The effect of component-resolved diagnosis on specific immunotherapy prescription in children with hay fever

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Background: Sensitization to profilins and other cross-reacting molecules might hinder proper specific immunotherapy (SIT) prescription in polysensitized patients with pollen-related allergic rhinitis (AR). In these patients, component-resolved diagnosis (CRD) might modify SIT prescription by improving the identification of the disease-eliciting pollen sources.
Objectives: We sought to measure the effect of CRD on SIT prescription in children with pollen-related AR.
Methods: Children (n = 651) with moderate-to-severe pollen-related AR were recruited between May 2009 and June 2011 in 16 Italian outpatient clinics. Skin prick test (SPT) reactivity to grass, cypress, olive, mugwort, pellitory, and/or Betulaceae pollen was considered clinically relevant if symptoms occurred during the corresponding peak pollen season. IgE sensitization to Phl p 1, Phl p 5, Bet v 1, Cup a 1, Art v 1, Ole e 1, Par j 2, and Phl p 12 (profilin) was measured by using ImmunoCAP. SIT prescription was modeled on SPT responses first and then remodeled considering also CRD according to GA^{2}LEN–European Academy of Allergology and Clinical Immunology guidelines and the opinions of 14 pediatric allergists.
Results: No IgE to the respective major allergens was detected in significant proportions of patients with supposed clinically relevant sensitization to mugwort (45/65 [69%]), Betulaceae (146/252 [60%]), pellitory (78/257 [30%]), olive (111/390 [28%]), cypress (28/184 [15%]), and grass (56/568 [10%]). IgE to profilins, polcalcins, or both could justify 173 (37%) of 464 of these SPT reactions. After CRD, the SPT-based decision on SIT prescription or composition was changed in 277 (42%) of 651 or 315 (48%) of 651 children according to the European or American approach, respectively, and in 305 (47%) of 651 children according to the opinion of the 14 local pediatric allergists.
Conclusions: In children with pollen-related AR, applying CRD leads to changes in a large proportion of SIT prescriptions as opposed to relying on clinical history and SPT alone. The hypothesis that CRD-guided prescription improves SIT efficacy deserves to be tested. (J Allergy Clin Immunol 2014;134:75-81.)

Key words: *Allergic rhinitis, children, component-resolved diagnosis, IgE, panallergens, pollen, profilin, specific immunotherapy*

Allergic rhinoconjunctivitis induced by pollens (pollen-related allergic rhinitis [AR]) affects millions of persons globally[1] and is particularly prevalent among children.[2] Allergen-specific immunotherapy (SIT) with pollen extracts can reduce pollen-related AR symptoms and prevent asthma comorbidity and is the only disease-modifying intervention.[3-5] Guidelines state that SIT efficacy requires proper matching of the SIT preparation against the pollen sources causing all or most symptoms in the individual patient.[6] Unfortunately, many patients with pollen allergy are

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Abbreviations used
AR: Allergic rhinitis
ARIA: Allergic Rhinitis and its Impact on Asthma
CRD: Component-resolved diagnosis
EAACI: European Academy of Allergology and Clinical Immunology
SIT: Specific immunotherapy
SPT: Skin prick test

today sensitized to many pollen sources with overlapping seasonality.[7] Moreover, many patients are sensitized to profilin or other highly cross-reacting molecules shared by many unrelated pollen sources and their extracts.[8] Thus the identification of the disease-eliciting pollen sources by means of extract-based skin prick tests (SPTs) in patients sensitized to multiple pollens with overlapping seasonality is often difficult.[9]

Measuring levels of IgE antibodies to major allergens makes it possible to decide whether SPT response positivity to a pollen source is "true" or "spurious" (ie, caused by corecognition of highly cross-reacting allergenic molecules).[10] Accordingly, it has been suggested that SIT to a pollen should be prescribed only when serum IgE antibodies to major allergenic molecular components of that pollen are detectable.[11] Hence component-resolved diagnosis (CRD) should make it possible to avoid either the isolated administration of irrelevant allergens or the "dilution" of the relevant ones in an SIT preparation.[12,13] For example, it was proposed that patients with SPT reactivity to grass pollen extracts should receive SIT for grass only in the presence of IgE antibodies to the major allergenic molecules Phl p 1, Phl p 5, or both.[14,15] Similarly, patients with SPT response positivity to the extract of pellitory, mugwort, Betulaceae, or olive should receive SIT only if they have IgE to Par j 2, Art v 1, Bet v 1, and Ole e 1, respectively.[14,15]

