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A multidisciplinary approach of outdoor aeroallergen selection for Skin Prick Testing in the geographical area of Greater Istanbul

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KEY WORDS

Pollen allergy; allergens; skin tests; environmental monitoring; symptoms.

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Doi

10.23822/EurAnnACI.1764-1489.188

IMPACT STATEMENT

A multidisciplinary approach is essential for the selection of the relevant allergens in the diagnostic workup of allergic diseases.

Summary

Background. Aeroallergen selection for skin prick testing and the interpretation of results need to be in line with allergenic sources of a specific geographic area. **Objective.** To identify aeroallergens for a skin test panel for the specific geographical area of Istanbul in a multidisciplinary approach based on aerobiological parameters, cross-reactivity patterns and clinical symptoms. **Methods.** Aerobiological parameters, cross reactivity patterns and the European Standard Skin Prick Test Panel determined allergen selection. Atopic adult patients ($n = 60$) compiled a questionnaire and were skin prick tested with 29 aeroallergens. Aerobiological sampling followed the requirements of the European Aerobiology Society. Results were statistically analyzed. **Results.** 65% of patients had positive skin reactions. Sensitization to at least one grass allergen was 30%. Key grass allergens were timothy grass (*Phleum pratense* L.) 25.8% and Johnson grass (*Sorghum halepense* (L.) Pers.) 22.6%; correlations between grass-sensitizations were significant at $p < 0.01$ and so was the correlation of Pooideae sensitization with symptoms and medication. Sensitization to at least one woody plant was 23%; to ash (*Fraxinus excelsior* L.) 8.1%; hazelnut (*Corylus avellana* L.), olive (*Olea europaea* L.) and mulberry (*Morus alba* L.) 6.5%; juniper (*Juniperus ashei* J. Buchholz) 4.8%. Correlations between Fagales allergen sensitizations were significant. Sensitization to at least one weed was 22%, sensitization to dock (*Rumex crispus* L.) 12.9%, ragweed (*Ambrosia artemisiifolia* L.), and mugwort (*Artemisia vulgaris* L.) 4.8%. Sensitization rates correlated significantly with the length of the Main Pollen Season. **Conclusions.** The European Standard Panel is suitable for the geographical area of Greater Istanbul, if it comprises Johnson grass and ash. Ragweed has become clinically relevant in this region. Mulberry and dock were exclusively associated to polysensitized individuals suggesting pan-allergen involvement.

Introduction

It is known that the presence of aeroallergens often correlates with symptom development with a non-linear dose-response relationship (1) and symptom inducing pollen concentrations vary from place to place (2). Atmospheric pollen concentrations

differ greatly in time and space because they are dependent on local vegetation and weather conditions (3). Moreover, biotic and abiotic particles from remote sources due to air mass movements add to the health burden through atmospheric transport (4, 5). Epidemiological studies investigate IgE mediated

sensitization patterns in atopic individuals to reveal allergens of clinical importance, since sensitized individuals often develop symptoms of allergic disease. The key here is to include all relevant allergens. In terms of outdoor aeroallergens, skin prick testing paired with aerobiological monitoring can be a means to detect the right allergens for a specific geographic area. In this regard, a standard test panel of 18 aeroallergen extracts for Europe has been proposed (6), whereby local exposure determines its adequacy (7). The current standard to detect local exposure to allergenic pollen and fungal spores is aerobiological monitoring using an automated volumetric Hirst type trap. Data exchange between trained aerobiologists and allergists needs to occur in regular time intervals to reflect environmental changes that directly affect the sources of allergens over time (8). Hereby it is essential to adhere to defined parameters that allow for comparison of calculations related to pollen concentrations, the definition of seasons, and to follow accepted terminology (9). This is even more so important in the light of climate change (10) and a crucial aspect to consider in the design of clinical trials on inhalant allergy (8).

Clinical experience in Istanbul indicated the need for an outdoor allergen skin test panel designed in accordance with local allergen exposure and cross reactivity patterns between species (7). The use of locally relevant panels is pressing in the metropolis Istanbul, since allergic rhinitis, food allergy, dermatitis, and asthma concern 3.3 million sufferers at an affected population rate of 21.2% (11).

