What we miss if standard panel is used for skin prick testing?

Ozlem Cavkaytar,[1] Betul Buyuktiryaki,[1] Erdal Sag,[2] Ozge Soyer[1] and Bulent E. Sekerel[1]

Summary

Background: **Although standard skin prick test (SPT) panels are crucial for routine investigation of sensitization in daily clinical practice, it has limitations in terms of missing allergens.**

Objective: **To find out sensitization rates (SR)s to additional panel of allergens and their relative contributions in allergic diseases.**

Methods: **SPTs with a battery of aeroallergens [tree pollen (*A.glutinosa, C.arizonica, J.communis, T.platyphyllos, R.pseudoacacia)*, weed pollen *(R.acetosa, U.dioica, A.artemisifolia)*, smut mix, yeast mix, storage mites (SM) (*B.tropicalis, L.destructor, T.putrescentiae, A.siro)*, mouse and budgerigar epithelia], were performed to 318 participants (6-18 years) who were previously identified to be sensitized to at least one of the aeroallergens found in standard battery.**

Results: **Forty percent of participants were sensitized to at least one additional aerollergen. Three most frequent sensitizations were to *B.tropicalis* (11.3%), *R.pseudoacacia* (9.7%) and *L.destructor* (8.2%). SR for tree pollen increased from 6.9% to 19.8%, for mites increased from 26.3% to 31.6% and for moulds increased from 5.3% to 9.4% with addition of respective group of other allergens to battery. Furthermore, higher rates for additional tree pollen sensitization was found among patients with "only AR" (21%) compared to patients with "only asthma" (4.6%, *p* =0.006), contrarily higher rates for SM sensitization was found among patients with "only asthma" (20%) compared to patients with "only AR" (3.2%, p =0.003)**

Conclusions: **Though some of sensitizations may occur due to cross-reactivity, almost 40% of sensitized children were also co-sensitized to the additional allergens tested. Physicians should consider further steps when a negative or inconsistent result is achieved through a standard skin test panel. *(Asian Pac J Allergy Immunol 2015;33:211-21)***

Keywords: *aeroallergen, animal dander, grass pollen, mould, sensitization, skin prick test, standard prick test panel, tree pollen, yeast*

Introduction

Skin prick testing (SPT) is the first-line interventional method used to diagnose Ig-E mediated allergic diseases for patients with respiratory symptoms.[1] The likelihood of having allergic rhinitis (AR) and/or allergic asthma is growing along with the rising incidence of aeroallergen sensitization in both children and adults.[2] SPT is reproducible, minimally invasive, relatively easy when performed properly, and allows for the testing of multiple allergens at once.[3] Interpretation of SPT results does not take much time; however, the concordance between sensitization and existence of symptoms is important.[4] Different allergens may cause different degrees of clinical relevance, which may even be true for the same allergen in different parts of the world.[2]

Another important issue concerning SPT is the battery of allergens used. The panel of aeroallergens is variable and generally depends on the prevalence of regional aeroallergens.[5] Due to potential cross-reactivities between aeroallergens, great effort is taken to develop the most cost-effective, least painful, and optimal panels showing maximum sensitization rates (SR)s.[6] In contrast, human activities in the developing world have an impact on global climate change via increasing air carbon dioxide (CO_2) levels and air temperature. These changes further alter pollen distribution, amount, germination rate, and allergenicity; they also lengthen the pollen season as well as the season for fungal spores.[7] An example of these changes is the

From 1. Department of Pediatric Allergy
2. Department of Pediatrics Hacettepe University, School of Medicine 06100, Sıhhıye, Ankara, Turkey
Corresponding author: Bulent Enis Sekerel
E-mail: b_sekerel@yahoo.com
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increase in the prevalence of ragweed sensitization throughout Europe.[8] It has been even shown that a considerable amount of Chinese patients with ragweed sensitization had increase in bronchial resistance regarding both immediate and late phase upon provocation with ragweed extract.[9] Climate changes result in novel patterns of atmospheric circulation that increase the risk of new sensitizations among the allergic population.[10,11] Frequent travelling for both private and business purposes, as well as migration, contribute to new sensitizations and result in the need for research in this field.[12]

Furthermore, the wider diversity of aeroallergen sensitization in younger children compared to adolescents and the increase in incidence of SRs with increasing age in children make the investigation of additional allergen sensitization in the paediatric age group feasible.[6,13]

Recently, our group defined the minimum optimal SPT panel regarding the number and diversity of allergen extracts required to detect a child with respiratory symptoms as sensitized in a tertiary referral center in Turkey located in cross section of eastern Europe and western Asia.[6] However, we are curious about the impact of global climate change, and we had not investigated the missing novel allergen sensitizations among our patients. For this purpose we developed an additional battery of aeroallergens composed of different types of pollen found in the aerobiological environment of Ankara[14] as well as other mite, mould and animal allergens. The rationale for the development of an additional battery was to detect extra aeroallergens with potential tendency to result in rhinitis and asthma symptoms. Therefore, the primary outcome of this study was to determine the frequencies of sensitizations to different groups of additional allergens in patients with a diagnosis of AR and/or allergic asthma who had been followed up and to compare the occurrence rates of sensitizations to these additional allergens with those of standard allergens belonging to respective groups. The secondary outcome was to explore the comparative contribution of additional allergen sensitization in different allergic diseases.

