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Early molecular biomarkers predicting the evolution of allergic rhinitis and its comorbidities: A longitudinal multicenter study of a patient cohort.

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Conflict of interest

S. Tripodi has received a lecture fee from Thermo Fisher Scientific (Phadia) and he is cofounder of TPS Production. A. Dondi has received consultancy fees from Charité University Hospital, Berlin, Germany. C. Mastrorilli has received fellowship from Italian Society of Pediatrics. G. Ricci has received a grant from Thermo Fisher Scientific (Phadia) for research support. P. M. Matricardi has received research support from TFS and lecture fees from TFS and Allergopharma. The rest of the authors declare that they have no relevant conflicts of interest.

Abstract

Background: Pollen-related seasonal allergic rhinoconjunctivitis (SAR) is a very frequent pediatric disease in westernized countries. Risk factors and disease phenotypes have been thoroughly examined in several cross-sectional studies. By contrast, only a few studies have examined disease evolution in patient cohorts. We investigated predictive biomarkers of disease evolution in a large cohort of children with SAR.

Methods: During 2015-2017 (follow-up), we re-examined 401 patients from those enrolled in 2009-2011 (baseline) by the "Panallergens in Pediatrics" study, a large multicenter survey of Italian children with SAR. Information on clinical history (standard questionnaire, AllergyCard®, TPS, Italy) and skin prick tests for inhalant and foods extracts (ALK-Abelló, Hørsholm, Denmark) was acquired as at baseline visit. Evolution in clinical and sensitization data of patients was analyzed over time, as well as their association with the main baseline characteristics and atopy risk factors.

Results: The average age of participants was 10.4±3.4 yrs at baseline and 16.2±3.6 yrs at follow-up. SAR persisted in 93.3% of patients at follow-up and became more frequently associated with asthma (from 36.7% at baseline, to 48.6% at follow-up) and oral allergy syndrome (OAS) (from 23.4% to 37.7%). Compared to baseline, the prevalence of skin sensitization to some pollens (*Phleum pratense*, *Corylus avellana*, *Platanus acerifolia*, *Artemisia vulgaris*) and vegetables (hazelnut, wheat and apple) significantly decreased at follow-up. Earlier onset of SAR and polysensitization at baseline were associated with incident

asthma at follow-up. The presence at baseline of serum IgE to the following allergen molecules were identified as biomarkers of clinical evolution: (1) Phl p 1, for persistence of SAR; (2) Phl p 5, for persistence of both, rhinitis and asthma; (3) Pru p 3, for new onset of asthma; (4) Bet v 1, for persistence of OAS.

Conclusions: SAR is clinically heterogeneous in its evolution from childhood to adolescence. The detection of serum IgE to specific molecules (Phl p 1, Phl p 5, Bet v 1, Pru p 3) may be useful as biomarkers to predict SAR persistence and future onset of comorbidities, such as asthma and/or OAS.

Keywords: Allergic rhinitis; Asthma; Bet v 1; Biomarkers; Children; comorbidities; IgE; longitudinal study; Phl p 1; Phl p 5; pollen; prediction; Pru p 3**.**

Introduction

Seasonal allergic rhinoconjunctivitis (SAR) induced by pollens (pollen-induced SAR) affects millions of people globally, with a particularly relevant prevalence in the pediatric age and high social and economic burden (1,2). The severity of SAR, its association with other allergic conditions (e.g. asthma, oral allergy syndrome - OAS), its response to pharmacotherapy and to allergen immunotherapy (AIT) can be widely different among patients (3). “Panallergens in Pediatrics” study (PAN-PED) was the first nationwide observational multicenter survey carried out by the I-PAN group with the aim to investigate the impact of sensitization to highly cross-reacting allergenic molecules on the diagnostic and therapeutic management of respiratory allergies in childhood (4,5). The baseline study recruited 1360 children affected by pollen-induced AR who had never undergone AIT in 16 pediatric outpatient clinics between May 2009 and June 2011. Patients’ clinical features and allergic sensitization have been widely described in the previous articles (6-11). In those suffering from grass pollen-induced SAR, we showed a wide heterogeneity of molecular IgE sensitization profiles to *Phleum pratense,* and we identified three main serological biomarkers: Phl p 1 as a marker of grass pollen allergy, Phl p 7 as a specific biomarker of asthma among SAR patients, and Phl p 12 as a stronger biomarker of OAS (11). The usefulness of these biomarkers in the diagnostic work-up of the child with grass pollen allergy needs to be tested in prospective studies of patient

