

BRIEF COMMUNICATION

How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area

J. Sastre^{1,2}, M. E. Landivar¹, M. Ruiz-García¹, M. V. Andregnette-Rosigno¹ & I. Mahillo³

¹Fundación Jiménez Díaz, Allergy Department, Fundación Jiménez Díaz, Madrid, Spain; ²CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain; ³Department of Epidemiology, ISS Fundación Jiménez Díaz, Madrid, Spain

To cite this article: Sastre J, Landivar ME, Ruiz-García M, Andregnette-Rosigno MV, Mahillo I. How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. *Allergy* 2012; DOI:10.1111/j.1398-9995.2012.02808.x.

Keywords

component-resolved diagnosis;
molecular allergy diagnosis; pollen allergy;
recombinant allergens;
specific immunotherapy.

Correspondence

Joaquín Sastre, Allergy Department,
Fundación Jiménez Díaz, Av. Reyes
Católicos 2, 28040 Madrid, Spain.
Tel.: +34-91-5498225
Fax: +34-91-5499498
E-mail: jsastre@fjd.es

Accepted for publication 01 February 2012

DOI:10.1111/j.1398-9995.2012.02808.x

Edited by: Pascal Demoly

Abstract

Background: The identification of disease-eliciting allergens is a prerequisite for accurate prescription of allergen-specific immunotherapy (SIT). The aim of this study was to determine whether molecular diagnosis (MD) may change indication and allergen prescription of SIT.

Methods: A total of 141 patients with allergic rhinoconjunctivitis and/or asthma sensitized to pollen with or without concomitant food allergy were included. Skin prick testing with a panel of aeroallergens and a microarray-based panel of allergens (ISAC[®]; Phadia, Sweden) was performed in all patients. Prior to learning the results of molecular diagnosis, three of the authors reached a consensus on the indication of SIT and use of allergens following EAACI recommendations, basing their judgment on clinical history and skin prick test results before and after obtaining the ISAC results. The agreement coefficient (kappa index) was used to analyze the results.

Results: Fifty-nine percent of the patients were women with a mean age of 31 ± 13.63 . Agreement in SIT indication before and after ISAC[®] results was found in only 62 (46%) patients ($\kappa = 0.1057 \pm 0.0413$). Concerning allergens used in the most common prescriptions before and after MD results, we obtained the following results: $\kappa = 0.117 \pm 0.0825$ for grass; $\kappa = 0.1624 \pm 0.0639$ for olive; $\kappa = 0.0505 \pm 0.0548$ for olive and grass; $\kappa = 0.1711 \pm 0.0471$ for grass and cypress; $\kappa = 0.1897 \pm 0.0493$ for grass and London plane; $\kappa = 1 \pm 0.0842$ for olive and cypress, and $\kappa = 0.3586 \pm 0.0798$ for other combinations.

Conclusions: There was very low agreement concerning indication and use of allergens for SIT before and after performing MD. This discrepancy emphasizes the usefulness of MD, at least in areas of complex sensitization to pollen, in determining correct indication of SIT.

Allergen-specific immunotherapy (SIT) is the only allergen-specific treatment of its kind (1). It can prevent progression of the disease and has a long-lasting therapeutic effect. As SIT is allergen-specific, the identification of the disease-eliciting allergen is a prerequisite for accurate prescription of treatment. Diagnostic tests based on allergen extracts use mixtures of various allergens, some of which are specific for the allergen source, while others are cross-reactive allergens of

various unrelated allergen sources. It may therefore be difficult or even impossible to identify the disease-causing allergen by performing such tests, particularly in patients who are sensitized to more than one allergen source. Sensitization to pollen from cypress, plane, olive, and grasses is common in our area (2), and these patients are frequently treated with SIT. Advances in molecular techniques applied to allergens have provided us with new tools such as recombinant allergens that can improve the accuracy of allergy diagnosis (3). The aim of this study was to assess whether molecular diagnosis of pollen allergy leads to changes in the indication and allergen prescription of SIT when compared to skin prick testing with commercial extracts.

Abbreviations

SIT allergen-specific immunotherapy; MD molecular diagnosis;
 κ kappa coefficient of agreement; SPT skin prick test.

