

Original article

GA²LEN skin test study III: Minimum battery of test inhalent allergens needed in epidemiological studies in patients

Background: The number of allergens to be tested in order to identify sensitized patients is important in order to have the most cost-effective approach in epidemiological studies.

Objective: To define the minimal number and the type of skin prick test (SPT) allergens required to identify a patient as sensitized using results of the new Pan-European GA²LEN skin prick test study.

Method: In a large Pan-European multicenter (17 centers in 14 countries) patient based study, a standardized panel of 18 allergens has been prick tested using a standardized procedure. Conditional approach allowed to determine the allergens selection.

Result: Among the 3034 patients involved, 1996 (68.2%) were sensitized to at least one allergen. Overall, eight allergens (grass pollen, *Dermatophagoides pteronyssinus*, birch pollen, cat dander, *Artemisia*, olive pollen, *Blatella* and *Alternaria*) allowed to identify more than 95% of sensitized subjects. However, differences were observed between countries, two allergens being sufficient for Switzerland (grass pollen and cat dander) as opposed to nine for France (grass pollen, *Dermatophagoides pteronyssinus*, olive pollen, cat dander, *Blatella*, cypress, dog dander, alder and [*Artemisia* or *Alternaria*]). According to country, up to 13 allergens were needed to identify all sensitized subjects.

Conclusion: Eight to ten allergens allowed the identification of the majority of sensitized subjects. For clinical care of individual patients, the whole battery of 18 allergens is needed to appropriately assess sensitization across Europe.

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Skin prick tests (SPT) represent the standard method to diagnose IgE-mediated allergic diseases (1) but there are advantages in harmonizing the standard panels of allergens used in different European countries. A first study has compared the different SPT procedures and sensitization rates at 29 allergy centers within the Global Allergy and Asthma European Network (GA²LEN) (2). Based on this study, a panel of 18 allergens was selected to be used in all European allergy centers. A new pan-European GA²LEN skin test study has been performed using this standardized panel of allergens in 17 centers in 14 countries (Heinzerling et al., in press). This study clearly showed that many allergens previously regarded as untypical for some regions in Europe have been underestimated (3). This could partly be related to changes in vegetation or climate in Europe or migration of citizens.

Skin prick testing is done (1) to identify sensitized subjects in epidemiological studies and (2) to identify the allergen responsible for IgE-mediated allergic disease in the clinical setting. For individual patient care all allergens which show a frequency of sensitization of more than 2% in patients are regarded as eligible for standard batteries. Epidemiological population based studies investigate whether IgE sensitization in general is present without having the wish to medically consult the individual patient. This question has been studied in the ECRHS (European Community Respiratory Health Survey) (4, 5). It was found that seven selected allergens (*Dermatophagoides pteronyssinus*, cat, grass, birch, olive pollen, *Alternaria*, and *Cladosporium*) allowed estimation of the prevalence of sensitized subjects in this population-based study (6).

However, such information is lacking in patient based surveys. The pan-European GA²LEN skin prick test study, a multicenter international cross-sectional survey (7) is a unique study which also answers this question

since a large number of well characterized subjects were studied in 14 countries, and standardized skin prick tests using the same 18 allergens and the same allergen extracts were tested in all centers.

The aim of the present study is thus to define the number and the type of skin prick test allergens required to identify a patient as sensitized using results of skin prick tests from the new pan-European GA²LEN skin test study, a large international multicenter study.

Materials and methods

Population and inclusion criteria

The new pan-European GA²LEN skin test study has been reported in detail (Heinzerling et al., in press). Briefly, this multicenter cross-sectional study included 17 centers in 14 countries. Patients consulting an outpatient clinic of one of the study centers because of a suspected IgE-mediated allergic disease were included.

