

The association of sensitization to inhalant allergens with allergy symptoms: the influence of bronchial hyperresponsiveness and blood eosinophil count

M. KERKHOF, J. P. SCHOUTEN and J. G. R. DE MONCHY*

Department of Epidemiology and Statistics, University of Groningen and *Department of Allergology, University Hospital Groningen, Groningen, The Netherlands

Summary

Background We investigated whether the association of allergy symptoms with sensitization to inhalant allergens depends on bronchial hyperresponsiveness, blood eosinophil count, or the degree and nature of sensitization.

Methods Data on asthma and rhino-conjunctivitis symptoms were obtained from 1904 subjects from a random sample of the Dutch population, aged 20–70 years by the ECRHS questionnaire. Total IgE and specific IgE to four inhalant allergens were measured using CAP System. Bronchial hyperresponsiveness (BHR) was defined as $PD_{20} \leq 2$ mg methacholine and 'high eosinophil count' as an eosinophil count in the highest quartile.

Results Forty-three percent of the subjects with specific IgE to inhalant allergens was asymptomatic. These subjects had a lower degree of sensitization than symptomatic sensitized subjects and had 'normal' prevalences of BHR and 'high eosinophil count'. Logistic regression showed that the presence of BHR increased the risk of having symptoms for subjects who were sensitized to indoor allergens. Low levels of specific IgE to indoor allergens were only associated with symptoms when BHR was present. Sensitization to outdoor allergens was associated with symptoms at all levels of specific IgE, independently of BHR or eosinophils.

Conclusion Our epidemiological data suggest that whether low levels of specific IgE to indoor allergens lead to allergic symptoms is probably determined by the concurrent existence of inflammation of the airways.

Keywords: allergic rhinitis, asthma, bronchial hyperresponsiveness, degree of sensitization, eosinophils, indoor allergens, outdoor allergens, sensitization

Clinical and Experimental Allergy, Vol. 30, pp. 1387–1394. Submitted 1 April 1999; revised 14 February 2000; accepted 9 March 2000.

Introduction

Sensitization to inhalant allergens is considered one of the key factors of asthma. Allergic rhinitis is the commonest manifestation of sensitization. Asthma and rhinitis frequently co-exist and allergic rhinitis may be a risk factor for developing asthma [1,2]. However, some epidemiological studies have shown that many subjects sensitized to

inhalant allergens experience no symptoms of asthma or allergic rhinitis [3]. Prevalences of asymptomatic sensitization are difficult to ascertain from the literature and little is known about the long-term consequences.

It has been hypothesized that pre-existing end organ hypersensitivity might predispose to the development of symptoms on exposure to allergic stimuli [4]. The severity of the early asthmatic response to allergen has in a laboratory setting been shown to depend on both the degree of bronchial responsiveness to histamine or methacholine and the amount of allergen-specific IgE [5–7]. On the other hand it has become increasingly clear that allergens can produce

Correspondence: M. Kerkhof, Department of Epidemiology and Statistics, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands.

eosinophilic inflammation of the airways and that chronic exposure is likely to be an important cause of both inflammation and the associated bronchial hyperresponsiveness [1,8–10]. Besides, allergen avoidance can lead to improvement of bronchial responsiveness in some patients [11,12]. Bronchial hyperresponsiveness is also frequently found in allergic rhinitis patients [1], although it occurs to a lesser degree in patients with rhinitis who have no symptoms of asthma [4,13,14].

Whether manifest disease occurs with sensitization may also depend on the type of allergen involved [15]. Differences in molecular size and biochemical properties between indoor and outdoor allergens may influence the intensity and site of the mucosal inflammation.

In this study we investigated whether the risk of having allergy symptoms for subjects who are sensitized to inhalant allergens is different depending on bronchial hyperresponsiveness, the degree and nature of sensitization, blood eosinophil count, and personal characteristics such as gender and age. We also studied in what way subjects who are asymptotically sensitized differ from nonsensitized subjects regarding bronchial responsiveness, blood eosinophil count, and the presence of aspecific respiratory symptoms.