International guidelines for SIT still do not incorporate CRD in the diagnostic procedure, leading to SIT prescription.[6,16] To date, only 2 recent studies in adults evaluated whether therapeutic decisions are modified by CRD.[16,17] Moreover, the above-mentioned CRD algorithm has never been systematically tested, and to our knowledge, studies focusing on children are not yet available. Therefore we analyzed the data set of a large population of Italian children with pollen-related AR who had never received SIT[18] to test whether CRD results influence the prescription of SIT modeled according to international guidelines or pragmatically proposed by a pool of 14 pediatric allergists.

METHODS

Study population

Panallergens in Pediatrics (PAN-PED) is the first nationwide observational multicenter survey carried out by the Italian Pediatric Allergy Network. The Italian Pediatric Allergy Network is a large group of Italian specialists in pediatric allergy[18-20] created to investigate the effect of sensitization to highly cross-reacting allergenic pollen molecules on the management of respiratory allergies in childhood. Children were enrolled in 16 pediatric outpatient clinics in 14 Italian cities in the Po valley (Milan, Verona, Parma, and Bologna), Central Italy (Empoli and Ascoli Piceno), the Tyrrhenian coast and inlands (Genoa, 4 centers in Rome, Naples, and Benevento), and Southern Italy and islands (Cagliari, Palermo, and Crotone) between May 2009 and June 2011. Criteria for eligibility were (1) age 4 to 18 years; (2) a history of pollen-induced AR, asthma, or both in one of the 2 last pollen seasons; and (3) positive skin prick test (SPT) responses to the relevant pollen extracts. Exclusion criteria were (1) previous SIT for any pollen allergen and (2) any other severe chronic disease. Recruited children's parents answered questionnaires, and patients underwent SPTs (see below) and a blood draw. Parents or tutors of all participants provided informed written consent to clinical investigations. The study design and procedures were approved by the ethics committee of each participating center.

Questionnaire and diagnostic criteria

Selected questions obtained from the following internationally validated questionnaires were administered to all participants: the International Study of Allergy and Asthma in Childhood,[21] Allergic Rhinitis and its Impact on Asthma (ARIA),[22] and the Global Initiative for Asthma.[23] A diagnosis of pollen-induced AR was made, as previously described,[18] in the presence of (1) nasal, eye, or both symptoms (apart from cold)[21] for at least 3 weeks during one of the 2 last pollen seasons and (2) a positive SPT response (wheal reaction of ≥3 mm) in accordance with clinical history and local pollination period. Pollen-induced AR was classified as mild or moderate to severe, as well as intermittent or persistent according to the ARIA classification.[22] An informatics platform (AllergyCARD; TPS Production, Rome, Italy) was used for data input.

SPTs

SPTs were performed with a panel of commercial extracts (ALK-Abelló, Milan, Italy), including timothy grass, olive, cypress, mugwort, pellitory, and Betulaceae (birch and/or hazel). Histamine (0.1 mg/mL) and glycerol solution were positive and negative controls, respectively. Morrow-Brown needles were used to prick the skin, and wheal reactions were read after 15 minutes. A wheal of 3 mm or greater (or ≥5 mm when indicated) after subtraction of the negative control was regarded as positive.[24] A hierarchy of relevance was assigned to each of the 6 pollen sources by the locally recruiting doctors. A positive skin reaction was considered clinically relevant if reported symptoms occurred during the peak season of the respective pollen registered during 2001-2010.

IgE assays

IgEs for allergenic molecules were tested in sera of patients with a wheal reaction of greater than 2 mm elicited by the corresponding allergenic source[18] by using the ImmunoCAP FEIA (TFS, Lund, Sweden). The following major allergenic molecules were selected, as previously suggested: Graminaceae (*Phleum pratense*, Phl p 1 and Phl p 5),[16] Oleaceae (*Olea europaea*, nOle e 1),[16] Cupressaceae (*Cupressus arizonica*, Cup a 1),[25] Betulaceae (*Betula verrucosa*, Bet v 1),[16] Urticaceae (*Parietaria judaica*, Par j 2),[16] and Compositae (*Artemisia vulgaris*, Art v 1).[26] Results were expressed in kilounits per liter and classified as positive if 0.7 kU/L or greater.

