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The additional values of microarray allergen assay in the management of polysensitized patients with respiratory allergy

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Abstract

Background: The IgE response is directed against specific components from an allergenic source. The traditional diagnostic methods use whole extracts, containing allergenic, nonallergenic and cross-reactive molecules. This may pose diagnostic challenges in polysensitized patients. Microarray techniques detect specific IgE against multiple molecules, but their value in term of additional information and economic saving has not been yet defined.

Objective: We assessed the additional diagnostic information provided by an allergen microarray in a large population of polysensitized subjects.

Methods: In this multicentre study, allergists were required to carefully record diagnosis and treatment of consecutive patients referred for asthma/rhinitis, using the standard methodology (history, skin prick test, IgE assay). Then, a microarray allergen assay was carried out. Clinicians were required to review their diagnosis/treatment according to microarray results.

Results: 318 allergic patients (30% reporting also nonrespiratory symptoms) and 91 controls were enrolled. The clinicians reported at least one additional information from the microarray in about 60% of patients, this resulting in therapeutic adjustments. In 66% of patients IgE to pan-allergens were detectable, being this clinically relevant in 38% of patients with polysensitization to pollens.

Conclusion: Microarray IgE assay represents an advancement in allergy diagnosis, as a third-level approach in polysensitized subjects, when the traditional diagnosis may be problematic.

The capacity of responding with the synthesis of specific IgE towards ubiquitous and innocuous substances is the distinctive characteristic of atopic subjects, which results from complex gene–environment interactions (1, 2). The subsequent IgE–allergen binding to the surface of mast cell and basophils is the trigger of the allergic reaction, that sustains respiratory-, food-, drug- and hymenoptera venom allergy. Therefore, since their discovery in 1966, the measurement of allergen-specific IgE represented a key diagnostic procedure

in allergic diseases. Specific IgE can be detected either *in vivo*, by skin prick test, or *in vitro* by specific IgE assays. Both methods usually employ whole natural extracts from allergenic sources which, despite a satisfactory standardization, contain a mixture of allergenic and nonallergenic proteins (3), whereas the IgE response is specifically directed only to some molecules (4). In addition, extractive preparations may contain cross-reactive components (5), which are highly conserved across widely different allergen sources (6, 7). This

may complicate the interpretation of the diagnostic results, especially in polysensitized subjects.

The introduction of highly purified natural and recombinant single allergenic molecules represented a step forward in the diagnosis of allergic diseases, allowing to distinguish between genuine IgE sensitizations and cross-reactivities (8). Another important advance was the introduction of the microarray technique, which allows to detect IgE antibodies against multiple allergenic components (either recombinant or natural purified) at the same time, using small amounts of serum (9, 10). One of the commercially available microarray techniques is the Immuno solid-phase allergen chip (ISAC), Phadia AB, Uppsala, Sweden, now Thermo Fisher Scientific, that in the version used in this study, detects specific IgE against 103 different allergenic molecules (now available with 112 molecules). Although the microarray technique represents a relevant evolution in the diagnostic approach (11), its positioning and value in real life is still not well defined (7). Also, the economic aspect of microarray may play a role, when compared with the standard diagnostic tests. Therefore, we performed a large population-based study to investigate the performance and the potential additional diagnostic values of the ISAC microarray in a real-life clinical setting, taking into account also the economic values.

Methods

Six Italian allergy units (Ancona, Bari, Cuneo, Florence, Genoa and Rome) participated in this prospective study. Each unit was requested to collect the demographic and clinical data of at least 50 patients referred for respiratory allergic diseases (rhinitis, asthma or both), and with at least two positive skin prick test, identified with commercial extracts. All patients underwent the standard diagnostic procedures, where skin prick test (SPT) was always the first approach, followed by specific IgE assay, as required. During the diagnostic work-up, serum samples were collected for the microarray ISAC assay. Twenty healthy controls per centre, with negative skin tests, were also included in the study. Based on clinical history and results of skin prick tests, allergists were asked to classify the patients, into one of the following classes: A: polysensitized patients with respiratory allergy, with only one clinically relevant sensitization, B: polysensitized patients with respiratory allergy, with more than one relevant sensitization (according to clinical history); C: polysensitized patients with respiratory allergy, but with suspected cross-reactivities (cross-reacting allergens possibly justifying the multiple sensitization), D: patients with respiratory allergy and reporting food-induced symptoms, E: patients not classifiable in the previous categories. This is what is currently done in clinical practice, using the standard diagnostic methods. Allergists prescribed the appropriate pharmacotherapy according to guidelines (12, 13), and allergen-specific immunotherapy (SIT), when indicated, according to recommendations (14). Clinicians had subsequently to review their first diagnosis and treatment, once they received the microarray results, and to recall those patients who had treatment/diagnosis changed. Clinicians were asked to grade the