Methods

Subjects

The subjects studied were from a random sample of 4522 adults aged 20–70 years from the general population of three areas in the Netherlands. The sample was stratified by gender and 10-year age groups. Participants underwent interviewed respiratory questionnaire, lung function testing, measurement of bronchial responsiveness by methacholine provocation, and venous blood collection for IgE and haematology.

Complete data were obtained from 1904 subjects in the period February 1992 to February 1993. The results of a postal screening questionnaire sent to all the subjects before they were invited to the examination showed that the prevalence of self-reported asthma or nasal allergy was not different in subjects of whom complete data were not obtained compared to the subjects with complete data.

Definitions

Data on symptoms and smoking were obtained by the ECRHS questionnaire [16]. Subjects were considered to have allergy symptoms when they reported symptoms of wheeze, cough, tightness in the chest, shortness of breath, a runny or stuffy nose, sneeze or itchy or watering eyes after exposure to animals, feathers, dust, trees, grass, flowers or

pollen or when they reported to have a nasal allergy. They were considered to have current asthma when they reported to have had asthma-attacks in the last 12 months. Subjects were considered to use asthma-medication when they used corticosteroids or bronchodilators for their breathing in the last 12 months. Subjects were supposed to be 'symptomatic' when they reported allergy symptoms or when they had current asthma or were using asthma-medication according to the definitions mentioned above. None of the asymptomatic-sensitized subjects had used oral antihistamines in the last 12 months.

Subjects were classified as current smokers if they had smoked at least 20 packs of cigarettes or 360 g of tobacco in their lifetime or at least one cigarette a day or one cigar a week for 1 year and had not stopped smoking in the preceding month.

Measurements

All measurements were performed according to the protocols of the European Community Respiratory Health Survey (ECRHS) [16] and the detailed methods have been published by the European Community [17].

Total and specific IgE were measured using the Pharmacia CAP System. The CAP was performed in the laboratories of Pharmacia diagnostics AB, Uppsala, Sweden. The assay is calibrated against the WHO Standard for IgE, with a range of 0.35–100 kU/L for specific IgE and 2–2000 kU/L for total IgE. Specific IgE was measured to four common inhalant allergens: house dust mite (*Dermatophagoides pteronyssinus*), cat, timothy grass, and birch. A specific IgE level 0.35–0.70 kU/L was defined as class 1, 0.70–3.5 kU/L as class 2, and > 3.5 kU/L as class ≥ 3 . House dust mite (*D. pteronyssinus*) and cat were grouped as 'indoor allergens' and timothy grass and birch as 'outdoor allergen' in logistic regression analysis.

Bronchial responsiveness was measured by methacholine inhalation using a Mefar dosimeter. Subjects were advised to avoid smoking for 1 h, using a β_2 -agonist or anticholinergic inhaler for 4 h or oral medication (β_2 -agonist, theophylline or antimuscarinic) for 8 h before the test. In symptomatic subjects methacholine challenge was performed according to a long protocol of doubling the cumulative dose of methacholine after a starting dose of 7.8 μ g up to a cumulative dose of 2 mg. In asymptomatic subjects a short protocol was followed in which doses were quadrupled after a starting dose of 15.6 μ g. The challenge was stopped if there was a 20% fall in FEV₁ from the control value in the best of two technically satisfactory manoeuvres performed after each inhalation. Subjects who had a decline in FEV₁ of 20% or more after 2 mg or less methacholine were classified as bronchial hyperresponsive.