Table 1 Agreement and disagreement in indication of immunotherapy based on skin prick test only and molecular diagnosis

Extract for SIT	Indication of SIT based on SPT	Indication of SIT based on MD	Number of patients with agreement of SIT (%)	Number of patients with disagreement of SIT	Kappa agreement for SIT based on SPT or MD
Grass	17	10	97 (68)	44 (32)	0.117 ± 0.0825 <i>P</i> = 0.0781
Olive	1	1	132 (93)	9 (7)	0.1624 ± 0.0639 <i>P</i> = 0.0055
Grass + olive	4	1	101 (71)	40 (29)	0.0505 ± 0.0548 <i>P</i> = 0.1782
Grass + cypress	0	1	132 (93)	9 (7)	0.1711 ± 0.0471 <i>P</i> = 0.0001
Grass + plane	0	1	133 (94)	8 (6)	0.1897 ± 0.0493 <i>P</i> = 0.0001
Olive + cypress	0	2	141 (100)	0 (0)	1 ± 0.0842 <i>P</i> < 0.0001
Other extracts	3	4	129 (91)	12 (9)	0.3586 ± 0.0798 <i>P</i> < 0.0001
Total	25	20	62 (46)	79 (54)	0.1057 ± 0.0413

SIT, specific immunotherapy; MD, molecular diagnosis.

Patients

A total of 141 patients with allergic rhinoconjunctivitis and/or asthma sensitized to pollen with or without concomitant food allergy were recruited from our outpatient clinic.

Skin prick tests (SPTs) with a panel of commercial common inhalants (all from ALK-Abelló, Madrid, Spain) were carried out. The panel included the following pollens: *Olea e*, *Platanus a*, *Cupressus a*, grass mix, *Cynodon d*, *Phragmites c*, *Artemisia v*, *Salsola k*, and *Plantago l*. Histamine (10 mg/ml) and glycerol-saline solution were used as controls. A skin test was considered positive if the wheal size was at least 3 mm greater than that of the negative control.

Specific IgE to molecular allergens

A microarray-based panel of 96 allergens (ISAC[®], Phadia, Sweden) was used. The panel included the following pollen allergens: *Ole e 1*, *Cup s 1*, *Cry j 1*, *Pla a 1*, *Pla a 2*, *Phl p 1*, *Phl p 5*, *Phl p 4*, *Phl p 6*, *rPhl p 11*, *Phl p 12*, *Cyn d 1*, *Sal k 1*, *Ahn g 1*, *Bet v 1*, *Cor a 1.0101*, *Amb a 1*, *Art v 1*, *Art v 3*, and *Par j 2*. Positive values were assessed according to the manufacturer's instructions. Sensitization to London plane or grass was considered when positivity to *Pla a 1* and/or *Pla a 2* or positivity to *Phl p 5* and/or *Phl p 6*, respectively.

Prescription of allergen immunotherapy

Prior to learning the results of molecular diagnosis, three of the authors reached a consensus on the indication of SIT and use of allergens, basing their judgment on clinical history. This initial assessment took into account the time of year in which respiratory symptoms developed according to the pollen calendar, and following the recommendations for immunotherapy established by the European Academy of Allergy

Table 2 Agreement between commercial extracts and recombinant or purified allergens of the main pollens found in the area of Madrid (Spain)

SPT/allergen molecule	Positive in ISAC	Negative in ISAC	Patients with agreement (%)
Olive/Ole e 1			
111 Positive SPT	80	31	100 (70)
30 Negative SPT	10	20	
Cypress/Cup s 1			
87 Positive SPT	72	15	115 (81)
54 Negative SPT	11	43	
Plane/Pla a 1			
102 Positive SPT	24	81	63 (44)
39 Negative SPT	2	39	
Plane/Pla a 2			
102 Positive SPT	57	48	87 (61)
39 Negative SPT	11	30	
Plane/Pla a 1 or Pla a 2			
102 Positive SPT	56	46	84 (60)
39 Negative SPT	11	28	
Grass/Phl p 1			
121 Positive SPT	105	16	114 (81)
20 Negative SPT	11	9	
Grass/Phl p 5			
121 Positive SPT	77	44	89 (62)
20 Negative SPT	8	12	
Grass/Phl p 5 or Phl p 1			
121 Positive SPT	110	11	119 (84)
20 Negative SPT	11	9	
Cynodon/Cyn d 1			
109 Positive SPT	96	13	115 (82)
32 Negative SPT	13	19	

SPT, skin prick test; ISAC[®], a microarray-based panel of allergens (Phadia, Uppsala, Sweden).

and Clinical Immunology (EAACI) Guidelines SPT results (4). After obtaining the result of ISAC[®], the same panel of doctors came up with a new indication and prescribed the use of allergens for immunotherapy. Determinations were considered to be in agreement if indication of SIT and allergen(s) used were the same before and after molecular diagnosis (MD) results were obtained. The agreement coefficient (κ index) was used to analyze results.

