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PEDIATRICS

Risk factors for persistence of asthma in children: 10-year follow-up

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Abstract

Objective: Risk factors related to the outcome of childhood asthma are not yet well established. We aimed to investigate the long-term outcome for children with asthma to determine the risk factors in predicting persistence of disease. *Methods:* Sixty-two children with asthma were evaluated retrospectively at the end of a 10-year follow-up. Patients were asked to complete a questionnaire requesting clinical information, and underwent physical examination, skin prick testing, a pulmonary function test and bronchial provocation testing. Immunologic parameters evaluated were allergen-specific IgE and IgG4 levels, and allergen-induced generation of $CD4^+CD25^+$ cells. *Results*: Mean age at final assessment was 15.9 ± 3.6 years, and duration of follow-up was 10.30 ± 1.27 years. Fifty percent of patients outgrew their asthma during the 10-year follow-up period. All the non-atopic patients outgrew their disease during the study period, whereas 67% of atopic patients did not. We identified two risk factors independently related to the persistence of symptoms: presence of bronchial hyper-responsiveness and presence of rhinitis. Atopic children who were in remission demonstrated significantly higher allergen-induced $CD4^+CD25^+$ T cells compared to healthy controls. *Conclusions*: Atopy, presence of rhinitis, positive and presence of bronchial hyper-reactivity are important risk factors for the persistence of asthma in children. Allergen-induced $CD4^+CD25^+$ T cells were higher in the atopic children who outgrew their disease, implicating an immunological mechanism of asthma remission in children.

Keywords

Asthma, atopy, bronchial hyper-reactivity, childhood, $CD4^+CD25^+$ T cell, persistence, remission, rhinitis

History

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Introduction

The long-term outcome for wheezy children has been investigated in several population-based cohorts [1–3]. Previously, three longitudinal patterns (early, late and persistent wheezers) were identified among children between 0 and 6 years of age [1]. Recently, six different longitudinal patterns of wheezing between birth and the age of 13 years were described, adding further complexity to the previous classification [3]. The general consensus now emerging is that, even in adults, asthma is unlikely to be a single disease entity, and strategies are being developed to distinguish different phenotypes and sub-phenotypes [4]. These observations pose the question whether various and overlapping phenotypes simply represent different time points in a single underlying pathological process in people with different predispositions [5].

Pathogenesis of atopic diseases has been explained by a dysregulation in the T Helper (Th1/Th2) balance. While Th2 cells infiltrate into the affected tissues of acute allergic tissue reactions, chronic allergic reactions may be characterized by the infiltration of both Th1 and Th2 cells. Meanwhile, the description of a subset of $CD4^+$ T cells named ''regulatory T cells'' (Tregs) has produced new insights into the concept of immune regulation [6]. Current concepts of allergy pathogenesis postulate that development of a healthy or an allergic immune response is determined by the ratio between Tregs, particularly Th2 and Th1 cells. In healthy individuals, $CD4^+CD25^+$ Tregs play an essential role in modulating and regulating immune responses by promoting tolerance [6,7]. Several studies have shown that the number and function of Tregs are impaired or altered in allergic patients, compared with healthy individuals [8–10]. However, the role of $CD4^+CD25^+$ Tregs in asthma phenotypes remains unclear.

In the current study, we aimed to investigate the long-term outcome of childhood asthma in patients followed up at a pediatric allergy and immunology clinic over a period of 10 years. In addition to evaluating roles of clinical parameters in predicting outcomes, we investigated whether different phenotypes, distinguished by persistence of symptoms and atopic sensitization, exhibited distinctive immunologic patterns, primarily in terms of the alterations in generation of allergen-induced $CD4^+CD25^+$ T cells and production of allergen-specific IgE and IgG4 antibodies.

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Methods

Subjects

We had previously evaluated 279 asthmatic children who had been followed up for 3 years at Marmara University Pediatric Allergy and Immunology Division [11]. We randomly selected 100 patients from this previous study subjects but we could reach 74 patients and 62 of them accepted to attend the study. In the current study, we re-evaluated 62 randomly selected patients, 10 years after the first admission. There was no statistically significant difference between those selected and the rest of the cases in terms of demographic properties ($p > 0.05$) (data not shown). Diagnosis and severity of allergic rhinitis and asthma were judged according to GINA and ARIA guidelines [12,13]. The diagnosis of asthma was based on symptoms of repetitive symptoms (wheeze, cough, breathlessness typically manifested by patterns of activity limitation), nocturnal symptoms/awakenings) and a strong family history of asthma in first degree relatives. The diagnosis of allergic rhinitis was based on symptoms of repetitive sneezing, nasal itching, secretion and/or obstruction apart from a cold episode. Atopy was defined as a positive prick test to allergens and/or *in vitro* method that detects antigen-specific IgE antibody [14]. Non-atopy was defined as negative skin test reactivity to allergens. Written consents were obtained from parents of all the children, and the study protocol was approved by the Institutional Review Board of Marmara University.

