutaline) when needed, at least 1 week before inclusion in the study.

Study design

The patients were included during September–December 2003 and visited Runby PHC four times during 8 weeks (baseline, 2, 4 and 8 weeks). The study was thus conducted outside the Swedish pollen season. At every visit, FE_{NO} was measured (mean value out of three measurements at an exhalation flow rate of 50 mL/s according to American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (19) with the NIOX® (Aerocrine AB, Stockholm, Sweden). Elizabeth Juniper's Asthma Control Questionnaire (ACQ) and a questionnaire about environmental exposure (Appendix 1) (allergen, cigarette smoke) were completed at every visit. Spirometry (Vitalograph, Spirotrac IV, Buckingham, England) with β_2 -agonist reversibility test was performed [forced vital capacity, forced expiratory volume in 1 s (FEV), FEV%, forced expiratory flow_{25–75}] and Elizabeth Juniper's standardised Asthma Quality of Life Questionnaire (AQLQ) was completed at the first and last visits. Asthma-related events including respiratory infections were registered at every visit. The patients were asked to keep diaries of daily medication intake. Allergy was confirmed with quantitative analyses of serum levels of IgE antibodies against cat, horse, dog, *Cladosporium* and mite (perennial allergens), and timothy, birch and mugwort (seasonal allergens) (ImmunoCAP™, Pharmacia Diagnostics, Uppsala, Sweden). Sensitivity was confirmed towards at least one perennial allergen in all study subjects (Table 1).

The ICS dose was adjusted according to the exhaled NO value following a stepwise algorithm (Table 2). Corticosteroid therapy was not withdrawn if the patient had used ICS at inclusion of the study (lowest allowed step = step 1).

Statistical analyses

Results are presented as mean $\pm$ standard error of the mean. For repeated measures (four visits), the change from baseline was tested using the Friedman test with Dunn's multiple comparison test, or repeated measures ANOVA with Tukey's multiple comparison test

Table 2. Treatment algorithm guided by FE_{NO}

ICS	Treatment step (μ g daily)				
	1	2	3	4	5
Budesonide	0	200	400	800	1600
Fluticasone	0	100	250	500	1000

A FE_{NO} value ≥ 22 ppb indicates a step-up and < 22 ppb indicates a step-down of treatment.

A step-down resulting in an increased NO value (≥ 22 ppb) was only allowed once during the study.

ICS, inhaled corticosteroids; FE_{NO} , expired nitric oxide.

when appropriate. For changes between baseline and last visit, Student's paired *t*-test was used, and for differences between subgroups, the unpaired *t*-test was employed, except for discrete variables (ICS use) where Mann–Whitney or Wilcoxon signed rank sum tests were used. For categorical data, for example exposure vs non-exposure, Fisher's exact test was employed. Correlation analysis has been used to test the hypothesis that there is a linear relationship between two variables (Pearson). FE_{NO} values were transformed into the corresponding logarithm before statistical evaluation. Correlation with IgE sensitisation was evaluated by using the sum of IgE antibody titres (kU/L) for either all eight allergens tested or subsets of allergens. Each test is performed at a significance level of 0.05. $P < 0.10$ is given to indicate a trend towards statistical significance. All statistical analyses were done with Graph Pad Prism (version 4.0c; GraphPad Software Inc., San Diego, CA, USA) on a Macintosh computer.

Results

One patient made an unscheduled visit during the study because of an upper respiratory tract infection, but no change in asthma therapy was needed. No other asthma-related events were registered during the study.

Relation between FE_{NO} and specific IgE at baseline

All patients were sensitised against at least one perennial allergen (Table 1). Cat was the dominant allergen (95% of subjects were sensitised), followed by dog and horse among the perennial allergens, and birch and timothy were the most common seasonal allergens (Table 3). There was a large scatter of IgE antibody titres for these allergens. Few subjects were sensitised to mugwort and *Cladosporium*.

