Emergence of pollen food allergy syndrome in asthmatic children in Paris

Christine Loraud, Charlotte Thibault de Ménonville, Mélisande Bourgoin-Heck, Nathalie Cottel, Stéphanie Wanin, Jocelyne Just

Emergence of pollen food allergy syndrome in asthmatic children in Paris

73 characters

Manuscript: 2482 words, 4 tables

Christine Loraud MD[1], Charlotte de Ménonville MD[1], Mélisande Bourgoin-Heck MD[1,2], Nathalie Cottel MD[1], Stéphanie Wanin MD[1], Jocelyne Just MD, PhD[1,2]

Affiliations

[1] Department of Allergology, Hôpital d'Enfants Armand Trousseau, APHP, 26 avenue du Dr Netter, 75012 Paris, France

[2] Sorbonne Universités, Equipe EPAR, Institut Pierre Louis d'Epidémiologie et de Santé Publique, UMR_S1136, INSERM Paris 06, Paris, France

Corresponding author

Jocelyne JUST

Department of Allergology—Centre de l'Asthme et des Allergies

Hôpital d'Enfants Armand Trousseau

26 avenue du Dr Arnold Netter

75012 Paris, France

Tel + 33 1 44 73 68 47

Fax: +33 1 44 73 53 15

jocelyne.just@aphp.fr

26

27

28

ABSTRACT

Background: Over the last few decades, the level of pollen from birch and homologous trees has increased in parts of Europe. Sensitization to birch pollen allergens (principally Bet v 1) has been associated with food cross-reactivity called pollen-food-allergy syndrome (PFAS).

Objective: To evaluate changes in allergic diseases due to IgE sensitization over 25 years in asthmatic children.

Methods: This was a cross-sectional retrospective study conducted in Paris. We analyzed two cohorts of asthmatic children with similar characteristics explored between 1993-1999 (old cohort=OC) and 2012-2018 (recent cohort = RC).

Results: 121 children were in the OC and 120 in the RC. An increase in sensitization to tree pollens was found especially for birch pollen which was 11.6% in the OC and 31% in the RC ($p=0.0002$). Allergic rhinitis prevalence was significantly higher in the RC than in the OC (96% vs 52%, respectively, $p<0.0001$). IgE-mediated food allergy increased from 6% to 16% in the OC and RC, respectively, ($p = 0.01$) mainly due to PFAS. In the RC, a higher mean Bet v 1-specific IgE level was observed in children with PFAS compared to children without (105.7 KU/L± 17.8 and 48.9 kU/L ± 15.7, respectively, $p<0.05$).

Conclusion: Allergic rhinitis and food allergy with tree-pollen sensitization has increased in Paris over 25 years mainly due to PFAS. Environmental factors could be responsible for these modifications as described in literature.

228 words

Key Words

Allergic rhinitis, Pollen-food-allergy syndrome, Asthma, Betv1 specific IgE

INTRODUCTION

With the rapid increase in the prevalence of allergic diseases over the last century, the World Health Organization (WHO) ranks allergy as the 4th chronic disease in the world after cancer, cardiovascular pathologies and AIDS (1). It is estimated that half of the world's population will be allergic by 2050.

Allergic asthma is the most common asthma phenotype in children (2). The percentage of patients in whom asthma is attributed to atopy varies from 35 to 50% depending on the study (3). Nevertheless, allergic rhinitis, atopic dermatitis and food allergy are strong risk factors for asthma inception and severity both in children and adults (2,4).

Moreover, pollens, and mainly birch pollen, are responsible for seasonal asthma attacks (5). In northern and central Europe, birch and other trees of the Betulaceae and Fagaceae families are the most dominant tree pollens and represent the most potent elicitors of allergy in early spring. Over the last few decades, the level of birch pollen and pollens from trees of the same group has increased in some parts of Europe (6).