Study design

At the 10th year assessment, patients were asked to complete a questionnaire requesting clinical information, including characteristics and severity of symptoms, use of medications, family history of allergic disorders and environmental risk factors. All patients underwent physical examination, skin prick testing (SPT), a pulmonary function test (PFT) and bronchial provocation testing; allergen-specific IgE and IgG4 levels were also measured.

After this initial assessment, 62 patients who were available for clinical evaluation were grouped into three categories, and, as a negative control, a fourth group comprising age-matched, non-atopic, healthy control children was included solely for comparing allergen-specific, allergen-induced generation of $CD4^+CD25^+$ cells. These groups were allocated by randomly selecting seven patients for each group, as follows:

Group 1 (atopic/symptomatic, $n = 7/31$): Patients with current asthma and rhinitis symptoms with SPT positivity to house dust mite (HDM) and positive bronchial hyper-responsiveness (BHR).

Group 2 (atopic/asymptomatic, $n = 7/15$): patients with no current asthma and rhinitis symptoms, with a positive SPT to HDM and negative BHR.

Group 3 (non-atopic/asymptomatic ($n = 7/16$): patients without asthma and rhinitis symptoms, negative SPT and BHR.

Group 4 (Control group, $n = 7$): Non-atopic healthy controls, age and gender-matched children confirmed by an ISAAC questionnaire, physical examination, SPT, PFT and bronchial provocation testing were recruited specifically to serve as negative controls for immunologic parameters.

Follow-up and therapy

Patients were followed up and treated according to the asthma treatment protocol at the Division of Pediatric Allergy, Marmara University. According to this asthma treatment protocol, patients with intermittent asthma received only inhaled beta-2-agonists on an as needed basis. Patients with persistent asthma were assigned to receive inhaled budesonide for a total daily dose of 800 mcg. At the end of 1 month, the dose was decreased to 400 mcg/day, if an improvement had been achieved. The dose was then tapered during follow-up visits (every 2–3 months) to the lowest dose needed to control symptoms [11].

Asthma remission and persistence

Asthma remission is defined as the absence of any asthma symptoms and medication for at least 12 months. Persistent asthma is defined as the presence of wheezing or any other exercise-related symptoms or use of rescue or controller medications within the last year of observation.

Skin prick testing

The cutaneous response to allergens and new sensitizations was assessed before treatment, and repeated at 3rd year and 10th year examinations. SPTs were performed with 20 common aeroallergens: mites, latex, molds, pollens, animal dander and insects (ALK-bello, Lainate, Italy), as described previously [15].

Pulmonary function and bronchial methacholine provocation test

Lung functions were assessed with a spirometer (Sensormedics, S3513; Sensormedics, CA). All patients were free of acute respiratory tract infection for 4 weeks, and required no bronchodilator therapy for 3 days prior to pulmonary function and bronchial methacholine provocation tests. A PC20 value of <8 mg/ml was considered positive for bronchial hyper-responsiveness. Positive BHR was further classified as severe BHR (PC20 <0.25 mg/ml), moderate BHR (between 0.25 and 2 mg/ml), mild BHR (between 2 and 8 mg/ml) and negative BHR (between 8 and 32 mg/ml) [16].