In this prospective observational study, we aimed to identify suitable outdoor allergens for a skin test panel to use in the geographical area of Greater Istanbul in a multidisciplinary approach by means of:

- a) allergen exposure through standardized aerobiological observations;
- b) correlation patterns in skin test results;
- c) patient symptoms.

Methods

Study area

The study area included most of European Istanbul, defined by a 30 km range of the monitoring site at 41°05'24.6"N 28°37'16.7"E in Büyükçekmece. The climate is between temperate and Mediterranean corresponding to the Köppen Geiger csa-type. The average annual temperature is 14.1 °C; the average annual rainfall is 747 mm (Climate-Data.org, 2020), and the main wind direction north-easterly. The vegetation in the surroundings of Istanbul pertains of the Euxine floristic region with Mediterranean elements (12). The agricultural land use consists in the growth of annual crops. The landscape is undulating not exceeding an elevation of 300 m above sea level.

Regional pollen and fungal spore monitoring

Regional pollen and fungal spore monitoring was performed with a volumetric Hirst-type pollen sampler at 10 m height on rooftop. We followed the minimum requirements of the European Aerobiology Society (EAS) (13). Data were sent to the European Allergen Network (EAN). The Main Pollen Season (MPS) and Main Spore Season (MSS) were defined according the 95% method. Annual Pollen and Spore Integrals (API_n and ASI_n) were expressed as pollen (p) × day/m³ and spore (s) × day/m³ (9).

Observational study

36 aeroallergens were selected according to: 1) the European Standard Panel (ESP) (6); 2) taking into consideration cross reactivity patterns (14, 15); and 3) own additions based on atmospheric pollen monitoring. Standardized extracts were used to obtain comparable results.

The study was approved by the ethic commission of Bakırköy Dr. Sadi Konuk Training and Research Hospital (approval number 2014/11/03). Upon their written informed consent on procedure and risks, 66 patients were admitted to testing. They were ≥ 18 years old, had symptoms of respiratory allergy, had lived in Istanbul for three years, were not immunocompromised and lived, worked or studied in the range of 30 km of the trap location (1). Patients were asked to stop anti-histamine medications more than five half-life prior testing. All participants compiled a questionnaire on symptoms and symptom severity. SPT was performed on the back on normal skin (7). At the end of the test series, the negative control (0.9% NaCl solution), and the positive control (histamine solution 1/1,000) were applied. The superficial layer of the skin was punctured using a tip of a Morrow Brown lancet (1 mm blade length), at the maximum acute angle. Bleeding disqualified the test. If a rapid exacerbation of an allergic reaction was observed at the puncture site, the solution was removed instantly. A test was positive if the largest diameter of the wheal was 3 mm above the negative control. Results were reported in five categories: grade 0 (no reaction); 1+ (erythema ≤ 15 mm, no oedema); 2+ (erythema > 15 mm, oedema < 3 mm); 3+ (oedema 3-6 mm); 4+ (oedema > 6 mm) to obtain the degree of sensitization. Tests were performed by a physician in a clinic located within the range of 30 km of the trap location.

Statistics

Statistics include descriptives (mean, SD) for quantitative and categorical data. We discerned skin test positive and negative groups as independent variables. For quantitative data we used a Mann Whitney U as hypothesis test. Non parametric correlation analysis (Spearman's rho) was used to identify relationships between airborne particle concentrations, symptom scores, medical scores and sensitization rates. We used a significance level of at least $\alpha = 0.05$. All analysis was performed with IBM SPSS Statistics 2.0.

Results

Clinical aspects

The mean age of participants was 31 years (SD 8) within a range of 18-52 years. 32 were female and 28 males. Respondents (n = 45) had suffered from allergy on average for 7.1 years (SD 7.5) ranging from 1-32 years previous to the testing day. The distribution of total symptom scores was the same ($p > 0.05$) among positive and negative tested participants (Mann Whitney U Test). However, there was a significant difference in the use of medication ($p < 0.05$) (**figure 1**).