The mean age of patients was 31 ± 13.63 years, and 59% were women. Agreement and disagreement are summarized in Table 1. Agreement in indication of SIT before and after ISAC[®] results occurred in only 62 (46%) patients ($\kappa = 0.1057 \pm 0.0413$).

Table 2 summarizes instances of agreement between commercial extracts and recombinant or purified allergens.

Selection of patients for SIT must take into consideration clinical parameters and confirmation of the presence of an IgE-mediated allergic sensitization. One factor that can limit the application and success of SIT is the correct identification of the disease-eliciting allergen.

Diagnostic tests are based on natural allergen extracts that are composed of mixtures of nonallergenic materials, major allergens, and cross-reactive allergens, thus making it difficult and often impossible to precisely identify the disease-eliciting allergen, particularly in patients sensitized to more than one allergen source. In such cases, it is important for the clinician to know whether a patient is co-sensitized to several allergen sources and needs SIT for each, or whether the patient is sensitized to several sources because of sensitization to cross-reactive components in each of the suspected allergen sources. Molecular diagnosis using recombinants or purified allergens can partially solve this problem and improve the diagnosis of allergy (3). In this study, we found that in 54% of patients, there was a disagreement in indication of SIT before and after ISAC[®] results. With regard to the extracts used for immunotherapy, the κ agreement coefficient varied from 1 for olive and cypress to <0.2 for the rest of the extracts, which is considered very weak from a clinical point of view. The most common indication of SIT was an extract of grass pollen: in this case, κ coefficient was only 0.11. When analyzing concordance of SPT results with recombi-

nants of purified allergens from the ISAC[®] test, we also found disagreement levels that varied from 40% with plane extract and positivity to *Pla a1* and/or *Pla a2* to 16% with grass extract and positive reaction to *Phl p1* and/or *Phl p 5*. This fact may be responsible for the high percentage of disagreement found when SIT was prescribed only on the basis of SPT results. This can be explained by false-negative results of SPT used in single application even when performed by skilled technicians and by technical problems in patients with low skin sensitivity (5, 6). However, it is also possible to find cases of false-positive and false-negative results when performing specific IgE to recombinants or purified allergens (7). Another factor that had a substantial influence and led to disagreement was the presence of sensitization to cross-reactive components such as profilin or polcalcin.

In summary, very low agreement was found concerning indication and use of allergens for SIT before and after performing MD. This great discrepancy makes the case for the usefulness of MD, at least in areas of complex sensitization to pollen, as a means of facilitating accurate prescription of pollen immunotherapy.

Acknowledgments

We thank Oliver Shaw for editorial assistance.

Funding

This study was funded by CIBERES (CIBER de Enfermedades Respiratorias), Instituto de Salud Carlos III of the Ministry of Science and Innovation, Spain.

Conflict of interest

Dr. Landívar, Dr. Ruiz-Garcia, Dr. Andregnette-Roscigno, and Dr Mahillo have no conflicts to disclose. Dr. Sastre reports having served as a consultant to Phadia, MSD, Novartis; FAES Farma and GSK, having been paid lecture fees by Novartis, GSK, Stallergenes, UCB and having received grant support from Phadia, GSK and ALK-Abello.

References

1. Zuberbier T, Bachert C, Bousquet PJ, Passalacqua G, Walter Canonica G, Merk H et al. GA² LEN/EAACI pocket guide for allergen-specific immunotherapy for allergic rhinitis and asthma. *Allergy* 2010;**65**:1525–1530.
2. Subiza J, Jerez M, Jiménez JA, Narganes MJ, Cabrera M, Varela S et al. Allergenic pollen pollinosis in Madrid. *J Allergy Clin Immunol* 1995;**96**:15–23.
3. Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010;**40**:1442–1460.
4. Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E, EAACI, Immunotherapy Task Force. Standards for practical allergen-specific immunotherapy. *Allergy* 2006;**61**(Suppl. 82):1–20.
5. Dreborg S. Methods for skin testing. EAACI Position paper. *Allergy* 1989;**44**(Suppl. 10):22–30.
6. Bousquet PJ, Chatzi L, Jarvis D, Burney P. Assessing skin prick tests reliability in ECRHS-I. *Allergy* 2008;**63**:341–346.
7. Wohrl S, Vigl K, Zehetmayer S, Hiller R, Jarisch R, Prinz M et al. The performance of a component-based allergen-microarray in clinical practice. *Allergy* 2006;**6**:633–639.