R.E. Rossi[1], G. Melioli[2], G. Monasterolo[3], C. Harwanegg[4], L. Rossi[2], G.W. Canonica[5], G. Passalacqua[5]

Sensitization profiles in polysensitized patients from a restricted geographical area: further lessons from multiplexed component resolved diagnosis

[1] Rete di Allergologia Regione Piemonte, Azienda Sanitaria Cuneo 1, Cuneo, Italy
[2] Laboratorio Centrale di Analisi, Istituto Giannina Gaslini, Genoa, Italy
[3] Laboratorio Analisi Ospedale di Savigliano e Fossano, Azienda sanitaria Cuneo 1, Cuneo, Italy
[4] Genosense, Vienna, Austria
[5] Allergy and Respiratory Diseases, University of Genoa, Italy - E-mail: passalacqua@unige.it

Key words

Sensitisation, specific IgE, micro-array

Corresponding author
Giovanni Passalacqua MD
Allergy and Respiratory Diseases,
University of Genoa
Padiglione Maragliano,
L.go R.Benzi 10
16132 Genoa, Italy
Tel. +390103538933
Fax +390103538904
E-mail: passalacqua@unige.it

Summary

Background: *The micro-array techniques for the detection of specific IgE has improved the diagnostic procedures for allergic diseases. This method also allows to define sensitisation profiles from an epidemiological point of view. We studied the sensitisation pattern in a population of polysensitized patients with respiratory allergy, living in a restricted geographical area in the north-west Italy.* ***Methods:*** *Consecutive patients with asthma/rhinitis, living in the province of Cuneo, and having at least two positive skin prick test for non related aeroallergens were studied by a microarray (Phadia, Milan Italy) which allowed to detect specific IgE against 103 different allergen components.* ***Results:*** *The 70 patients included had specific IgE towards a mean of 4.3 allergens/patient (range 2-12 allergens). Concerning pollens, 63 (90%) had specific IgE to at least one genuine grass pollen allergen, 32 (45.7%) had Ole e 1 specific IgE antibodies, although olive tree is not present in the area. A relevant percentage of sensitisation to mite was found (47,1%). True co-sensitisation to grass-pollen allergens/Bet v 1/Ole e 1 was observed in 15 individuals (21.4%). Pru p 1, resulted to be a sensitising allergen in 23 patients (32.85%), 4 of whom were co-sensitised to Pru p 3 and/or Art v 3.* ***Conclusion:*** *A detailed knowledge of the sensitisation pattern may have relevant implications for the prescription of specific immunotherapy. Moreover, sensitisation to PR-10 (or profilin), frequently associated to oral allergy syndrome, in some cases could hide the sensitisation to LTPs which are clinically more relevant.*

Introduction

Allergen-based microarray is a diagnostic tool for the detection of specific IgE towards numerous allergenic components simultaneously. This multiplexing allows, for instance to discriminate between cross-reactive allergens and genuine sensitisation markers. In fact, the allergen extracts, largely used in standard diagnostic procedures, provide no information about the disease-eliciting molecule(s), and this may represent a problem, particularly in polysensitized patients (1, 2). One of the advantages of the microarray technology is that it allows to compare in-

dividual sensitisation profiles in one single sample (3). This may have a great impact on epidemiological ground as recently demonstrated in a population-based study (4). In this study, a commercial microarray immunoassay bearing 103 natural and recombinant allergen molecules was utilized for specific IgE detection. We studied the sensitisation profile, by means of microarray, in a population of patients living in a restrict geographical area (Cuneo, Northwest Italy), who were presumably exposed to the same aeroallergens.

Material and methods

This retrospective study involved consecutive patients, referred to the Allergy Unit for respiratory allergy symptoms (rhinitis/asthma), associated or not with other allergic diseases, from January 2009 to May 2010. The diagnosis of rhinitis or asthma was made according to current guidelines (5, 6). All patients had to have positive skin tests to at least two not related aeroallergens (for example, mites and birch). In addition the patients had to live within the province of Cuneo (about 6,900 square kilometres), located south of the Alps and east of France, in the north-western part of Italy.