Airborne pollen and fungal spore concentrations

To understand the relationship between prevalence of sensitization and aerobiological features, we report the following aerobiological parameters: season start and end date, the length of the Main Pollen and Main Spore Season (MPS and MSS), the Annual Pollen and Spore Integral (APIn and ASIn), the ratio of the APIn on the total APIn and the maximum daily concentration of relevant airborne particles monitored during 2015 (**table I**). Besides single allergens, the table also includes data on pollen types not tested as single allergens and groups of interrelated pollen allergens at a higher level of systematic hierarchy for better interpretation.

Prevalence of sensitization and symptom development

Out of 66 patients 60 were eligible for SPT evaluation. Six were removed since they had a histamine reaction below grade 3. None of the patients reacted to saline solution, the negative control, while 65% demonstrated a positive skin reaction. We found 15% of participants each sensitized to one or two allergens; 3.3 % were

sensitized to 3 allergens; 13.3% to 4; 1.7% to 5; 8.3% to 6; 6.7% to 7 and 1.7% to 10 allergens each. Sensitization rates correlated significantly ($p < 0.05$) with the length of the MPS. Consult again **table I** for sensitization rates to outdoor allergens tested.

Grasses

Grasses were the main sensitizers; 30% of participants were sensitized to at least one grass allergen. We found significant correlations ($p < 0.01$) between sensitizations to all grass allergens. Also, the correlation between the total grass allergen sensitization score and the total medical score ($p < 0.01$) and the total symptom score ($p < 0.05$) were significant. In detail, meadow grass (*Poa pratensis* L.), timothy grass (*Phleum pratense* L.) and sweet vernal grass (*Anthoxanthum odoratum* L.) correlated significantly with the total symptom score, while sensitization to Bermuda grass (*Cynodon dactylon* L.) Pers) and Johnson grass (*Sorghum halepense* L.) Pers.) did not. We found a significant correlation between the total medical score and sensitization to *Pooideae* ($p < 0.01$), as well as to *Panicoideae* ($p < 0.05$) allergens but not to *Chloroideae*. All grass allergens, except Bermuda grass, correlated significantly with total eye symptoms ($p < 0.01$) and total nose symptoms ($p < 0.05$). Meadow grass displayed the strongest correlation with nose symptoms ($p = 0.018$). There was no correlation with the total lung symptom score. In grass sensitizations grade 4+ prevailed (**figure 2**). Note that meadow grass had the highest relative rate of grade 4 reactions with respect to the sensitization rate.

Woody plants

Woody plants sensitized 23% of participants. Ash (*Fraxinus excelsior* L.) was the main sensitizer in this group. We did not

Figure 1 - Total symptom scores (left) and total medical scores (right) of SPT positive and negative participants.