Table 1 Characteristics of subjects, stratified by the presence of symptoms of asthma or allergy

		Without symptoms	With symptoms
Number		1232	672
Female gender		45%	52%*
Mean age		45 years	42 years*
Highest class of specific IgE	class 1	106 (9%)	60 (9%)
	class 2	107 (9%)	76 (11%)
	class ≥ 3	49 (4%)	210 (31%)*
Specific IgE HDM	class 1	69 (6%)	50 (7%)
	class 2	74 (6%)	64 (10%)
	class ≥ 3	37 (3%)	120 (18%)*
Specific IgE cat	class 1	10 (1%)	22 (3%)
	class 2	6 (1%)	37 (6%)
	class ≥ 3	0	28 (4%)*
Specific IgE timothy grass	class 1	61 (5%)	45 (7%)
	class 2	31 (3%)	58 (9%)
	class ≥ 3	8 (1%)	87 (13%)*
Specific IgE birch	class 1	26 (2%)	43 (6%)
	class 2	26 (2%)	44 (7%)
	class ≥ 3	8 (1%)	54 (8%)*
Geometric mean total IgE (SD)		24 (4) kU/l	45 (5) kU/L*
High eosinophils		225 (18%)	200 (30%)*
Bronchial hyperresponsive		184 (15%)	189 (28%)*
Current smoking		483 (39%)	221 (33%)*
Other respiratory symptoms†		47 (4%)	111 (17%)*

* $P < 0.05$. †Attacks of shortness of breath at night, wheeze without a cold or reporting of problems with breathing.

For measurement of blood eosinophil count venous blood was drawn into collection tubes containing ethylenediaminetetraacetic acid anticoagulant. Blood eosinophil counts were performed in four hospital laboratories: two laboratories with Technicon H1 (Technicon instruments Corp., Tarrytown, NY, USA), one laboratory with Technicon H6000, and one with a Bürker-Türk counting chamber. Results of the Bürker-Türk counting chamber were substantially different from the other methods. Besides, geometric mean blood eosinophil counts were significantly different between men and women. Therefore 'high eosinophils' was defined as a blood eosinophil count in the highest quartile by gender: $210 \times 10^6/L$ for men and $160 \times 10^6/L$ for women when the Technicon method was used and $140 \times 10^6/L$ for men and $120 \times 10^6/L$ for women when the Bürker-Türk counting chamber was used.

Data analysis

Chi-square tests were used to compare prevalences. One-way analysis of variance (ANOVA) followed by Scheffé's test was used to compare means of the logarithm of total IgE and Mann-Whitney test was used to compare means of age. To

study the relationship between respiratory symptoms and sensitization to inhalant allergens with simultaneously adjustment for other explaining factors, multiple logistic regression analysis was performed with 'asthma or allergy symptoms' as dependent variable. Tests for interaction were used to examine whether the association between sensitization and allergy symptoms was influenced by the presence of bronchial hyperresponsiveness, a high blood eosinophil count and personal characteristics as gender and age. A significance level of 0.05 was used.

Since there were significantly different associations for subjects with and without bronchial hyperresponsiveness, the results are presented after stratification by bronchial hyperresponsiveness.

Results

Thirty-five percent of the subjects reported symptoms (allergy symptoms, current asthma or the use of asthma-medication). Table 1 shows the characteristics of symptomatic and asymptomatic subjects. Fifty-one percent of the symptomatic subjects had specific IgE to any of the four inhalant allergens, against 21% of the asymptomatic

Table 2. Characteristics of subjects, stratified by the presence of specific IgE to any of the four inhalant allergens (positive class ≥ 1), and the presence of symptoms of asthma or allergy

	specific IgE +		specific IgE –	
	symptoms –	symptoms +	symptoms –	symptoms +
Number	262	346	970	326
Female gender	114 (44%)	174 (50%)	445 (46%)	178 (55%)*
Mean age	42 years	39 years*	46 years*	45 years*
Highest class of specific IgE				
Class 1	106 (41%)	60 (17%)*		
Class 2	107 (41%)	76 (22%)*		
Class ≥ 3	49 (19%)	210 (61%)*		
Number of positive tests				
1	187 (71%)	159 (46%)*		
2	58 (22%)	99 (29%)*		
3 or 4	17 (6%)	88 (25%)*		
Geometric mean total IgE	66 kU/L	99 kU/L*	18 kU/L*	19 kU/L*
High eosinophils	44 (17%)	120 (35%)*	181 (19%)	80 (25%)*
Bronchial hyperresponsive	33 (13%)	121 (35%)*	151 (16%)	68 (21%)*
Current smoking	109 (42%)	106 (31%)*	374 (39%)	115 (35%)
Other respiratory symptoms†	12 (5%)	64 (19%)*	35 (4%)	47 (14%)*