cohorts. Meanwhile, the evolution of SAR and the progression of allergic sensitization in childhood are still lacking of evidence and there are no validated or generally accepted candidate biomarkers to predict the clinical response to AIT (12). The present study aims at investigating the evolution of clinical features and allergic sensitization of the previous recruited patients of the PAN-PED cohort and analyzing their association with the main baseline characteristics of patients. The final target is therefore to identify potential predictive factors for clinical evolution of SAR.

Materials and methods

Study design and population - The prospective follow-up study (PAN-PED FU) was performed in 11 pediatric outpatient clinics from eight Italian cities in Northern Italy (Milan, Verona, Parma and Bologna), Central Italy (Empoli and Rome), and Southern Italy (Naples and Benevento) between March 2015 and December 2017. Six of the original 16 recruiting centers (Genoa, Ascoli Piceno, Ostia, Crotone, Cagliari, Palermo) of the PAN-PED cohort didn't participate in the present follow-up study. Criteria for eligibility to the original study (2009-2011) were: (i) age 4–18 yrs; (ii) a history of pollen-induced AR and/or asthma in one of the two last pollen seasons; (iii) positive skin prick tests (SPT) for the relevant pollen extracts. Criteria for eligibility to this follow-up study (2015-2017) were: (i) participation to PAN-PED study in the years 2009-2011 and (ii) signed informed consent, (iii) absence of any other severe chronic disease. Recruited children's parents answered to a standardized questionnaire, and patients underwent skin prick tests (SPTs) and a blood sample collection, as for the baseline study. The PAN-PED FU study design and procedures have been approved by the ethical committee of S. Orsola-Malpighi Hospital of Bologna (103/2009/O/Oss, EM 178/2014/O and 210/2015/U) and of all the participating centers. Patients or their parents or tutors of all participants signed an informed consent before being involved in the clinical investigations.

Questionnaire - An updated version of the electronic Clinical Record Form (e-CRF) already used at baseline (AllergyCARD®, Technology Projects & Software Productions [TPS], Rome, Italy) was used for data input and collection in the follow-up study. This new version of AllergyCARD included a standardized questionnaire to assess health-related quality of life (HRQoL) (13), questions about pharmacotherapy drugs administered in the previous pollen season and its efficacy on symptoms, allergy related to lipid transfer protein (LTP) in patients who referred allergic reactions to peach, and informations about AIT and its subjective efficacy on AR symptoms for patients who underwent AIT after the baseline study. Questionnaires were administered to all participants by well-trained physicians. A diagnosis of current pollen-induced AR was made in the presence of nasal and/or eye symptoms (apart from cold) for at least 3 weeks during the last pollen seasons. The annual period of symptoms was defined as the months with symptoms in the 12 months preceding the recruitment visit. In presence of a doctor-diagnosed pollen-induced asthma, symptoms severity were classified according to Global Initiative for Asthma - GINA (14). Oral allergy syndrome (OAS) was defined as the occurrence of immediate oral itching with or without angioedema of the lips and or the tongue following the ingestion of the food, as previous described (7). The presence of other allergic symptoms (doctor-diagnosed urticaria/angioedema, anaphylaxis, gastrointestinal symptoms) after food ingestion has been investigated in the last 5 years.