importance of the additional information obtained from ISAC as 'remarkable', 'partial' or 'none', for diagnostic and therapeutic aspects.

This study involved standard diagnostic procedures, and it was simply notified to the local ethics committees, according to the national law. All patients signed an informed consent, as per routine, for the anonymous treatment of personal data.

Skin prick testing and specific IgE

Skin tests were performed at each centre, with the same technique and extracts, according to current recommendations (15). The standard panel of commercial extractive preparations (ALK-Abello, Milan, Italy) included mites, grass, olive, Parietaria, birch, cypress, ragweed, mugwort, cat and dog dander, *Alternaria* and *Aspergillus*. Histamine (1%) and diluent served as positive and negative controls, respectively. A wheal reaction ≥ 3 mm was considered positive. No recombinant allergen preparation was used in this standard diagnostic work-up.

Blood samples were collected by venipuncture. Blood was clotted, and serum obtained by centrifugation was sent to a central laboratory to perform the assay. Aliquots were stored at -30°C . The specific IgE assay, with extractive allergens, was performed by a commercial immunoenzymatic method (Phadia AB, Uppsala, Sweden, now Thermo Fisher Scientific) using the same allergen sources used for skin tests. Results were expressed as kU/l, and values greater than 0.35 kU/l were considered positive.

ISAC microarray

Immune solid-phase allergen chip tests were performed following the manufacture's recommendations (Thermo Fisher Scientific, Milan, Italy), as previously described (11). Briefly, 0.02 ml of serum was incubated on the slide microchip containing 103 allergen spots (either recombinant or highly purified). After one-hour incubation, slides were washed, and a monoclonal anti-IgE antiserum labelled with a fluorochrome was added, then incubated for another hour. Slides were then analysed using a Laser Scan Confocal microarray reader (LuxScan 10K/A, CapitalBio, Beijing, China). Analysis of the results was automatically performed using a Microarray Image Analyser, being all samples identified by a bar code, and without any intervention of the technicians. All slides were also visually scored to detect the presence of defects (e.g. smears of fluorescence or drops interfering with the reading). Only in these cases, a manual correction (or refusal of the result) was decided. The results were reported as ISAC standardized units (ISU), considering as negative an $\text{ISU} < 0.35$.

Definition of additional values

Additional value was considered any information of clinical relevance achieved through ISAC, that could have not been achieved by traditional SPT or routine-specific IgE assay,

such as the identification of specific IgE against cross-reacting molecules (profilins, lipid transfer proteins, Bet v 1 homologs, bromelain, parvalbumins, tropomyosin, serum albumin, calcium-binding proteins and peanut-associated components). Another added value was the possibility to distinguish between true and false polysensitizations. A true polysensitization occurs where there are specific IgE against genuine components of different allergenic sources. False polysensitization occur when genuine components are recognized only for one or more of the allergens positive at SPT, and pan-allergens are also recognized. Remarkable added value was any information obtained by ISAC (and not available using standard diagnostics procedures) that could impact on the management of the patient in term of accuracy of the diagnosis, or specific immunotherapy (SIT) prescription.