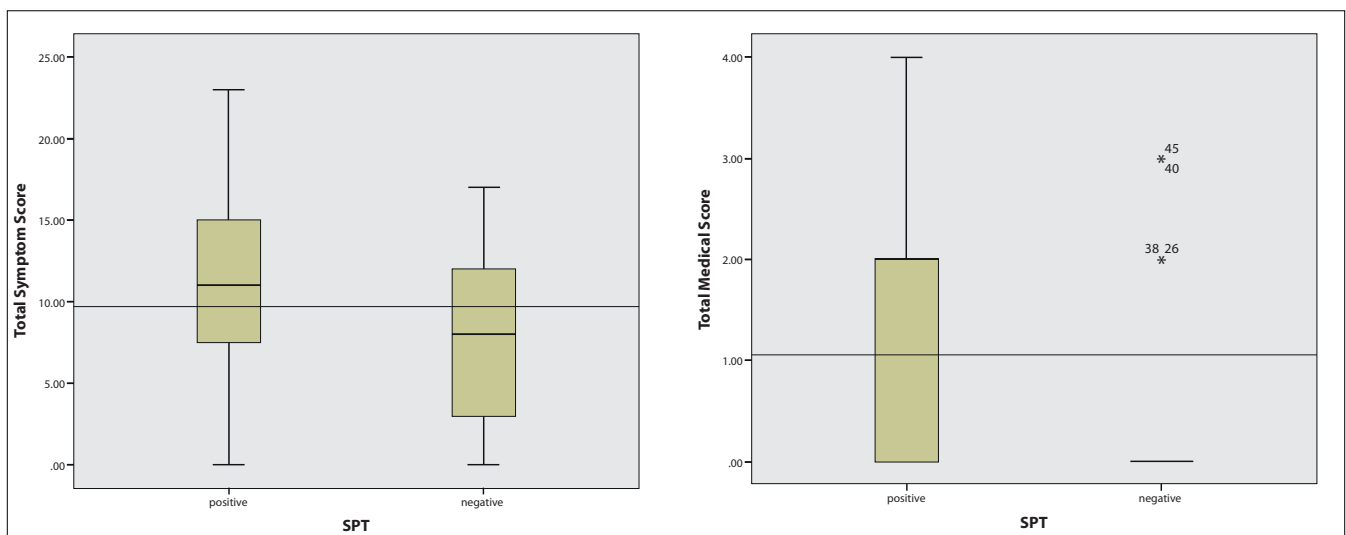


Table 1 - Allergens, respective airborne particles, aerobiological parameters (where applicable) and sensitizations.

Allergen	Airborne particles at determination level	Start date:	End date:	MPS (days)	APIn (pollen × day/m ³)	APIn/total pollen (%)	Maximum daily concentration (pollen/m ³)	SPT positive (n) ^a	Sensitization (%)
Grasses									
Phleum pratense	Poaceae	26.04.	23.09.	151	901.00	11.32	66.00	18	30.00
Sorghum halepense	Poaceae							16	25.80
Anthoxanthum odoratum	Poaceae							14	22.58
Cynodon dactylon	Poaceae							13	20.97
Poa pratensis	Poaceae							12	19.35
								9	14.52
Woody plants									
Corylus avellana	Corylus	02.02.	24.03.	51	33.00	0.41	5.00	14	23
Alnus glutinosa	Alnus	24.02.	23.03.	28	62.00	0.78	7.00	4	6.67
-	Carpinus	24.03.	02.06.	71	241.26	3.03	65.72	1	1.67
Betula alba	Betula	09.04.	06.05.	28	19.00	0.24	4.00	-	-
Betulaceae ^b		02.02.	02.06.	121	355.26	4.46		2	3.33
-	Fagus	17.04.	16.06.	61	37.82	0.47	31.62	7	11.67
Quercus rubra	Quercus	21.04.	29.05.	39	657.52	8.26	62.00	-	-
-	Castanea	09.06.	24.07.	46	20.06	0.25	3.10	2	3.33
Fagales ^b		02.02.	24.07.	173	1425.92	17.91	65.72	-	-
Fraxinus excelsior	Fraxinus	09.02.	11.05.	92	91.14	1.14	6.82	9	15.00
Olea europaea	Olea	15.05.	13.06.	30	235.76	2.96	66.34	5	8.33
Oleaceae ^b		09.02.	13.06.	122	326.90	4.11	66.34	4	6.67
Morus alba	Morus	15.04.	21.05.	36	169.26	2.13	16.74	9	15.00
Acer negundo	Acer	06.03.	26.04.	51	31.62	0.39	4.34	4	6.67
Populus alba	Populus	14.04.	23.03.	23	34.72	0.44	19.22	2	3.33
Salix alba	Salix	31.03.	10.05.	41	95.48	1.19	11.16	1	1.67
Platanus occidentalis	Platanus	14.04.	28.04.	15	349.06	4.38	79.98	0	0
Celtis occidentalis	Celtis	21.02.	21.02.	1	1.86	0.02		0	0
Juniperus ashei	Cupressaceae	22.02.	21.05.	89	1808.05	22.71	422.22	1	1.67
								2	3.33
Weeds									
Rumex crispus	Polygonaceae	17.04.	26.06.	71	46.66	0.59	7.44	13	22
Amaranthus retroflexus	Amaranthaceae	25.05.	24.09.	123	257.47	3.23	31.00	8	13.33
Chenopodium album	Amaranthaceae							2	3.33
								1	1.67