Sensitization and skin prick tests

In the present study, the following commercial extracts for skin prick tests were carried out: *Alternaria* (1 : 20 G/V), *Aspergillus fumigatus* (10 000 BE/ml, ALK Abello, Hørsholm, Denmark), *Cladosporium herbarum* (10 000 BE/ml, Allergopharma), cat (10 HEP, ALK Abello), dog (10 HEP, ALK Abello), *Dermatophagoides pteronyssinus* (100 IR/ml, Stallergènes, Antony, France), *Dermatophagoides farinae* (100 IR/ml, Stallergènes), *Blatella* (1 mg/ml, Leti, Barcelona, Spain), and pollens of alder (50 000 SBE/ml, Allergopharma, Darmstadt, Germany), *Ambrosia* (1 : 100 G/V, ALK Abello), *Artemisia* (100 IR/ml, Stallergènes) (50 000 BE/ml), birch (50 000 SBE/ml, Allergopharma), cypress (*Cupressus sempervirens*) (100 IC/ml, Stallergènes), hazel (50 000 BE/ml, Allergopharma), plane tree (100 IC/ml, Stallergènes), olive (100 IR/ml, Stallergènes), grass mix (10 HEP, meadow oat grass, cock's foot grass, meadow fescue, perennial rye grass, timothy grass, smooth meadow grass, ALK Abello) and *Parietaria* (10 HEP, ALK Abello). A histamine positive

Abbreviations: ECRHS, European Community Respiratory Health Survey; GA²LEN, Global Allergy and Asthma European Network.

control and a negative control were also tested (ALK Abello). Central distribution of allergen extracts ensured use of equal allergen batches from the same companies. The allergen extracts were randomly selected and donated by GA²LEN sponsors

Results were recorded according to a standard operating procedure after 15 min. The largest and perpendicular diameter of the wheal for each of the allergens was measured and the following value calculated: largest + perpendicular diameter/2. Although in epidemiologic studies the cutoff of a positive skin test was proposed to be > 0 mm (7), in the present study the cutoff for a positive skin test was 3 mm (1). Subjects with at least one positive skin prick test were considered as sensitized.

Statistical analysis

The aim of the study was to define the number and the type of skin prick test allergens required to identify a subject as sensitized. Thus, a step-by-step conditional approach was proposed in order to classify the allergens from the one that give the highest increase in prevalence of sensitization to the one that gives the lowest. That procedure started by defining the most prevalent allergen. Then, to identify the next allergen that gives the highest increase in sensitization prevalence, the allergen that gives the highest prevalence of sensitization was looked for in the subgroup of subject not sensitized for the previous allergen. In other words, the allergen that gives the highest probability of sensitization knowing that subjects are not sensitized to the previous allergen tested. The procedure was repeated until none of the resulting allergen induced a change in prevalence. The prevalence of sensitization for the whole population was computed at each step.

The analysis was first performed grouping all subjects, and then by countries. In that particular case, centers were grouped by country. For each analysis, the number and type of allergens needed to identify at least 95% of sensitized subjects was observed. Finally, patients for whom at least one allergen was not tested or the result was not available were excluded from the analysis. The analysis was conducted using STATA 9 (StataCorp LP, College Station, TX, USA).

Results

A total of 3034 patients was included in the study, involving 1305 (43.0%) men, aged 33 (21–47) years. The demographic characteristics are reported in detail in a previous paper (Heinzerling et al., in press). Among them 106 (3.5%) had to be excluded since the results for all allergens prick tested were not available, restricting the analysis to 2928 patients.

Among the 2928 patients, 1996 (68.2%) were sensitized to at least one allergen (Table 1). Prevalence of sensitization ranged from 37.1% when only one allergen was tested, grass pollen (i.e. the most prevalent) to 68.2% when all allergens were tested. Eight allergens (grass pollen, *Dermatophagoides pteronyssinus*, birch pollen, cat dander, *Artemisia*, olive pollen, *Blatella* and *Alternaria*) out of 18 allowed to identified more than 95% of sensitized subjects. At a population level, seven allergens (*Dermatophagoides farinae*, cypress pollen, alder, plane pollen, *Cladosporium* and hazel pollen) induced less than a 0.5% increase in sensitization prevalence.