Results

Three hundred and eighteen patients (170 women, mean age 37 years, age range 12–78 years) and 91 healthy controls (72 women, mean age 40 years, age range 15–83 years) were included in the study. All the patients suffered from respiratory allergy. Of them, 47% had rhinitis only, 52% asthma and rhinitis and 1% asthma alone. Notably, 95 patients (30%) reported in their clinical history also nonrespiratory symptoms including urticaria (36 patients), angioedema (19), oral allergy syndrome (51), gastrointestinal tract complaints (14), atopic eczema (4) and anaphylaxis (6 patients). All the controls were totally symptom free and had a negative clinical history.

Before ISAC, the patients were classified as follows: 56 class A, 176 class B, 44 class C, 34 class D and 8 class E. After ISAC, classification remained unchanged in 135 (42%) patients, whereas 183 patients (58%) could be reclassified, as detailed in Table 1. In particular, the 8 class E (nonclassifiable) patients could be placed into another class. Class A (polysensitized with only one clinically relevant sensitization) and class B (true polysensitized patients) were reduced in number. On the contrary, class C (polysensitized patients with suspected cross-reactivity) and class D (sensitized to inhalants and foods) became more represented, because ISAC allowed to identify sensitizations, not detectable by SPT and specific IgE assay.

At the ISAC test, 211 subjects had IgE against at least one of the following cross-reacting molecules: parvalbumin (12

patients), profilin (52), lipid transfer proteins (76), Bet v 1 (134), bromelain (15), tropomyosin (10), serum albumin (9) and calcium-binding proteins (12). As an example, among 135 patients with SPT and IgE positivity to grass mix, timothy grass and birch, only 83 resulted to be truly polysensitized, whereas in the remaining 52 (38%), the multiple positivity was due to cross-reacting proteins.

Before ISAC, SIT was indicated in 32 patients. After ISAC, the prescription was changed in 3 of those patients by adding a new extract and was newly prescribed in further 85 patients (31 with a single extract and 54 with two or more extracts). Concerning the prescription of environmental control measures or prophylaxis, almost 60% of previous decisions (based on SPT and sIgE) were modified. The majority of suggestions for environmental control concerned house dust mite (53%), cat (40%), dog (25%), moulds (8%) and latex (6%).

Table 2 shows the results of microarray for cross-reacting components that cannot be identified by traditional SPT and sIgE assays. In these cases, relevant additional information, as compared with the usual diagnostic procedures, was detected in 221/318 patients (69.4%), with more than 2 information achieved in 13.5% of patients (Table 3). In a relevant proportion of patients with grass sensitization (assessed by SPT, sIgE and Phl p 1 and/or Phl p 5 positivity), the absence of a sensitization to profilins represented a clear indication of genuine polysensitization. Table 4 details the additional diagnostic information obtained by the use of ISAC as judged by clinicians. In almost 1/3 of cases, relevant additional information, in terms of a more confident diagnostic and therapeutic approach, was achieved. More in detail, in a subgroup of 42 patients (13.2%), the additional information was considered of special clinical relevance. In 7 cases, it concerned the

Table 2 N and percentage of pts found positive to pan-allergens

Cross-reacting allergens	Number	%
Profilins	35	11.0
LTP	51	16.0
Bet v 1 homolog	88	27.7
Bromelain	9	2.9
Parvalbumins	5	1.6
Tropomyosin	6	1.8
Serum albumin	9	2.8
Ca++-binding proteins	10	3.1
Peanut	8	2.5

Table 1 Patients' classification before and after ISAC (see text). In light grey, the number of patients who were not reclassified after the ISAC test

		After ISAC				
		A	B	C	D	E
Before ISAC	Total	33	117	99	69	0
A	56	13	32	4	7	0
B	176	15	72	54	35	0
C	44	0	6	32	6	0
D	34	2	5	9	18	0
E	8	3	2	0	3	0

Table 3 Additional information, related to cross-reacting allergens, added by ISAC to the standard diagnostics (pan-allergens)

	Number	%
Overall	221	69.4
1 additional information	102	31.1
2 additional information	82	24.8
3 additional information	36	10.8
4 additional information	7	2.1
5 additional information	2	0.6

Table 4 Additional information obtained by the use of ISAC, as judged by clinicians