Quantification of serum total IgE and allergen-specific antibodies

Serum total IgE level was measured with the immulite method, both at referral and at the 3rd year visit. The IgE and IgG4 anti-Der-p-1-specific antibody contents in serum were measured by ELISA. Briefly, ELISA plates (Maxisorb, Roskilde, Denmark) were coated with 10 μg/ml Der-p-1 overnight and left in phosphate buffered saline (PBS) at 4 °C. Uncoated parts were blocked with PBS, pH: 7.4, containing 3% Top-Block and 1% TWEEN20 (both from Sigma-Aldrich Co., Buchs, Switzerland). Standards and serum samples were added in eight-step 1:2 serial dilutions in duplicates, and incubated 2 h at 4 °C. Biotinylated anti-IgEmAb 6–7 (Novartis, Basel, Switzerland) and peroxidase-labeled ExtrAvidine (Sigma Chem. Co., St Louis, MD) were used to develop IgE anti-Der-p-1. Anti-IgG4 mAb PJ4 (Oxoid Ltd., Basingstoke, UK), and peroxidase-labeled anti-mouse Ig

antibodies (Tago AG, Burlingame, CA) were used in IgG4 anti-Der-p-1 ELISA. Specific antibody binding to coated plates was controlled with hydrolyzed milk powder and human serum albumin (Sigma Chem. Co). Mouse and human mAbs to different antigens were used as negative controls. Uncoated parts were blocked with PBS pH: 7.4 containing 3% Top-Block and 1% TWEEN 20. A pooled serum from four Der-p-1-allergic patients was used as a standard. High amounts of Der-p-1-specific antibody containing serums were selected separately for each of the antibody isotypes or subtypes. Cut-off for allergen-specific antibodies was determined by mean + 3 SD of absorbance obtained in control wells (eight wells for each antibody), to which only dilution buffer (1% BSA in PBS) was added.

PBMC isolation and culture

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll (BiochromKG, Berlin, Germany) density gradient centrifugation of peripheral venous blood. Cells were washed three times and resuspended in RPMI 1640 medium supplemented with mM sodium pyruvate, 1% MEM non-essential amino acids and vitamins, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-mercaptoethanol (all from Life Technologies, Basel, Switzerland) and 10% heat inactivated FCS (Sera-Lab, Sussex, UK). PBMC were stimulated with PHA (5 μg/ml), Der-p-1 (5 μg/ml), and OVA (5 μg/ml) for 5 days.

T cell isolation

Pure T cells were isolated from Der-p-1 stimulated PBMCs using human Pan T cells isolation kit (MiltenyiBiotec). Briefly, anti-CD14, anti-CD16, anti-CD19, anti-CD56, anti-CD36, anti-CD123 and anti-CD235a were added to PBMCs in order to get pure T cells without B cells, NK cells, dendritic cells, monocytes, granulocytes and erythroid cells. After purification, T cells were stimulated with anti-CD2/3/28 antibodies for 3 days. Viability of T cells was assessed by uptake of 1μM ethidium bromide, and flow cytometric (BD Biosciences, San Jose, CA) analysis for $CD4^+CD25^+$ T cells was performed.

Statistical analysis

Values are presented as mean ± SD and median (range), unless otherwise specified. Comparisons for quantitative variables were performed by parametrical analysis, independent-sample *T* test and one-way ANOVA tests for non-related samples. Comparisons at two different times were carried out using the paired-samples T for related samples. By using persistence of asthma symptoms as a dependent variable in a multiple logistic regression model, independent predictors of persistence of symptoms were determined. Variables associated univariately were included in the multiple regression model (enter stepwise selection). Significance was set at $p < 0.05$. SPSS 13.0 (SPSS, Inc., Chicago, IL) was used for analysis.

Results

Characteristics of study population

We studied 62 patients (34 male, 28 female) with a mean age at referral and duration of follow-up of 5.49 ± 3.25 and 10.30 ± 1.27 years, respectively. Mean age at final assessment was 15.9 ± 3.6 years. Among 62 children, seven (11%) children had ≥1 asthma attack in the last year. Mean FEV1 value of the patient population was 87 ± 15% while mean $MEF_{25–75}$ value was 74 ± 22%. Analyzing changes in atopic sensitization over time revealed that, among these atopic children, 44 (71%) conserved their atopic status, whereas seven (11%) turned out to be non-atopic at final examination. Nine patients remained non-atopic throughout the study period, while two were initially non-atopic but had become sensitized by the time of the final examination. At current evalution, 46 patients were sensitized to at least one aeroallergen and all sensitized patients had HDM sensitization, 15 of them had multiple sensitizations (11 pollens, 2 pollens and animal danders, 2 molds).

All of the patients received inhaled budesonide, 18 of them received a combination therapy with a long-acting agonist and/or a leukotriene receptor antagonist. Thirty-one out of 62 (50%) outgrew their disease at the end of 10 years of follow-up. These 31 patients had not had any symptoms during the previous 2 years, the mean duration of asymptomatic period for these patients being 56.61 ± 23.50 months (24–95 months). Among this group, the mean age of cessation of therapy was 11.13 ± 3.8 years.