The polysensitisation status was firstly established by skin prick tests (SPT), which were carried out with commercial extracts of: *Phleum pratense*, (60 μg/ml Phl p 5), *Parietaria judaica* (6 μg/ml Par j 1), birch (45 μg/ml Bet v 1), olive (60 μg/ml Ole e 1), mugwort (135 μg/ml Art v 1), ragweed (30 HEP), Cypress (30HEP), cat dander (60 μg/ml Fel d 1) mites (40 μg/ml Der p 1/Der f 1 and 20 μg/ml Der p 2/Der f 2), (Alk-Abellò, Lainate, Milan, Italy). Commercial food allergen extracts were also used in those patients with a suspicion of food allergy. No recombinant allergens were utilized for in vivo diagnosis, and the tests were performed according to current guidelines (7)

Sera of all patients were analysed for the presence of specific IgE to allergen components by the allergen-microarray (ImmunoCAP_ISAC® version CRD-103, Phadia, Milan, Italy) according to manufacturer's guidelines. Different groups of 103 allergenic molecules are spotted on the allergen chip. The IgE-allergen reaction is revealed by a fluorimetric reaction that is read by an automatic analyser. The intensity of the reaction, grossly corresponding to the amount of specific IgE is expressed, for each allergen, into ISAC Standard Units (ISU).

Results

Seventy polysensitized patients (27 male, 43 female, mean age 28,7 years, range 12,3-64,6 years), with a history of respiratory allergy were included. All patients had rhinoconjunctivitis with mild (n=29), moderate (n=8) or severe (n=1) asthma. Atopic dermatitis was present in 24 patients (all aged 20 years or less), oral allergy syndrome (OAS) in 20, acute urticaria in 9; gastroenteric complaints in 12, and anaphylaxis in one patient. The positive SPT were: 2 in 2 patients, 3 in 10 patients, 4 in 8 patients and 5 or more in 50 subjects.

The 70 patients had specific IgE towards 302 allergens in total (mean 4.3 allergens per patient, range 2-12 allergens). Concerning pollens, 63 (90%) had specific IgE to at least one genuine grass pollen allergen, 32 (45.7%) had Ole e 1 specific IgE antibodies, and 27 (38%) were Bet v 1 positive. A relevant percentage of sensitization to mite was found (Group 1 and/or Group 2 allergens): 33/70 patients (47,1%). Sensitization to Fel d 1, involved 24 patients (34.3%). The positive results of SPT and the correspondent positivity of genuine sensitisation molecular markers are summarized in Table 1. It is clear that for some allergens, the presence of cross reactive proteins causes an excess positive skin tests, in particular for ragweed, mugwort and olive. The overall percentage of sensitizations to inhalant allergens is summarized in Figure 1.

True co-sensitization to grass-pollen allergens/Bet v 1/Ole e 1 was observed in 15 individuals (21.4%). This finding might have implication on allergen immunotherapy selection. Co-sensitization to mite allergens and Fel d 1 was observed in 17 patients (24.2%) (this may indirectly suggest that the presence of cat could promote house-dust mite development).

All the 17 patients with specific IgE to profilin were sensitized to grass pollen allergens. Among food allergens the Bet v 1 homolog, Pru p 1, accounted for the higher percentage of sensitization in 23 patients (32.85%). Out of these 23 patients, 4 (5.7%), were co-sensitized to Pru p 3 and/or Art v 3, and two patients had also specific IgE antibodies to profilins (Fig. 2). This finding is important because a sensitization to PR-10 (or profilin), frequently associated to oral allergy syndrome, in some cases could hide the sensitization to LTPs which are clinically more relevant. There were 6 sensitizations to Pru p 3 and 6 to Art v 3, associated in 3 individuals. One patient was positive to Cor a 8, and he was also positive to Art v 3 and Pru p 3.. Sensitization to clinically relevant allergens such as Ara h 1, Ara h 3, Ber e 1, Ses e 1, Act d 2, Hev b 6

Table 1 - Number of patients with positive skin test and correspondent positivity to genuine sensitisation markers

SPT	Timothy	Bermuda	Birch	Olive	Cypress	Ragweed	Mugwort
N Positive	64	64	32	39	11	12	17
Markers of Genuine sensitisation	Phl p 1, 2, 5,6, 11	Cyn d 1	Bet v 1	Ole e 1	Cup a 1	Amb a 1	Art v 1
N Positive	63	55	26	31	18	2	5
SPT	Parietaria	Latex	Alternaria	Mite 1	Mite 2	Cat	Dog
N Positive	18	6	7	36	36	25	19
Markers of Genuine sensitisation	Par j 2	Hev b 6	Alt a 1	Der p/f 1	Der p/f 2	Fel d 1	Can f 1
N Positive	14	2	10	27	32	23	6

Figure 1 - Prevalence of specific IgE sensitisation to the major inhalant allergens (upper panel) and to tree pollen allergens (lower panel)