Skin prick tests - Allergic sensitization was assessed by SPTs using the same panel of aeroallergens (*Phleum pratense, Cynodon dactylon*, *Parietaria judaica*, *Plantago lanceolata, Chenopodium album*, *Artemisia vulgaris*, *Salsola kali, Ambrosia artemisiifolia*, *Cupressus arizonica*, *Betula verrucosa*, *Platanus acerifolia*, *Olea europea*, *Corylus avellana*) and food allergens (peach, apple, wheat, soybean, peanut, hazelnut), profilin and latex used in the previous study (ALK-Abelló, Hørsholm, Denmark). In addition, patients underwent SPT also for *Dermatophagoides pteronyssinus*, cat, and alternaria. Histamine dihydrochloride 10 mg/ml and glycerol saline solution were used as positive and negative controls, respectively. Morrow-Brown needles were used to prick the skin, and the wheal reactions were read after 15 min. A wheal ≥3 mm was regarded as positive (15).

Serological biomarkers at baseline **–** Serum total IgE levels and specific IgE (sIgE) levels to known panallergens (Phl p 7, Phl p 12, Bet v 1, Pru p 3,) to the main *P. pratense* molecular allergens (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 11) and to the perennial allergens *Dermatophagoides pteronyssinus*, *Alternaria alternata* and cat dander had been tested at baseline using a fluorescence enzyme immunoassay (FEIA) with capsulated cellulose polymer solid-phase (Immuno CAP®) coupled allergens (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden). Results were expressed in kU/L and a cut-off point of 0.35 kU/L has been used for positivity.

Statistical analysis - Mean and standard deviation (sd) were used to summarize quantitative data and number of subject (n) and percent (%) to summarize categorical data. Change in binomial data (presence of allergic symptoms, positivity at SPT) between baseline and follow up was tested by McNemar Test, change in quantitative data was tested by paired T test. The analysis of predictive factors of clinical evolution of allergic rhinitis and its main comorbidities (asthma and OAS) was performed by grouping patients in several groups: i) *persistent* if symptoms were present both at baseline and follow-up, ii) *remittent* if symptoms were present at baseline study but not at follow-up; iii) *absent* if patient never had the symptoms, neither at baseline nor at follow-up (only for SAR comorbidities); iiii) *incident* if patients had no symptoms at baseline but had them at follow-up (only for SAR comorbidities). We analysed the correlation between the clinical evolution of AR, allergic asthma and OAS and individual or aggregated serological biomarkers tested at baseline: total IgE levels, sIgE response to known panallergens (Phl p 12, Bet v 1, Pru p 3), sIgE response to the main *P. pratense* molecular allergens, number of *P. pratense* molecules recognized by IgE, sIgE to *Dermatophagoides pteronyssinus*, *Alternaria alternata* and cat dander. Several univariable logistic regression was implemented to evaluate relationship between clinical predictor factors, SPT, sIgE and symptoms evolution separately in Rhinitis remission, Asthma remission, Asthma incident, OAS remission and OAS incidence, odd ratio (OR) and its 95% confidence interval were reported (CI 95%). SAS 9.4 was used for all analysis and a p value <0.05 was considered statistically significant.

Results

Clinical evolution of AR and its comorbidities **-** The study population included 401 patients (64.3% males) out of the 1024 (60.6% males) recruited by the 11 participating centers in the baseline study in the "PAN-PED" project **(Table 1)**. Patients participating in the follow-up study had higher familiar atopy from the father's side, and an increase of other food-related allergic symptoms, particularly urticaria/angioedema and gastrointestinal symptoms, than those not participating at the follow-up study **(Table e1)**. The mean age was 10.4 ± 3.4 yrs at baseline and 16.2 ± 3.6 yrs at follow-up. At baseline, all the patients were suffering from AR, 36.7% of them had asthma, 23.4% oral allergy syndrome (OAS) and 32.2% other food-related allergic symptoms **(Table 1)**. AR persisted at follow-up in 93.3% of patients, whose symptoms period within a year did not significantly change. Compared to baseline, the prevalence of asthma (from 36.7% to 48.6%, $p<0.001$) and OAS (from 23.4% to 37.7%, $p<0.001$) significantly increased, while the prevalence of other food-related allergic symptoms significantly decreased (from 32.2% to 23.4%, $p=0.001$). The overall increase in the prevalence of asthma at follow-up takes into account both cases of remissions and new onset of disease. In fact, among the 147 patients (36.7% of the whole sample) suffering from asthma at baseline, 33/147 (22.4%) experienced the remission of symptoms. Meanwhile, among the 254 patients without a diagnosis of asthma at baseline, 81/254 (31.9%) developed asthma in the following years. Among the 94 patients (23.4% of the whole sample) suffering from OAS at baseline, 31 (33%) experienced the remission of symptoms, meanwhile among the 307 without OAS at baseline 88 (28.7%), developed OAS during the following years **(Table 1)**.