Characteristics of patients according to resolution of symptoms

Patients with symptoms during the previous 12 months

Thirty-one out of 62 patients continued to have asthma symptoms (current asthma) during the previous 12 months. Univariate analyses revealed that factors related to current asthma were positive family history of allergy ($p = 0.041$), atopic sensitization ($p < 0.0001$), presence of bronchial hyperresponsiveness ($p < 0.0001$) and persistent symptoms of rhinitis ($p < 0.0001$). Compared to those with no current symptoms, patients with current asthma had longer duration of symptoms at referral, higher IgE levels at baseline and lower PC20 value at final assessment (Table 1). In the logistic regression analysis, BHR, persistent symptoms of rhinitis were found to be independently associated with persistence of symptoms (Table 2). In addition, patients with current asthma were found to have significantly lower initial FEV1, PEF and $MEF_{25–75}$ values at baseline, which continued to be low at final assessment (Figure 1, Table 1).

Factors related to age at onset and recovery from symptoms

The age at onset of symptoms in atopic asthmatics was higher than that of the non-atopic patients ($p = 0.01$, Figure 2A). Among the factors evaluated, only atopy and environmental tobacco smoke (ETS) were found to be related to delayed resolution of symptoms. Ten-year follow-up of children with asthma revealed that atopic sensitization has a significant impact on the persistency of asthma, in that all non-atopics recovered by 18 years of age, with a recovery rate of 70% before age of 10 years. On the other hand, only 33% of those with atopy recovered, and only 25% before 10 years (Figure 2B). Patients with atopy had an older age of resolution of symptoms than non-atopics (9.26 years versus

Table 1. Characteristics of the study population and risk factors for presence of asthma symptoms during last year.

		Presence of symptoms during last year		
	Whole group ($n=62$)	No ($n=31$)	Yes ($n=31$)	p
Age (years), mean (±SD)	15.9 (3.6)	15.2 (3.4)	16.6 (3.7)	0.131
Gender (female), *n* (%)	28 (45.1)	14 (45.1)	14 (45.1)	1
Age at onset of symptoms (years), mean (±SD)	3.74 (3.14)	3.42 (3.38)	3.91 (2.62)	0.529
Age at diagnosis (years), mean (±SD)	5.7 (3.16)	5.27 (3.37)	6.14 (2.94)	0.291
Duration of symptoms prior to therapy (years), mean (±SD)	2.5 (1.9)	2.06 (1.78)	2.83 (2.10)	0.126
Age at cessation of treatment, mean (±SD)	11.1 (3.8)	10.48 (3.86)	12.1 (3.57)	0.135
Family history of allergy, *n* (%)	33 (53)	12 (39)	21 (68)	0.041
Atopic dermatitis, *n* (%)	14 (23)	4 (13)	10 (31)	0.127
Persistent symptoms of rhinitis, *n* (%)	31 (50)	2 (7)	29 (94)	<0.001
Initial IgE level (mg/dl), mean (±SD)	541 (578)	299 (440)	713 (509)	0.003
IgE level (mg/dl) at 3rd year follow-up, mean (±SD)	619 (851)	193 (347)	1004 (302)	0.009
Der-p-1 specific IgE, mean (±SD)	750 (970)	312 (620)	1126 (1066)	0.002
Der-p-1 specific IgG4, mean (±SD)	177 (239)	94 (157)	250 (276)	0.015
Initial prick test positive, *n* (%)	51 (82)	21 (68)	30 (97)	0.006
Final prick test positive, *n* (%)	46 (74.1)	15 (48.4)	31 (100)	<0.001
Initial MEF25-75 (% predicted), mean (±SD)	74 (22.7)	88 (23.4)	66 (18.9)	0.04
MEF25-75 (% predicted) at 3rd year follow-up, mean (±SD)	87 (33.6)	97 (37.2)	81 (30.6)	0.24
MEF25-75 (% predicted) at 10th year, mean (±SD)	105 (28.7)	118 (24.9)	92 (26.8)	0.002
Initial FEV1 (% predicted), mean (±SD)	87 (15.1)	93 (14.3)	83 (114.9)	0.44
FEV1 (% predicted) at 3rd year follow-up, mean (±SD)	89 (15.6)	94 (17.8)	86 (15.6)	0.22
FEV1 (% predicted) at 10th yearfollow-up, mean (±SD)	97 (13.8)	101 (8.5)	92 (16.4)	0.006
FEV1 reversibility (%), mean (±SD)	6.7 (7.1)	5.9 (3.1)	7.5 (9.6)	0.273
PC20 value, mean (±SD)	5.2 (7.9)	9.8 (9.9)	1.3 (1.8)	<0.001
Bronchial provocation test positive, *n* (%)	40 (65)	10 (32)	30 (97)	<0.001

FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; MEF25-75, mid-expiratory flow; PC20, concentration of methacholine causing FEV1 to decrease by 20%; IgE, immunoglobulin E; Der-p-1, *Dermatophagoidespteronyssinus*.

Table 2. Logistic regression analysis of risk factors and their association with presence of symptoms during last year.

			95% confidence interval	
	p Value	Odds ratio	Lower	Upper
Bronchial hyper-responsiveness	0.009	30.195	2.456	942.811
Rhinitis	0.032	9.331	1.430	149.127
Family history of allergy	0.216	3.077	0.625	1.298
Atopic sensitization	0.998	4.423	0.001	1.027
Initial IgE level	0.497	1.001	0.999	1.002
Age at onset of symptoms	0.694	0.934	0.625	1.298

12.14 years, $p=0.048$). Age at resolution of symptoms was significantly higher among patients exposed to ETS (11.3 years versus 8.47 years, $p=0.036$) compared to those who were not.

Immunologic assessments

Compared to those with no current symptoms, patients with current asthma had higher Der-p-1-specific IgE and IgG4 antibody levels at final assessment (Table 1). In both atopic groups, whether symptomatic or not, percentages of Der-p-1 stimulated $CD4^+CD25^+$ T cells were significantly higher than in an unstimulated condition ($p<0.05$). In the non-atopic group and healthy controls, there was no significant difference in percentages of $CD4^+CD25^+$ T cells in response to Der-p-1 stimulation. Atopic children sensitized to HDM who outgrew their symptoms were distinguished by significantly higher Der-p-1 induced $CD4^+CD25^+$ T cells than healthy controls (Figure 3).

Discussion

In this article, we present a 10-year follow-up of children with asthma, 50% of them with continuing symptoms at a mean age of 15.9 years. We identified two risk factors independently related to the persistence of symptoms: presence of bronchial hyper-responsiveness and presence of rhinitis. All the non-atopic patients outgrew their disease during the study period, while 67% of atopic patients did not, indicating the importance of atopy for persistency. Additionally, our results revealed that those atopic children who outgrew their disease had significantly higher numbers of allergen induced $CD4^+CD25^+$ T cells.

Non-specific BHR is a common characteristic of asthma and is shown to be a risk factor in the development and outcome of asthma [17–19]. But the methacholine test for BHR is still not a perfect test due to variability in its sensitivity and specificity. These variations in sensitivity and specificity of methacholine challenge test have been attributed to different study populations and different methacholine particle sizes [20–22]. As recently showed by Naji et al reporting that the variability in the particle size produced by different nebulizers had effected methacholine PC20 value [22]. In the Childhood Asthma Management Program (CAMP) study, which included 1041 asthmatic children, remission was associated with lack of sensitization and allergen exposure, milder symptoms, older age, higher FEV1 and less bronchial hyper-responsiveness [23]. In another 25-year follow-up study of a community sample of children with asthma, 25% reported current disease [24]. Factors measured at the age of 7 years that independently predicted current asthma were female sex, history of eczema, low PFT, a positive family history of asthma, late onset (after 2 years) and frequent wheezing. In a birth cohort from New