Methods

Study population

The study was performed in the outpatient clinic of the paediatric allergy department of Hacettepe University İhsan Dogramaci Childrens' Hospital, which is one of the few referral centers for paediatric allergy in Turkey. All patients aged between 6-18 years who were admitted between March 2013 and August 2013 were invited to participate in the study. These patients had been referred to the paediatric allergy department before due to recurrent respiratory symptoms and had been diagnosed with allergic asthma and/or AR in accordance with their respective guidelines.[15,16] The children with recurrent wheezing symptoms and reversible airway obstruction after bronchodilator administration either clinically and/or as a result of at least a 12% improvement in FEV_1 were diagnosed with asthma. The patients diagnosed with AR had two or more symptoms of sneezing, watery rhinorrhea, nasal obstruction, and nasal pruritus that lasted for more than one hour on most days. Moreover, their symptoms coincided with a history of seasonal and perennial allergies and sensitization to SPTs.

Before enrollment, all the participants had been found to be sensitized to at least one of the aeroallergens used in a standard battery, which included grass pollen mix (*Phleum pratense, Poa pratensis, Dactylis glomerata, Lolium perenne, Festuca pratensis, Avena sativa, Cynodon dactylon*), weed pollen mix *(Parietaria judaica, Artemisia vulgaris, Plantago, Chenopodium, Salsola kali)*, tree pollen mix *(Salix caprea, Ulmus campestris, Quercus robur, hazel, Betula alba, Populus alba, Platanus vulgaris, Olea europaea)*, house dust mites (HDM) (*Dermatophagoides pteronyssinus, Dermatophagoides farinae*), animal dander (cat and dog), moulds (*Alternaria alternata, Cladosporium herbarum*), and cockroach *(Blattella germanica)* (Stallergenes, Anthony, France).

Hacettepe University is a tertiary referral center and located in central Anatolia. Not only the patients from local environment but also from different cities within Turkey are referred to paediatric allergy department. The study participants were classified into three according to the climatic conditions where they live in Turkey depending upon Köppen-Geiger climate map.[17] These climatic conditions were Csb (warm temperate, dry and warm summer), Csa (warm temperate, dry and hot summer) and Cfb (fully humid and warm temperate with warm summer).

Skin prick test procedures

Epidermal SPTs were performed with the following additional panel of aeroallergens in

Table 1. Extracts for novel allergen prick test panel

Allergen	Company
Budgerigar epithelia	Allergopharma, Reinbek, Germany
Mouse epithelia	Allergopharma, Reinbek, Germany
Cupressus arizonica	ALK; Hölshorm, Denmark
Ambrosia artemisifolia	ALK; Hölshorm, Denmark
Alnus glutinosa	Stallergenes; Antony, France
Juniperus communis	Stallergenes; Antony, France
Rumex acetosa	Stallergenes; Antony, France
Tilia platyphyllos	Stallergenes; Antony, France
Urtica dioica	Stallergenes; Antony, France
Blomia tropicalis	Stallergenes; Antony, France
Acarus siro	Stallergenes; Antony, France
Lepidoglyphus destructor	Stallergenes; Antony, France
Tyrophagus putrescentiae	Stallergenes; Antony, France
Negative control (diluent)	Stallergenes; Antony, France
Positive control (histamin dihydrochloride)	Stallergenes; Antony, France

the study group (Table 1): tree pollen (*Alnus glutinosa, Cupressus arizonica, Juniperus communis, Tilia platyphyllos, Robinia pseudoacacia*), weed pollen (*Rumex acetosa, Urtica dioica, Ambrosia artemisifolia*), moulds (smut mix composed of *Ustilago species*), and yeast mix (*Saccharomyces cerevisae, Saccharomyces minor*), as well as mites composed of storage mites (SM) (*Blomia tropicalis, Lepidoglyphus destructor, Tyrophagus putrescentiae, Acarus siro*), animal epithelia (mouse and budgerigar), and histamine (10 mg/ml of histamine phosphate) as positive and 0.9% sterile saline as negative controls. The patients who had been initially found to be sensitized to pollen mix extracts in the standard battery were additionally investigated for sensitization to each of the pollen included in this "mix" of extracts in order to detect concurrent additional allergen SRs. Highest diameters for indurations for the respective allergens was measured both horizontally and vertically and were classified as "sensitization" if the mean induration diameter was 3 mm greater than that of the negative control. In order to exclude cross-reactive allergen hypersensitivity for respective group of tree pollen and mites in standard and additional panel the size of the wheals for both group of allergens were compared in each patient. For a patient, if the size of the wheal for a particular allergen in the additional panel was equal to or greater than the respective group of allergen in the standard panel, the sensitization for that particular allergen was defined as "true sensitization" for that patient. Single-use test devices (Stallerpoint, Anthony, France) were used for prick testing. SPTs were performed by experienced and standardized personnel at our institution.[18] This study was conducted according to the principles expressed in the Declaration of Helsinki; it was approved by the Ethics Committee of Hacettepe University, and parents provided written informed consent.

Statistical analysis

Age, sex, diseases (only asthma, only AR, or AR and asthma), additional and standard sensitizations to grass, weed, tree pollen, mites, moulds, and animal dander of the patients were recorded accordingly. Additional sensitizations were grouped as "additional allergen sensitization" and "additional allergen sensitization only" according to the presence or lack of concurrent sensitizations in a standard battery for the respective allergen groups. As an example, if a participant were to be sensitized to one of the additional tree pollen without any sensitization to any of the tree pollen used in a standard battery, then he/she would be classified in "additional tree pollen allergen sensitization only" subgroup. Descriptive statistics was used to determine sensitization prevalences for different allergens and allergen groups. Incidences of sensitizations to additional or standard allergens in different disease groups were compared by chi-square test with the Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (IBM SPSS Statistics, Chicago, IL, US). Multivariate analysis and odds ratios (OR)s with relevant 95% confidence intervals (CI)s were calculated in order to adjust the SRs of the patients for additional panel of tree pollen and mites according to their age, gender, geographic distribution and disease states (AR and asthma). A p-level <0.05 was considered significant.

