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Component resolved diagnosis in real life: the risk assessment of food allergy using microarray-based immunoassay

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Key words

Food allergy; component-resolved diagnostics; risk-assessment; food allergenic molecules

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Summary

Background. *The development of component-resolved diagnostics constitutes a potential breakthrough in food allergy testing, as detection of specific IgE (sIgE) to individual allergens may make it possible to establish the risk of a mild versus severe reaction.* ***Objective.*** *To compare allergists' risk assessment based on the current decision making process with that of virtual allergen-oriented risk assessment through microarray-based immunoassay.* ***Patients and Methods.*** *An observational, real-life study was performed on 86 adults with food allergy. The prescription of epinephrine was the surrogate marker of a severe reaction. In the same patients, the prescription of epinephrine based on the current decision making of the allergist and the independently established allergen-oriented risk assessment determined by microarray-based immunoassay were compared.* ***Results.*** *Fair degree of agreement between the specialists' risk assessment and that of the microarray-based immunoassay (k index 0.372 (95% CI: 0.185-0.559) $p < 0.001$) was documented. Three causes of discrepancy emerged: the poor sensitivity of the allergen microarray-immunoassay (51.9%), the differences in risk assessment established by the specialist and the microarray-immunoassay (33.3%), the non-inclusion of the causative allergen in the microarray-immunoassay platform (14.8%).* ***Conclusion.*** *Improvement of the diagnostic accuracy of microarray-immunoassay, combined with marrying its results to clinical information, could one day soon lead to changes in clinical practice in food allergy.*

Introduction

Food allergies impair quality of life and can culminate in life-threatening reactions, whose prevalence affects an estimated 2-4% of the general adult population (1, 2), with some studies suggesting that the last few decades have seen a rise in the number of cases in industrialized countries (3, 4).

Even more striking is the increase in the number of patients in industrialized countries who think they have a food allergy and seek medical assistance (5).

Diagnosis of food allergy is necessary both to prevent severe reactions and to avoid unnecessary dietary restrictions; unfortunately, the complex mixture of allergenic proteins of diagnostic extracts for skin and serological tests makes testing inaccurate and hence unable to predict the likelihood and, in particular, the severity of a future reaction (6).

As a result, in current clinical practice, in most patients combining their history with the results of skin testing or immunoassay is a necessary step towards reaching an accurate diagnosis, though controlled food challenge, which is time-consuming and labour intensive, remains the diagnostic gold standard and may sometimes be required (6).

The development of component-resolved diagnostics (CRD) is a potential breakthrough in food allergy testing, as the detection of specific IgE (sIgE) to individual allergens in diagnostic extracts could signify diagnostic improvement,

helping to establish the real risk of either a mild or severe reaction (7).

A further advancement in this field is the combination of CRD and microarray technology, which allows testing a panel of one hundred inhalant and food allergens, including recombinant and purified native allergens with very small quantities of serum (8,9). This technology holds promise for a significant change in the management of food allergic patients, depicting in a single step the virtual allergen-oriented risk assessment in less time and with fewer resources than the current diagnostic workup.

Possible changes in clinical practice in allergy through allergen microarray-immunoassay are currently under investigation both in food allergy (10) and in the prescription of specific immunotherapy (11).

In consideration of this, our aim was to compare the risk assessment that resulted from the allergist's current decision-making approach with that of the allergen-oriented approach reached by means of microarray-based immunoassay.

Methods

Study design

Observational, cross-sectional study performed in a real-life setting. Patients with a case history suggestive of food allergy to several food allergens or of respiratory and food allergy to several inhalant and food allergens were eligible for the study.

Diagnosis was carried out by four trained allergists involved in the study through their usual diagnostic workup, namely combining patient case history obtained using a standardized questionnaire with skin test results and, in case, the immunoassay.

Skin prick testing with commercial extracts (Alk Abellò, Madrid, Spain) was performed in all patients. The basic list of food allergens included almond, anisakis, apple, celery, cod, egg white, gliadin, hazelnut, milk, parsley, peanut, profilin, tomato, peach, sunflower, soy, shrimp, walnut and wheat. Any further skin tests used in current clinical practice (prick test for inhalant allergens, prick test with food allergens not on the basic list, prick by prick test with fresh food) were allowed if the physician deemed it necessary for the diagnosis. Histamine (10mg/ml) and glycerol-saline solution were used as controls. A skin test was considered positive if the mean wheal diameter ≥ to 3 mm was greater than the negative control.

Detection of sIgE, both for food extract or for allergenic molecules (ImmunoCAP, Phadia, Uppsala, Sweden) were added to the diagnostic workup if the physician judged that it was necessary for an accurate diagnosis.

The use of allergen microarray-immunoassay was disallowed to specialists. On completion of the diagnostic workup, the diagnosis and the decision whether or not to prescribe epinephrine were recorded. The allergist's decision was taken as the study's gold standard. The prescription of epinephrine was considered a surrogate marker of the risk assessment, as it is mandatory for severe, potentially life-threatening reactions, whereas an avoidance diet is prescribed for food allergy irrespective of the severity of the reaction.