→ Allergen	Airborne particles at determination level	Start date:	End date:	MPS or MSS (days)	APIIn and ASIn (pollen or spores × day/m ³)	APIIn or SPIn/total pollen or spores (%)	Maximum daily concentration (pollen or spores/ m ³)	SPT positive (n) ^a	Sensitization (%)
Artriplex canescens	Amaranthaceae							1	1.67
Salsola kali	Amaranthaceae							1	1.67
Parietaria judaica	Urticaceae	05.04.	22.08.	140	291.33	3.66	17.98	0	0
Plantago lanceolata	Plantaginaceae	23.04.	08.11.	200	112.36	1.41	8.06	2	3.33
Artemisia vulgaris	Artemisia	14.07.	05.10.	84	80.24	1.01	7.44	3	5.00
Ambrosia artemisiifolia	Ambrosia	06.08.	01.10.	57	628.17	7.89	137.64	3	5.00
Xanthium strumarium	Xanthium	09.08.	14.09.	37	29.37	0.37	4.34	2	3.33
Asteroidae ^b		14.07.	05.10.	84	737.79	9.27		8	13.33
Fungal spores									
Alternaria alternata	Alternaria	23.05.	10.11.	172	3881.1	48.76	145.86	2	3.33
Control									
Saline								0	0.00
Histamine								60	100.00

^ato ≥ 1 allergen of the group; ^bsummarized for interpretation.

detect a significant correlation between olive (*Olea europaea* L.) and ash. Correlations between species within the order Fagales were all significant. None of tested tree species correlated with the total symptom score. When looking at sensitization to mulberry, we found significant correlations not only with genetically related hackberry (*Celtis occidentalis* L.), but also to unrelated poplar (*Populus alba* L.), oak (*Quercus rubra* L.), box elder (*Acer negundo* L.), and Johnson grass.

Weed allergen

Weed allergen sensitization to at least one weed was 22%. Dock (*Rumex crispus* L.) was the most prominent sensitizer. The mean level of the wheal and flare reaction was 1.83. We detected a significant correlation with symptoms and problems related to the eyes ($p < 0.01$) and with sensitizations to grasses (at least $p < 0.05$), Johnson grass excluded. With respect to *Asteroidae* pollen allergens, none of the participants was polysensitized to ragweed (*Ambrosia artemisiifolia* L.), mugwort (*Artemisia vulgaris* L.) or cocklebur (*Xanthium strumarium* L.). Cocklebur sensitization, however, correlated significantly with other weed species (**table II**). Ragweed induced a grade 4, mugwort a level 3 and cocklebur a grade 2 reaction. None of the *Asteraceae* allergens was significantly correlated with the total symptom score and neither with the total medical score. Ragweed positive tested participants complained about eye and nose symptoms, though. Amaranth (*Amaranthus retroflexus* L.) was the main sensitizer among the *Amaranthaceae* family displaying grade 1 and 2 reactions. Plantain (*Plantago lanceolata* L.) displayed a grade 1 reaction, while we did not detect any sensitization to wall pellitory.

Fungal sensitization

Sensitization to *Alternaria alternata* ((Fries) Keissler) did not correlate with the total symptom score, only the correlation with the total nose score was significant ($p < 0.05$).

Discussion

To issue a recommendation for a skin test panel to use in this geographic area, we reframed the ESP panel in a multidisciplinary approach according to locally relevant allergens, cross reactivity patterns and symptoms. The limitation of our work is the sample size.