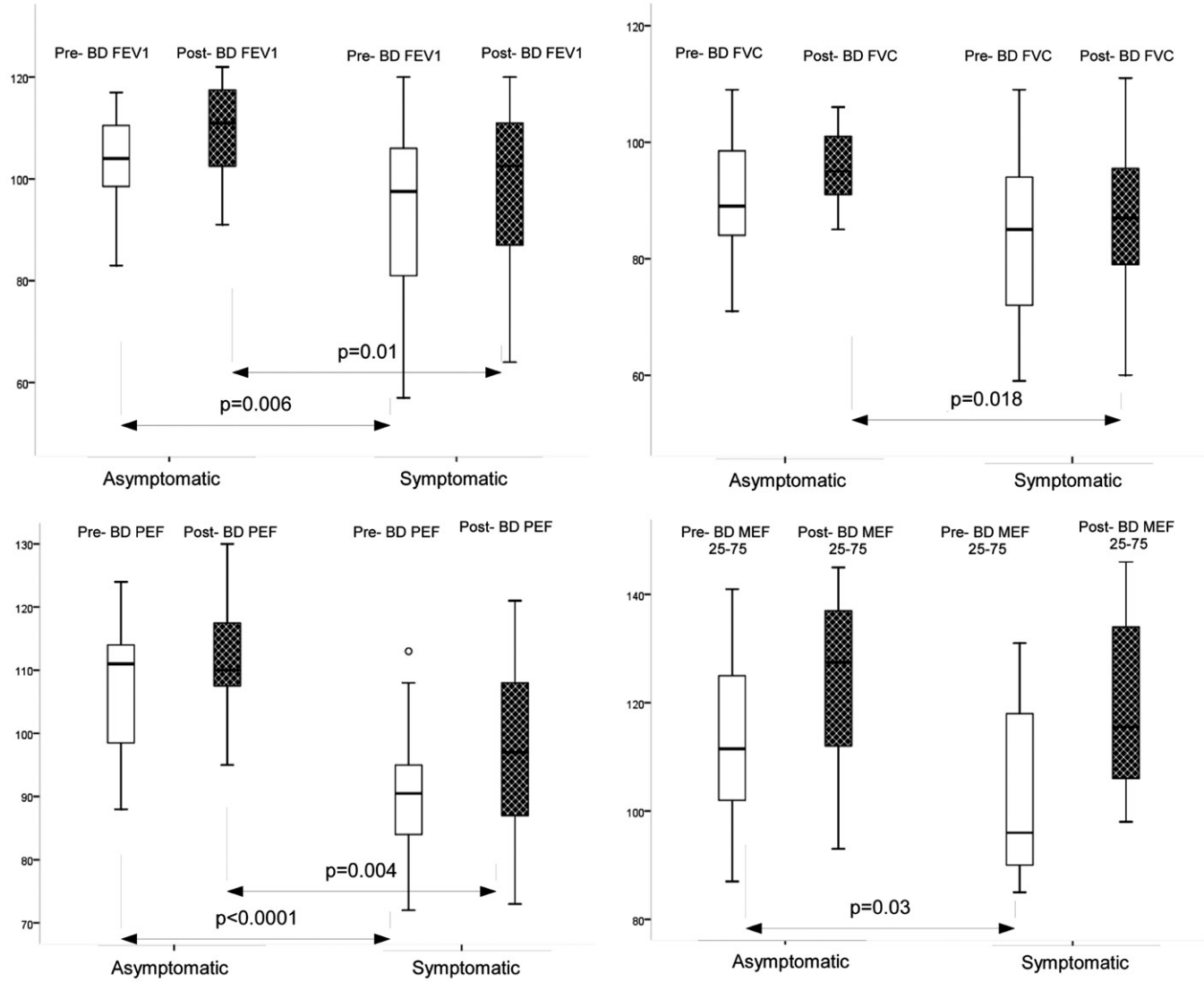

Figure 1. Pre- and post-bronchodilator spirometric measurements of patients according to presence of symptoms during last year. BD: Bronchodilator. Percent predicted values are indicated.