Results

This study included 350 patients who were known to have standard allergen sensitization and who had also been assessed on routine follow-up visits between March and August 2014 and then invited for the determination of additional allergen

Table 2. Demographical characteristics of the study group (n=318)

Age (years)	11.5 (8.9-14.1)
Sex (Male) (%)	189 (59.1)
Asthma n(%)	256 (80.3)
AR n(%)	253 (79.3)
Only Asthma n(%)	65 (20.4)
Only AR n(%)	62 (19.5)
Asthma and Allergic Rhinitis n(%)	191 (59.9)
Age for emergence of asthma symptoms (years)*	5 (2.5-7)
Age for emergence of alleric rhinitis symptoms (years)*	6 (4.5-9)
Sensitization rates for Standard prick test battery n(%)	
Grass pollen mix	231 (72.2)
Weed pollen mix	55 (17.2)
Tree pollen mix	22 (6.9)
House dust mite (Dermatophagoides spp†.)	84 (26.3)
Moulds (Claudosporium or Alternaria)	17 (5.3)
Animal dander (Cat or dog)	58 (18.1)
Cockroach	8(2.5)

*Median (Interquartile range)

† *Dermatophagoides spp: D. Pteronyssinus or D. farinae*

sensitization. Seventy-five percent of the 32 patients who declined to participate in the study cited the reason as being a lack of time, and the remaining students rejected due to their desire to abstain from pain. A total of 318 children and adolescents with sensitization to at least one of the allergens in a standard battery took part in the study. Demographic characteristics of the study group (Table 2) did not differ from nonparticipants (data not shown). Among the study group, the three most frequent sensitizations in a standard battery were to grass pollens, house dust mites, and animal dander (Table 2), as was shown before.[6] Forty percent of the whole study population (n = 124) were found to be sensitized to at least one of the aeroallergens in the additional battery. The three most frequent sensitizations among additional panel of allergens were to *Blomia tropicalis* (11.3%), *Robinia pseudoacacia* (9.7%), and *Lepidoglyphus destructor* (8.2%, Figure 1, Table 3).

Prevalence of sensitization to at least one of the additional tree pollen (16.3%) was higher than that of tree pollen used in a standard battery (6.9%) (Figure 2). There also was a relatively high number of patients who were sensitized to additional panel of tree pollen but who showed no sensitization to tree pollen used in the standard battery (n = 41, 12.9%), the rate of which was much higher than "additional allergen sensitization only" groups for other classes of allergens (Table 3). The frequency of tree pollen sensitization increased to 19.8% with the addition of additional panel of tree pollen to the battery (Figure 2). Patients diagnosed with "only asthma", "only AR" and "asthma and AR" had different frequencies of sensitizations to one of the additional tree pollen (4.6%, 25%, and 8.8%, respectively, $p = 0.015$), whereas no difference was shown for that of tree pollen in the standard battery (Table 3). Additionally, more patients with a diagnosis of AR (n =49, 15.4%) demonstrated sensitization to one of the additional tree pollen compared to patients diagnosed with "only asthma" (n = 3, 4.6%, $p = 0.004$, Table 3). Additional tree pollen sensitization was also higher among patients diagnosed with "only AR" (21%) compared to patients diagnosed with "only asthma" (4.6%, $p = 0.006$). The difference for the frequencies of patients sensitized to "additional tree pollen only" among all three disease groups was also significant ($p = 0.048$). The particular additional tree pollen contributing to this difference was *R. pseudoacacia,* which has the most frequent SR of 9.7% among additional tree pollen. Concurrent sensitization to grass pollen was seen in 90%, 72%, and 85% of the patients who were sensitized to *R. pseudoacacia, C. arizonica, and T. platyphyllos,* respectively.

Nearly 10% of the entire study population were sensitized to additional weed pollen, whereas 6.3% had no sensitization to weed pollen used in the standard battery (Figure2). There was no difference for different disease groups regarding the prevalence of both additional weed pollen and those used in the standard battery. Among additional weed pollen, *Ambrosia artemisiofilia* caused the highest rate of sensitization (Table 3). Concurrent sensitization to grass pollens was seen in 94%, 86%, and 86% of the patients who were sensitized to A. artemisiophilia, *R. acetosa,* and *U. dioica,* respectively.

There were 84 patients (26.3%) sensitized to HDM; 52 patients (16.3%) were sensitized to additional panel of mites (storage mites), 17 of whom (5.3%) had no sensitization to HDM. In all, 101 patients were sensitized to mites with the addition of SM to the battery, which raised the