Birch pollen allergens induce IgE cross-reactivity, predominantly observed in relation to Bet v 1, including food cross-reactivity which is called pollen-food-allergy syndrome (PFAS). PFAS typically consists of an immediate mild local reaction (oral food syndrome) including itching, tingling and angioedema of the lips, tongue, and throat. It rarely causes systemic reactions such as anaphylaxis. In the north of Europe, the principal PFAS mechanism results in cross-reactivity toward molecular components of tree pollens and food allergens mainly due to the presence of PR-10 proteins. Recent studies suggest that PFAS might be more frequent in childhood than previously recognized (7).

The aim of our study was to evaluate changes in allergic diseases, and specially PFAS, over 25 years in children with asthma.

METHODS

Study design and population

This was a cross-sectional retrospective study conducted in the Department of allergology in a French pediatric hospital (Trousseau Hospital, Paris).

The study population consisted of two random cohorts of children aged 7-15 years and admitted to the department for a follow-up assessment of their asthma between 1993-1999 (old cohort = OC) or 2012-2018 (recent cohort = RC). The inclusion criteria were: (1) sensitization to at least one aeroallergen; (2) living in Paris or neighbouring suburbs (departments 92, 93 and 94). The study data were extracted from medical information gathered during the hospital stay and entered in a computerized database.

Study data

The following data were recorded and compared between the two cohorts:

1. Demographic characteristics: age, sex, geographic distribution (Paris or its suburbs) and whether the child was overweight (including obesity) defined by a Body Mass Index (BMI = weight/ height^2) >IOTF-25 (International Obesity Task Force) threshold.
2. Personal and family atopic diseases: allergic rhinitis and active atopic dermatitis (defined by a flare-up within the previous year) mostly assessed by questions from the International Study of Asthma and Allergies in Childhood (8).
3. Asthma characteristics:
    a. Proximal limitation of expiratory airflow was defined by a forced expiratory volume 1 second/forced volume vital capacity ratio (FEV_1/FVC) <0.9 and/or prebronchodilator FEV_1 <80%
    b. Long-term treatment prescribed on discharge especially inhaled corticosteroids (ICS) and Long-Acting-Beta2-Agonists (LABA). ICS doses were classified

according to the Global Initiative for Asthma (9): low (200-500 µg beclometasone), medium (500-1000 µg beclometasone) or high dose (>1000 µg beclometasone).

4. Biological inflammatory atopic markers were measured in peripheral blood through: blood eosinophilia (cell counting by automated Sysmex, France), total immunoglobulins E (IgE) and specific IgE measured by ImmunoCAP™ (Phadia, Uppsala, Sweden). Bet v 1 specific IgE levels were available in children in the RC if they tested positive to birch-pollen specific IgE.
5. Allergenic sensitization was defined as a positive skin prick test (wheal allergen ≥3 mm in the absence of a positive reaction to the negative control), and confirmed by positive specific IgE ≥0.35 kU/L (ImmunoCAP®; Phadia, Uppsala, Sweden) (10). The battery of allergens explored included the common allergens (house dust mites (HDM), cat and dog dander, grass and birch pollen, *alternaria alternata*, cow's milk proteins, egg, and peanut) and allergens detected as being responsible for symptoms and/or present in the environment.
6. IgE-mediated food allergy was defined by relevant symptoms of food allergy occurring within 6 hours following consumption of the food allergen associated with an allergic sensitization to the same allergen (11).

Ethics

The study was declared to the French "Commission Nationale d'Informatique et Libertés" under the reference number MR-5714060420 and a letter of information was sent to the patients' family. Informed consent was not required because of the retrospective nature of the analysis.

Statistical analysis

Analysis was performed per patient. Quantitative variables are reported as mean ± SD

and compared by unpaired t-test. If the sample was small equality of variances was tested with Fisher's test and the Welch t-test was used if variances differed. Categorical variables are reported as count and proportions. Statistical significance was considered at the $p<0.05$ level. Statistical analysis was performed using Graphpad Prism version 7.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Characteristics of the two cohorts

One hundred twenty-one children were included in the OC and 120 in the RC.

Demographic characteristics (Table I): There was no significant difference regarding age, sex ratio, number of overweight children. The geographical distribution was homogenous between Paris and the suburbs.