Grass pollen sensitization and allergy

It was taken care to include representative allergens from three grass subfamilies *Pooideae*, *Panicoideae* and *Chloroideae* – all found in the environs of Istanbul and its hinterland (16). Johnson and Bermuda grass can extend the grass pollen season into autumn (17). In relation to other pollen types, airborne grass pollen concentrations were comparably high. As grasses are strong sensitizers, the chance of developing hay fever is increased in atopic patients during their flowering. In fact, symptoms of rhino-conjunctivitis

Figure 2 - Grass allergen sensitization (%) with respect to species subfamilies across sensitized subjects and the grade (4+ the strongest, 1+ the weakest) of the skin test reaction.

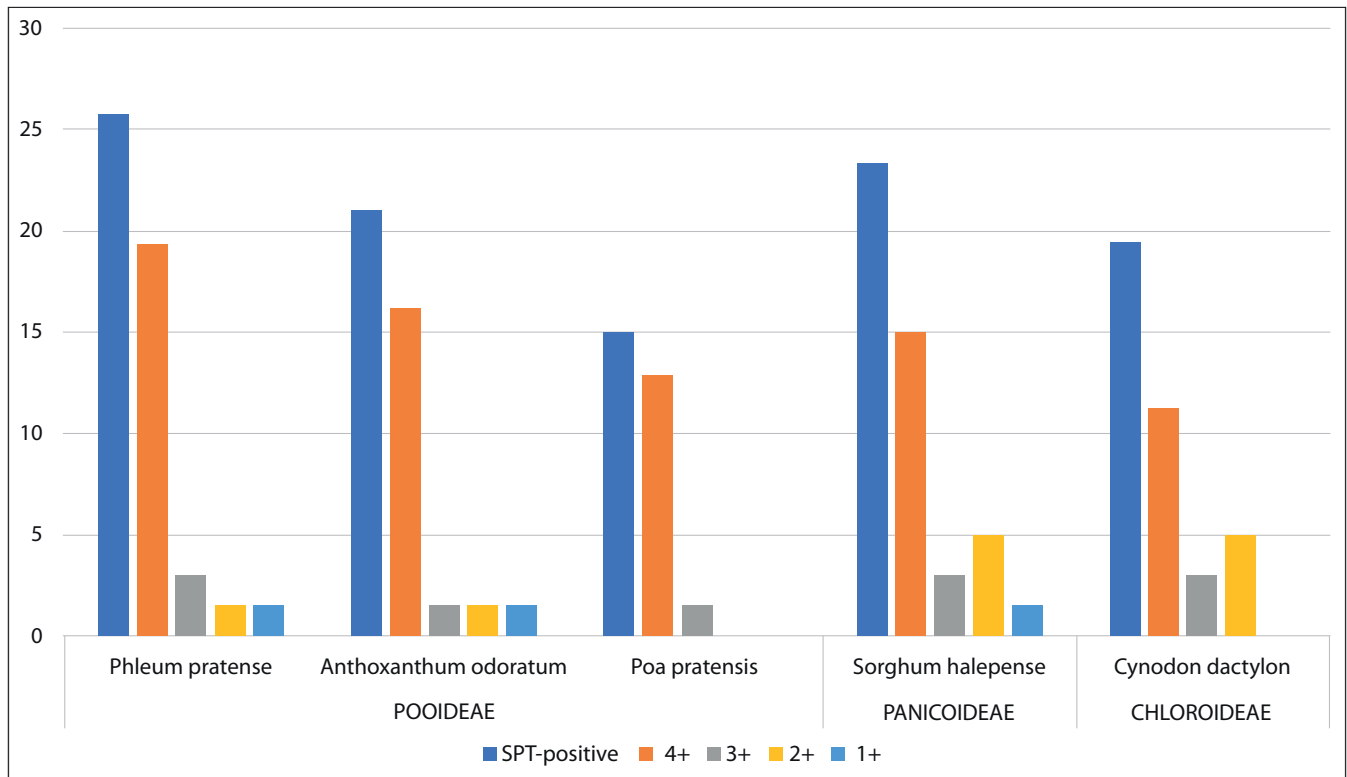


Table II - Correlations between weed sensitizations.