*Significantly ($P < 0.05$) different from asymptomatic-sensitized group (specific IgE positive, no symptoms). †Attacks of shortness of breath at night, wheeze without a cold or problems with breathing.

subjects (Table 1). Especially specific IgE class 3 or higher was found frequently in symptomatic subjects (31%), while this was rare among those who did not report symptoms (4%).

Specific IgE to inhalant allergens was detected in 32% of subjects. Forty-three percent of these sensitized subjects reported no symptoms. Thus, the overall prevalence of asymptomatic sensitization to inhalant allergens was 14%. Twenty-six percent of the sensitized subjects reported to have allergy symptoms of the lower airways as well as rhino-conjunctivitis symptoms. A similar proportion of 27% had symptoms of rhino-conjunctivitis without involvement of the lower airways. Only four percent of these sensitized subjects reported symptoms of the lower airways without rhino-conjunctivitis.

Compared to subjects with specific IgE who did report symptoms, asymptomatic-sensitized subjects had a lower level of total and specific IgE and had more frequently specific IgE to only one allergen (Table 2). They also had lower prevalences of a high blood eosinophil count and bronchial hyperresponsiveness and they were more often current smoker.

The only differences between the asymptomatic sensitized subjects and the 'healthy' control group (without sensitization and symptoms) were a higher mean total IgE and a younger mean age. Prevalences of other respiratory symptoms, like attacks of shortness of breath at night,

wheeze without a cold or reporting of problems with breathing were not different between asymptomatic sensitized subjects (5%) and the control group (4%), whereas sensitized subjects with symptoms had a significantly higher prevalence of 19% of these symptoms.

Logistic regression analysis showed that the odds-ratio's of having symptoms increased with the level of specific IgE. The odds ratios were the highest for specific IgE class ≥ 3 for both indoor and outdoor allergens.

In bronchial hyperresponsive subjects, class 2-specific IgE to indoor allergens was significantly associated with symptoms (odds ratio = 3.3; 95% CI 1.2–9.5), whereas class 1-specific IgE was not (odds ratio = 1.5; 95% CI 0.5–4.3). Bronchial hyperresponsive subjects with a high blood eosinophil count had a higher risk of symptoms than those who did not have a high blood eosinophil count, whether they were sensitized to indoor allergens or not (Table 3). A high blood eosinophil count did not significantly modify the association of specific IgE to indoor allergens with symptoms in bronchial hyperresponsive subjects, as it turns out from a non-significant interaction term of indoor specific IgE with high eosinophils (odds ratio = 1.5; 95% CI 0.4–5.9).

In subjects who were not bronchial hyperresponsive, specific IgE levels lower than class 3 to indoor allergens were not associated with having symptoms. A high blood eosinophil count without sensitization to indoor allergens

Table 3. Odds ratio's and 95% confidence intervals of the association between symptoms of asthma or allergy and different classes of specific IgE to indoor (house dust mite or cat) and outdoor (birch or timothy grass) allergens obtained from multiple logistic regression models, stratified by bronchial hyperresponsiveness (BHR = PD₂₀ ≤ 2 mg methacholine)