We found a significant correlation between the sum of IgE antibody titres for all allergens as well as peren-

Table 3. Distribution of IgE sensitisation in the patients ($n = 20$)

Allergen	No. of positive patients	Range of IgE levels (kU/L)
Cat	19	0.37–25.1
Dog	15	1.31–13.2
Birch	13	0.84–45.5
Timothy	12	0.44–21.6
Horse	10	0.39–26.9
Mite	6	0.37–68.4
Mugwort	2	0.80–0.90
<i>Cladosporium</i>	2	1.50–2.70

IgE, immunoglobulin E.

nial allergens vs FE_{NO} at baseline, but no correlation between FE_{NO} and the sum of IgE antibody titres for seasonal allergens (Table 4). When studying the correlation between FE_{NO} and the individual allergens, it was found that cat, timothy and mite IgE titres were much more closely related to FE_{NO} at baseline ($r > 0.35$, $P < 0.10$ for all) than IgE titres for the other five allergens ($r < 0.20$, $P > 0.4$ for all). These groups of allergens were denominated dominant and non-dominant allergens, respectively, and the sum of IgE titres for these subsets was also used in the correlation analysis (Table 4). A highly significant correlation between dominant IgE titres and FE_{NO} was noted at baseline. All significant correlations between FE_{NO} and IgE titres seen at baseline had disappeared at the 8 weeks follow up (Table 4).

Effect of FE_{NO} -guided steroid treatment

At baseline, eight subjects (40%) were not taking ICS and none used the highest dose. Seventeen out of 20 patients (85%) had elevated exhaled NO values (≥ 22 ppb) at baseline (Table 1). FE_{NO} changed signifi-

cantly at visit 3 and 4 compared with baseline at the group level. The average FE_{NO} level decreased by 38% between baseline and last visit (Fig. 1A).

At last visit, all subjects had ICS therapy and eight were treated with the highest allowed dose. Three patients decreased the corticosteroid dose, two patients stayed at the same dose and fifteen patients increased the dose of ICS. The mean daily dose of ICS increased by 147% (Fig. 1B).

The mean ACQ score decreased significantly from 1.0 at baseline to 0.7 at the second visit ($P < 0.05$). The mean score was still numerically reduced compared with baseline at visits 3 and 4 but no longer significantly. Total mean score for AQLQ and all separate domains did not change significantly. Four out of 32 questions increased significantly ($P < 0.05$), all from the symptom domain. Seven subjects showed a clinically relevant change (≥ 0.5 units) in total AQLQ score.

None of the spirometry measures, including β_2 -agonist reversibility, changed significantly during the study.

Characterisation of patients with persistently elevated FE_{NO} at last visit

Nine patients (45%) still had a clearly elevated FE_{NO} value at the last visit, whereas the remaining showed normal NO values (< 22 ppb) (Table 5). There was no difference in the mean ICS use between these subgroups at first visit but there was a significant rise of the corticosteroid dose between the first and last visit in the group with persistently elevated FE_{NO} , and the change in ICS dose at last visit was significantly higher compared with the normalised group. The average FE_{NO} value decreased significantly in the normalised subgroup but not in the non-normalised group. There was no difference between the groups with regard to gender (three men in each group) and self-reported adherence to ICS treatment (95% and 96% in the normalised and non-normalised group, respectively).

Table 4. Correlations between IgE titres and exhaled NO

	Visit 1		Visit 4	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
All (8)	0.48	0.03	0.22	0.36
Perennial (5)	0.47	0.04	0.38	0.10
Seasonal (3)	0.32	0.17	−0.07	0.77
Dominant (3)	0.59	0.007	0.36	0.12
Non-dominant (5)	0.08	0.74	−0.10	0.66

Correlation coefficients (*r*) and *P* values for the relation between sums of IgE antibody titres (no. of allergens given in brackets) and FE_{NO} at baseline and last visit (Pearson). Significant correlations are indicated in bold. Dominant allergens were cat, mite and timothy, and non-dominant allergens were dog, horse, birch, mugwort and mould (see text for explanation).

IgE, immunoglobulin E; FE_{NO} , expired nitric oxide.