	<i>Artemisia</i>	<i>Salsola</i>	<i>Ambrosia</i>	<i>Chenopodium</i>	<i>Plantago</i>	<i>Xanthium</i>	<i>Amaranthus</i>	<i>Rumex</i>	<i>Atriplex</i>
<i>Artemisia</i>	1.000	-0.030	-0.053	-0.030	.376**	-0.043	-0.043	-0.090	-0.030
<i>Chenopodium</i>	-0.030	-0.017	-0.030	1.000	.689**	.689**	.689**	.293*	-0.017
<i>Plantago</i>	.376**	-0.024	-0.043	.689**	1.000	.466**	.466**	0.168	-0.024
<i>Xanthium</i>	-0.043	-0.024	-0.043	.689**	.466**	1.000	1.000**	.450**	-0.024
<i>Amaranthus</i>	-0.043	-0.024	-0.043	.689**	.466**	1.000**	1.000	.450**	-0.024
<i>Rumex</i>	-0.090	-0.051	-0.090	.293*	0.168	.450**	.450**	1.000	.337**
(n)	60	60	60	60	60	60	60	60	60

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

were predominantly induced by grass allergens and participants sought relieve with medication. Timothy grass played hereby an important role in cross-sensitizations. Its major allergen's Phl p 1 genetic sequence displays 90% homology to allergens from other Pooideae-subfamily species (18), which explains the high prevalence of polysensitization among grass sensitized individuals. Johnson grass (*Panicoidae*) showed to be a weaker sensitizer than species from the *Pooideae* subfamily. Nevertheless, high sensitization prevalence and monosensitization to this grass species indicate the need for inclusion in test panels and even more so in mixtures for immunotherapy. Bermuda grass seemed to play a minor role in our study sample. All in all, *Pooideae* subfamily allergens are the main culprits for grass pollen sensitization. In contrast to past (19, 20), and recent (21) studies concerning sensitization rates in atopic patients in the Marmara Region, where Istanbul is located, we refrained from including cereal allergens in our panel in accordance with the ESP. Advancements in molecular allergology, in fact, have shown that including cereals in test panels along with wild species is redundant. Cereals are wild species derived and, hence, carry homologue allergens (22). Due to their polyploid nature, cereals may have increased allergen content and induce stronger reactions in sensitized patients. Most cereals produce lower pollen concentrations per anther, and larger pollen than anemophilous wild grass species due to self-pollination. They appear rarely in routine pollen monitoring, as they do not become airborne easily due to their weight and size. Furthermore, most cereals (with the exception of rye, *Secale* and maize, *Zea*) are self-pollinating and very little pollen is dispersed (23). In fact, cereal pollen mostly stays in the surroundings of the fields and is connected to occupational allergy, concerning mainly field workers who are in direct contact with this crop (24).

Woody plant pollen sensitization and allergy

The lead tree pollen allergen was ash, despite low airborne concentrations in 2015. Although common in Istanbul's public space, we explain the low APIn at our location by annual fluctuations in pollen concentrations, possibly due to masting phenomena (25), which we can support with our own unpublished data. Moreover, ash was reportedly among the main pollen contributors in central Istanbul between 2005 and 2006 (26). According to our results, sensitization to olive tends to be less important than to genetically related ash. The climate in Istanbul is not strictly Mediterranean and there are no olive cultivars in the study area. Therefore, allergen exposure needed for sensitization might simply be limited. For Mediterranean areas olive is considered an important allergen and recommended in the ESP (6). In our sample, hazel was the lead *Betulaceae* allergen. Cross-reactivity between species of the Fagales order, and more prominently within *Betulaceae* members, is well known and linked to shared major allergens (Bet v 1) (14, 15). This explains why we detected sensitization rates to hazel and

birch despite comparably low concentrations in the air, while related hornbeam (*Carpinus* ssp.) concentrations were high. Sensitization to mulberry was exclusively found in polysensitized patients. Whether pan-allergen sensitization, possibly due to non-specific lipid transfer proteins such as Mor n 3, may play a role, needs to be further investigated (27). Although *Cupressaceae* exposure was high, juniper sensitization occurred only in weakly and multiply sensitized patients. Rhinoconjunctivitis has been associated with *Cupressaceae* (*Cupressus sempervirens* and *Juniperus communis*) sensitization, which we also observed in our study (28). Interestingly, we did not detect sensitization to *Platanus orientalis*, which we either link to the small sample size or to a reduced performance of the allergen extract used.