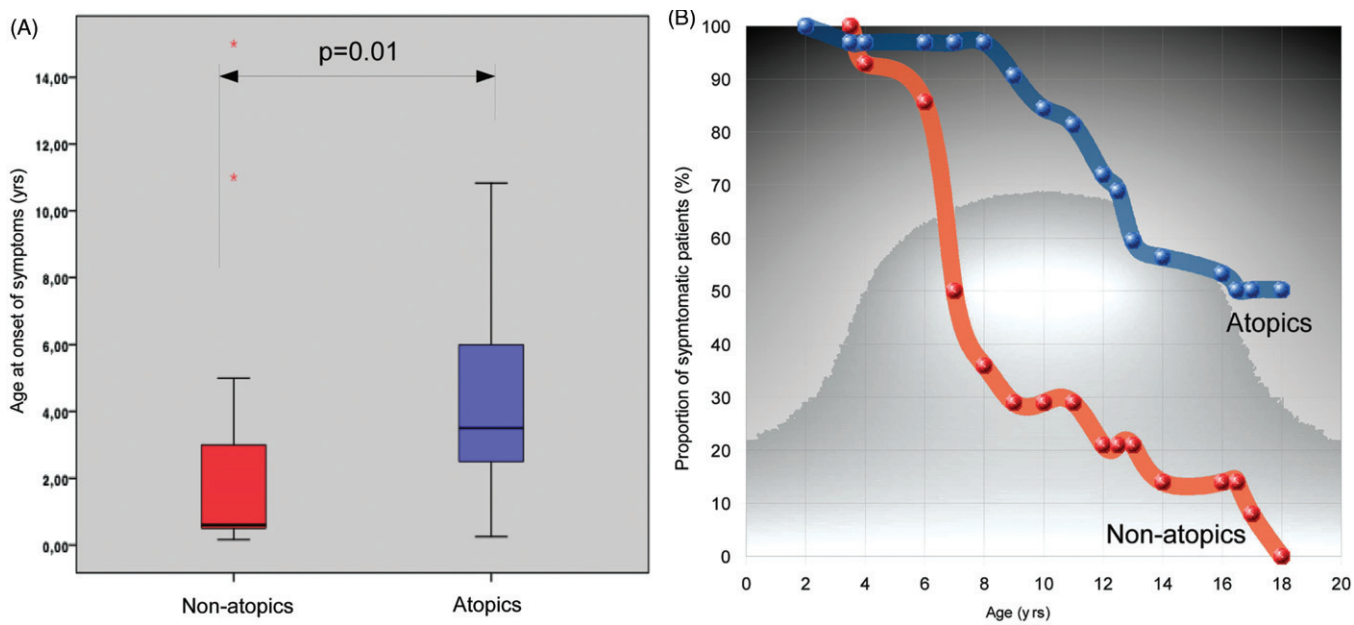

Figure 2. Patterns in presentation of asthma symptoms in relation to age. (A) The age at onset of symptoms in atopic asthmatics was higher than the non-atopic patients ($p = 0.01$, Figure 2A). (B) Proportion of symptomatic children at each age according to the presence of atopy. *Significance $p < 0.05$.

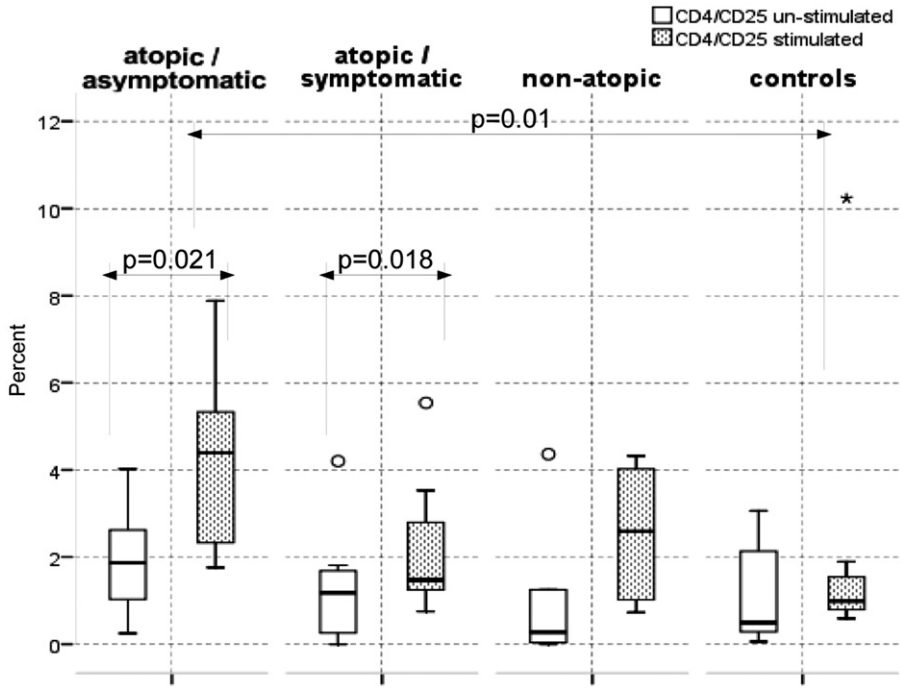

Figure 3. Comparison of $CD4^+CD25^+$ cells between four groups. Percentages of $CD4^+CD25^+$ cells obtained with no stimulation and after Der-p-1 are demonstrated. Atopic children sensitized to HDM who outgrew their symptoms were distinguished by significantly higher Der p-1 induced $CD4^+CD25^+$ T cells than healthy controls ($p < 0.05$). *Significance $p < 0.05$.