Predictors	BHR (<i>n</i> = 373)		no BHR (<i>n</i> = 1531)	
	OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value
Specific IgE to indoor allergens*				
No	1		1	
Class 1	1.5 (0.5–4.3)	0.44	1.1 (0.7–1.8)	0.64
Class 2	3.3 (1.2–9.5)	0.03	1.1 (0.7–1.7)	0.77
Class ≥ 3	5.1 (1.9–13.3)	<0.01	3.7 (2.1–6.6)	<0.01
Specific IgE to outdoor allergens†				
No	1		1	
Class 1	2.3 (0.9–6.1)	0.07	1.4 (0.9–2.3)	0.13
Class 2	2.2 (0.8–6.4)	0.15	2.0 (1.2–3.3)	0.01
Class ≥ 3	9.6 (2.0–45.0)	<0.01	16.6 (8.7–31.7)	<0.01
High eosinophils and				
Neg. specific IgE to indoor class ≥ 1	2.3 (1.2–4.5)	0.01	1.3 (1.0–1.8)	0.10
Pos. specific IgE to indoor class ≥ 1	3.5 (1.1–10.9)	0.03	2.3 (1.3–4.1)	<0.01
Current smoking	0.7 (0.4–1.2)	0.21	0.8 (0.6–1.0)	0.03
Total IgE (natural log)	1.1 (0.9–1.3)	0.35	1.0 (0.9–1.1)	0.94
Female gender	2.1 (1.2–3.6)	<0.01	1.3 (1.1–1.7)	0.02
Age per 10 years	0.8 (0.7–1.0)	0.09	1.0 (0.9–1.1)	0.37

*House dust mite or cat, †timothy grass or birch.

was not significantly associated with symptoms (odds ratio = 1.3; 95% CI 1.0–1.8) in subjects who were not bronchial hyperresponsive (Table 3). However, subjects with a high blood eosinophil count had a significantly higher risk of symptoms than those who did not have a high blood eosinophil count when they were sensitized to indoor allergens (Table 3). The interaction term of a high eosinophils with sensitization to indoor allergens had an odds ratio of 1.7 (95% CI 0.9–3.4).

Regarding specific IgE to outdoor allergens, bronchial hyperresponsiveness and blood eosinophil count did not influence the association between sensitization and symptoms.

In a grouped model without stratification by bronchial hyperresponsiveness, sensitization to outdoor allergens was significantly associated with symptoms at all levels of specific IgE (not shown).

The associations between sensitization and symptoms were not different for males and females. Age did not modify these associations either.

Discussion

This study shows that the presence of bronchial hyperresponsiveness or a high blood eosinophil count increases

the risk of having symptoms of asthma or allergy for subjects who are sensitized to indoor allergens. Low levels of specific IgE to indoor allergens are only associated with symptoms of asthma or allergy when this goes together with bronchial hyperresponsiveness or a high blood eosinophil count. The association of specific IgE to outdoor allergens with symptoms is not elevated by the presence of bronchial hyperresponsiveness or influenced by a high blood eosinophil count.

The relation between symptoms, sensitization and markers of inflammation may be two sided. On the one hand sensitization to inhalant allergens may cause inflammation of the airways. On the other hand pre-existing inflammation of the airways may increase the effect of sensitization. Our results suggest that subjects with specific IgE class ≤ 2 to indoor allergens only become symptomatic when pre-existing inflammation of the lower airways is present indicated by the presence of bronchial hyperresponsiveness or eosinophilia. Class ≤ 2 levels of specific IgE to indoor allergens probably do not cause inflammation of the airways, since subjects with these low levels of specific IgE did not show higher prevalences of bronchial hyperresponsiveness or eosinophilia than nonsensitized subjects, which would be expected when there were a causative relationship (Fig. 1). Specific IgE levels class 3 or higher are probably involved