Table 1. Prevalence of sensitization, allergen ordered from the most prevalent to the allergen with the least increase in identifying additional sensitized subjects

	Prevalence n (%)	Increase in identifying sensitized patients by adding allergen in prick test	
		n	%
Grass pollen	1085 (37.1%)		
<i>Dermatophagoides pteronyssinus</i>	1523 (52.0%)	438	14.96
Birch pollen	1666 (56.9%)	143	4.88
Cat dander	1763 (60.2%)	97	3.31
<i>Artemisia</i>	1818 (62.1%)	55	1.88
Olive pollen	1850 (63.2%)	32	1.09
<i>Blatella</i>	1878 (64.1%)	28	0.96
<i>Alternaria</i>	1902 (65.0%)	24	0.82
Dog dander	1924 (65.7%)	22	0.75
<i>Parietaria</i>	1942 (66.3%)	18	0.62
<i>Ambrosia</i>	1957 (66.8%)	15	0.51
<i>Dermatophagoides farinae</i>	1968 (67.2%)	11	0.37
Cypress pollen	1977 (67.5%)	9	0.31
<i>Aspergillus</i>	1986 (67.8%)	9	0.31
Alder	1991 (68.0%)	5	0.17
Plane pollen	1993 (68.1%)	2	0.07
<i>Cladosporium</i>	1995 (68.1%)	2	0.07
Hazel	1996 (68.2%)	1	0.03

Cells in gray: More than 95% of sensitized subjects are identified.

Using the same allergen order, it appeared that with the eight allergens, 95% of sensitized subjects were identified in nine out of 14 countries (Table 2). Some local allergen, not included in the previous eight allergens, had an important impact. For instance, this is case for *Ambrosia* in Hungary or *Parietaria* in Italy.

Performing the analysis at a country level, the number and type of allergens required to identify an individual as sensitized was found to be dependent on the country (Table 3). For instance, in Switzerland, testing two allergens (grass pollen and cat dander) was sufficient to identify over 95% of sensitized subjects. In most countries (Belgium, Finland, Germany, Greece, Hungary, Italy, The Netherlands, Poland and Portugal) it was found that testing five to six allergens identified 95% of sensitized subjects. In some countries there was a need for additional allergens (seven in Austria and nine in France). Finally, only five allergens are sufficient to identify 90% of sensitized subjects for all countries except France (in that specific case, seven allergens are needed).

The most frequent allergens were grass pollen (one of the three most prevalent allergens in 12 out of 14 countries), *Dermatophagoides pteronyssinus* (8) and birch pollen (9). For five countries, these three allergens induced the highest prevalence of sensitization, and two of them are involved or four other countries (Table 3). At a country level, some allergens such as dog dander for Finland or *Parietaria* for Italy had an important impact.

Table 2. Prevalence of sensitization according to the country, allergens ordered from the most prevalent to the allergen with the least increase in identifying additional sensitized subjects under condition (same order used for all countries)