GA²LEN–European Academy of Allergology and Clinical Immunology and alternative prescription models

Prescription of SIT was modeled according to the recently published GA²LEN–European Academy of Allergology and Clinical Immunology (EAACI) pocket guide.[6] Briefly, a subject was eligible for SIT if his or her pollen-related AR symptoms were (1) moderate to severe according to ARIA classification, (2) associated with SPT sensitization to pollen sources against which SIT is effective (timothy grass, birch, mugwort, olive, cypress, and pellitory), and (3) occurring during the local peak of pollen exposure.[6] In the European model, when 4 or more clinically relevant sensitizations were detected, the 3 most relevant allergens were selected on the basis of the opinion of the locally recruiting doctor.[6] Three additional SIT prescription models were taken into account (Table I). In the American model the number of allergenic sources to be mixed was unlimited.[27,28] In the monoallergenic model only the most important allergenic source was allowed,[28,29] and in the monosensitization model only patients with clinically relevant sensitization to 1 pollen source were eligible for SIT. All 4 prescription models described above were applied, again taking CRD into account, as previously proposed,[14] to measure the effect of CRD on SIT prescription. Briefly, SPT sensitization was considered irrelevant for SIT if not confirmed by a positive (≥0.7 kU/L) result to IgE testing to the respective major allergenic protein or proteins.[14]

TABLE I. Criteria for SIT prescription

Model	Patient's clinically relevant sensitization	Number of allergenic extracts
A. European	Monosensitization or polysensitization	Not more than 3
B. American	Monosensitization or polysensitization	As many as the clinically relevant sensitization
C. Monoallergenic	Monosensitization or polysensitization	Only 1
D. Monosensitization	Monosensitization	Only 1

SIT prescription by pediatric allergists

Fourteen doctors expressed their opinions on SIT prescription to achieve insight into the effect of CRD in real-life conditions. Each doctor received the data (clinical history and SPT responses) of a random subsample of patients living in his or her own geographic area and then expressed an opinion on SIT prescription: yes or no on SIT and, if yes, details on allergen-specific SIT composition. Afterward, each doctor received information on the qualitative (positive/negative) data and, if results were positive, the concentration of IgE antibodies against Phl p 1, Phl p 5, Bet v 1, Art v 1, Cup a 1, Par j 2, and Ole e 1 of the same patients; then they were asked to express whether they had changed their decision on SIT prescription and its composition.

Statistics

Atopic sensitization, disease characteristics, SIT prescription, and the effect of CRD on SIT prescription were analyzed by using descriptive statistics. Differences were tested by means of ANOVA if quantitative and by using the χ^2 test if qualitative. A P value of less than .05 was considered significant.

RESULTS

Study population

The target population has been thoroughly described elsewhere.[18] Of the original population of 695 children with moderate-to-severe AR, 651 (94%) had a complete data set for the present study. The major characteristics of the population sample are shown in Table II. According to the ARIA classification, AR was intermittent in 131 (20%) of 651 patients and persistent in 520 (80%) of 651 patients. Most patients had AR symptoms from March to July, and a smaller proportion also had AR symptoms from August to October.[18] Most patients were sensitized to timothy grass (91%) and olive (62%), whereas 47%, 44%, and 40% were sensitized to Betulaceae, cypress, and pellitory, respectively, and only 25% were sensitized to mugwort. Skin sensitization to grass pollen was almost always (96%) consistent with symptoms of AR during the peak season (Table III), whereas this was true for only 64% and 40% of skin sensitizations to cypress and mugwort, respectively. The vast majority of the patients were polysensitized: clinically relevant pollen monosensitization was observed in only 133 (20%) patients, whereas 182 (28%) had clinically relevant sensitization to 4 or more pollen sources.

Consistency between SPT and CRD results

The discordance rate between SPT and conventional (extract-based) IgE to pollens was quite low, ranging from 2.5% for grass to 11.7% for mugwort and 11.8% for cypress (see Table E1 in this article's Online Repository at www.jacionline.org). By contrast, inconsistency between SPT and CRD results ranged from 10% for grass pollen to 69% for mugwort (Table III). Only 56 (10%) of the 568 patients with clinically relevant sensitization to grass pollen did not show IgE antibodies specific for the major allergens Phl p 1 and Phl p 5. By contrast, 28% and 30% of the patients with clinically relevant sensitization to olive and pellitory did not have IgE antibodies for Ole e 1 and Par j 2, respectively, and IgE levels to Bet v 1 or Art v 1 were negative in more than 50% of the patients with apparently clinically relevant skin sensitization to the extract of Betulaceae or mugwort, respectively (Table III). A consistent proportion, but not all, of the inconsistencies between SPT and CRD results were associated with IgE sensitization to profilin (Phl p 12) or polcalcin (Phl p 7, Table III).