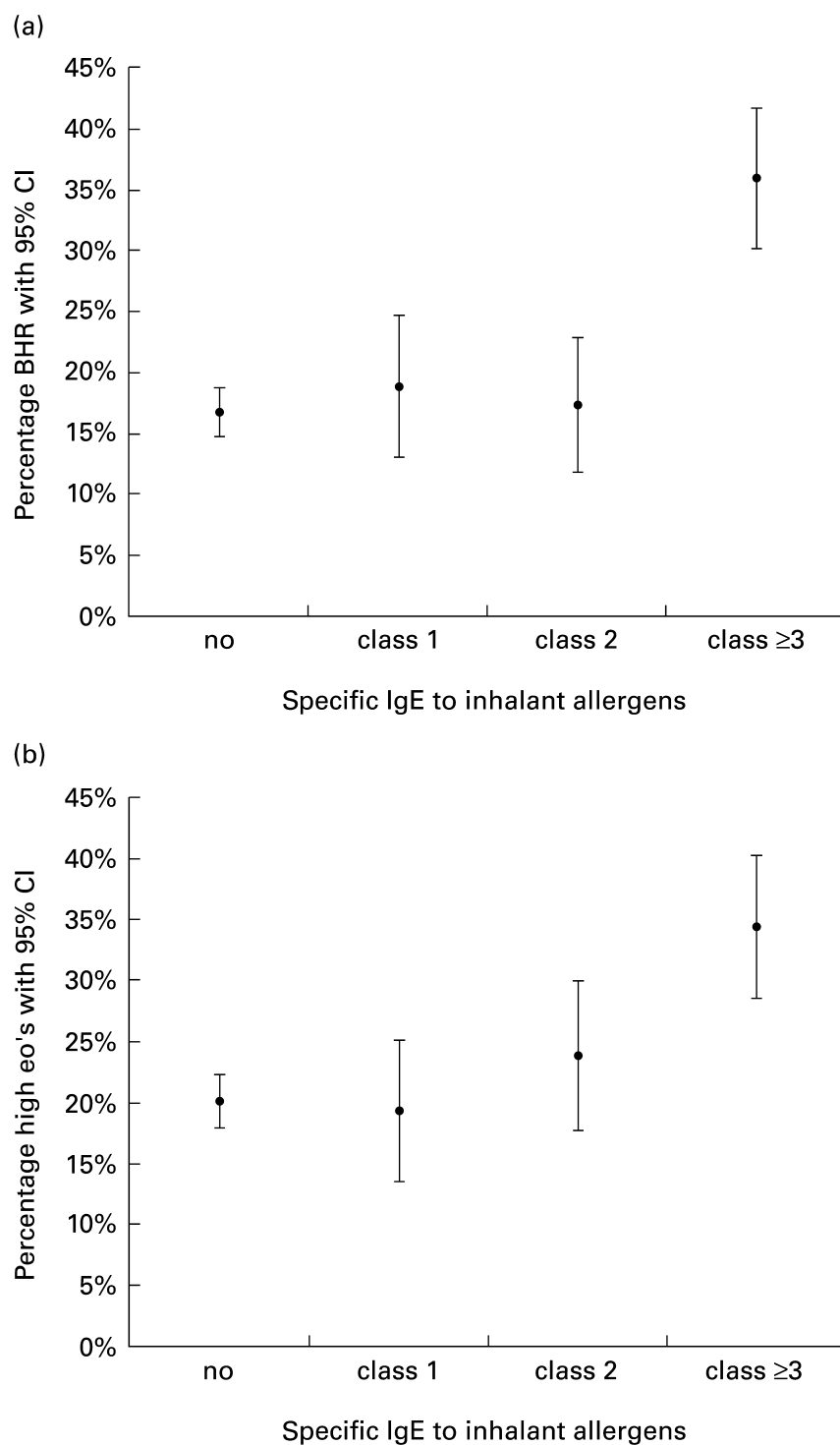


Fig. 1. The prevalence of bronchial hyperresponsiveness (a) and high eosinophils (b) for different classes of specific IgE to inhalant allergens

in the process of the development of inflammation, since higher prevalences of bronchial hyperresponsiveness and a high blood eosinophils count were found in subjects with these levels of specific IgE.

The fact that so many sensitized subjects do not experience

symptoms suggests that, next to IgE, other factors are involved in the development of allergic disease. Various cells are associated with inflammatory events characteristic of atopic allergy and asthma and especially T lymphocytes may be of particular significance [18].