Country	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Austria	56 (27.7%)	75 (37.1%)	87 (43.1%)	93 (46%)	97 (48%)	100 (49.5%)	102 (50.5%)	104 (51.5%)	105 (52%)	105 (52%)	105 (52%)	106 (52.5%)	107 (53%)	.	.	.	.	.
Belgium	50 (25%)	75 (37.5%)	86 (43%)	88 (44%)	89 (44.5%)	89 (44.5%)	90 (45%)	90 (45%)	91 (45.5%)	91 (45.5%)	91 (45.5%)	92 (46%)	94 (47%)	95 (47.5%)	.	.	.	.
Denmark	122 (68.5%)	145 (81.5%)	160 (89.9%)	162 (91%)	167 (93.8%)	167 (93.8%)	168 (94.4%)	168 (94.4%)	169 (94.9%)	169 (94.9%)	169 (94.9%)	170 (95.5%)	170 (95.5%)	170 (95.5%)	170 (95.5%)	171 (96.1%)	.	.
England	71 (54.2%)	93 (71%)	97 (74.1%)	99 (75.6%)	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Finland	35 (20.7%)	44 (26%)	57 (33.7%)	68 (40.2%)	71 (42%)	71 (42%)	77 (45.6%)	78 (46.2%)	81 (47.9%)	81 (47.9%)	81 (47.9%)	81 (47.9%)	81 (47.9%)	82 (48.5%)	.	.	.	.
France	47 (23.5%)	94 (47%)	94 (47%)	101 (50.5%)	104 (52%)	110 (55%)	112 (56%)	114 (57%)	117 (58.5%)	117 (58.5%)	118 (59%)	119 (59.5%)	122 (61%)	124 (62%)	126 (63%)	.	.	.
Germany	125 (31.8%)	158 (40.2%)	203 (51.7%)	212 (53.9%)	218 (55.5%)	218 (55.5%)	226 (57.5%)	232 (59%)	234 (59.5%)	234 (59.5%)	234 (59.5%)	234 (59.5%)	234 (59.5%)	236 (60.1%)	237 (60.3%)	237 (60.3%)	238 (60.6%)	.
Greece	107 (49.3%)	142 (65.4%)	143 (65.9%)	152 (70.1%)	156 (71.9%)	166 (76.5%)	167 (77%)	172 (79.3%)	172 (79.3%)	175 (80.7%)	177 (81.6%)	178 (82%)	178 (82%)	179 (82.5%)	180 (83%)	.	.	.
Hungary	99 (38.2%)	126 (48.7%)	134 (51.7%)	148 (57.1%)	167 (64.5%)	168 (64.9%)	168 (64.9%)	171 (66%)	173 (66.8%)	173 (66.8%)	184 (71.1%)	185 (71.4%)	186 (71.8%)	186 (71.8%)	186 (71.8%)	186 (71.8%)	187 (72.2%)	.
Italy	45 (19.6%)	109 (47.4%)	111 (48.3%)	124 (53.9%)	128 (55.7%)	133 (57.8%)	133 (57.8%)	133 (57.8%)	135 (58.7%)	145 (63%)	145 (63%)	145 (63%)	146 (63.5%)	146 (63.5%)	146 (63.5%)	147 (63.9%)	.	.
Poland	83 (41.7%)	108 (54.3%)	122 (61.3%)	131 (65.8%)	134 (67.3%)	134 (67.3%)	134 (67.3%)	136 (68.3%)	140 (70.4%)	140 (70.4%)	140 (70.4%)	140 (70.4%)	141 (70.9%)	142 (71.4%)	.	.	.	.
Portugal	60 (35.5%)	136 (80.5%)	140 (82.8%)	148 (87.6%)	150 (88.8%)	151 (89.4%)	154 (91.1%)	155 (91.7%)	157 (92.9%)	161 (95.3%)	161 (95.3%)	164 (97%)	164 (97%)	165 (97.6%)	166 (98.2%)	166 (98.2%)	166 (98.2%)	167 (98.8%)
Switzerland	93 (80.2%)	96 (82.8%)	101 (87.1%)	103 (88.8%)	103 (88.8%)	103 (88.8%)	103 (88.8%)	103 (88.8%)	103 (88.8%)	103 (88.8%)	103 (88.8%)	104 (89.7%)	.	.	.	.	.	.
The Netherlands	92 (34.7%)	122 (46%)	131 (49.4%)	134 (50.6%)	135 (50.9%)	141 (53.2%)	145 (54.7%)	147 (55.5%)	148 (55.9%)	149 (56.2%)	150 (56.6%)	151 (57%)	.	.	.	.	.	.
Total	1085 (37.1%)	1523 (52%)	1666 (56.9%)	1763 (60.2%)	1818 (62.1%)	1850 (63.2%)	1878 (64.1%)	1902 (65%)	1924 (65.7%)	1942 (66.3%)	1957 (66.8%)	1968 (67.2%)	1977 (67.5%)	1986 (67.8%)	1991 (68%)	1993 (68.1%)	1995 (68.1%)	1996 (68.2%)