TABLE II. Characteristics of 651 Italian children with moderate-to-severe AR

Male sex, no. (%)	443 (68)
Age (y), mean (SD)	10.7 (3)
Race, no. (%)	
White	646 (99.2)
Black	3 (0.5)
Asian	2 (0.3)
Familial atopy, no. (%)	
Father	240 (37)
Mother	292 (45)
Smoke exposure, no. (%)	
Father	273 (42)
Mother	162 (25)
AR	
Age at onset (y), mean (SD)	5.4 (3.0)
Disease duration (y), mean (SD)	5.3 (3)
Months/year with symptoms, mean (SD)	4.7 (1.8)
ARIA classification (quality), no. (%)	
Sneezers	519 (80)
Blockers	360 (55)
ARIA classification, no. (%)	
Moderate-to-severe intermittent	131 (20)
Moderate-to-severe persistent	520 (80)
Asthma, no. (%)	253 (39)
Oral allergy syndrome, no. (%)	157 (24)
Skin sensitization to pollens (≥3 mm), no. (%)	
Grass	592 (91)
Betulaceae	309 (47)
Olive	405 (62)
Pellitory	259 (40)
Mugwort	163 (25)
Cypress	287 (44)
Serum total IgE (kU/L, geometric mean (SE)	389 (2.8)
IgE sensitization (≥0.7 kU/L), no. (%)	
Mite	335 (51)
Cat	201 (31)
Alternaria species	176 (27)

CRD effect on SIT prescription (European model)

To measure the effect of CRD on SIT prescription, we applied the GA^{2}LEN-EAACI guidelines to the whole population of patients with a 2-step procedure based on clinical history,

TABLE III. Clinically relevant sensitization not confirmed by means of CRD in 651 Italian children with moderate-to-severe AR by allergenic source

	SPT response ≥3 mm		SPT positivity: Clinically relevant fraction		Relevant SPT response positivity not confirmed by CRD			With IgE to profilins and/or polcalcins	
	No.	Percent	No.	Percent	Lack of IgE to:	No.	Percent	No.	Percent
Grass pollen	592	91	568/592	96	Phl p 1, Phl p 5	56/568	10	6/56	11
Olive	405	62	390/405	96	Ole e 1	111/390	28	30/111	27
Pellitory	259	40	257/259	99	Par j 2	78/257	30	29/78	37
Cypress	287	44	184/287	64	Cup a 1	28/184	15	11/28	39
Betulaceae	309	47	252/309	82	Bet v 1	146/252	60	71/146	49
Mugwort	163	25	65/163	40	Art v 1	45/65	69	26/45	58

TABLE IV. The effect of molecular diagnosis* on SIT prescription† based on SPT responses in 651 children with moderate-to-severe AR by allergen source

	Prescribed after SPT						Not prescribed after SPT						All				
	Total (n = 651)		Prescribed after CRD		Not prescribed after CRD		Total		Not prescribed after CRD		Prescribed after CRD		Total	Confirmed after CRD		Not confirmed after CRD	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	No.	Percent	No.	Percent
Grass pollen	568	87	512	90	56	10	83	13	83	100	0	0	651	595	91	56	9
Olive	347	53	247	71	100	29	304	47	287	94	17	6	651	534	82	117	18
Pellitory	232	36	165	71	67	29	419	64	414	99	5	1	651	579	89	72	11
Cypress	122	19	108	89	14	11	529	81	506	96	23	4	651	614	94	37	6
Betulaceae	152	23	66	43	86	57	499	77	489	98	10	2	651	555	85	96	15
Mugwort	31	5	10	32	21	68	620	95	616	99	4	1	651	626	96	25	4
All allergens	1452		1108	76	344	24	2454		2395	98	59	2	3906	3503	90	403	10