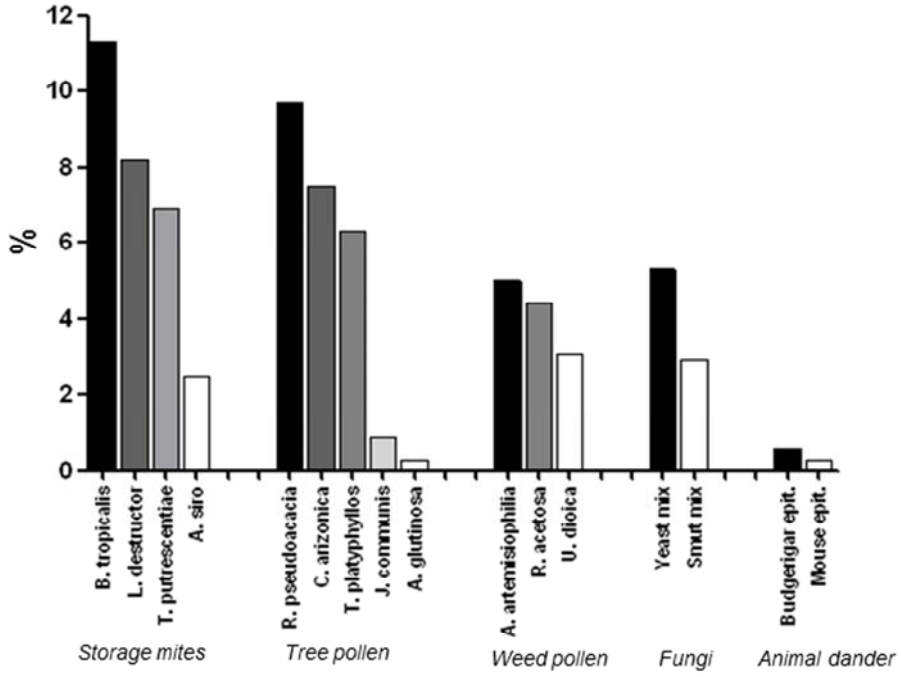

Figure 1. Sensitization rates for additional panel of allergens. Percentage of the patients with positive skin prick tests with allergens in novel battery among study participants (n =318) *Skin prick test with smut was performed in 2/3rd (n=210) of the patients.*

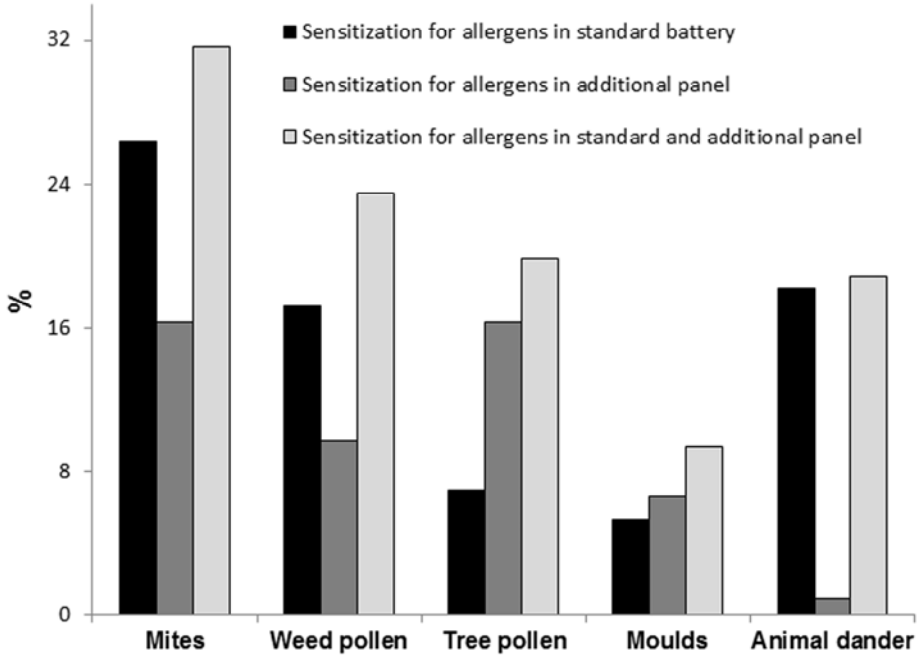

Figure 2. Comparison of standard battery and additional allergen panel. Percentage of the patients with positive skin prick tests with respective group of allergens in both standard and additional prick test battery. Common sensitization denotes the percentage of the patients found to be sensitized to respective group of allergens in both standard and additional battery.

frequency of mite sensitization to 31.6%. The prevalence of SM sensitization was different among patients with "only asthma" (20%), "only AR" (3.2%), and "asthma and AR" (19.4%, p = 0.008, Table 3). More patients who had been diagnosed with asthma (n = 50, 15.7%) had sensitization to at least one of the SM compared to patients diagnosed with "only AR" (n = 2, 3.2%, p = 0.002, Table 3). Storage mite sensitization was also higher among patients diagnosed with "only asthma" (20%) compared to patients diagnosed with "only AR" (3.2%, p = 0.003). *B. tropicalis* was found to be the most frequent SM (78%) that existed with HDM sensitization, whereas *A. siro* was the least frequent (data not shown).

Furthermore the pattern of additional tree pollen sensitization and additional mite sensitization according to the clinical profile (allergic rhinitis/asthma) were adjusted for other factors like age, gender and the geographical region where the patients lived. Multivariate logistic regression analysis by using age, gender, geographic regions, allergic rhinitis and asthma as covariates revealed that only AR remained as a risk factor for additional tree pollen sensitization (OR: 4.964, 95% CI: 1.495-16.479, p = 0.009) and only asthma remained as a risk factor for additional mite sensitization (OR: 5.341, 95%CI: 1.25-22.77, p = 0.024).

As a result of comparison of the size of the wheals for respective group of mites and tree pollen in standard and additional panel; 40 of 52 (76.9%) patients with a sensitization to SM and 50 of 52 (96.2%) patients with a sensitization to additional panel of tree pollen were defined to have "true sensitization". Furthermore, "true" SR for additional mites and additional panel of tree pollen were significantly different for patients diagnosed with "only asthma", "only AR" and "asthma and AR" (p = 0.045, p = 0.022, respectively). More patients with a diagnosis of asthma had "true sensitization" to additional mites compared to patients with "only AR", (p =0.013). More patients with a diagnosis of AR had "true sensitization" to additional panel of tree pollen compared to patients with "only asthma", (p = 0.006).

Yeast and smut sensitization were detected in 17 (5.3%) and 6 (2.9%) of the patients, respectively, and a majority of them had asthma either with or without AR (Table 3). Forty-seven percent of the patients with yeast sensitization had concurrent sensitizations to moulds used in the standard battery (data not shown), whereas only 15% of the patients with smut sensitization exhibited the same characteristics. Mould sensitization increased from 5.3% to 9.4% with the implementation of additional panel of allergens to the battery (Figure 2).

