



# Optimal Skin Prick Test Panel for Detecting Respiratory Allergens in Children: A Retrospective Study

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## ABSTRACT

**Aim:** The skin prick test (SPT) is the standard tool for determining respiratory allergen sensitizations. Different allergen sensitization patterns have been observed within countries and regions according to geographical and seasonal variations. This study aims to identify the sensitization pattern of children in different age groups and to define the minimum number and type of allergen extracts in an SPT to detect a sensitized child.

**Materials and Methods:** This retrospective study was conducted in the Outpatient Clinic of the Pediatric Allergy, Immunology, and Pulmonology Unit of a tertiary Children's Hospital from October 2019 to December 2020. Children aged between 2 and 18 years suspected of inhalant allergy with the presence of clinically relevant symptoms were included. The results of SPT were collected from medical records to determine the optimal panel to cover 95% of the sensitized children.

**Results:** A total of 1821 patients with SPT results were evaluated. Forty-three patients (2.4%) were excluded from the study because some allergen extracts did not apply. Consequently, 1778 children (male/female ratio of 1.33) were included in the study. The median age (interquartile range) was 8 years (2-18). The most common sensitizations were to grasses (*Lolium perenne* and *Poa pratensis*), trees (*Olea europaea* and *Fraxinus excelsior*), cereals (*Avena sativa* and *Hordeum vulgare*), animal dander (cat and dog), and weeds (*Plantago lanceolata* and *Ambrosia artemisiifolia*). The rate of sensitization tended to increase with age. Applying an SPT that included six allergen extracts for 2-5 years, five for 6-11 years, and four for 12-18 years was sufficient to identify 95% of sensitized children.

**Conclusion:** A test panel with six allergen extracts was sufficient to identify most of the sensitized children and adolescents suspected of allergy and had clinically relevant symptoms. An SPT with fewer allergen sources was required to detect older sensitized children than younger children.

**Keywords:** Aeroallergens, children, respiratory allergy, sensitization, skin prick test

## Introduction

Atopic diseases have become increasingly prevalent in recent years and constitute a considerable proportion of pediatric outpatient visits (1). Aeroallergen sensitization

is a significant factor in the development of these chronic illnesses and the increase in their prevalence. Although serum allergen-specific immunoglobulin E (IgE) can provide an indication when diagnosing IgE-mediated diseases, the skin prick test (SPT) has superior sensitivity and positive

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predictive value in comparison to specific IgE blood tests (1,2).

Some studies have revealed that aeroallergen sensitization changes with seasonal and geographical variations (3-6). However, most of these studies were concerned with the prevalence and distribution of airborne allergens and, thus, they did not reflect the patients' sensitization patterns. In the pan-European GA2LEN skin test study, 14 participating countries responded to this question, but this study's subjects consisted primarily of adults (7,8). In addition, some allergens are rarely observed in certain regions (2). Therefore, a limited number of allergen extracts may be used in children. Currently, no standardized test panel has been established across Europe. Determining the optimal number of allergen extracts to detect sensitized patients is more cost-effective and less painful for children. Therefore, it is advantageous from an economic and scientific view point.

This study aimed to investigate the aeroallergen sensitization patterns of children in different age groups and to determine the optimal number and type of SPT allergens needed to identify a child as being sensitized.

## Materials and Methods

This retrospective study was carried out in the Outpatient Clinic of the Pediatric Allergy, Immunology, and Pulmonology Unit at a tertiary Children's Hospital in İzmir between October, 2019 and December, 2020. While the clinic serves as a referral center for the Aegean region of the country, patients may also be admitted on request; thus, it serves as both a primary and a tertiary health care center. Children aged 2 to 18 years with a suspected inhaled allergy and, in addition, a high likelihood of clinically relevant symptoms underwent SPTs. Patients were excluded if they had a negative histamine control or missing data. This study was approved by the Medical Research Ethics Committee of the Ege University Faculty of Medicine (approval no: 19-10.1T/34, date: 16.10.2019).