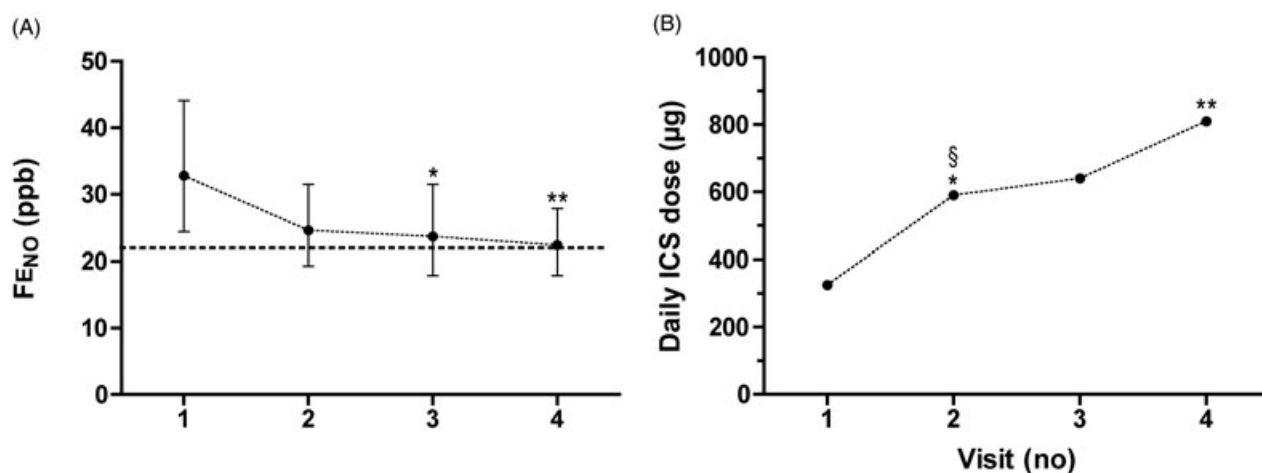


Figure 1. Changes in the fraction of expired NO (FE_{NO}) and inhaled corticosteroid (ICS) use over visits 1–4 in 20 patients with allergic asthma. (A) Geometric mean (95% confidence interval) of FE_{NO}. **P* < 0.05, ***P* < 0.01 compared with visit 1 (one-way ANOVA). Horizontal line indicates cut-off between normal and elevated (22 ppb). (B) Arithmetic mean of daily ICS use since last visit. §*P* < 0.05 compared with previous visit, and **P* < 0.05, ***P* < 0.01 compared with visit 1 (Friedman).

Table 5. Characteristics for the subgroups that were normalised and not normalised, respectively, with regard to exhaled NO

	Visit no.	Normalised [‡] (<i>n</i> = 11)	Non-normalised (<i>n</i> = 9)
Exposure (no) [§]	Visit 4	3 (27%)	8 (89%)***
IgE (kU/L)	Visit 1		
All (8)		16.1 ± 4.1	43.3 ± 11.9*
Perennial (5)		10.7 ± 3.9	28.9 ± 7.0*
Dominant (3)		6.6 ± 1.9	26.8 ± 9.7*
Cat		3.1 ± 1.3	12.3 ± 3.6*
Seasonal		5.5 ± 2.2	14.4 ± 6.9
Positive (no) [¶]	Visit 1		
Horse		2 (18%)	8 (89%)***
FE _{NO}	Visit 1	26.3 ± 3.3	57.1 ± 12.6**
	Visit 4	16.5 ± 1.1	35.0 ± 3.4****
	Δ	−9.8 ± 2.9 ^{†††}	−22.1 ± 11.6 [†]
ICS use	Visit 1	382 ± 109	289 ± 111
	Visit 4	636 ± 188	1089 ± 209
	Δ	+255 ± 83	+800 ± 180 ^{†††*}
AQLQ	Visit 1	5.6 ± 0.3	5.7 ± 0.2
	Visit 4	6.1 ± 0.2	5.9 ± 0.3
	Δ	+0.5 ± 0.2 [†]	+0.2 ± 0.2

P* < 0.10, *P* < 0.05, ****P* < 0.01, *****P* < 0.001 compared with normalised group (Fisher, Mann–Whitney or unpaired *t*-test).

[†]*P* < 0.10, ^{††}*P* < 0.05, ^{†††}*P* < 0.01 for change between visit 1 and visit 4 (Wilcoxon or paired *t*-test).

[‡]FE_{NO} < 22 ppb at visit 4.

[§]Regular or recent exposure to a relevant allergen, or an ongoing respiratory tract infection.