Sensitization to mouse and budgerigar epithelia was detected in only one and two patients, respectively, which represented a much lower prevalence than that of sensitizations to animal dander used in the standard battery (Table 3).

Discussion

In this study, sensitizations to additional panel of allergens were detected at a frequency of 40% in a group of patients who had been previously identified to be sensitized to at least one of the aeroallergens found in the standard battery. The major allergens revealed by the standard battery were grass pollen and house dust mites. Grass pollen and HDM sensitizations were significantly more frequent in patients with AR and asthma, respectively. We detected an increase in the frequency of sensitizations to tree pollen (12.9%), mites (5.3%), weed pollen (6.3%), and moulds (4.1%) with the addition of extra allergens to the battery. Particularly, the addition of extra tree pollen (*R. pseudoacacia*, *C. arizonica,* and *T. platyphyllos*) and SM to the standard battery not only increased the SRs for the respective group of allergens but also resulted in the differential determination of patients with symptoms of AR and asthma, respectively. Therefore, the extension of the standard battery to include these additional allergens would be practical in order to identify causative factors resulting in respiratory allergic diseases in patients with relevant clinical histories. Furthermore, the inclusion of additional panel of moulds would also be useful due to less cross-reactivity with fungal allergens used in the standard battery. On the other hand, the addition of extra panel of animal dander would have no impact.

The worldwide variation as well as the within-country differences in rates of allergic diseases in childhood suggest that environmental factors may be significantly important in the development of allergic diseases in this age group.[19] A variation in SRs for different types of aeroallergens in separate European countries[20] as well as variability in the clinical significance depending on the type of allergen and the country[2] made this hypothesis evident. Climate change observed during the past few decades has had an obvious effect on pollen amounts, allergenicity, and distribution and extent of

Table 3. Frequencies of sensitizations to standard and additional panel of allergens (percentage of positive skin prick test results according to diagnoses)

Allergen	Patients sensitized with the given allergen n (%)							
	Whole study population n=318	**OnlyAsthma n=65**	**Only AR n=62**	**Asthma and AR n=191**	**p^1**	**p^2**	**p^3**	**p^4**
Grass pollen mix	**231 (72.4)**	31 (47.7)	55 (88.7)	144 (75.4)	**<0.001**	**<0.001**	**0.001**	**<0.001**
House dust mite	**84 (26.3)**	24 (36.9)	6 (9.7)	54 (28.3)	**0.002**	0.031	**0.001**	**<0.001**
D. pteronyssinus	82 (25.7)	23 (35.4)	6 (9.7)	53 (27.7)	**0.003**	0.047	**0.001**	**0.001**
D. farinae	72 (22.6)	20 (30.8)	5 (8.1)	47 (24.6)	**0.006**	NS	**0.002**	**0.001**
Additional panel of mites	**52 (16.3)**	13 (20)	2 (3.2)	37 (19.4)	**0.008**	NS	**0.002**	**0.003**
B. tropicalis	36 (11.3)	8 (12.3)	2 (3.2)	26 (13.6)	NS	NS	**0.025**	NS
L. destructor	26 (8.2)	6 (9.2)	1 (1.6)	19 (9.9)	NS	NS	**0.036**	NS
T. putrescentiae	22 (6.9)	3 (4.6)	1 (1.6)	18 (9.4)	NS	NS	NS	NS
A.siro	8 (2.5)	-	1 (1.6)	7 (3.7)	NA	NS	NS	NS
Additional mite sensitization only	**17 (5.3)**	3 (4.6)	2 (3.2)	12 (6.3)	NS	NS	NS	NS
Tree pollen mix	**22 (6.9)**	1(1.5)	7 (11.3)	14 (7.3)	NS	NS	NS	0.024*
Additional panel of tree pollen	**52 (16.3)**	3 (4.6)	13 (21)	36 (18.8)	**0.015**	**0.004**	NS	0.006
R. pseudoacacia	31 (9.7)	1 (1.5)	6 (9.7)	24 (12.6)	**0.035**	**0.012**	NS	0.045*
C.arizonica	24 (7.5)	2 (3.1)	8 (12.9)	14 (7.3)	NS	NS	NS	0.04*
T.platyphyllos	20 (6.3)	2 (3.1)	3 (4.8)	15 (7.9)	NS	NS	NS	NS
J.communis	3 (0.9)	-	-	3 (1.6)	NA	NA	NA	NA
A.glutinosa	1 (0.3)	-	-	1 (0.5)	NA	NA	NA	NA
Additional tree pollen sensitization only	**41 (12.9)**	3 (4.6)	9 (14.5)	29 (15.1)	**0.048**	**0.026**	NS	NS
Weed pollen mix	**55 (17.2)**	7 (10.8)	13 (21)	35 (18.3)	NS	NS	NS	NS
Additional panel of weed pollen	**31 (9.7)**	3 (4.6)	5 (8.1)	23 (12)	NS	NS	NS	NS
A.artemisifolia	16 (5)	1 (1.5)	3 (4.8)	12 (6.3)	NS	NS	NS	NS
R.acetosa	14 (4.4)	2 (3.1)	3 (4.8)	9 (4.7)	NS	NS	NS	NS
U. dioica	10 (3.1)	2 (3.1)	1 (1.6)	7 (3.7)	NS	NS	NS	NS
Additional weed pollen sensitization only	**20 (6.3)**	3 (4.6)	2 (3.2)	15 (7.9)	NS	NS	NS	NS
Moulds	17 (5.3)	7 (10.8)	2 (3.2)	8 (4.2)	NS	**0.029**	NS	NS
A.alternata	15 (4.7)	6 (9.2)	2 (3.2)	7 (3.7)	NS	NS	NS	NS
C.herbarum	9 (2.8)	4 (6.2)	-	5 (2.6)	NA	NS	NS	NA
Additional panel of moulds	**21 (6.6)**	4 (6.2)	1(1.6)	16 (8.4)	NS	NS	NS	NS
Yeast mix.	17 (5.3)	3 (4.6)	1 (1.6)	13 (6.8)	NS	NS	NS	NS
†Smut mix	6 (2.9)	2 (4.0)	-	4 (3.1)	NS	NS	NS	NS
Additional mould sensitization only	**13 (4.1)**	2 (3.1)	-	11 (5.8)	NA	NS	NS	NA
Animal dander	**58 (18.2)**	13 (20)	11 (17.7)	33 (17.3)	NS	NS	NS	NS
Cat	53 (16.6)	13 (20)	10 (16.1)	29 (15.2)	NS	NS	NS	NS
Dog	20 (6.3)	4 (6.2)	4 (6.5)	11 (5.8)	NS	NS	NS	NS
Additional panel of animal dander	**3 (0.9)**	1 (1.5)	1 (1.6)	1 (0.5)	NS	NS	NS	NS
Budgerigar	2 (0.6)	1 (1.5)	-	1 (0.5)	NA	NS	NA	NA
Mouse	1 (0.3)	-	1 (1.6)	-	NA	NS	NA	NA
Additional animal dander sensitization only	**2 (0.6)**	-	1 (1.6)	1 (0.5)	NA	NA	NS	NA
Cockroach	**8 (2.5)**	1 (1.5)	2 (3.2)	5 (2.6)	NS	NS	NA	NS