Asthma characteristics (Table II): There was no significant difference in proximal limitation of expiratory airflow which was present in nearly 30% of the population in the two cohorts. The most commonly prescribed long-term discharge treatment mainly corresponded to step 3 and 4 of the GINA guidelines for the majority of the children in both cohorts.

Atopic inflammatory biomarkers (Table II)*:* Blood eosinophilia and serum total IgE were at similarly high levels in both cohorts.

Changes in sensitization to inhaled allergens (Table III)

The three most frequent allergens were HDM, animal dander and grass pollen, in a similar proportion for the two cohorts. Children in the RC were three-times more likely to have a tree pollen sensitization ($p = 0.0002$) and especially for birch (9.9% in the OC vs 29% in the RC, $p = 0.0001$) and oak (5% in the OC vs 16% in the RC, $p = 0.005$). There was a slight decrease in sensitization to perennial allergens in the RC compared to the OC: HDM ($p = 0.0001$) and *alternaria alternata* ($p = 0.04$).

Changes in atopic diseases except food allergy (Table IV)

Children in the RC were twice more likely to have allergic rhinitis compared to those in the OC ($p < 0.0001$) and a family history of asthma ($p = 0.005$). There was no difference in the percentage of children with a personal history of atopic dermatitis or with active atopic dermatitis between the two cohorts.

Changes in Pollen-Food-Allergy Syndrome

A significant increase in IgE-mediated food allergy was observed in the two cohorts: 6% in the OC versus 16% in the RC ($p = 0.01$). The difference was mainly due to PFAS related to tree pollens sensitization. In the OC, only one of the seven children with IgE-mediated food allergy had PFAS (due to cross reactivity between oak pollen and apple, kiwi, peach, hazelnut). In the RC, ten children with IgE-mediated food allergy had PFAS: four with isolated PFAS and six with PFAS associated with another IgE-mediated food allergy. More precisely, in the RC: one child had a PFAS due to a cross reactivity between isolated oak pollens and soybean; four children due to a cross reactivity between birch and oak pollens and hazelnut, peanut, carrot, kiwi; one child due to a cross reactivity between birch and plane tree pollens and hazelnut, kiwi and banana; and four children due to a cross reactivity between isolated birch pollen and apple, celery, peanut, hazelnut.

In the RC, in children with tree pollens sensitization a higher mean Bet v 1 IgE level was observed in children with PFAS compared to children without (105.7 KU/L ± 17.8 and 48.9 kU/L ± 15.7, respectively, $p < 0.05$), as well as a tendency for elevated mean oak IgE levels in children with PFAS versus those without (22.5 kU/L ± 4.6 ($n = 5$) and 16.2 kU/L ± 4.7 ($n = 15$), respectively, $p = 0.4$).

DISCUSSION

This study of two cohorts of asthmatic children showed a huge increase in allergic rhinitis since the year 2000 due to sensitization to tree pollens associated with an increase in the number of cases of PFAS.

Changes in allergic sensitization to inhaled allergens observed in the two cohorts

Several studies, mostly conducted in adults, have shown a similar increase in tree pollens sensitization. A study of adult asthmatic patients performed in Belgium (12) found rates similar to ours with an increase in sensitization to birch pollen from 13% (1975-1979) to 34% (1992-1995) with no increase in sensitization to grass or artemisia pollen. Similarly, a study conducted in northern Sweden in the general population also found an increase in sensitization to birch pollen albeit lower than that found in ours: 13% in 1994 to 18% in 2009 (13). In the same manner, a Danish study reported an increase in birch pollen sensitization in the general population from 12.1% to 13.7% between 1990 and 1998 (14).

Several other studies have explored changes in allergic sensitization with different conclusions. Rönmark's study (15), conducted in northern Sweden between 1996 and 2006 in 7–8-year-old children, showed that the prevalence of positive skin prick tests increased from 21% in 1996 to 30% in 2006. The allergens tested were birch, timothy, mugwort, dog, cat, horse, HDM, cladosporium and *alternaria alternata.* Despite an increase in the prevalence of allergic sensitization of all allergens tested, no increase in the prevalence of symptoms of asthma, rhinitis or eczema was found.