[¶]Positive, IgE > 0.35 kU/L.

FE_{NO}, expired nitric oxide; ICS, inhaled corticosteroids; AQLQ, Asthma Quality of Life Questionnaire.

Δ = Change between visit 1 and 4.

The subgroup with persistently elevated NO levels was characterised by significantly higher titres of IgE antibodies against perennial and dominant allergens, and a significantly higher proportion of subjects were sensitised against horse. Also, significantly more patients in this group were recently or regularly exposed to relevant allergens (primarily pet allergens) or had an ongoing upper respiratory infection at the last visit (Table 5). Only one patient had symptoms of an infection at this time point, in the non-normalised group. However, if this patient was omitted from the statistical evaluation, the difference in the proportion of exposed subjects between the subgroups was still significant ($P < 0.05$). Furthermore, the non-normalised subgroup had significantly higher FE_{NO} values at baseline.

There was a statistical trend towards an increase in the AQLQ total mean score in the group with normalised FE_{NO} , whereas such trend was lacking in the non-normalised subgroup (Table 5). Mean ACQ score and results from spirometry, including β_2 -agonist reversibility, did not show any significant differences between subgroups.

Discussion

In this study, including adult patients with allergic asthma, we found a significant correlation between allergen-specific serum IgE antibody titres against all eight allergens, perennial and dominant allergens and FE_{NO} . The correlation disappears after a high-dose ICS regimen. Despite high ICS doses, nine subjects still had an elevated FE_{NO} at the end of the study. This group had significantly higher titres of specific-IgE antibodies and was, according to answers from the questionnaire about exposure, more exposed to allergens that they were sensitised to.

The use of an objective marker of inflammation may be helpful in the treatment of patients with allergic asthma and FE_{NO} seems to be a reliable marker for eosinophilic inflammation (2, 7) and can now be measured in the primary health care setting with an easy and reproducible method. At baseline, 17 out of 20 patients had elevated FE_{NO} , which indicates that many patients with perennial allergic asthma have an ongoing chronic eosinophilic inflammation in the airways and possibly could benefit from start or restart with ICS treatment or to get higher doses.

There are several possible reasons for the high NO levels. For example, poor adherence to treatment, bad inhalation technique, allergen exposure or suboptimal dose adjustment according to standard management therapy, may all result in persistent inflammation and elevated NO levels.

FE_{NO} has, in different ways and in many studies, shown a relation to atopic sensitisation. Studies have, for example, shown a correlation between FE_{NO} and total serum IgE levels (9, 20), the number of positivities in skin prick tests (9, 10, 21), the wheal size in skin prick test for mite (22) and specific IgE antibody titres for mite (23). Natural (24, 25) or controlled (26) exposure to a relevant allergen seems to elevate FE_{NO} and, vice versa, avoidance results in a lowering of exhaled NO (24). In this study, we could see a significant correlation at baseline between the sum of specific IgE antibody titres for all allergens and perennial allergens vs FE_{NO} , but no correlation between FE_{NO} and the sum of specific IgE antibody titres for seasonal allergens. This is a logical result, since the study was done entirely out of the pollen season and it indicates that the subjects were exposed to perennial allergens that they were sensitised to. Interestingly, cat, timothy and mite IgE titres (dominant) were much more closely related to FE_{NO} than IgE titres for the other five allergens tested (non-dominant). It is common to get exposed to cat and mite allergens, and the pollen season for timothy is longer compared with the other two seasonal allergens in the study (birch and mugwort), and ended just before the start of this study. Atopic asthma is characterised by bronchial eosinophilic inflammation which correlates with local NO production (2, 7, 8). This type of inflammation can also be present in patients with non-atopic asthma or chronic obstructive pulmonary disease, but to a lesser degree (1, 28). Treatment with corticosteroids reduces the eosinophilic inflammation and FE_{NO} decreases concomitantly (29, 30). FE_{NO} levels are likely to be low in the absence of eosinophilic inflammation, with the exception of rhinovirus infections (30). In the present study, after treating bronchial inflammation with higher ICS doses, all significant correlations between FE_{NO} and serum IgE titres seen at baseline had disappeared. Thus, the correlation between IgE sensitisation and exhaled NO levels can be disassociated by anti-inflammatory treatment, supporting the view that exhaled NO is a marker of inflammation and not a marker of atopy *per se*. This supports the view that an elevated exhaled NO value in an atopic subject should not be considered normal merely based on the atopic status.