*The molecules taken into consideration are Phl p 1 and Phl p 5 (grass pollen), Ole e 1 (olive), Par j 2 (pellitory), Cup a 1 (cypress), Bet v 1 (Betulaceae), and Art v 1 (mugwort).
†Based on the European prescription model (A; see the Methods section and Table I).

pollen calendars, and SPT responses first and on CRD results as well afterward. According to the guidelines, only the 651 patients affected by moderate-to-severe AR were considered eligible for SIT (Table IV). Grass pollen SIT was prescribed in 568 (87%) of the 651 patients, and SIT to grass pollen was the most common prescription. This high frequency was justified by the extremely high prevalence of sensitization to grass pollen in this population (Table III) and by the fact that grass pollen was always the first or second most important allergenic source in all participating Italian cities. Approximately one half and one third of the patients received a prescription of olive- and pellitory-specific SIT, respectively. The prescription of an SIT with cypress or Betulaceae applied to one fifth of the patients. Finally, according to the European guidelines, only 5% of the children would have received mugwort immunotherapy. Only 76% of these prescriptions survived the screening with CRD. In particular, 90% of prescriptions for grass pollen and cypress were confirmed after CRD. This figure decreased to 71% for olive and pellitory and to 43% and 32% for Betulaceae and mugwort, respectively (Table IV). When a sensitization detected based on SPT responses was not confirmed by means of CRD in a patient with 3 or more clinically relevant sensitizations, the corresponding pollen extract was replaced by the pollens scoring fourth, fifth, or even sixth in the local hierarchy of clinical relevance. However, this event was not frequent and produced only 59 new prescriptions (Table IV).

Role of the SPT cutoff point and disease severity

Although international guidelines indicate that a cutoff of 3 mm is a good decision point for SIT prescription,[6] many doctors use a higher cutoff point to increase diagnostic specificity before starting a long and quite expensive treatment, such as SIT. We therefore tested the effect of CRD on SIT prescription based on the European model modified by the assumption of a 5-mm cutoff point for SPT response positivity. Interestingly, the discordance rate between SPT and CRD decreased considerably when a 5-mm cutoff point was used for all the allergenic sources taken into account (Fig 1). This was mostly due to the fact that most of the SPT reactions not confirmed by CRD had a wheal diameter of 3 or 4 mm only and were on average smaller than the SPT reactions confirmed by means of CRD (4.1 ± 1.9 vs 7.1 ± 3.1 mm, $P < .001$).

CRD effect on SIT prescription (alternative models)

To test the virtual effect of CRD on the prescription of SIT by doctors with different prescription habits, we repeated the theoretic exercise by applying the American, monoallergenic, and monosensitization models (see Table E2 in this article's Online Repository at www.jacionline.org). Interestingly, the results obtained with the American model overlapped substantially with those obtained with the European model, with the obvious exception that no new prescriptions were possible in

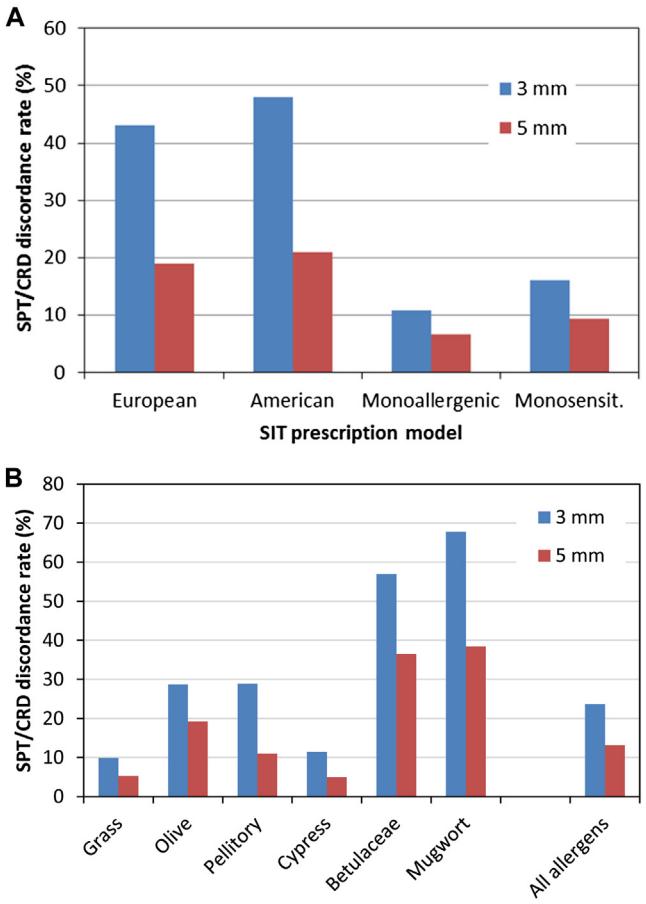

FIG 1. CRD's effect on SIT prescription based on SPTs with extracts by SPT cutoff. **A,** By prescription model. **B,** By allergenic source.