Zealand, participants were assessed at 9, 11, 13, 15, 18, 21 and 26 years of age [25]. Approximately one-third of study members (35%) with asthma in remission at 18 years of age relapsed by 21 years or 26 years of age. Atopy, BHR and lower FEV1/FVC ratio at 18 years of age were significant independent factors for a poor prognosis. Bronchial hyper-responsiveness to cold dry air at 6 years of age was a risk factor for asthma by 22 years of age in the Tucson Children's Respiratory Study [26]. Our findings provided further proof for the previously indicated risk factors, namely, atopy and BHR.

In a cohort study of childhood asthma followed up to adulthood, female sex, smoking and atopy appeared to be associated with persistence of asthma, and earlier age of onset was associated with greater risk of relapse [27]. The Melbourne Asthma Study investigated the long-term outcome of wheezing in a cohort of children from 7 years of age until their 40's, before the availability of inhaled steroids. Persistence of symptoms into adulthood was associated with the severity of symptoms in childhood; a detailed analysis of the allergic features of these subjects at all reviews up to age 35 years indicated that the presence of an atopic condition increased the risk of more severe asthma in adult life [28,29]. The Multicenter Allergy Study (MAS) recruited 815 unselected newborns and 499 newborns with high risk of atopy, with a follow-up of 58% by age 13 years. They stratified children with current wheezing at 5–7 years of age by means of concurrent sensitization. Sensitization was a strong risk factor for persistence of wheezing during schoolage, with 46% of sensitized wheezers at early schoolage having persistent symptoms at 13 years of age, compared with 10% of non-sensitized wheezers [30].

In this 10-year follow-up study, 50% of patients had persisting asthma symptoms. Analyses of patterns of outgrow in our cohort revealed two distinct phenotypes: one with an early onset transient course, and the other, late onset persistent asthma associated with atopy. Among patients who outgrew their symptoms, atopy and passive exposure to ETS were the two critical factors related to a delay in recovery from symptoms. Regarding the age of resolution of symptoms, children with atopy began to outgrow their symptoms after 10 years of age, as opposed to non-atopic counterparts, about 70% of whom had already recovered before this age (Figure 2).

In the past decade, only a few studies have focused on the role of Tregs in common allergic diseases, some suggesting a pathogenic [31] and others a protective role in asthma [32,33]. It is suggested that Tregs may suppress allergic responses to inhaled antigens such as grass or birch pollen [34,35]. It has been previously shown that children who outgrew milk allergy had higher frequencies of circulating $CD4^+CD25^+$ T cells and decreased *in vitro* proliferative responses to bovine β-lactoglobulin, compared to those whose allergy persisted [36]. Furthermore, depletion of CD25+ cells from PBMCs of tolerant children led to an increase in *in vitro* proliferation against β-lactoglobulin, suggesting that mucosal induction of tolerance of dietary antigens is associated with the induction of $CD4^+/CD25^+$ cells. The role of $CD4^+CD25^+$ Treg cells in the manifestation of allergy was studied by Wang et al. in HDM sensitized asthmatic patients. They found that house dust mite (HDM) sensitized asymptomatic asthmatic patients had higher percentages of both $CD4^+CD25^+$ Treg cells than those from HDM sensitized asthmatic patients [37]. Similarly, in our study group, atopic children who outgrew their asthma demonstrated a 3-fold higher percentage of Der-p-1-stimulated $CD4^+CD25^+$ T cells compared to atopic children whose symptoms persisted (Figure 3).

In conclusion, our 10-year follow-up of children with asthma revealed that the status of atopy in patients determines persistence of asthma, in that all non-atopics recovered by 18 years, most of them before the age of 10 years. On the other hand, asthma persisted in two-thirds of those with atopy. Two risk factors independently were found to be related to the persistence of symptoms: presence of bronchial hyper-responsiveness and presence of rhinitis. Among those with

atopic asthma who recovered, allergen induced $CD4^+CD25^+$ T cells were higher, implicating the importance of therapeutic strategies targeting induction of such cells.

Declaration of interest

None of the authors declared a conflict of interest.

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