Our results suggest that bronchial hyperresponsiveness plays a role in allergic disease concerning indoor allergens rather than outdoor allergens, which is in keeping with the findings of Omenaas *et al.* [19]. From studies in many parts of the world, it is clear that asthma is associated with sensitization to indoor allergens rather than with sensitization to pollen [8,20]. Differences in molecular size and biochemical properties between indoor and outdoor allergens may influence the intensity and site of the mucosal inflammation. Pollen particles are somewhat larger than those of house dust or animal origin and are more easily trapped in the nose. The induction of mucosal inflammation by timothy grass and birch may be mainly present in the upper airways. Nevertheless, seasonal exposure to pollen allergens may have some relation with bronchial hyperresponsiveness [21,22]. Absence of pollen exposure at the time of examination in a part of the group of subjects may have caused differences between indoor and outdoor sensitization. However, when our analyses were performed in a subgroup of subjects who were examined in the period of exposure to outdoor allergens, the results were not different.

A lower amount of exposure to allergens in subjects who have no symptoms, despite their sensitization to inhalant allergens, may be one of the factors responsible for the absence of symptoms. Since symptomatic persons tend to reduce exposure to relieve their symptoms, the influence of the amount of exposure could not be evaluated in our cross-sectional study design.

Our findings that the risk of symptoms increases with the degree of sensitization are in line with those of Gergen *et al.* [15] who showed that in younger subjects, the prevalence of asthma and rhinitis increased with an increasing number of positive skin tests to inhalant allergens.

Almost all of the individuals with specific IgE to indoor allergens were sensitized to house dust mite (94% of specific IgE class ≥ 3 , 91% of class 2 and 75% of class 1). Specific IgE to cat, without specific IgE to house dust mite was very rare which made it impossible to study the independent effect of different levels of specific IgE to cat allergens on symptoms in logistic regression analysis.

We have chosen a broad definition of the presence of allergy symptoms, including asthma or the use of asthma medication. In the case of perennial allergens, there may not be an immediate or easily recognized association between exposure and characteristic symptoms, resulting in a false-negative history. These patients may have other respiratory symptoms such as attacks of shortness of breath at night, without a cold or other problems with breathing. However, prevalences of these other respiratory symptoms were equal among asymptomatic sensitized and 'normal' subjects. Thus, it is unlikely that many subjects who had mild asthmatic symptoms with sensitization were classified as asymptomatic.

We did not have accurate information about the current presence of eczema or skin allergy, only about the lifetime prevalence of these disorders. The lifetime prevalence of eczema was not significantly associated with sensitization to inhalant allergens in subjects who had no allergy symptoms of the respiratory tract.

Twenty-five percent of the subjects who were not sensitized to inhalant allergens were classified as symptomatic. A part of them will be sensitized to allergens not tested in our study, but most of them will have symptoms that are not IgE-mediated.

Eosinophil counts were measured in different ways in different laboratories. The Bürker-Türk counting chamber method was used in one area in the south-east of the Netherlands, while the other two areas (one in the south-west and one in the north) used the Technicon method. Quartiles of eosinophils were calculated for both methods separately, under the assumption that the distributions of eosinophil count will be equal among both populations. There were no relevant differences in the prevalences of 'high eosinophils' between both areas measured with the Technicon method. Thus, we don't expect the distribution in the other area, in which the Bürker-Türk counting chamber was used, to be substantially different.

In summary, our epidemiological data suggest that low levels of specific IgE to indoor allergens, i.e. house dust mite, are of no clinical importance when no concurrent signs of inflammation of the airways are present. Low levels of specific IgE to outdoor allergens, however, may lead to symptoms without the presence of bronchial hyperresponsiveness or a high blood eosinophil count. Asymptomatic sensitization, which occurs in a substantial part of the population, is not related to positive markers of inflammation and might be harmless.

Acknowledgements

Supported by the Dutch Ministry of Welfare, Public Health, and Culture and The Netherlands Asthma Foundation.