### Aeroallergen Panel and SPT

An SPT is performed as a routine procedure in our clinic on the forearm of all children with clinical symptoms suggesting allergy. A standard panel consisted of 30 allergen extracts (Allergopharma, Germany), including house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), animal dander (cat, dog, and horse), *Blattella germanica*, grass pollens (*Dactylis glomerata*, *Phleum pratense*, *Lolium perenne*, *Poa pratensis*, and *Festuca pratensis*), weed pollens (*Artemisia vulgaris*,

*Urtica dioica*, *Parietaria officinalis*, *Plantago lanceolata*, and *Ambrosia artemisiifolia*), tree pollens (*Alnus glutinosa*, *Ulmus scabra*, *Populus alba*, *Olea europaea*, *Fraxinus excelsior*, *Tilia cordata*, and *Acer pseudoplatanus*), cereal pollens (*Hordeum vulgare*, *Avena sativa*, and *Secale cereale*), and molds (*Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Penicillium notatum*), histamine as a positive control (10 mg/mL of histamine phosphate), and 0.9% sterile saline as a negative control. The selection of aeroallergens was based on their availability. The SPTs in our study were evaluated 15-20 minutes after their application. A positive result was defined as a wheal diameter  $\geq 3$  mm compared to the negative control. The presence of sensitization for a patient was determined as a positive SPT result for at least one of the allergen extracts tested in our study.

### Statistical Analysis

Quantitative data were reported as mean  $\pm$  standard deviation or median with an interquartile range. The prevalence of sensitization to each allergen extract was determined using frequencies and percentages. A step-by-step conditional approach was used to classify allergens from the one which caused the highest increase in the prevalence of sensitization to the one which gave the lowest increase. First, the most prevalent allergen was determined. Then, the highest prevalence of sensitization was investigated in the subgroup of subjects not sensitized to the previous allergen in order to detect the following allergen with the highest increase in the prevalence of sensitization (8). This procedure was repeated for the whole study group until the number and type of allergens needed were determined in order to identify at least 95% of sensitized subjects. Furthermore, the same process was repeated with the patients allocated by their age subgroups: 2-5 years, 6-11 years, and 12-18 years. Statistical analysis was performed using IBM SPSS V.20.0 (SPSS, Chicago, IL).

## Results

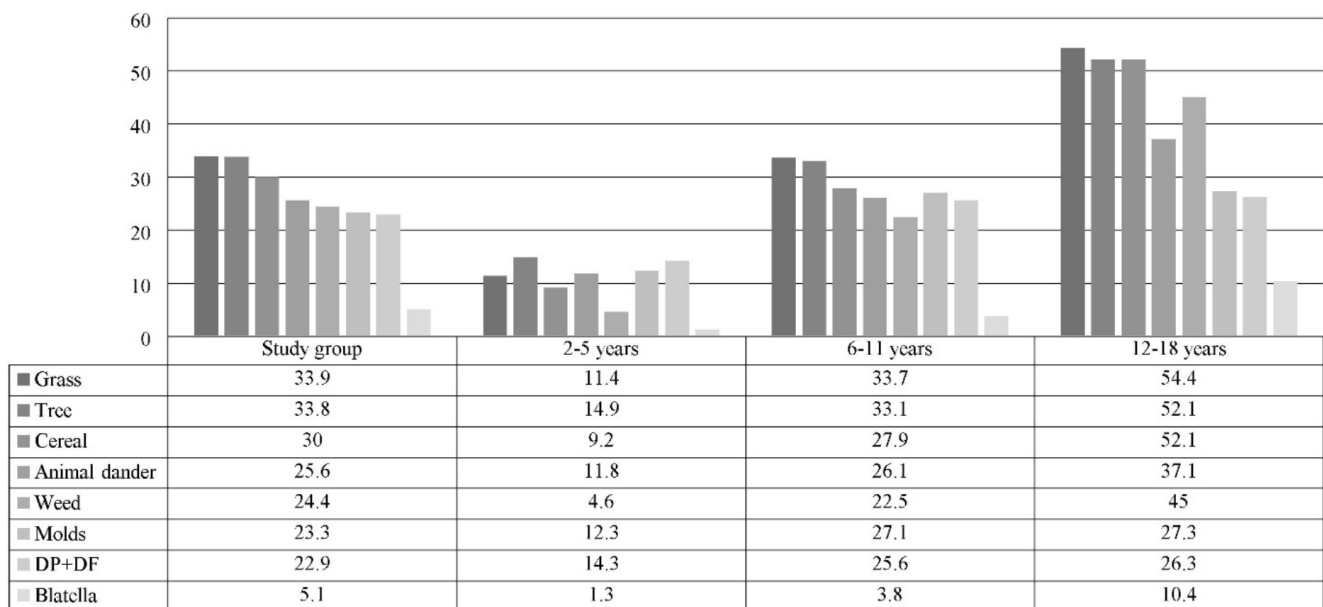
A total of 1,821 patients with SPT results were evaluated. Forty-three patients (2.4%) were excluded because some allergen extracts had not been applied. Finally, 1,778 children (male/female ratio of 1.33) with a median age of 8 years (5.0-12.0) were included in this study. The sensitization rate was higher in those children aged 12-18 years than in those aged 2-5 or 6-11 years (77.0% vs. 38.6% and 60.8%, respectively). Multiple sensitizations to aeroallergen extracts were found in 815 (45.8%)