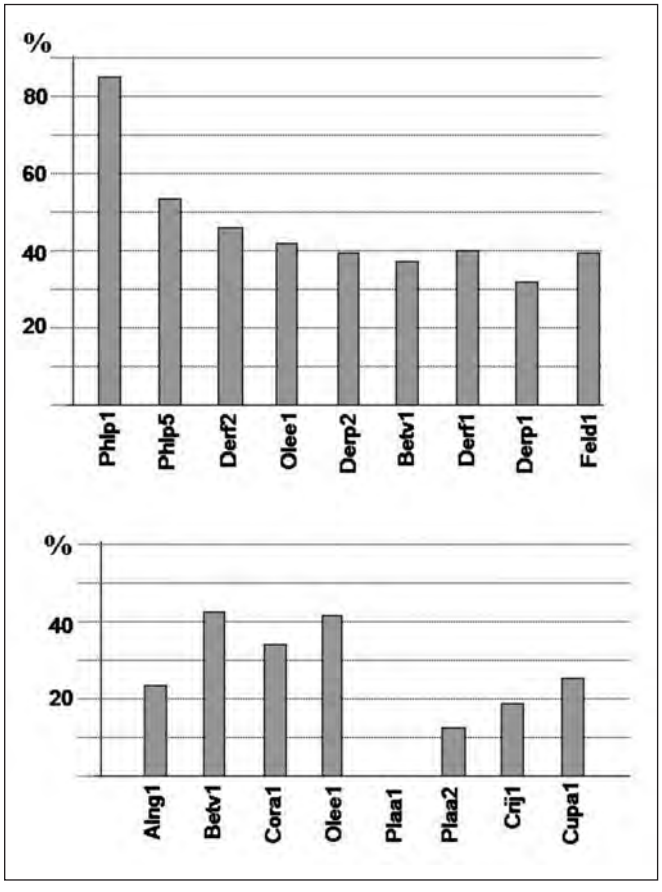
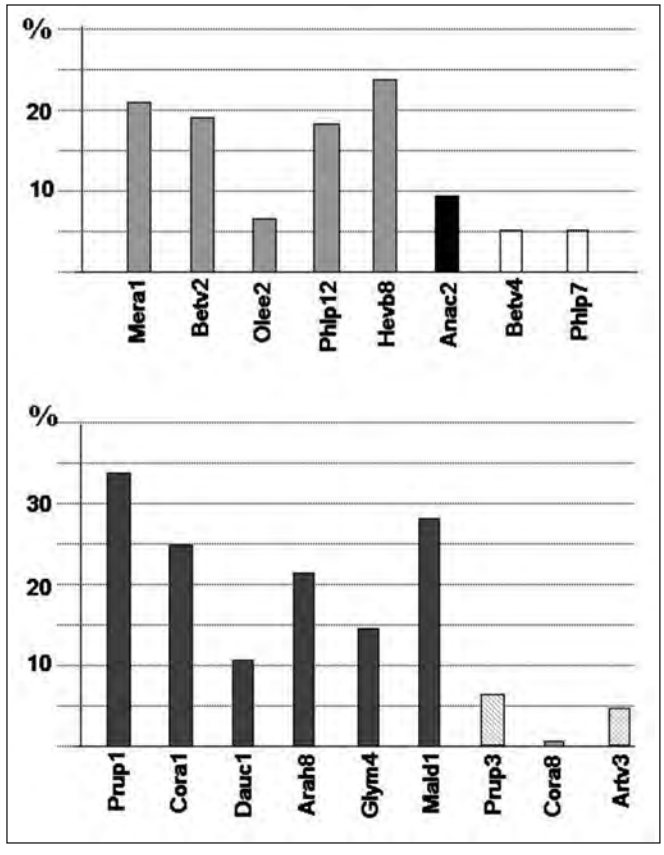


Figure 2 - Percentage of sensitisation to cross-reactive plant and plant food allergens. Upper panel: light grey= profilins, black= CCD, white= calcium binding proteins. Lower panel: dark grey= PR-10 proteins, speckled= Lipid Transfer Proteins



were observed only sporadically. Among animal allergen foods 5 subjects had positive IgE results to horse serum albumin Equ c 1 (7.14%) and 2 subjects had specific IgE to Bos d 8 (casein). No sensitization to other relevant plant food allergens (i.e. Tri a 19, Ara h 2) could be documented.

Discussion

The introduction of multiplexed IgE detection systems represented a substantial advance in the aetiological diagnosis of allergic diseases, expecially in polysensitized patients or individuals with food-plant syndromes, who may pose problems, for instance, in recommending appropriate avoidance measures or in prescribing specific immunotherapy (3, 9). In addition, the "microarray" systems can provide a not negligible support in epidemiological studies, by identifying the relevant allergenic components which sensitize patents with specified characteristics. The ISAC method is a type of microarray that allows to detect simultaneously in a small sample of serum, the presence of specific IgE towards 103 allergenic components. Recently, a study involving 16,408 patients, clearly defined, by means of the ISAC assay, the prevalence of sensitizations towards clinically relevant allergens (4). That study can be considered in a broad sense "a cross-section of the Italian allergic population" and represents an useful basis for epidemiological comparisons.