References

- 1 Corren J. Allergic rhinitis and asthma: how important is the link? *J Allergy Clin Immunol* 1997; 99:S781–S786.
- 2 Braman SS, Barrows AA, DeCotiis BA, Settiple GA, Corrao WM. Airway hyperresponsiveness in allergic rhinitis. A risk factor for asthma. *Chest* 1987; 91 (5):671–4.
- 3 Baldacci S, Modena P, Carozzi L *et al.* Skin prick test reactivity to common aeroallergens in relation to total IgE, respiratory symptoms, and smoking in a general population sample of northern Italy. *Allergy* 1996; 51:149–56.
- 4 Bryant DH, Burns MW. The relationship between bronchial histamine reactivity and atopic status. *Clin Allergy* 1976; 6:373–81.

- 5 Bryant DH, Burns MW. Bronchial histamine reactivity: its relationship to the reactivity of the bronchi to allergens. *Clin Allergy* 1976; 6 (6):523–32.
- 6 Cockcroft DW, Murdock KY, Kirby J, Hargreave F. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987; 135:264–7.
- 7 Crimi E, Balbo A, Voltolini S, Troise C, Brusasco V, Negrini AC. Is the allergen-inhalation challenge predictive of the severity of seasonal asthmatic exacerbations? *Allergy* 1993; 48:202–6.
- 8 Platts-Mills TA, Sporik RB, Chapman MD, Heymann PW. The role of indoor allergens in asthma. *Allergy* 1995; 50:5–12.
- 9 De Monchy JG, Kauffman HF, Venge P, Koëter GH, Jansen HM, Sluiter HJ, De Vries K. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985; 131 (3):373–6.
- 10 O'Byrne PM, Hargreave FE, Kirby JG. Airway inflammation and hyperresponsiveness. *Am Rev Respir Dis* 1987; 136:S35–7.
- 11 Platts-Mills TA, Tovey ER, Mitchell EB, Moszoro H, Nock P, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 25, 1982; 2 (8300): 675–8.
- 12 van Velzen E, van den Bos JW, Benckhuijsen JA, van Essel T, de Bruijn R, Aalbers R. Effect of allergen avoidance at high altitude on direct and indirect bronchial hyperresponsiveness and markers of inflammation in children with allergic asthma. *Thorax* 1996; 51 (6):582–4.
- 13 Muller BA, Leick CA, Smith RM, Suelzer MT, Richerson HB. Comparisons of specific and nonspecific bronchoprovocation in subjects with asthma, rhinitis, and healthy subjects. *J Allergy Clin Immunol* 1993; 91:758–72.
- 14 Bonavia M, Crimi E, Quaglia A, Brusasco V. Comparison of early and late asthmatic responses between patients with allergic rhinitis and mild asthma. *Eur Respir J* 1996; 9:905–9.
- 15 Gergen PJ, Turkeltaub PC. The association of individual allergen reactivity with respiratory diseases in a national sample: data from the second National Health and Nutrition Examination Survey. 1976–80 (NHANES II). *J Allergy Clin Immunol* 1992; 90:579–88.
- 16 Burney PGJ, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994; 7:954–60.
- 17 European Commission Directorate General XII. Protocol for the European Community Respiratory Health Survey. Office for Official Publications L-2920, Luxembourg, 1993.
- 18 Kay ABT. lymphocytes and their products in atopic allergy and asthma. *Int Arch Allergy Appl Immunol* 1991; 94 (1–4):189–93.
- 19 Omenaas E, Bakke PS, Eide GE, Elsayed S, Gulsvik A. Serum house dust mite antibodies: predictor of increased bronchial responsiveness in adults of a community. *Eur Respir J* 1996; 9:919–25.
- 20 Platts-Mills TA, Thomas WR, Aalberse RC, Vervloet D, Chapman MD. Dust mite allergens and asthma: report of a second international workshop. *J Allergy Clin Immunol* 1992; 89 (5):1046–60.
- 21 Boulet LP, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE. Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J Allergy Clin Immunol* 1983; 71 (4):399–406.
- 22 Rak S, Lowhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. *J Allergy Clin Immunol* 1988; 82 (3 Part; 1):470–80.