Evolution of atopic sensitization **-** The mean number of positive SPTs reactions slightly but significantly decreased, from 5.4 ± 2.8 at baseline to 5.2 ± 2.8 at follow-up ($p=0.021$). In particular, sensitization to *P. pratense*, *C. album*, *P. acerifolia, C. avellana, A. artemisiifolia,* and the prevalence of highly polysensitized patients decreased at follow-up **(Table 2)**. Similarly, sensitization to food allergens, in particular for hazelnut, wheat and apple, remarkably decreased at follow-up. In contrast, the prevalence of sensitization to cat (from 34.9% to 45.1%, $p<0.001$) significantly increased at follow-up **(Table e2)**. Overall, no variations

in the prevalence of patients with 4 or more positive SPTs to indoor and/or pollen allergens were observed **(Table 2)**.

Predictive biomarkers of clinical evolution – We analysed the association and predictive value of biomarkers of atopic sensitization at baseline and the clinical evolution (persistence/remission and new onset) of (A) allergic rhinitis, (B) asthma, and (C) oral allergic syndrome (OAS).

- ***Predictive biomarkers of persistent or remittent AR -*** Polysensitization to pollen and indoor allergens, a positive SPT to grass pollen and genuine sensitization to Phl p 1, to Phl p 5 were all significantly associated with persistence of AR at follow-up **(Figure 1, Table e3)**. By contrast, gender, age at onset, yearly duration of symptoms, asthma- or OAS-comorbidity at baseline were not associated with remission or persistence of AR symptoms at follow-up **(Figure 1, Table e3)**.

- ***Predictive biomarkers of persistent or incident asthma -*** Persistence of asthma at follow-up was weakly, but significantly associated with a higher spreading of the sIgE response to *Phleum pratense* molecules at baseline, and with a higher prevalence of sensitization to Phl p 5 **(Figure 2, Table e4)**. Incident asthma was instead associated with an earlier onset of AR symptoms, a higher number of positive SPT to indoor allergens and to airborne allergens in general, a higher total IgE levels and presence of sIgE to Pru p 3 at baseline **(Figure 2, Table e4).**

- ***Predictive biomarkers of persistent or incident OAS –*** Persistency of oral allergy syndrome (OAS) was associated with female gender, with a longer duration of AR symptoms, asthma at baseline and with IgE sensitization to Bet v 1 at baseline **(Figure 3a, Table e5)**. Incident OAS at follow-up was associated with polysensitization to airborne allergens **(Figure 3b, Table e5**).

Discussion

This longitudinal, prospective study examined the clinical evolution of seasonal allergic rhinitis and its predictors in 401 Italian pediatric patients followed for about 6 years. To our knowledge, this is one of the few studies identifying various biomarkers of AR remission and of incident or persistent asthma and OAS in children with seasonal allergic rhinitis.

Absence of IgE to Phl p 1 predicts AR remission - We found an extremely low rate (only 7%) of AR remission at follow-up. A lower atopic status and the absence of sIgE sensitization to the major *P. pratense* molecular allergens **Phl p 1** was found to be associated with remission of AR at follow-up. Phl p 1 is a marker of genuine sensitization to grass pollen **(16, 17)** and by far the most important initiator of grass pollen allergy. We **(18,19)** and others **(20)** have already shown that grass pollen allergic patients are rarely not sensitized to Phl p 1. The present study adds that the lack of sIgE to Phl p 1, an infrequent condition also in this patient cohort, is a predictor of remission of grass pollen allergy in Italian children.