the American model. When the monoallergenic model was applied, almost all the subjects (627/651 [96%]) still received a vaccine and in most cases (563/627 [90%]) vaccine against grass pollen. When the monosensitization model was applied, only 133 subjects were treated when the prescription was based only on SPT responses. In this case, however, the introduction of CRD caused a sharp increase in SIT prescriptions (from 133 to 199, +50%) because many presumptive polysensitized patients became monosensitized. In all 3 alternate models, the effect of CRD was reduced by simply increasing the cutoff point for SPT response positivity from 3 to 5 mm (data not shown).

CRD effect on modeled SIT prescription at the patient level

Analyses were also aimed at describing the proportion of patients whose SIT prescriptions would have been affected by CRD. According to the European model, the prescription would have been different in 277 (42.5%) of 651 patients after SPT and CRD compared with after SPT alone. The proportion is slightly higher (315/651 [48.4%]) when the American model of SIT prescription is followed (Table V). In contrast, the effect of CRD on SIT prescription would be much less if a cutoff point of 5 mm had been applied (19.4% in the European model and 21% in the American model, Fig 1).

CRD effect on SIT prescription by participating doctors

Prescription of SIT in the whole population sample was submitted to the "virtual" decision of 14 doctors participating in the study. Among the 508 of the 651 patients who would have received an SIT prescription when evaluated only on the basis of clinical history, pollen calendars, and SPT responses, 170 (33%) would have received SIT with a different composition, and a further 52 (10%) would have received no SIT at all (Table VI). Of the 143 patients with no prescription when evaluated only on the basis of clinical history, pollen calendars, and SPT responses, 83 (58%) would have instead received an SIT prescription after considering the results of IgE testing against the major allergenic proteins. Overall, in 305 (47%) of 651 patients, the decision about SIT would have been changed after additional *in vitro* testing for allergenic molecules. The changes affected the different pollen sources in different ways. Interestingly, grass pollen was proportionally by far the most frequent newly introduced SIT and the least cancelled based on CRD results. Withdrawal of prescription was rather frequent (51%) for olive, followed by mugwort and Betulaceae (40% and 38%), cypress (24%), and pellitory (19%, Table VI).

DISCUSSION

In a clinic-based population of 651 Italian children with pollen-related AR, we found that the measurement of serum specific IgE levels to the major allergenic molecules of pollens (CRD) can very frequently modify the decision to provide allergen-specific SIT based on the same clinical information and clinical history integrated with SPT responses with pollen extracts. These results were obtained not only by applying 4 different theoretic prescription and pragmatic models for prescribing SIT but also by asking 14 local pediatric allergists for their opinions on SIT prescription in each patient.

The extent of changes in SIT prescriptions after considering CRD, concerning more than 40% of the examined patients, is somewhat impressive and deserves a careful analysis and explanation. It is well known that extracts from pollen sources contain panallergens, such as profilins, polcalcins, and lipid transfer proteins, which are highly cross-reactive and responsible for skin test reactions to many pollen sources with widely overlapping seasons, so that clinical history and SPT responses with extracts are not sufficient to discriminate the pollen or pollens eliciting symptoms.[8-12] Accordingly, approximately one fourth of our patients reacted to profilin,[18] a figure consistent with previous observations in Italy[30] and other Mediterranean countries.[14,15] Moreover, sensitization to profilin was linked to pollen polysensitization in our[18] and other[8-10] study populations. Here we show that CRD-driven changes in SIT prescription based on international guidelines[6] and on previously proposed algorithms[14] are only partially explained (Table III) with profilin sensitization. As a consequence, additional explanations must be found, and we cannot exclude that other highly cross-reacting molecules might have contributed to confound the results of SPTs based on allergenic extracts. On the other hand, the evidence that the diameter of the positive wheal reactions was significantly larger in SPTs confirmed by CRD than in those not confirmed by CRD is of great interest. Most national and international guidelines[6] suggest a 3-mm wheal cutoff point to evaluate SPT response positivity in the diagnostic process leading to SIT