Serum samples were collected from each participant and a commercial microarray-base immunoassay, which allows 103 airborne and food allergenic molecules (ISAC, Phadia, Uppsala, Sweden) to be tested at once, was performed in the university hospital's general laboratory, according to the manufacturer's recommendations.

Briefly, reaction sites were incubated with 20 mL of patient sera for two hours. After rinsing, washing and drying, allergen-specific IgE complexes were stained with a fluorescence-labelled anti-human IgE for one hour. After further washings, a laser scanner took fluorescence readings, and results were translated into numeric data by comparison with a reference serum standardized against ImmunoCAP IgE. As a consequence, the results, expressed as standardized ISAC units (ISU/L), are indirectly linked to the World Health Organization IRP 75/502 IgE standard.

Levels > 0.3 ISU/L were considered positive, according to the manufacturer's recommendations.

A physician, blinded to the allergist's decision making, prescribed epinephrine on the basis of the most recently published list of foods which trigger anaphylaxis in Italy (12) and of the available information on the risk of severe reactions associated with the allergenic molecules in the ISAC panel (7,9,13).

The prescription of epinephrine, for example, was allowed if a peach allergy was associated with the detection of Pru p 3 sIgE levels, or if a peanut allergy was associated with detection of Ara h1, Ara h2 sIgE levels, and so on (7,9,13).

To enhance the sensitivity of the virtual risk assessment, sIgE levels greater than a threshold level of 0.3 ISU/L were considered as significant.

On completion of the study, an independent referee compared the allergists' prescription of epinephrine against that suggested by the allergen microarray-immunoassay.

All data were collected between January 1st 2010 and June 30th 2010.

The study was approved by the local Ethics Committee of Azienda Ospedaliero-Universitaria "Ospedali Riuniti" of Ancona. Our patients underwent a routine medical examination and provided an oral informed consent before commencing the study and giving a blood sample.

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Science (SPSS) version 13.0 for Windows. Descriptive statistics were presented as numbers and percentages for qualitative variables. The agreement coefficient (k index) was used to

analyze the results. The degree of concordance between the two methods of epinephrine prescription was assessed using Landis and Koch's interpretive scale for k values: 0.81-1.00, almost perfect; 0.61-0.80, substantial; 0.41-0.60, moderate; 0.21-0.40, fair; 0.00-0.20, slight; and < 0.001, poor. Statistical significance was set at < 0.05.

Results

One hundred and four patients were enrolled, 18 of whom were excluded as they failed to meet the selection criteria. **Table 1** presents the main demographic and clinical features of the patients. In the diagnostic workup of the allergist, besides skin prick testing, almost two-thirds of the patients were tested for sIgE. In more than two-thirds of the patients, the risk assessment of the allergist and that of the microarray-based immunoassay agreed: in 23 cases neither suggested prescribing epinephrine, while in 36 both suggested its prescription (**table 2**). The k index was = 0.372 (95% CI: 0.185-0.559) p < 0.001. Three causes of discrepancy emerged (**table 2**). Poor sensitivity of the microarray-based immunoassay accounted for more than fifty percent of the divergent risk assessments: in 14 patients, allergists documented the role of food allergens and sometimes even of allergenic molecules in severe reactions, whereas ISAC yielded a negative score for the equivalent allergenic molecules in its platform (**table 3**). The discordance between the virtual risk assessment of microarray-based immunoassay and that of the physicians for the same allergenic molecule was the second cause of discrepancy. Consistent with the detection of Pru p 3 sIgE levels, the virtual risk assessment of ISAC suggested the need to prescribe epinephrine in six patients, whereas, consistent with their case histories (food-induced oral allergy syndrome or mild symptoms), the allergists did not take this decision. In the remaining three patients, the combination of their case histories (food-induced oral allergy and dyspnoea in one patient and food-induced oral allergy syndrome plus systemic urticaria in the other two) with skin test result in one patient, and skin test results plus sIgE for the allergen extracts in the other two, suggested epinephrine prescription to the doctor. The virtual ISAC risk assessment denied this decision, as sIgE against profilin, sIgE against Bet v1-like homologous allergens and sIgE against profilin plus sIgE against Bet v1-like homologous allergens were found in the first, second and third patient respectively.

The last cause of discordance arose from the non-inclusion of certain food allergens in the platform in the microarray-based immunoassay: while the ISAC test was negative, the diagnostic workup of the allergists revealed the role of bell pepper, pistachio, sesame and peanut (Ara h 9) respectively in four patients with severe reactions.