NS: Non-significant NA: Not applicable *Not significant when Bonferoni Correction was performed †Only 210 patients could be skin prick tested with smut mix.

p^1 denotes the comparison of patients with only asthma vs. only AR vs. asthma and AR

p^2 denotes the comparison of patients with only asthma v.s. AR ± asthma

p^3 denotes the comparison of patients with only AR vs. asthma ± AR

p^4 denotes the comparison of patients with only AR. vs.only asthma.

pollen season[21]. Longer periods of sunshine, strong winds, and high daily mean temperatures enhance the risk for high pollen counts, but humidity has little impact.[14] Long distance transports also may result in symptoms and sensitization to an aeroallergen among allergic individuals living far away from the source of the allergen.[11] Finally, the increased transportation rates for both work and leisure contribute to the enhanced diversity of aeroallergens that can cause respiratory symptoms in patients living in a specific area, by the way additional aeroallergen sensitizations may emerge among patients with symptoms of allergic respiratory diseases.

Additional panel of tree pollen sensitization has been shown to have a particular impact in patients with symptoms of AR. Black locust (*R. pseudoacacia)* pollen is especially important and is mainly used as an ornamental tree. *R. pseudoacacia* includes a considerable amount of panallergens like profilin, polcalcin, and 1,3-β-glucanase, giving rise to high rates of cross-reactivity between taxonomically different pollen.[22,23,24,25] However, in the study by Compes et al., a nasal challenge with *R. pseudoacacia* pollen may induce allergic symptoms in sensitized individuals, although these individuals were also sensitized to these panallergens.[22] In our study, relatively high SRs for *R. pseudoacacia* may be a result of cross-reactivity with grass pollen due to the panallergens, however this finding is still expected to be clinically important.

Birch, hazel, and alder have a great potency to induce allergenic symptoms in this group of allergenic trees throughout Europe with the highest degree particularly in central and northern Europe.[11] However, SR to these three tree pollen were low (<2%) (data not shown) among the whole study group. On the other hand, the relatively high SR for cypress pollen is interesting because it is mainly seen in the Mediterranean areas, as in olive and Parietaria pollen.[11] Cupressaceae have been recognized as one of the pollen responsible for increasing pollinosis in Italy[26], France[27], and Israel[28] during the last few decades. Although our center is mainly located in central Anatolia, SR to *O. europea* and *P. judiaica* was found to be 3.4% and 1.3%, respectively, SR to *C. arizonica* pollen was 7.5% in our study. Although cypress pollen cross-react with grass pollen and some other tree pollen to a high extent[29], cypress is in charge of polinosis during winter when no other allergenic plants are flowering[11], which clarifies the clinical importance of cypress sensitization.

In a previous study, it was shown that tree pollens comprised 85% of pollen grains in the atmosphere of Ankara observed as an average over a three-year period;[30] however, the most frequent aeroallergen sensitization detected in our study was grass pollen. Although pollen grains belonging to *Pinacae* were shown to be the most abundant among tree pollen in Ankara,[23,30] the SRs for additional panel of tree pollen (*R. pseudoacacia*, C. *arizonica,* and *T. platyphyllos)* were greater than that of *Pinaceae.* The amount of pollen grains for Tilia was shown to be one of the lowest among tree pollen in Ankara;[30] nonetheless, *T. platyphyllos* sensitization was detected at a considerable rate in our study. According to a 10 year pollen count in Ankara it was previously shown that the most prevalent pollen types belonged to Pinaceae (22.4%), Cupressaceae (13.8%), Populus (12.4%).[31] Although grass pollen were responsible for high SRs in that study, the percentage of grass pollen count was lower (11.6%) compared to tree pollen count[31]. Besides, the percentage of Robinia pollen count was 4%. This finding indicates that the allergenic potential of specific pollen does not entirely depend on its concentration in the atmosphere and the particular tree pollen found in the additional panel namely *R. pseudoacacia*, *C. arizonica* and *T. platyphyllos* pollen are potential causes of allergic respiratory diseases in patients living in and around Ankara.