Clinicians' judgements on the relevance of added information*	New information related to diagnosis Number (%)	A more confident diagnostic approach Number (%)	New information related to the patient's disease Number (%)	More confident in management of disease Number (%)
To some extent	220 (69)	227 (71)	232 (73)	232 (74)
Remarkable	87 (27)	295 (93)	299 (95)	286 (90)
None	–	–	–	–

*Multiple answers allowed.

presence of IgE to components possibly associated with food allergy, in 11, an unexpected sensitization to profilins or LTPs, in 8, the presence of allergens potentially responsible for an oral allergy syndrome, in 10 specific indications related to food intake could be given, and in 6 cases, a previously unknown sensitization to insect venom was diagnosed. Patients were always informed of those positivities, and appropriate advice was given according to the clinical history.

All the controls were totally asymptomatic with negative SPT resulted and specific IgE assays, whereas at the ISAC procedure, some weak positivities (<1 ISU) mainly to mite, *Aspergillus*, *Parietaria* and grass components, were identified in around 20% of subjects. Of course, these subjects would require a clinical follow-up.

Discussion

The introduction of a novel diagnostic approach requires a careful analysis not only of the technical characteristics, but also of its potential additional usefulness in clinical practice, and of the economic aspects. Microarray techniques in allergy diagnosis were indeed described 10 years ago, but only in the last few years, those techniques became part of the diagnostic armamentarium in allergy practice. The good laboratory performance of these assays was clearly defined (11, 16, 17), but their actual impact and role in clinical practice remains a matter of debate, and still under evaluation (18, 19). Other molecular approaches, based on assays (either SPT or specific IgE detection) using single allergenic components, are routinely available. However, the costs and availability of those components make difficult the detection of sensitization to numerous allergenic molecules at a same time. In addition, they do not allow to detect unsuspected (or undeclared), and possibly harmful sensitizations. In this context, the availability of a multiplexed assay capable of identifying specific IgE towards about 100 different allergen components is intriguing, in particular in polysensitized patients where an accurate aetiological diagnosis is complex, due to the presence of sensitizations to both genuine allergens and cross-reacting components.

On this background, we evaluated the magnitude of the advantages achieved by the use of ISAC microarray in everyday clinical practice, and the conditions where this technology becomes advantageous economically. Clinicians were asked to perform their diagnosis in polysensitized patients with respiratory allergy using the standard techniques and subsequently to revise the diagnosis/treatment

according to the ISAC results. Overall, the use of the microarray lead to an improvement in the accuracy of diagnosis and appropriateness of treatment in a variable percentage ranging from 25% to 50% of patients, on the basis of the level of accuracy used for the molecular diagnosis. This is in agreement with a previous similar work, where the concordance in immunotherapy prescriptions before and after ISAC was less than 50% (20).

The cost of the procedure remains a possible main drawback. In general, clinical presentation and patient's history usually allow allergists to define the relevant sensitizations. Nevertheless, a significant number of cases of polysensitized patients cannot be fully identified using only those parameters, and in these cases, a more sophisticated approach is needed. In this context, it can be expected that when too many single recombinant allergens are required to define an accurate sensitization profile, ISAC maybe preferable, in terms of costs. Notably, the cohort enrolled in this study was represented by patients with only respiratory allergy as reason for referral; thus, this aspect becomes even more relevant in patients with concomitant diseases, such as reported adverse reaction to food or in children (21, 22). Finally, from a technical point of view, ISAC may be less sensitive than the ImmunoCAP for some molecules, such as Art v 1 or Amb a 1 (11, 23); thus, the risk of false-negative results may occur.

In conclusion, ISAC have shown additional clinical values in a real-life setting in polysensitized patients (24–26). Nonetheless, it must be kept in mind that revealing a sensitization with a laboratory test does not automatically imply overt allergy, and therefore, a detailed clinical evaluation still remains the mainstay of the diagnostic process, leaving the microarray as a third-level diagnostic approach.

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Conflict of interest

All the signing authors were part of a scientific board specifically convened by Phadia AB/Thermo Fischer Scientific.

Author contributions

All the authors have equally contributed in the clinical work, analysis of the data and preparation of the manuscript.

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