children and this tended to increase with age (Table I). The distribution of aeroallergen sources among the sensitized children is presented in Figure 1.

The most common allergens were Lolium (32.6%), Poa (32.0%), Phleum (31.8%), Dactylis (31.3%), and Olea (31.3%; Table II). Applying five allergen extracts (Lolium, D. pteronyssinus, cat, Alternaria, and Olea) was sufficient to detect 95% of all sensitized patients. Furthermore,

we showed that testing with six allergen extracts for children aged 2 to 5 years (D. pteronyssinus, Olea, cat, Alternaria, Poa, and D. farinae) and five for those aged 6 to 11 years (Lolium, D. pteronyssinus, cat, olea, and Alternaria) identified 95% of SPT sensitization. Finally, four allergen extracts (Lolium, D. pteronyssinus, cat, and Alternaria) were required to detect 95% of sensitized children between 12 and 18 years of age (Table III).

	All patients n=1778	2-5 years n=456	6-11 years n=813	12-18 years n=509
<b>Age, year</b>				
Mean ± SD	8.9±4.1	4.1±0.9	8.2±1.7	14.4±1.9
Median (IQR)	8.0 (5.0-12.0)	4.0 (4.0-5.0)	8.0 (7.0-10.0)	14.0 (13.0-16.0)
<b>Gender, n (%)</b>				
Male	1016 (57.1)	258 (56.6)	478 (58.8)	280 (55.0)
Female	762 (42.9)	198 (43.4)	335 (41.2)	229 (45.0)
<b>SPT, n (%)</b>				
Positive	1062 (59.7)	176 (38.6)	494 (60.8)	392 (77.0)
Mono-sensitization	247 (13.9)	84 (18.4)	119 (14.6)	44 (8.6)
2 allergens	179 (10.1)	41 (9.0)	82 (10.2)	56 (11.0)
≥3 allergens	636 (35.7)	51 (11.2)	293 (36.0)	292 (57.4)
SD: Standard deviation, IQR: Interquartile range, SPT: Skin prick test				



**Figure 1.** Distribution of aeroallergen extracts among sensitized children for the whole study group and age subgroups  
DP: *Dermatophagoides pteronyssinus*, DF: *Dermatophagoides farinae*

**Table II.** Distribution of the frequencies of sensitization for each allergen used in the skin prick tests in the whole study population and by age subgroups