1: Grass, 2: *Dermatophagoides pteronyssinus*, 3: Birch pollen, 4: Cat dander, 5: *Artemisia*, 6: Olive pollen, 7: *Blatella*, 8: *Alternaria*, 9: Dog dander, 10: *Parietaria*, 11: *Ambrosia*, 12: *Dermatophagoides farinae*, 13: *Cypripes* pollen, 14: *Aspergillus*, 15: Alder, 16: Plane pollen, 17: *Cladosporium*, 18: Hazel.

Cumulative frequency and percent are reported.

Cells in gray: More than 95% of sensitized subjects are identified.

Table 3. Prevalence of sensitization according to the country, allergens order from the most prevalent to the less under condition for each country

Country	n	1	2	3	4	5	6	7	8	9	10	11	12	13
Austria	202	56 (27.7%)	75 (37.1%)	87 (43.1%)	93 (46%)	97 (48%)	100 (49.5%)	103 (51%)	104 (51.5%)	105 (52%)	106 (52.5%)	107 (53%)	.	.
Belgium	200	59 (29.5%)	75 (37.5%)	86 (43%)	89 (44.5%)	91 (45.5%)	92 (46%)	93 (46.5%)	94 (47%)	95 (47.5%)	.	.	.	.
Denmark	178	122 (68.5%)	145 (81.5%)	160 (89.9%)	165 (92.7%)	167 (93.8%)	168 (94.4%)	169 (94.9%)	170 (95.5%)	171 (96.1%)	.	.	.	.
England	131	71 (54.2%)	93 (71%)	98 (74.8%)	99 (75.6%)	.	.	.	.	.	.	.	.	.
Finland	169	48 (28.4%)	63 (37.3%)	70 (41.4%)	75 (44.4%)	78 (46.2%)	79 (46.7%)	80 (47.3%)	81 (47.9%)	82 (48.5%)	.	.	.	.
France	200	70 (35%)	94 (47%)	102 (51%)	107 (53.5%)	110 (55%)	113 (56.5%)	116 (58%)	119 (59.5%)	121 (60.5%)	123 (61.5%)	124 (62%)	125 (62.5%)	126 (63%)
Germany	393	125 (31.8%)	178 (45.3%)	203 (51.7%)	211 (53.7%)	219 (55.7%)	227 (57.8%)	232 (59%)	234 (59.5%)	235 (59.8%)	235 (59.8%)	.	.	.
Greece	217	107 (49.3%)	142 (65.4%)	157 (72.4%)	165 (76%)	170 (78.3%)	173 (79.7%)	175 (80.6%)	176 (81.1%)	177 (81.6%)	178 (82%)	179 (82.5%)	.	.
Hungary	259	137 (52.9%)	160 (61.8%)	166 (64.1%)	175 (67.6%)	178 (68.7%)	181 (69.9%)	183 (70.7%)	184 (71%)	185 (71.4%)	186 (71.8%)	187 (72.2%)	.	.
Italy	230	86 (37.4%)	126 (54.8%)	133 (57.8%)	138 (60%)	141 (61.3%)	143 (62.2%)	144 (62.6%)	145 (63%)	146 (63.5%)	147 (63.9%)	.	.	.
Poland	199	83 (41.7%)	108 (54.3%)	122 (61.3%)	131 (65.8%)	136 (68.3%)	138 (69.3%)	140 (70.4%)	141 (70.9%)	142 (71.4%)	.	.	.	.
Portugal	169	113 (66.9%)	136 (80.5%)	148 (87.6%)	153 (90.5%)	157 (92.9%)	159 (94.1%)	161 (95.3%)	162 (95.9%)	163 (96.4%)	164 (97%)	165 (97.6%)	166 (98.2%)	167 (98.8%)
Switzerland	116	93 (80.2%)	100 (86.2%)	103 (88.8%)	104 (89.7%)	.	.	.	.	.	.	.	.	.
The Netherlands	265	92 (34.7%)	122 (46%)	131 (49.4%)	137 (51.7%)	142 (53.6%)	145 (54.7%)	148 (55.8%)	150 (56.6%)	151 (57%)	.	.	.	.