Predictors of persistent or incident asthma – In our patient cohort, a bigger molecular spreading of *P. pratense* IgE responses combined with higher IgE to **Phl p 5** at baseline were associated with persistent asthma. Phl p 5 is a less common sensitizing agent in grass pollen allergic patients than Phl p 1 **(17)**. However, IgE to this molecule provoke stronger degranulation of Mast cells and Basophils **(21)** and has been already associated with more severe disease and asthma **(22)**.

Higher total IgE levels, polysensitization to airborne allergens, IgE to indoor allergens and to Pru p 3 were associated with incident asthma at follow-up. A later onset age of AR was found to be associated with a lower risk to develop asthma, independently of patient's atopic status, and confirmed by multivariate analysis. Most of these associations just confirm remote or recent observations. A higher degree of atopic sensitization **(23,24,25)**, sensitization to mites and other indoor or perennial allergens **(23, 25, 26)**, and

higher total IgE **(27, 28, 29)** are all independent predictors of asthma. A recent population-based birth cohort study identified different cross-sectional sensitization patterns and their longitudinal trajectories (30): the strongest predictor of asthma at age 16 years was IgE sensitization to dust mite molecules, while the strongest early life predictor of subsequent asthma was IgE sensitization to grass/cat molecules. In the MAS birth cohort study, a higher spreading of IgE sensitization to a comprehensive panel of *Dermatophagoides pteronyssinus* allergenic molecules was associated with asthma **(31)**. This study has also revealed that earlier onset of IgE sensitization to indoor allergens is a predictor of incident asthma **(31)**.

Interestingly, the present study adds sIgE to **Pru p 3** to the long list of asthma predictors in children with seasonal allergic rhinitis. Pru p 3 is a lipid transfer protein (LTP) and it is a marker of food allergy and higher risk of anaphylactic reactions **(32, 33)**. IgE sensitization to other nonspecific lipid transfer proteins (nsLTPs), such as Art v 3 and Pla a 3 (mugwort and plane tree nsLTPs, respectively), have been associated with respiratory symptoms in nsLTP-positive patients (34). Our study cannot discriminate whether IgE sensitization to Pru p 3 is causally associated with asthma or is simply a useful proxy of more severe atopic propensity, which in turn is causally associated to allergic inflammation of the lower airways and asthma. This question deserves further investigation.

Predictors of persistent or incident oral allergy syndrome – sIgE to **Bet v 1** at baseline was associated with persistent or incident OAS at follow-up. sIgE to Bet v 1 is a well-known qualitative and quantitative marker of OAS **(35, 36, 37)**. In the Swedish BAMSE cohort study, Bet v 1 was identified as a risk molecule, and as the most prevalent PR-10 protein, with higher amount of specific IgE (25, 38). The sensitization to this molecule in early childhood was associated with severe allergic rhinitis to birch pollen at 16 years of age and with OAS **(38)**. Another interesting outcome of our study was the association of asthma at baseline with persistent OAS. Moreover, both, persistent asthma and persistent OAS were associated with polysensitization to airborne allergens at baseline. Together, these data suggest the presence of a cluster of children with polysensitization and allergy multimoribidity, i.e. atopic disease affecting the upper and lower

airways and the oral cavity. A similar phenotype has been recently described in a broader study of Garcia-Aymerich et al. from the MEDALL study group **(39)**.