	<b>Study population n=1,778 n (%)</b>	<b>2-5 years n=456 n (%)</b>	<b>6-11 years n=813 n (%)</b>	<b>12-18 years n=519 n (%)</b>
<b>House dust mites</b>				
<i>D. pteronyssinus</i>	385 (21.7)	61 (13.4)	195 (24.0)	129 (25.3)
<i>D. farinae</i>	372 (20.9)	57 (12.5)	191 (23.5)	124 (24.4)
<b>Animal danders</b>				
Cat	434 (24.4)	54 (11.8)	200 (24.6)	180 (35.4)
Dog	226 (12.7)	21 (4.6)	92 (11.3)	113 (22.2)
Horse	36 (2.0)	4 (0.9)	16 (2.0)	16 (3.1)
<i>Blatella germanica</i>	90 (5.1)	6 (1.3)	31 (3.8)	53 (10.4)
<b>Grass pollens</b>				
<i>Lolium perenne</i>	579 (32.6)	48 (10.5)	258 (31.7)	273 (53.6)
<i>Poa pratensis</i>	569 (32.0)	49 (10.7)	252 (31.0)	268 (52.7)
<i>Phleum pratense</i>	565 (31.8)	47 (10.3)	247 (30.4)	271 (53.2)
<i>Dactylis glomerata</i>	556 (31.3)	46 (10.1)	241 (29.6)	269 (52.8)
<i>Festuca pratensis</i>	549 (30.9)	46 (10.1)	243 (29.9)	260 (51.1)
<b>Weed pollens</b>				
<i>Plantago lanceolata</i>	341 (19.2)	14 (3.1)	141 (17.3)	186 (36.5)
<i>Ambrosia artemisiifolia</i>	196 (11.0)	8 (1.8)	83 (10.2)	105 (20.6)
<i>Artemisia vulgaris</i>	184 (10.3)	6 (1.3)	75 (9.2)	103 (20.2)
<i>Parietaria officinalis</i>	115 (6.5)	1 (0.2)	45 (5.5)	69 (13.6)
<i>Urtica dioica</i>	85 (4.8)	0 (0)	29 (3.6)	56 (11.0)
<b>Tree pollens</b>				
<i>Olea europaea</i>	557 (31.3)	59 (12.9)	243 (29.9)	255 (50.1)
<i>Fraxinus excelsior</i>	466 (26.2)	50 (11.0)	200 (24.6)	216 (42.4)
<i>Alnus glutinosa</i>	161 (9.1)	9 (2.0)	73 (9.0)	79 (15.5)
<i>Tilia cordata</i>	158 (8.9)	5 (1.1)	61 (7.5)	92 (18.1)
<i>Ulmus scabra</i>	149 (8.4)	6 (1.3)	67 (8.2)	76 (14.9)
<i>Populus alba</i>	133 (7.5)	4 (0.9)	60 (7.4)	69 (13.6)
<b>Cereal pollens</b>				
<i>Avena sativa</i>	508 (28.6)	37 (8.1)	213 (26.2)	258 (50.7)
<i>Hordeum vulgare</i>	496 (27.9)	34 (7.5)	210 (25.8)	252 (49.5)
<i>Secale cereale</i>	465 (26.2)	30 (6.6)	195 (24.0)	240 (47.2)
<b>Molds</b>				
<i>Alternaria alternata</i>	359 (20.2)	52 (11.4)	194 (23.9)	113 (22.2)
<i>Cladosporium herbarum</i>	131 (7.4)	12 (2.6)	61 (7.5)	58 (11.4)
<i>Aspergillus fumigatus</i>	89 (5.0)	7 (1.5)	42 (5.2)	40 (7.9)
<i>Penicillium notatum</i>	48 (2.7)	4 (0.9)	20 (2.5)	24 (4.7)

**Table III.** The suggested test panel for different age groups and the percentage of sensitization by certain aeroallergen extracts