TABLE V. The effect of molecular diagnosis* on SIT prescription† based on SPT responses in 651 children with moderate-to-severe AR by prescription model

SIT after SPT	No					Yes							SIT changed decision	
SIT after SPT and CRD	All	No SIT		SIT		All	No SIT		Modified		Identical			
Prescription model	No.	No.	Percent	No.	Percent	No.	No.	Percent	No.	Percent	No.	Percent	No.	Percent
European	24	24	100	NA	—	627	38	6.1	239	38.1	350	55.8	277	42.5
American	24	24	100	NA	—	627	38	6.1	277	44.2	312	49.8	315	48.4
Monoallergenic	24	24	100	NA	—	627	38	6.1	32	5.1	557	88.8	70	10.8
Monosensitized	518	432	83	86	16.6	133	18	13.5	0	0.0	115	86.5	104	16.0

NA, Not applicable.
*The molecules taken into consideration are Phl p 1 and Phl p 5 (grass pollen), Ole e 1 (olive), Par j 2 (pellitory), Cup a 1 (cypress), Bet v 1 (Betulaceae), and Art v 1 (mugwort).
†Based on the prescription model (B; see the Methods section and Table I).

TABLE VI. Effect of CRD* on SIT prescription made on the basis of SPT responses by doctors in 651 Italian children affected by hay fever

SIT after SPT	Not prescribed						Prescribed								All	
	All (n = 651)		No SIT		SIT		All (n = 651)		No SIT		Changed SIT composition		Unchanged SIT composition		Changed SIT (n = 651)	
SIT after SPT and CRD	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Grass pollen	215	33	135	63	80	37	436	67	74	17	NA	NA	362	83	154	24
Olive	538	83	484	90	54	10	113	17	58	51	NA	NA	55	49	112	17
Pellitory	542	83	498	92	44	8	109	17	21	19	NA	NA	88	81	65	10
Cypress	618	95	578	94	40	6	33	5	8	24	NA	NA	25	76	48	7
Betulaceae	612	94	591	97	21	3	39	6	15	38	NA	NA	24	62	36	6
Mugwort	636	98	632	99	4	1	15	2	6	40	NA	NA	9	60	10	2
All allergens	3161	100	2918	92	243	8	745	100	182	24	NA	NA	563	76	425	11
Patients	143	22	60	42	83	58	508	78	52	10	170	33	286	56	305	47

NA, Not applicable.
*The molecules taken into consideration are Phl p 1 and Phl p 5 (grass pollen), Ole e 1 (olive), Par j 2 (pellitory), Cup a 1 (cypress), Bet v 1 (Betulaceae), and Art v 1 (mugwort).

prescription. Our results provide the biological evidence that larger wheal reactions are more "specific" and support the idea that a higher cutoff point should be considered when SPT responses are used to drive SIT prescription.[6]

To improve the general validity of our study, we examined different prescription models and settings. Notably, the effect of CRD on SIT did not substantially change by applying either the European or the American guidelines, suggesting that with both approaches the implementation of CRD would have a similar effect in this Mediterranean population. Similarly, the global effect of CRD on SIT prescriptions by 14 physicians was very similar (47%) to the theoretic effect produced by the European (42%) or American (48%) approach. In both cases the highest absolute number and lowest relative frequency of corrections concerned grass pollen SIT, whereas the prescription of SIT for olive and Betulaceae presented the highest frequencies of corrections (Table VI). However, 83 patients would have received an SIT prescription from these doctors only after their IgE sensitization to major allergenic molecules (particularly Phl p 1 or Phl p 5) was demonstrated by CRD, and this contributed to a net increase in the overall SIT prescription rate (Table VI).

Our study has several implications for clinical practice. Even though CRD has been available for more than a decade, guidelines for SIT still largely ignore this diagnostic approach. Our findings suggest that a more precise description of the patient's sensitization profile before SIT is prescribed should be taken into account. To take this further, we need controlled studies comparing the efficacy of SIT in children whose prescription changes as a result of CRD and who are randomized to receive conventional or CRD-guided SIT. Cost/benefit studies should also evaluate whether the immediate additional costs implied by molecular analysis are justified in the long term. In this context it is important to evaluate also whether the increase in the cutoff point of SPT response positivity would also be useful from a clinical and economic standpoint. Our results suggest that a higher cutoff point of SPT-induced wheal reactions (eg, 5 mm) should be used to take decisions when a confirmatory CRD assay cannot be implemented.