In our geographical area, Phl p 1 allergen was the more common sensitization (more than 90% of subjects), followed by Phl p 5 (more than 50% of individuals). Those figure differ from the study by Scala et al (4), where sensitizations to Phl p 1 and Phl p 5 were found in 37.94% and 21.94% of patients, respectively. This clearly reflects the local flora, as in our area grasses are largely present, and this is not true in all regions of Italy. Looking at mites, which are ubiquitous, the rate of sensitization was about 40% in our study and 38.7% in the Scala study. The fourth more represented allergen resulted to be Ole e 1, a marker of true sensitization to the Oleaceae family. This was quite surprising, since olive tree is absolutely rare in our area, where birch is the predominant tree. However, in this geographic area, other Oleaceae are largely represented. These include Privet (Lig v 1), Ash (Fra e 1), Forsythia (For v 1) and lilac (Syr v 1), which are employed as ornamental plants. Moreover, other proteins from different sources with a high homology to Ole e 1 may account for the high percentage of sensitization towards Ole e 1 of these subjects. These proteins include the grass pollen component Phl p 11 (in the present study 22 subjects, 22.8%, had specific IgE to group 11 grass pollen), the plantain protein Pla a 1 and the corn protein Zea m 13 (10).

Interestingly, a co-sensitization to grass birch and olive was observed in 21.4% patients, and this might have an implication in the prescription of an appropriate immunotherapy. Similarly, the sensitization to PR-10 or profilin, frequently associated to oral allergy syndrome, in some cases masked the sensitization to LTPs (e.g. Pru p 3), which are clinically more relevant (11).

An interesting findings of this study is that the stronger allergens, capable of stimulate high levels of specific IgE, are in descending order Phl p 1, Phl p 5, Der f 2, Der p 2, Bet v 1, Der f 1, Der p 1, Ole e 1 and Fel d 1 (Fig. 2). These data should be taken into account by allergen extract producers to provide clinicians with improved products for diagnosis and immunotherapy. Also, the role of corss-reacting proteins in causing an excess positive skin tests (i.e for ragweed and mugwort), should be taken into account. Finally, patients selected on the basis of their respiratory symptoms, resulted sensitized to potential harmful food allergens other than LTPs such as 2S albumin Ber e 1 from Brazil nut (n=1); 11S Globulin, Ara h 3 from peanut (n=1); Taumathin like protein Act d 2 from kiwi and Tropomyosin from Crustaceans (n=2).

In conclusion, component resolved diagnosis and particularly ImmunoCAP-ISAC may be a precious diagnostic tool in allergic patients with positive skin tests towards more than 3/4 allergen extracts and symptoms scarcely related to allergen exposition.

References

1. Bonds RS, Midoro-Horiuti T, Goldblum R. A structural basis for food allergy: the role of cross-reactivity. Curr Opin Allergy Clin Immunol 2008; 8: 82.
2. Mari A. When does a protein become an allergen? Searching for a dynamic definition based on most advanced technology tools. Clin Exp Allergy 2008; 38: 1089-94.
3. Ferrer M, Sanz ML, Sastre J, et al. Molecular diagnosis in allergology: application of the microarray technique. J Investig Allergol Clin Immunol 2009; 19 (Suppl 1): 19-24.
4. Scala E, Alessandri C, Bernardi ML, et al. Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. Clin Exp Allergy 2010; 40: 911-21.
5. Bousquet J, Van Cauwenberge P, (Eds). Allergic Rhinitis and its Impact on Asthma (ARIA). J Allergy Clin Immunol 2001; 108 (suppl 5): 147-334.

6. Global Initiative on Asthma. www.ginasthma.org last accessed July 2010
7. Dreborg S, Frew A. Position paper: Allergen standardization and skin tests. Allergy 1983; 48 (suppl 14): 49-82.
8. Sastre J. Molecular diagnosis in allergy. Clin Exp Allergy 2010; 30: 1-15.
9. Weber RW. Cross reactivity of pollen allergens: impact on immunotherapy. Ann Allergy Asthma Immunol 2007; 99: 203-12.
10. Marknell DeWitt A, Niederberger V, Lehtonen P, et al. Molecular and immunological characterization of a novel timothy grass (Phleum pratense) pollen allergen, Phl p 11. Clin Exp Allergy 2002; 32: 1329-40.
11. Flinterman A, Flinterman AE, Akkerdaas JH, Knulst AC, van Ree R, Pasmans SG. Hazelnut allergy: from pollen-associated mild allergy to severe anaphylactic reactions. Curr Opin Allergy Clin Immunol 2008; 8 (3): 261-5.