A study conducted in Denmark by Thomsen SF et al. (16) evaluated atopic sensitization by skin prick test reactivity from 1986 and 2001 in two random population samples of children aged between 7-17 years. There was a non-statistically significant decline in the prevalence of sensitization to most allergens (birch, horse, dog, cat, HDM) with a statistically significant decrease in mugwort and *alternaria alternata* sensitizations. In the same study, the prevalence of sensitization to grass pollens remained stable.

Changes in atopic diseases observed in the two cohorts

We found a statistically higher prevalence of allergic rhinitis and family atopy in the RC compared to OC as reported by other authors. Conversely, the prevalence of atopic dermatitis remained unchanged contrary to other findings in the literature (17). This is probably because we only noted atopic dermatitis that was active during the study period and because atopic dermatitis is often in remission at 7 years which was the minimum age for inclusion in our study (18).

Changes in Pollen-Food-Allergy Syndrome observed in the two cohorts

Our study showed a 3-fold increase in PFAS in the RC compared to the OC.

The first description of PFAS dates back as far as 1942 (19) (20). In 1995, the term PFAS was used to better characterize the pathogenesis (21) and relationship principally to the PR-10 family.

In our study, children sensitized to tree pollens with PFAS had higher Bet v1 IgE levels compared to patients without ($p < 0.05$). This result is in accordance with the study by Asero R. et al. (22) who showed that birch pollen allergic patients with PFAS were more likely to have asthma and higher specific IgE levels to birch pollen than patients without. In the same manner, the Italian study by Ciprandi et al. (23) conducted in 245 adults sensitized to Bet v 1 with allergic rhinitis, showed that patients with PFAS had higher Bet v 1 levels and more severe symptoms of allergic rhinitis than patients without. Our results are also in agreement with the Swedish birth cohort study by Westman et al. (24) who found that the risk of later onset or persistence of symptoms of allergic rhinitis to birch pollen increased with increasing levels of Bet v 1 specific IgE.

Although less described in literature, oak pollen allergy can also cause food allergy toward the PR-10 proteins. However, oak pollen is close to birch pollen: birch pollen allergy

immunotherapy can be effective for other tree pollens especially alder, hazel and oak, a group of trees that we call the "birch homologous group" (25).

Environmental factors to explain the changes in allergen sensitization

The plane tree is by far the most planted tree in Paris currently, mainly as an alignment tree, against 4 330 oak trees and 2 413 birch trees. The plane tree is an aerobiological polluter which can also be a provider of food allergies (26). While the high allergenic potential of the birch is well known by urban planning departments, that of oak and plane trees, although lower, is less so as is their role in food allergies. It is important that urban developers as well as the general public are made aware of their allergenic risk and consequently restrict the planting of such trees through diversification. Introducing diversity in landscaping is a simple way of reducing the concentration of pollen of the same species in the air (27).

Climate change, with the marked increase in the earth's temperature observed over the last 50 years, has had an impact on birch pollen for several decades now (28) (29). The rates of change in annual pollen cycles have been shown to be associated with the rates of change in the annual cycles of several meteorological parameters with overall warmer temperatures (29). In Beck's study in Munich, pollen from birch exposed to higher ozone levels induced larger wheals and flares in skin prick tests compared to lower ozone-exposed pollen suggesting an allergenicity increasing effect of ozone (30).

The decrease in perennial allergen sensitization found in our study could be explained by the improvement of the indoor environment and recent measures taken against HDM and molds (15).

Strengths and limits of the study

The main limits of our study lie in the fact that it is retrospective and that all the patients were recruited from one center albeit a tertiary care center for allergic diseases. However, atopic disease was assessed by standardized questionnaires in both cohorts to minimize the risk of

misinterpretation, and allergic sensitization was measured by validated parameters – skin prick tests and specific IgE levels – in the same way. Furthermore, the two cohorts in our study were homogenous phenotypes: a predominance of boys, a high rate of overweight patients and atopic comorbidities, and the same severity of asthma. Finally, the homogeneous geographic distribution of our population in Paris and suburbs makes it possible to compare allergen sensitization within a similar environment, in particular with tree plantations which can affect the development of allergies.