In Figure 2 it is obviously seen that the rate for tree pollen sensitization has increased to a SR higher than two times the original one with the addition of extra tree pollen particularly *R.pseudoacacia, C. arizonica and T. platyphyllos,* however addition of the other group of extra allergens do not end up with such an increase in SRs. This result suggests us that the tree pollen mix used in standard panel should be revised and these extra allergens should be added to the standard panel.

Sensitization rates for a additional weed pollen only group does not seem to be as high as the SR for additional tree pollen only group. But the SR for *Ambrosia artemisiofilia* is the highest among them relevant with the tendency for increased SR throughout Europe.[9] As there is minor to negligible cross-reactivity between ragweed and mugwort[32] relatively high SRs to ragweed as an additional allergen maybe clinically important.

Currently, the importance of fungus sensitizations is increasing in patients with asthma,

especially in the severe asthma subgroup.[33] With its associated increase in temperature and CO_2 levels, global climate change also has an impact on fungal sensitization by increasing fungal spore production and environmental fungal antigen levels.[34,35] In this study, the SR to moulds increased from 5.3% to 9.4% with the addition of extra moulds to the battery, and there was a tendency for the increased SRs to moulds in patients with asthma symptoms. Therefore, the addition of extra moulds to the battery would be particularly important in patients with lower respiratory tract symptoms in allergy practice.

Storage mites are known to only exist in rural settings, and they have been implicated as one of the major causative factors for asthma and AR in rural areas in earlier studies.[36] In Turkey, it was previously reported that living in a village in the first few years of life was associated with SM sensitization in the elder years.[37] In contrast, SMs are also found in house dust, and there is increased evidence that SM sensitization also exists in urban populations.[38] In our study, the addition of SM to the aeroallergen SPT panel increased the SR for mites by 5.3%. Sensitization to SM is detected more frequently in patients with asthma, so it would be important to use the extracts of SM in any allergic work up of patients with asthmatic symptoms and to query these patients for clinically related symptoms.

The inclusion of already sensitized patients may be one limitation to this study. We included atopic patients with respiratory tract symptoms in order to determine the impact of the additional panel of allergens in this clinically symptomatic group of individuals. In this way, we would like to decide the most practical and useful additional allergen(s) to use to expand the standard battery in our center. Additionally, most of the pollen-sensitized patients were sensitized to grass pollen. Grass pollen sensitization may give rise to cross-reactivity with weed and tree pollen, and grass pollen sensitization may increase the risk for tree pollen sensitization.[39] However, the real cross-reactivity rates for grass pollen sensitization and weed or tree pollen can not be promptly determined because the study population was composed of patients who had already been identified as sensitized and was therefore not a sample from the general population.

In conclusion, though standard SPT panels are the most cost-effective method in daily practice, a practicing allergist must understand what these panels cover and what they do not. The results of this study indicated that further steps are warranted when a negative or inconsistent result is achieved through a standard skin test panel. Based upon the knowledge that an optimal panel is good but may not be the best, an extended panel is essential for difficult/uncontrolled cases. Our data demonstrate that the implementation of additional panel of allergens, especially additional tree pollen for patients with symptoms of AR; storage mite and additional moulds for patients with symptoms of asthma to the standard battery used in a tertiary allergy referral center would be beneficial to detect more sensitized patients and allow them to take precautions to decrease allergen exposure. Determination of the SR to these additional allergens in the general population and exploration of cross-reactivities in the light of the clinical history and provocation tests will be further steps to define the clinical importance of these additional panel of allergens.

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Conflict of interest

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References

1. Bousquet J, Heinzerling L, Bachert C, Papadopoulos NG, Bousquet PJ, Burney PG et al; Global Allergy and Asthma European Network; Allergic Rhinitis and its Impact on Asthma. Practical guide to skin prick tests in allergy to aeroallergens. Allergy 2012;67:18-24.
2. Burbach GJ, Heinzerling LM, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe. Allergy 2009;64:1507-15.
3. Cox L, Williams B, Sicherer S, Oppenheimer J, Sher L, Hamilton R et al. Pearls and pitfalls of allergy diagnostic testing: report from the American College of Allergy, Asthma and Immunology/ American Academy of Allergy, Asthma and Immunology Specific IgE Test Task Force. Ann Allergy Asthma Immunol 2008;101:580-92.
4. Carr TF, Saltoun CA. Chapter 2: Skin testing in allergy. Allergy Asthma Proc 2012;33(Suppl 1):S6-8.
5. Eigenmann PA, Atanaskovic-Markovic M, O'B Hourihane J, Lack G, Lau S, Matricardi PM, et al. Testing children for allergies: why, how, who and when: an updated statement of the European

Academy of Allergy and Clinical Immunology (EAACI) Section on Pediatrics and the EAACI-Clemens von Pirquet Foundation. Pediatr Allergy Immunol 2013;24:195-209.