	n	%	Cumulative %
<b>2-5 years</b>			
D. pteronyssinus	61	34.7	34.7
Olea europaea	51	29.0	63.7
Cat	24	13.6	77.3
Alternaria alternata	18	10.2	87.5
Poa pratensis	12	6.8	94.3
D. farinae	3	1.7	96.0
Others	7	4.0	100
<b>6-11 years</b>			
Lolium perenne	258	52.2	52.2
D. pteronyssinus	119	24.1	76.3
Cat	52	10.5	86.8
Olea europaea	27	5.5	92.3
Alternaria alternata	18	3.6	95.9
Others	20	4.1	100
<b>12-18 years</b>			
Lolium perenne	273	69.6	69.6
D. pteronyssinus	65	16.6	86.2
Cat	26	6.6	92.8
Alternaria alternata	13	3.3	96.1
Olea europaea	6	1.5	97.6
Others	9	2.4	100
<b>Whole study group</b>			
Lolium perenne	579	54.5	54.5
D. pteronyssinus	235	22.1	76.6
Cat	109	10.3	86.9
Alternaria alternata	61	5.7	92.6
Olea europaea	38	3.6	96.2
Blatella germanica	9	0.8	97.0
Aspergillus fumigatus	7	0.7	97.7
D. farinae	5	0.5	98.2
Phleum pratense	3	0.3	98.5
Others	16	1.5	100

## Discussion

This study determined that grass pollens were the most common allergen source in children, followed by tree and cereals pollens. While sensitization to tree pollens and house dust mites was more common in those children aged 2-5 years, we found that sensitization to grass and tree

pollens was more frequent in those aged 6-11 and 12-18 years. Furthermore, the application of an SPT involving a limited number of allergen extracts (4-6) was sufficient to identify 95% of the sensitized children.

Geographical and climate variations play a crucial role in sensitization patterns. These relationships were revealed in several extensive studies, such as the European Community Respiratory Health Survey and the International Study of Asthma and Allergies in Childhood (5,6). Turkey has wide geographical diversity, containing Mediterranean, Black Sea, and Turanian climate regions. The climatic condition of this study setting is classified as Csa (warm temperature, fully humid, hot summer) according to the Köppen-Geiger climate map (9). Earlier studies under the same climatic conditions demonstrated that grass pollens were the most common sources of aeroallergens, followed by weed and tree pollens (10,11). Our results were similar to those of most European countries, including Denmark, the UK, Greece, Poland, and the Netherlands, where sensitization to grass pollens varied between 19.5 and 69.9%. However, we noted that house dust mites were the leading allergen in some countries, including Portugal, France, Italy, and Belgium (7).

Studies on determining the optimal and most cost-effective SPT panel began with the population-based European Community Respiratory Health Survey. The authors showed that seven allergen extracts (D. pteronyssinus, cat, grass, birch, olive, Alternaria, and Cladosporium) were adequate for epidemiological studies in Europe (6,12). Subsequently, the GA2LEN study group conducted patient-based research and found that a similar test panel of eight allergen extracts was sufficient to detect more than 95% of sensitized patients. However, they highlighted that there may be some differences in the number of allergen extracts between countries (e.g., two allergen extracts in Switzerland and nine allergen extracts in France) (8). We propose a test battery which includes five allergen extracts (Lolium, D. pteronyssinus, cat, Alternaria, and Olea) as being sufficient to identify 95% of sensitized children in our study population. Our results agreed with those of most European countries. Surprisingly, in another study involving 2,457 children conducted in the center of Turkey, the authors showed that a test panel of 12 allergen extracts identified more than 95% of the patients (2). As mentioned earlier, this discrepancy could be attributed to the different climate conditions and geographical location. While our study was conducted in the coastal part of the country, the other study was conducted in central

Anatolia, defined as Dsb (Mediterranean-influenced warm-summer humid continental climate) according to the Köppen-Geiger climate map. Another explanation might be associated with the fact that their patient population came from distinct geographical locations, such as from the East, North, and South of Turkey, resulting in increased heterogeneity in allergen sources. A birth cohort of preschool-age children determined that four allergen extracts were sufficient to identify 94% of sensitized subjects (13). Şahiner et al. (2) determined that the minimum number of allergens in an SPT was 12, 8, and 7 for preschool, school, and adolescence patients, respectively. Sensitization to aeroallergens is related to environmental exposures, and it is also inevitable to encounter specific allergens as a child grows older. Consequently, the spectrum of allergen sources is more uniform in older children than in younger ones, as shown by the increase in the rate of all allergen sources with age in our study. This predominance decreases the number of allergen extracts necessary to reach a total sensitization rate of 95%.