Conclusion

Allergic rhinitis, sensitization to trees and PFAS has increased in Paris and the surrounding suburbs over 25 years as shown in our two cohorts of asthmatic children of the same severity. Environmental factors could be responsible for these modifications as mentioned in literature.

REFERENCES

1. Platts-Mills TAE. The allergy epidemics: 1870-2010. J Allergy Clin Immunol. 2015;136(1):3-13.
2. Just J. Atopy is important in the management of asthma. Paediatr Respir Rev. 2013;14(2):92-5.
3. Arbes SJ, Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: results from the Third National Health and Nutrition Examination Survey. J Allergy Clin Immunol. 2007;120(5):1139-45.
4. Gough H, Grabenhenrich L, Reich A, Eckers N, Nitsche O, Schramm D, et al. Allergic multimorbidity of asthma, rhinitis and eczema over 20 years in the German birth cohort MAS. Pediatr Allergy Immunol. 2015;26(5):431-7.
5. Canova C, Heinrich J, Anto JM, Leynaert B, Smith M, Kuenzli N, et al. The influence of sensitisation to pollens and moulds on seasonal variations in asthma attacks. Eur Respir J. 2013;42(4):935-45.
6. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. Allergy. 2019;74(7):1237-48.
7. Mastrorilli C, Cardinale F, Giannetti A, Caffarelli C. Pollen-Food Allergy Syndrome: A not so Rare Disease in Childhood. Medicina (Kaunas). 2019;55(10).
8. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J. 1995;8(3):483-91.
9. Reports [Internet]. Global Initiative for Asthma - GINA. [2020]. https://ginasthma.org/reports/
10. Bernstein IL, Storms WW. Practice parameters for allergy diagnostic testing. Joint Task Force on Practice Parameters for the Diagnosis and Treatment of Asthma. The American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol. 1995;75(6 Pt 2):543-625.
11. Heinzerling L, Mari A, Bergmann K-C, Bresciani M, Burbach G, Darsow U, et al. The skin prick test – European standards. Clin Transl Allergy. 2013;3:3.
12. Stevens WJ, Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS. Is the prevalence of specific IgE to classical inhalant aeroallergens among patients with respiratory allergy changing? Evidence from two surveys 15 years apart. Acta Clin Belg. 2003;58(3):178-82.
13. Warm K, Lindberg A, Lundbäck B, Rönmark E. Increase in sensitization to common airborne allergens among adults - two population-based studies 15 years apart. Allergy Asthma Clin Immunol. 2013;9(1):20.
14. Linneberg A, Nielsen NH, Madsen F, Frølund L, Dirksen A, Jørgensen T. Increasing prevalence of specific IgE to aeroallergens in an adult population: two cross-sectional surveys 8 years apart: the Copenhagen Allergy Study. J Allergy Clin Immunol. 2000;106(2):247-52.
15. Rönmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundbäck B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. J Allergy Clin Immunol. 2009;124(2):357-63, 63.e1-15.
16. Thomsen SF, Ulrik CS, Porsbjerg C, Backer V. Skin test reactivity among Danish children measured 15 years apart. J Asthma. 2006;43(2):151-3.
17. Asher MI, Montefort S, Björkstén B, Lai CKW, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006;368(9537):733-43.
18. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. J Allergy Clin Immunol. 2003;112(6 Suppl):S118-127.