1: Grass, 2: *Dermatophagoides pteronyssinus*, 3: Birch pollen, 4: Cat dander, 5: *Artemisia*, 6: Olive pollen, 7: *Blatella*, 8: *Alternaria*, 9: Dog dander, 10: *Parietaria*, 11: *Ambrosia*, 12: *Dermatophagoides farinae*, 13: Cypress pollen, 14: *Aspergillus*, 15: Alder, 16: Plane pollen, 17: *Cladosporium*, 18: Hazel.

Results are expressed in frequencies and percents.

Cells in gray: More than 95% of sensitized subjects are identified.

Empty cells: Additional allergen did not change the prevalence.

Allergens tested are reported in the table thereafter

Country	1	2	3	4	5	6	7	8	9	10	11	12	13
Austria	gra	derp	bir	cat	art	dog	oli	cyp/pla	bla	alt	derf	.	.
Belgium	derp	gra	bir	dog	cyp	bla	art	derf	asp	.	.	.	.
Denmark	gra	derp	bir	art	dog/cat	dog/cat	derf	pla	bla	.	.	.	.
England	gra	derp	cat	bir/ald/pla	.	.	.	.	.	.	.	.	.
Finland	dog	bir	bla	derp	cat	asp/cla	gra	alt	art	.	.	.	.
France	derp	gra	oli	cat	bla	cyp	dog	ald	asp/art	asp/art	alt	derf	amb
Germany	bir	derp	gra	cat	bla	alt	art	dog	asp	ald/pla	cla	.	.
Greece	gra	derp	oli	alt	par	cat/dog	amb	asp	bla	art	derf	ald	.
Hungary	amb	cat	gra	derp	art	alt	oli	cyp	cla	dog	derf	.	.
Italy	derp	par	oli	cat	gra	dog	bir/ald/haz	art/amb	cyp	pla	.	.	.
Poland	gra	derp	bir	dog	cat	alt	art	cyp	asp	.	.	.	.
Portugal	derp	gra	par	dog	derf	bir	bla	art	asp	cat	ald	haz	alt
Switzerland	gra	cat	derf	bir/ald/haz	.	.	.	.	.	.	.	.	.
The Netherlands	gra	derp	bir	oli	bla	amb	cat	derf	asp/par	.	.	.	.

1: Grass, 2: *Dermatophagoides pteronyssinus*, 3: Birch pollen, 4: Cat dander, 5: *Artemisia*, 6: Olive pollen, 7: *Blatella*, 8: *Alternaria*, 9: Dog dander, 10: *Parietaria*, 11: *Ambrosia*, 12: *Dermatophagoides farinae*, 13: Cypress pollen, 14: *Aspergillus*, 15: Alder, 16: Plane pollen, 17: *Cladosporium*, 18: Hazel.

ald: Alder, alt: *Alternaria*, amb: *Ambrosia*, art: *Artemisia*, asp: *Aspergillus*, bir: Birch pollen, bla: *Blatella*, cat: Cat dander, cla: *Cladosporium*, cyp: Cypress pollen, derf: *Dermatophagoides farinae*, derp: *Dermatophagoides pteronyssinus*, dog: Dog dander, gra: Grass pollen, haz: Hazel, oli: Olive pollen, par: *Parietaria*, pla: Plane pollen.

Cumulative frequency and percent are reported.

Cells in gray: The impact of these allergens on sensitization prevalence is the same. The order proposed is arbitrary.

Cells with more than one allergen: The impact of all these allergens is similar. Each of them can substitute the others.

Finally, a limited number of allergen were sufficient to identify sensitized persons in a clinical population. With the testing of 13 allergens, all sensitized subjects were identified. Additional allergens did not change the measured sensitization prevalence (Table 3).