Despite a considerable increase of ICS doses after altogether three dose adjustments, it was still not possible to normalise FE_{NO} in 9 out of 20 subjects. These subjects had a marked persistent elevation of exhaled NO, in spite of using a 70% higher mean daily dose of ICS and having increased their mean ICS use more than the normalised group. In this study we used a cut-off point of 22 ppb for normal vs abnormal NO

values. The use of a cut-off at this level is supported by recent studies in large samples of healthy, non-atopic adult subjects that have shown that normal median or mean exhaled NO levels at an exhalation flow rate of 50 mL/s are well below 20 ppb (32–34). Furthermore, healthy subjects stay at this level with very little variation when measured over a 3-week observation period (33). It may be argued that the non-normalised group showed a change in FE_{NO} that exceeded that of the normalised group. However, the mean change in the non-normalised group was not statistically significant and was highly driven by two patients showing a very large reduction in FE_{NO} (>50 ppb). In spite of this large reduction, these two patients still were markedly elevated at the last visit (>40 ppb).

Obvious reasons for persistently elevated NO levels include poor adherence to steroid treatment and inadequate inhalation technique. However, reported adherence to treatment was generally very good during the study and there was no difference between the groups. Also, inhalation technique was checked during spirometry and found to be adequate in both groups. Other possible explanations are high nitrate intake (35) or respiratory tract infections, at least rhinovirus infections (36). Nitrate intake (lettuce etc.) was asked for at all visits. It was found to be generally low and did not explain our group differences. Only one patient had signs of an ongoing respiratory tract infection at the last visit, and constituted one case in the non-normalised group.

When we look further at the non-normalised group, we could see that seven out of nine subjects were exposed to a sensitising allergen before last visit. The non-normalised group was also more sensitised, the patients had significantly higher titres of specific IgE antibodies against all eight allergens, perennial and dominant allergens compared with the normalised group. It seems that a high level of specific IgE antibodies against an allergen, in combination with exposure to this allergen, makes it difficult or maybe impossible to normalise FE_{NO} with ICS. Besides the seven allergen-exposed subjects, one subject had an upper respiratory tract infection, and only for one remaining subject, the reason was unclear why FE_{NO} was still elevated.

A recently published study (18) looking at children with moderate to severe atopic asthma showed that some patients have persistently high FE_{NO} levels despite high ICS doses, and FE_{NO} could not be reduced by improving the inhalation technique or increasing ICS doses. Based on our findings, we suggest that allergen exposure should always be considered as a possible

explanation to why patients would not lower their FE_{NO} despite the use of high steroid doses. It will be important in future studies on FE_{NO} , to investigate if the subjects are atopic, to what degree they are sensitised, for example, by measuring IgE titres quantitatively and to estimate the degree of exposure to sensitising allergens.

We conclude that there is a relationship between allergen-specific serum IgE antibody titres and FE_{NO} , especially for perennial allergens. The relationship disappears after a high ICS dose regimen, indicating that FE_{NO} relates to bronchial inflammation and not to IgE antibody levels *per se*. Exposure to relevant allergens has a great impact on FE_{NO} and is an important factor to consider when adjusting ICS therapy, according to FE_{NO} , in patients with allergic asthma. Thus, in patients with allergic asthma, not only anti-inflammatory treatment but also the degree of IgE sensitisation and allergen load must be taken into account when interpreting exhaled NO as a marker of airway inflammation.

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Appendix 1. Environmental exposure questionnaire

Environmental exposure was assessed with the following questions:

1. Do you regularly get exposed to smoke from cigarettes (almost daily since at least 1 month), at home, at work or anywhere else? – if yes, how many hours per day on average?;
2. Do you have any pet animal at home? – if yes, what sort of animal?;
3. Do you regularly (at least once a week since at least 1 month) get exposed to furred animals outside your home? – if yes, what sort of animal?;
4. Do you get window pane condensation at home during the winter?

Relevant allergen exposure was defined as ‘yes’ to question no. 2 and/or 3.