Table 1 *- Demographic and clinical profile of the patients (n = 86)*

Variable	
Gender	
Male	40 (46.5%)
Female	46 (53.5%)
Age (years)	
Mean	28
Range	15-67
Median	28
Symptoms	
Urticaria/angioedema	56 (64.4%)
Oral Allergic Symptom (OAS)	25 (28.7%)
Dyspnoea	17 (19.5%)
Gastrointestinal symptoms	9 (10.3%)
Anaphylactic shock	14 (16.1%)
Glottis oedema	2 (2.3%)
Respiratory allergy	
Yes	73 (84.8%)
No	13 (15.2%)
Diagnostic tests	
Commercial extracts skin prick test	86 (100%)
Prick by prick test	43 (49.4%)
Dosage specific IgE	59 (67.8%)
All diagnostic tests in a single patient	30 (34.5%)
Number of positive skin test for food	
<3	22 (19%)
≥3	64 (81%)

Table 2 *- Congruence between the physician work up and the virtual risk assessment of the microarray-base immunoassay and causes of disagreement*

Agreement in prescription of epinephrine	
Yes	59 (68.6%)
No	27 (31.4%)
Causes of disagreement	
Insufficient sensitivity of the microarray-based immunoassay	14 (51.9%)
Different risk assessment of the doctor and microarray-based immunoassay	9 (33.3%)
Allergen missing in the platform of the microarray-based immunoassay	4 (14.8%)

Table 3 *- Foods associated with severe allergic reactions detected by the diagnostic work up of the allergist but not by ISAC. The diagnostic tests used by allergist were indicated*

	Allergen	Skin Prick Test	sIgE for allergenic extract	sIgE for allergenic molecules
1	Egg, Cow milk	x	x	
2	Nuts, Peach	x	x	
3	Shrimp, Egg white, Cow milk	x	x	
4	Anisakis simplex	x	x	
5	Cod	x	x	
6	Peach, Apple, Celery, (Pru p 3)	x	x	x
7	Cow milk, Anisakis simplex	x	x	
8	Peach, Nuts	x		
9	Peach, Nuts, (Pru p 3)	x	x	x
10	Peach, apple, (Pru p 3)	x	x	x
11	Peach, nuts, (Pru p 3)	x	x	x
12	Nuts, fish	x	x	
13	Cow milk	x		
14	Peach	x	x	

Discussion

In a selected patient population with a case history suggestive of food allergy to several foods or of concomitant respiratory and food allergies, a weak but significant degree of agreement, 0.372 (95% CI: 0.185-0.559) $p < 0.001$, was found between the risk assessment of the allergists and that virtually established by the microarray-based immunoassay.

Whereas a weak result in a randomized clinical trial is often predictive of the failure of its translation to a real world setting, a weak result in the real world could nevertheless be promising, provided that the teething problems of a technology still in its infancy are addressed and overcome.

We found that the problems associated with virtual risk assessment of food allergy by ISAC were technological and conceptual. Technologically, the non-inclusion of certain allergenic molecules in the ISAC platform apart, we found that sometimes, for the same allergen, sIgE detection by ISAC was less sensitive than the combining of the skin and serological test results routinely used by the allergist; whether this results from the poor diagnostic ability of the allergenic molecules in the ISAC platform, from the high operator-dependent management of the instrument is an open question.

These difficulties will likely be overcome in time through technological improvements and adjustments, while the conceptual limitation of the risk assessment for a single allergenic molecule seems less easily surmountable.

Fundamentally, the allergenic molecule-oriented risk assessment arises from the fact that different degrees of risk for a severe reaction are associated with the detection of sIgE against different allergenic molecules coexisting in the same food (13).

Consistent with this scenario, in our model of virtual risk assessment the detection of Pru p 3 sIgE, which is associated with the most severe allergic reactions to peach, required the prescription of epinephrine: unfortunately decision making of this type reduced the specificity of the epinephrine prescription as, even though peach LTP-hypersensitivity is associated with the highest risk for anaphylaxis in Italy (22% of food-induced anaphylaxis), only 7% of peach LTP-hypersensitive patients manifest this syndrome (12).

Moreover, in the case of Pru p3, the serum level of sIgE seems unable to predict either the presence of the clinical allergy (14) or the severity of the reaction (15). Only association with other allergenic molecules seems indicative of a decreased risk for severe reactions (16).

Even though the risk assessment for other allergenic molecules appears to be more reliable (7,9), similar results were found for other allergenic molecules (Bet v1-homologous food allergy) of vegetal origin (17).

As a result, ISAC is currently able to provide an allergen-oriented risk assessment between different molecules in the same food, but the risk assessment of the single allergenic molecule needs to be supported by clinical information to improve reliability. We speculate that a software combining clinical information, obtained by standardized questionnaire, with ISAC results could partially overcome this problem.

Our study has several limitations, which include the small size of the patient sample and the over-simplified model of food allergy management.

Overall, the assumption that the allergists' prescription of epinephrine was the gold standard is questionable, as both the discussion on the appropriate use of adrenaline remains open (18,19), and the allergists' conclusions in regard to a severe food reaction might be erroneous, as they were not validated by a controlled food challenge.

Indeed, in the three patients whose results were discordant with those of ISAC, the prescription of epinephrine by allergists seems to derive from an incorrect diagnosis or from the allergists' defensive medicine.

In conclusion, our results suggest that the allergen-oriented risk assessment in food allergic patients by ISAC is still pre-

mature for current clinical practice. However, the combining of a robust improvement of its diagnostic accuracy and a close linking of its results with clinical information could lead to changes in clinical practice in food allergy, in the not too distant future.

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