We have to acknowledge some limitations of our study. First, our conclusions apply to settings with high pollen exposure for prolonged seasonal periods, such as those of Mediterranean countries, and the study should be repeated in other geographic areas. In Northern Europe the prevalence of IgE sensitization to profilins is lower, and the peak seasons of different pollens are shorter and can be better discriminated.[31,32] Therefore the effect of CRD on SIT prescription might be lower in Northern than in Southern Europe.

Second, for this study, we purposely examined only the molecules already proposed in an algorithm for CRD-driven SIT prescription.[16] We cannot exclude that sensitization to other major or minor allergenic proteins might be responsible for the bulk of symptoms in a given patient.

Third, the models we have applied might not reproduce real-world conditions. However, the survey conducted among 14 different Italian pediatric allergists largely confirmed the results obtained by applying the theoretic prescription models.

Finally, we examined only children, and therefore specific studies among adults should be performed to test whether the same conclusions applied to older groups.

In conclusion, our findings suggest that in countries with high and prolonged exposure to many allergenic pollen sources, a higher cutoff of SPT response positivity should be suggested and CRD should be considered as a diagnostic step after SPTs with extracts. This conclusion might be useful to update national and international guidelines on the prescription of SIT in patients with pollen-related AR. Further work is needed to test the hypothesis that CRD modifications of SIT prescription can improve its clinical efficacy and cost-effectiveness.

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Clinical implications: CRD, when the internationally proposed algorithms are applied, modifies the decision on SIT prescription in a large proportion of children affected by pollen-related AR.

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TABLE E1. Discordance rate between extract-based IgE assays and SPT responses

	SPT ≥3 mm (extract)	IgE <0.35 kU/L (extract)	
	No.	No.	Percent
Grass pollen	592	15	2.5
Olive	405	26	6.4
Pellitory	259	16	6.2
Cypress	287	34	11.8
Betulaceae	309	24	11
Mugwort	163	19	11.7

TABLE E2. Effect of molecular diagnosis* on SIT prescription based on SPT responses in 651 children with moderate-to-severe AR by allergen source and prescription model

	Model A (European)						Model B (American)						Model C (monoallergenic)						Model D (monosensitization)					
	Yes			No			Yes			No			Yes			No			Yes			No		
SIT after SPT	**All**	**No**		**All**	**Yes**		**All**	**No**		**All**	**Yes**		**All**	**No**		**All**	**Yes**		**All**	**No**		**All**	**Yes**	
SIT after SPT and CRD	**No.**	**No.**	**Percent**	**No.**	**No.**	**Percent**	**No.**	**No.**	**Percent**	**No.**	**No.**	**Percent**	**No.**	**No.**	**Percent**	**No.**	**No.**	**Percent**	**No.**	**No.**	**Percent**	**No.**	**No.**	**Percent**
Grass pollen	568	56	10	83	0	0	568	56	10	83	NA	—	563	55	10	88	0	0	100	10	10	551	62	11
Olive	347	100	29	304	17	6	390	111	28	261	NA	—	19	6	32	632	14	2	13	3	23	638	9	1
Pellitory	232	67	29	419	5	1	257	78	30	364	NA	—	32	4	13	619	11	2	13	2	15	638	8	1
Cypress	122	14	11	529	23	4	184	28	15	467	NA	—	7	1	14	644	6	1	5	1	20	646	5	1
Betulaceae	152	86	57	499	10	2	252	146	58	399	NA	—	6	4	67	645	1	0	2	2	100	649	2	0
Mugwort	31	21	68	620	4	1	65	45	69	586	NA	—	0	0	—	651	0	0	0	0	—	651	0	0
All allergens	1452	345	24	2454	59	2	1716	464	27	2160	NA	—	627	70	11	3279	32	1	133	18	14	3773	86	2

NA, Not applicable.

*The molecules taken into consideration are Phl p 1 and Phl p 5 (grass pollen), Ole e 1 (olive), Par j 2 (pellitory), Cup a 1 (cypress), Bet v 1 (Betulaceae), and Art v 1 (mugwort).