On the other hand, the prevalence of positive skin wheal reactions to wheat, hazelnut and apple extracts declined over years in our study population, while the prevalence of OAS increased, especially in subjects with sIgE to Bet v 1 at baseline. This outcome leads to hypothesize that the diagnostic and predictive value of in vitro assays defined at molecular level is much higher than that of in vivo SPT performed with vegetable food extracts. We are currently investigating this hypothesis, that has been also addressed by a recent study in a pediatric population of Spanish children (40). The BAMSE cohort study has shown that, though general IgE sensitization to foods decreases between childhood and adolescence, the sensitization to plant food allergens remains analogous, while milk and egg sensitization decreases (41). The Tucson cohort study also reported a high rate of remission of skin sensitization to many allergens during adolescence (42). Therefore, our study is further reinforcing the concept that the evolution of IgE responses from childhood to adolescence is a very dynamic process characterized by new sensitization and remissions generating a great variety of IgE sensitization profiles at population levels in different ages **(19, 41-43).**

Limitations - We have to acknowledge some limitations of our study. First our study population is a subset of a population of Italian pollen allergic children. The generalizability of our outcomes to populations of different ages and geographic areas is therefore questionable. Second, for economic reasons, we have chosen to examine IgE sensitization to a few, although relevant, allergen molecules with a singleplex approach, while a broader evaluation with a microarray would have been more informative. Third, this longitudinal study is based on two time points only, so that our analysis is limited in its power and we are cannot say how stable are the phenotypes observed at baseline in 2009-2011 and at follow-up in 2015-2017.

Conclusions - The present follow-up study highlighted the heterogeneous clinical course of SAR and its comorbidities from childhood to adolescence and adulthood. The evidence of a low rate of AR remission and a significant increase of AR comorbidities at follow-up remarks the importance to find predictive biomarkers of clinical evolution. The detection of serum sIgE to Phl p 1, Phl p 5, Pru p 3 and Bet v 1 can be useful as clinical biomarkers for evolution of SAR and its comorbidities.

Authors' contribution

GR, PMM, ST and FC contributed to the conception and design of the study. FC, ST, SP, AD, RB, CC, AC, RC, LC, PC, GDC, MMDG, IDI, ADRB, MG, AG, CM, VM, SP, IS, EV, VV, AMZ, GB, and GR contributed to the acquisition of data. VP and FC performed the statistical analysis of data under the supervision of PMM. FC, VP, EK and PMM contributed to the interpretation of the results and in drafting the manuscript. All authors revised and approved the final version of the manuscript.

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Tables

Table 1. Characteristics of the population sample at baseline and follow-up.

	Baseline N=401	**Follow-up N=401**	***p value***
Males (n, %)	258 (64.3%)		
Age (years) (mean, SD)	10.4 (3.4)	16.2 (3.6)	
Geographic area of Italy			
North (n, %)	193 (48.1)		
Center (n, %)	142 (35.4)		
South (n, %)	66 (16.5)		
Familial atopy			
Father (n, %)	180 (44.9)		
Mother (n, %)	157 (39.2)		
Allergic rhinitis (n, %)	401 (100)	374 (93.3)	
Age at onset (years) (mean, SD)	5.2 (2.8)		
Months/year with symptoms (mean, SD)	4.1 (1.7)	4.2 (2.2)	
Asthma* (n, %)	147 (36.7)	195 (48.6)	**<0.001**
Remittent		33 (8.2)	
Persistent		114 (28.4)	
Incident		81 (20.2)	
Absent		173 (43.1)	
Oral allergy syndrome** (n. %)	94 (23.4)	151 (37.7)	**<0.001**
Remittent		31 (7.7)	
Persistent		63 (15.7)	
Incident		88 (21.9)	
Absent		219 (54.6)	
Other food-related allergic symptoms** (n, %)	129 (32.2)	94 (23.4)	**0,001**
Anaphylaxis (n, %)	21 (5.2)	21 (5.2)	1,000
Urticaria/angioedema (n, %)	93 (23.2)	72 (18)	**0,046**
Gastrointestinal symptoms (n, %)	41 (10.2)	39 (9.7)	0,789

* Doctor-diagnosed Asthma;

** Doctor-diagnosed Oral allergy syndrome or other food-related allergic symptoms.

Table 2. Atopic sensitization assessed by skin prick test to pollens and vegetable food allergens at baseline and follow-up in 401 patients included in the study.