19. Tuft L, Blumstein GI. Studies in food allergy: II. Sensitization to fresh fruits: Clinical and experimental observations. Journal of Allergy.1942;13(6):574-82.
20. Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. Clinical & Experimental Allergy. 1987;17(1):33-42.
21. Valenta R, Kraft D. Type I allergic reactions to plant-derived food: A consequence of primary sensitization to pollen allergens. Journal of Allergy and Clinical Immunology. 1996;97(4):893-5.
22. Asero R, Massironi F, Velati C. Detection of prognostic factors for oral allergy syndrome in patients with birch pollen hypersensitivity. J Allergy Clin Immunol. 1996;97(2):611-6.
23. Ciprandi G, Comite P, Ferrero F, Bignardi D, Minale P, Voltolini S, et al. Birch allergy and oral allergy syndrome: The practical relevance of serum immunoglobulin E to Bet v 1. Allergy Asthma Proc.2016;37(1):43-9.
24. Westman M, Lupinek C, Bousquet J, Andersson N, Pahr S, Baar A, et al. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. J Allergy Clin Immunol. 2015;135(5):1199-1206.e1-11.
25. Couroux P, Ipsen H, Stage BS, Damkjaer JT, Steffensen MA, Salapatek AM, et al. A birch sublingual allergy immunotherapy tablet reduces rhinoconjunctivitis symptoms when exposed to birch and oak and induces IgG4 to allergens from all trees in the birch homologous group. Allergy. 2019;74(2):361-9.
26. Miralles JC, Caravaca F, Guillén F, Lombardero M, Negro JM. Cross-reactivity between *Platanus* pollen and vegetables. Allergy. 2002;57(2):146-9.
27. Le Réseau National de Surveillance Aérobiologique (R.N.S.A.) [Internet]. [2020]. https://www.vegetation-en-ville.org/
28. D'Amato G, Vitale C, Lanza M, Molino A, D'Amato M. Climate change, air pollution, and allergic respiratory diseases: an update. Curr Opin Allergy Clin Immunol. 2016;16(5):434-40.
29. Bruffaerts N, De Smedt T, Delcloo A, Simons K, Hoebeke L, Verstraeten C, et al. Comparative long-term trend analysis of daily weather conditions with daily pollen concentrations in Brussels, Belgium. Int J Biometeorol. 2018;62(3):483-91.
30. Beck I, Jochner S, Gilles S, McIntyre M, Buters JTM, Schmidt-Weber C, et al. High environmental ozone levels lead to enhanced allergenicity of birch pollen. PLoS ONE. 2013;8(11):e80147.

Table I: Demographic characteristics

	Old Cohort	Recent Cohort	p value*
Number of patients	121	120	-
Age, (years)	10.6 ± 0.2	10.2 ± 0.2	0.1
Male, nb. (%)	78 (64)	84 (70)	0.4
Overweight †, nb. (%)	21 (19) n=110	22 (18) n = 118	0.9
Geographical distribution, nb. (%)	n = 121	n = 120	
Living in Paris (75)	60 (50)	51 (43)	0.3
Living in suburbs	61 (50)	69 (57)	0.3
-All inhabitants of 92 / 92 close to Paris (<5km)	10 (8) / 9 (7)	3 (2.5) /3 (2.5)	-
-All inhabitants of 93 / 93 close to Paris (<5 km)	26 (21) / 17 (14)	34 (28) / 21 (18)	-
-All inhabitants of 94 / 94 close to Paris (<5 km)	25 (21) / 24 (17)	32 (26.5) / 27 (23)	-

Abbreviations: Admitted to the department for a follow-up assessment of their asthma between 1993-1999 (Old Cohort) or 2012-2018 (Recent Cohort).
Definitions: * Statistical significance was considered at the p <0.05 level; † Overweight (included obesity): Body Mass Index > IOTF-25 (International Obesity Task Force) threshold

Table II: Asthma characteristics

	Old Cohort	Recent Cohort	P value*
Number of patients	121	120	-
Early onset of asthma†, nb. (%)	64 (54)	80 (67)	0.07
Proximal limitation of expiratory airflow ‡, nb. (%)	30 (31) n= 97	34 (30) n= 114	0.9
Prebronchodilator FEV1 < 80 % (%)	29 (30) n= 97	23 (22) n= 106	0.2
Uncontrolled asthma § (%)	82 (70) n= 116	77 (64) n= 120	0.3
Discharge treatment, nb. (%) :			
No ICS	15 (13) n = 118	14 (12) n= 118	0.8
ICS only, low doses (200-500 µg beclometasone)	29 (26) n = 112	28 (24) n = 118	0.7
ICS low or medium doses + LABA / ICS only medium doses (500-1000 µg beclometasone)	56 (50) n = 111	63 (53) n = 118	0.7
ICS high doses (>1000 µg beclometasone)	11 (10) n = 111	10 (8) n = 118	0.7
Blood eosinophilia count ¶ (cells/mm^3) mean ± SD	624 ± 35	605 ± 38	0.7
Total IgE count ‖(kUI/L) mean ± SD	638 ± 67	755 ± 85	0.3