6. Sahiner UM, Civelek E, Yavuz ST, Büyüktiryaki AB, Tuncer A, Şekerel BE. Skin prick testing to aeroallergen extracts: what is the optimal panel in children and adolescents in Turkey? Int Arch Allergy Immunol 2012;157:391-8.
7. Levetin E, Van de Water P. Changing pollen types/ concentrations/distribution in the United States: fact or fiction? Curr Allergy Asthma Rep 2008;8:418-24.
8. Burbach GJ, Heinzerling LM, Rohnelt C, Bergmann KC, Behrendt H, Zuberbier T; GA(2)LEN study. Ragweed sensitization in Europe - GA(2)LEN study suggests increasing prevalence. Allergy 2009;64:664-5.
9. Lu D, Zhau P, Yu Q, Zhang C. Airway provocation test with ragweed pollen extract in Chinese asthmatics. Asian Pac J Allergy Immunol. 1994 Dec;12:125-9.
10. Cecchi L, Morabito M, Paola Domeneghetti M, Crisci A, Onorari M, Orlandini S. Long distance transport of ragweed pollen as a potential cause of allergy in central Italy. Ann Allergy Asthma Immunol 2006;96:86-91.
11. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, et al. Allergenic pollen and pollen allergy in Europe. Allergy 2007;62:976-90.
12. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. Allergy 2009;64:1498-506.
13. Stern DA, Lohman IC, Wright AL, Taussig LM, Martinez FD, Halonen M. Dynamic changes in sensitization to specific aeroallergens in children raised in a desert environment. Clin Exp Allergy 2004;34:1563-9.
14. Kizilpinar I, Civelek E, Tuncer A, Dogan C, Karabulut E, Sahiner UM et al. Pollen counts and their relationship to meteorological factors in Ankara, Turkey during 2005-2008. Int J Biometeorol 2011;55:623-31.
15. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al; World Health Organization; GA(2)LEN; AllerGen. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 2008;63 (Suppl 86):8-160.
16. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. Updated 2011. www.ginasthma.org/ uploads/users/files/GINA Report 2011.pdf.
17. Kottek M, Grieser J, Beck C, Rudolf B, Rubel F. World map of the Koeppen-Geiger climate classification updated. Meteorol Z 2006;15:263.
18. Buyuktiryaki B, Sahiner UM, Karabulut E, Cavkaytar O, Tuncer A, Sekerel BE. Optimizing the use of a skin prick test device on children. Int Arch Allergy Immunol 2013;162:65-70.
19. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 1998;351:1225-32.
20. Heinzerling L, Frew AJ, Bindslev-Jensen C, Bonini S, Bousquet J, Bresciani M et al. Standard skin prick testing and sensitization to inhalant allergens across Europe--a survey from the GALEN network. Allergy 2005;60:1287-300.
21. Songnuan W. Wind-pollination and the roles of pollen allergenic proteins. Asian Pac J Allergy Immunol. 2013;31:261-270.
22. Compes E, Hernandez E, Quirce S, Palomares O, Rodríguez R, Cuesta J, et al. Hypersensitivity to black locust (Robinia pseudoacacia) pollen: "allergy mirages". Ann Alergy Asthma Immunol 2006;96:586-92.
23. Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P et al. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 1992;175:377-85.
24. Palomares O, Villalba M, Quiralte J, Polo F, Rodríguez R. 1,3-β-glucanases as candidates in latex-pollen vegetable food cross-reactivity Clin Exp Allergy 2005;35:345-51.
25. Ledesma A, Villalba M, Batanero E, Rodríguez R. Molecular cloning and expression of active Ole e 3, a major allergen from olive-tree pollen and member of a novel family of Ca+2 -binding proteins (polcalcins) involved in allergy. Eur J Biochem 1998;258:454-59.
26. Italian Association of Aerobiology. An epidemiological survey of Cupressaceae pollenosis in Italy. J Invest Allergol Clin Immunol 2002;12:287-92.
27. Caimmi D, Raschetti R, Pons P, Dhivert-Donnadieu H, Bousquet PJ, Bousquet J, et al. Epidemiology of cypress pollen allergy in Montpellier. J Invest Allergol Clin Immunol 2012;22:280-5.
28. Geller-Bernstein C, Waisel Y, Lahoz C. Environment and sensitization to cypress in Israel. Allergie et immunologie 2000;32:92-3.
29. Sposato B, Scalese M. Prevalence and real clinical impact of Cupressus sempervirens and Juniperus communis sensitisations in Tuscan "Maremma", Italy. Allergol Immunopathol 2013;41:17-24.
30. Kaplan A. Predominant aeroallergen pollen grains in the atmosphere of Ankara, Turkey. Allergy 2004;59:670-2.
31. Can IH, Islam A, Karasoy DS, Samim EE. Does regional pollen load affect the prevalance of clinical allergy to those pollen groups? J Laryngol Otol 2010;124:297-301.
32. Weber RW. Patterns of pollen cross-allergenicity. J Allergy Clin Immunol. 2003:112;229-39.
33. Knutsen AP, Bush RK, Demain JG, Denning DW, Dixit A, Fairs A, et al. Fungi and allergic lower respiratory tract diseases. J Allergy Clin Immunol 2012;129:280-91.
34. Wolf J, O'Neill NR, Rogers CA, Muilenberg ML, Ziska LH. Elevated atmospheric carbon dioxide concentrations amplify Alternaria alternata sporulation and total antigen production. Environ Health Perspect 2010;118:1223-8.
35. Hollins PD, Kettlewell PS, Atkinson MD, Stephenson DB, Corden JM, Millington WM, et al. Relationships between airborne fungal

spore concentration of Cladosporium and the summer climate at two sites in Britain. Int J Biometeorol 2004;48:137-41.

36. Cuthbert OD, Brostoff J, Wraith DG, Brighton WD. 'Barn allergy': asthma and rhinitis due to storage mites. Clin Allergy 1979; 9:229-36.
37. Celebioglu E, Ozturk AB, Comert S, Karakaya G, Kalyoncu AF. Storage mite sensitisation is associated with early life village residence in Turkey. Allergol Immunopathol (Madr). 2013;41:402-6.
38. Van Hage-Hamsten M, Johansson E. Clinical and immunologic aspects of storage mite allergy. Allergy 1998;53:49-53.
39. Vieira FA, Ferreira EN, Cruz AA. Grass allergy increases the risk of tree pollen sensitization: a warning to urban planners. J Allergy Clin Immunol 1998;102:700-1.