Allergenic extract	Baseline		Follow-up		*p value**	*p value^*
	%	Wheal (mm, SD)	%	Wheal (mm, SD)		
Pollens						
Phleum pratense	93,0	7.0 ± 3.2	86.5	6.8 ± 2.6	**<0.001**	0.228
Cynodon dactylon	76.1	5.5 ± 2.4	75.3	5.8 ± 2.3	0.753	0.217
Olea europea	60.3	6.1 ± 3.0	62.1	6.1 ± 2.6	0.486	0.989
Plantago lanceolata	66.1	5.4 ± 2.8	68.1	5.7 ± 2.7	0.462	0.265
Chenopodium album	67.2	4.7 ± 1.9	54.6	4.9 ± 2.4	**<0.001**	0.341
Cupressus arizonica	37.7	5.2 ± 2.2	45.9	5.8 ± 2.2	**<0.001**	**0.017**
Betula verrucosa	41.1	5.0 ± 1.8	39.2	5.1 ± 1.9	0.419	0.632
Platanus acerifolia	37.9	4.8 ± 2.1	31.7	5.0 ± 2.4	**0.017**	0.529
Parietaria judaica	30.4	5.9 ± 2.8	29.2	6.3 ± 2.7	0.541	0.281
Corylus avellana	46.7	2.2 ± 2.5	38.2	2.0 ± 2.4	**0.03**	0.268
Artemisia vulgaris	27.2	4.8 ± 2.1	20.7	4.9 ± 1.7	**0.009**	0.753
Salsola kali	29.2	4.1 ± 1.5	27.2	4.4 ± 2.6	0.424	0.432
Ambrosia artemisiifolia	21.4	4.1 ± 1.7	26.9	4.3 ± 1.5	0.168	0.458
All (mean, SD)**		5.4 ± 2.8		5.2 ± 2.8	**0.021°**	
Monosensitized	7.8		6.2		0.304	
*2-4 pollen***	33.1		35.9		0.169	
*> 4 pollen***	59.2		55.9		0.107	
*> 6 pollen***	35.8		32.2		**0.043**	
Vegetable foods						
Peanut	31.4	4.7 ± 2.7	30.2	5.1 ± 2.8	0.629	0.232
Hazelnut	29.4	4.9 ± 2.2	20.4	4.9 ± 2.9	**<0.001**	0.859
Peach	17.5	5.3 ± 2.4	15.7	6.1 ± 2.3	0.336	**0.048**
Wheat	23.7	6.5 ± 3.0	7.5	3.9 ± 1.6	**<0.001**	**0.003**
Apple	13.5	5.4 ± 2.3	9.7	5.1 ± 1.8	**0.032**	0.565
Soybean	8.5	4.2 ± 1.5	5.2	4.5 ± 1.5	0.058	0.555
> 3 foods	10.7		7,0		**0.016**	
Profilin	31.2	5.8 ± 2.4	30.4	5.6 ± 2.0	0.736	0.548
Latex	1.1	3.6 ± 0.9	3.5	4.4 ± 3.7	0.206	0.678

**p value is referred to the percentage of patients with wheal diameter ≥ 3 mm, except for ° that are referred to mean number of positive SPT.*

^p value is referred to mean value and is calculated by mixed model taking in account that some patients are the same at baseline and at follow up and some not.

*** Skin reactions to pollens (wheal ≥3 mm). SPT extracts included: grass pollen (P. pratense or C. dactylon), B. verrucosa, C. arizonica, P. acerifolia, O. europaea, P. judaica, Compositae pollen (A. vulgaris or A. artemisiifolia), P. lanceolata, S. kali, C. avellana, C. album.*

Legends to figures

Figure 1. Predictive factors of allergic rhinitis remission in 401 Italian children with pollen allergy (univariate analysis).

Figure 2. Predictive factors of asthma (a) remission and (b) new onset in 401 Italian children with pollen allergy (univariate analysis).

Figure 3. Predictive factors of oral allergy syndrome for (a) remission and (b) new onset in 401 Italian children with pollen allergy (univariate analysis).