Abbreviations: Admitted to the department for a follow-up assessment of their asthma between 1993-1999 (Old Cohort) or 2012-2018 (Recent Cohort).
Definitions: ICS, inhaled corticosteroid; LABA, Long-Acting-Beta2-Agonist; Total-IgE, total immunoglobulin E *Statistical significance was considered at the $p < 0.05$ level † asthma onset before 3 years of age ‡defined by $FEV_1/FVC < 0.9$ in children § uncontrolled asthma as defined as GINA guidelines ¶ cell counting by automated Sysmex®, France ‖ measured by ImmunoCAP™ (Phadia, Uppsala, Sweden)

Table III: Changes in allergic sensitization to inhaled allergens

	Old cohort	Recent cohort	p value*
Number of patients	121	120	-
Sensitization to inhaled allergens †			
House dust mites, nb. (%)	**113 (93)**	**91 (76)**	**0.0001**
Dermatophagoides pteronyssinus	**113 (93)**	**91 (76)**	**0.0001**
Dermatophagoides farinae	**6 (5)**	**17 (14)**	**0.02**
Animal dander, nb. (%)	53 (44)	58 (48)	0.5
Cat	41 (34)	48 (40)	0.3
Dog	20 (16)	28 (23)	0.2
Horse	7 (5.7)	4 (3.3)	0.4
Rabbit	2 (1.6)	0	0.2
Hamster	3 (2.5)	2 (1.6)	0.7
***Alternaria alternata*, nb. (%)**	**38 (31)**	**24 (20)**	**0.04**
Aspergillus, nb. (%)	5 (4.1)	3 (2.5)	0.5
Grass pollen, nb. (%)	50 (41)	45 (37.5)	0.5
Artemisia pollen, nb. (%)	2 (1.6)	3 (2.5)	0.6
Trees, nb. (%)	**14 (11.6)**	**37 (31)**	**0.0002**
Birch	**12 (9.9)**	**35 (29)**	**0.0001**
Oak	**6 (5)**	**19 (16)**	**0.005**
Plane tree	1 (0.8)	1 (0.8)	0.6
Cypress	0	1 (0.8)	0.3
Ash	0	1 (0.8)	0.3

Abbreviations: Admitted to the department for a follow-up assessment of their asthma between 1993-1999 (Old Cohort) or 2012-2018 (Recent Cohort).
Definitions: IgE, immunoglobulin E * Bold characters means p-value reaching statistical significance ($p<0.05$); † Sensitization to inhalant allergen was defined as a specific IgE level ≥ 0.35 kU/L, measured by ImmunoCAP™ (Phadia, Uppsala, Sweden).

Table IV: Personal and family history of atopic diseases

	Old Cohort	Recent Cohort	p value*
Number of patients	121	120	-
History of atopic dermatitis † nb. (%)	54 (45)	53 (44)	0.9
Active atopic dermatitis† ‡, nb. (%)	14 (11)	17 (14)	0.6
Allergic rhinitis, nb. † (%)	**63 (52)**	**111 (96)**	**<0.0001**
Family history of atopy §, nb. (%)	**45 (37)**	**84 (70)**	**<0.0001**
Family history of asthma, nb. (%)	**39 (32)**	**60 (50)**	**0.005**

Abbreviations: Admitted to the department for a follow-up assessment of their asthma between 1993-1999 (Old Cohort) or 2012-2018 (Recent Cohort).
Definitions: * Bold characters means p-value reaching statistical significance ($p<0.05$); † Assessment by the questions from the International Study of Asthma and Allergies in Childhood (ISAAC); ‡ defined by flare-up in the last year; § defined as food allergy, eczema or allergic rhinitis