Weed pollen sensitization and allergy

Dock, the main weed sensitizer in our study, appeared only in polysensitized profiles. It remains obscure, whether sensitization to dock is genuine, or rather linked to a pan-allergen. Specific *Rumex* allergens were not found in the WHO/IUIS allergen nomenclature database (<http://www.allergen.org/>). We explain the correlation of dock with allergy symptoms with simultaneous sensitization to grass allergens of respective patients. Weed sensitization was always connected to multiple sensitizations, regardless of inter-family relationships. *Amaranthus retroflexus* best represented the *Amaranthaceae* allergens, which is plausible also in terms of pollen exposure. Wall pellitory is an important allergen in Mediterranean cities (29, 30) and a substantial contributor of allergenic pollen in our study area. It grows extensively on old stone walls and represents the main species on Istanbul's walls (31). However, we did not detect any sensitization to wall pellitory probably due to an underrepresentation in the sample. Since sensitization of wall pellitory was detected in Turkish Thrace (20), and allergen exposure is given in Istanbul, we deem this allergen relevant for the region. Ragweed was the strongest sensitizer among weeds bearing the potential to become more relevant in Istanbul, if not managed. Known stands in Istanbul happened to lay within the study area (32). Besides, there is evidence that ragweed pollen incurs through long distant transport from across the Black Sea, especially the Ukraine and the Crimean peninsula into the Marmaras (33). In Ankara, on the other hand, where ragweed pollen has been observed from the 1990s onwards with increasing concentrations over the years (34), the prevalence in susceptible children and adolescents has reached 16% (35). We did not detect polysensitization within *Asteroidae* allergens (14, 15). Cocklebur and plantain seem to be minor sensitizers.

Alternaria sensitization

It was low in our study sample, yet there was some clinical indication with respect to nose symptoms. This spore type prevails in summer months when temperatures are high.

Conclusions

Our recommendation towards outdoor allergens to use in Greater Istanbul and possibly Turkish Thrace confirm the ESP with the following emphasis: a grass panel for SPT should include at least timothy grass and meadow grass from the *Pooideae* subfamily, as well as Johnson grass from the *Panicoideae* subfamily. Johnson grass is especially important, if symptoms of late summer and autumn grass pollinosis occur. If the practitioner envisions treatment in form of allergy immunotherapy, the use of a wide range of grass allergens, possibly in mixtures including Johnson and Bermuda grass is advised. We recommend refraining from the use of cereal pollen extracts or mixtures of it when testing patients with a diagnosis of grass pollinosis. In terms of tree allergens, the addition of *Fraxinus excelsior* to the ESP is recommended. Instead of *Cupressus sempervirens*, a stronger *Cupressaceae* allergen such as *Juniperus ashei* might be used. We proved that the allergic population of Istanbul has started to become sensitized to neophytic ragweed. If this trend continues, the prevalence of ragweed allergy in Istanbul is likely to increase. It needs to be further investigated if mulberry and dock testing could identify pan-allergen sensitizations. Fungal spore allergens may be used as in ESP.

We, lastly, emphasize the importance of the collaboration with aerobiologists to facilitate an informed selection of potential outdoor allergens according to standardized parameters for testing and research purposes. Our findings not only facilitate successful diagnosis and treatment of hay fever in the specific geographical area of Istanbul but also ensure the inclusion of relevant outdoor allergens in future epidemiological studies.

Fundings

There was no financial support for the conduct of the research and/or preparation of the article. Thus, there was no involvement of a funder in any part of the present work.

Acknowledgments

We would like to thank Stallergenes Turkey for supplementing standardized test extracts free of charge.

Conflict of interests

The authors declare that they have no conflict of interests.

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