Our results showed that the sensitization to cereal and weed pollens was 30% and 24.4%, respectively. Reports from our country showed that cereal sensitization ranged between 32.1% and 45% (2,10,11), while weed sensitization ranged between 7% and 23% (10,14). When we consider Ambrosia sensitization, which was tested in both the GA2LEN study and our study, it was 11% in this study. On the other hand, the sensitization to Ambrosia in Greece and Portugal, where the Mediterranean climate is dominant, as is the case Izmir, ranged between 11.7% and 12.4%, and it reached 53.8% in Hungary (7). Most European countries are characterized as climate regions of Cfb (warm temperature, fully humid and warm summer) and Dfb (snow, fully humid and warm summer) according to the Köppen-Geiger climate map (9). Therefore, the sensitization to weed and grass pollens is higher in those countries in comparison to Turkey. *Olea europaea* was another major allergen in our study and it was identified in 31.3% of sensitized children. Our result was supported by previous studies conducted in the Aegean region, which determined the *Olea* sensitization rate to be between 24% and 30% (10,15). Higher *Olea* sensitization may be associated with the fact that *Olea* plantations are more common in Izmir, which is located on the west coast of the country, in comparison to the center of the country.

In this study, sensitization to house dust mites (*D. pteronyssinus* or *D. farinae*) was found to be 22.9%. This

result was consistent with previous studies in our country (2,10,14,16). According to the GA2LEN skin test study, sensitization to house dust mites was rare in Central and Western Europe (7). In contrast, it was notably higher in Nordic and Mediterranean countries (32.7% in Greece and 68.8% in Portugal for *D. pteronyssinus*) (7). Although cat ownership is low in our country (17), cat sensitization was determined to be 24.4% in the present study. As pet ownership is more common in Europe, the sensitization rates there were higher than our results (49.3% in Denmark and 42.1% in Switzerland) (7). Many cat-allergic patients became sensitized by environmental exposure to cats (18,19). *Alternaria* and *Cladosporium* were the most prevalent molds, with 20.2% and 7.4%, respectively. From a population-based study in the United States, *Alternaria* sensitization was reported to be 36% (20). In Europe, sensitization rates were low in many countries, but high levels were observed in Hungary and Finland (18.6% for *Alternaria* and 7.1% for *Cladosporium*), as in our study (7). An implication of these findings may suggest having a particular test battery for each clinic related to the region. In addition, further studies focusing on the relationship between aeroallergen sensitization and their clinical relevance need to be considered.

### Study Limitations

This study had some limitations. Firstly, this was a patient-based study focusing on children with clinical allergy symptoms, so the results may not be in line with those of epidemiological studies. Secondly, this study investigated only sensitization patterns and did not determine the relationship between the allergen and its clinical relevance. As it was a single-center study, some allergen sources may have been omitted, resulting in a decrease in the generalizability of the findings of this study. Nevertheless, the center admits patients from all parts of the Aegean region.

### Conclusion

Identifying the sensitization patterns of children in different age groups can help select the allergen extracts to be used in SPT panels, thus preventing the use of those allergen extracts which are unnecessary. Regarding those cities with a Mediterranean climate, a standardized 6-allergen extract panel might be sufficient to determine 95% of sensitized children.

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## Ethics

**Ethics Committee Approval:** This study was approved by the Medical Research Ethics Committee of the Ege University Faculty of Medicine (approval no: 19-10.1T/34, date: 16.10.2019).

**Informed Consent:** This was a retrospective study.

## Authorship Contributions

Concept: A.E., G.K.Ö., F.G., E.D., Design: A.E., G.K.Ö., F.G., E.D., Data Collection and/or Processing: A.E., G.K.Ö., F.G., E.D., Analysis or Interpretation: A.E., G.K.Ö., F.G., E.D., Literature Review: A.E., G.K.Ö., F.G., E.D., Editing of the Manuscript: A.E., G.K.Ö., F.G., E.D., Writing: A.E., G.K.Ö., F.G., E.D.

**Conflict of Interest:** The authors declared that there were no conflicts of interest.

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