Discussion

Based on a multicenter international study involving 3034 subjects suspected to suffer from an allergic disease were tested by a standardized skin prick test with a standardized allergen battery of 18 allergens. To only identify sensitized subjects the number of allergens can be limited. Depending on the country, two to nine allergens out of 18 were sufficient to identify 95% of sensitized subjects; while four to 13 allergens were required to identify 100% of sensitized subjects. However, the number and the type of allergens were highly correlated to the country, and local allergens were often of relevance.

Some limitations to the study should be taken into consideration. Even if the study was the first large study on a pan European scale using a common battery of allergenic extracts, the number of subjects tested was restricted, ranging from 116 to 393 per center. However, the study design, the sample size and the population (patients consulting for symptoms) allowed to observe uncommon sensitization (<1%). Another limitation could be the number of centers by country, which was restricted (one to two centers per country). That probably reinforced the impact of some local allergens or allergens highly represented in the area.

The present study focused only on the sensitization and did not explore the clinical relevance of the sensitization, i.e. the link between sensitization to a specific allergen and symptoms. Sensitization was based on skin prick tests as the standard method for assessing an IgE-mediated allergy (1, 10, 11), extracts from selected specified manufacturers being used. It is possible that using other allergens extracts the results of the study may differ, but this is unlikely except for cypress pollen and some molds which are notoriously difficult to standardize (12). However, mold sensitization is usually less common than pollen, mites or pet danders.

The number of allergens needed to identify 95% of sensitized subjects varied widely between countries. These differences could be due to the impact of allergens observed in the country, in particular for tree pollen. Another explanation could be the age of the tested population. It appeared that around one-third of patients enrolled in the Mediterranean countries were children under 14 whereas no children were included in Nordic countries.

The impact of some allergens was lower than expected. For instance, *Dermatophagoides farinae*, usually a prevalent allergen, had not a major impact in the present

study on sensitization prevalence. This was probably the consequence of the cross reactivity with *Dermatophagoides pteronyssinus*. The later one was more prevalent, and many patients were sensitized to both of them. Cypress pollen also had a lower impact than expected, particularly in Mediterranean countries. In Montpellier, around 30% of the tested patients have positive skin prick tests to *Juniperus ashei* (8), a Cupressaceae species closely related to *Cupressus sempervirens* whereas far fewer patients have positive tests for the later one. It is therefore likely that *Cupressus sempervirens* extracts are weaker than those of *Juniperus ashei*. Thus, the low impact of cypress pollen in the study could be related to the low allergen potency of *Cupressus sempervirens* pollen extract.

Although the list of allergen species relevant for a particular country is closely related to the vegetation and climate, surprising results were observed, with, for example, cypress pollen in Belgium, *Ambrosia* pollen in the Netherlands and alder pollen in France (Montpellier). These results may be due to testing of subjects who have migrated around Europe or other countries, or to cross reactivity. It is common that in a country like France, people commonly migrate during their life and are exposed to three different climates (Mediterranean, Atlantic and Alpine) resulting in mixed sensitization patterns. Finally, some differences were observed between centers located in the same geographic area such as Vienna (Austria) and Budapest (Hungary). In Vienna, *Ambrosia* pollen does not count whereas in Budapest it was the first ranking allergen and accounted for over 50% of sensitizations. The method used (conditional approach) may be partly related to this difference since only 8.3% of patients were sensitized to ragweed in Austria, and none (or very few) of them were monosensitized. Thus, they were accounted with other allergens.

In conclusion, the present study confirmed previous findings (9). Similar to population-based epidemiologic studies, a limited number of allergens allowed the identification of the majority of sensitized subjects in patients based studies. However for individual patients, this does not mean that you are able to detect the relevant allergens with this approach. Thus for clinical practice, the whole battery of 18 allergens is needed to appropriately assess allergens affecting at least 1–2% of patients across Europe.

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