Expert Review of Clinical Immunology

ISSN: 1744-666X (Print) 1744-8409 (Online) Journal homepage: informahealthcare.com/journals/ierm20

Novel *in silico* technology in combination with microarrays: a state-of-the-art technology for allergy diagnosis and management?

Giovanni Melioli, Giovanni Passalacqua & Giorgio W Canonica

To cite this article: Giovanni Melioli, Giovanni Passalacqua & Giorgio W Canonica (2014) Novel *in silico* technology in combination with microarrays: a state-of-the-art technology for allergy diagnosis and management?, Expert Review of Clinical Immunology, 10:12, 1559-1561, DOI: 10.1586/1744666X.2014.978761

To link to this article: https://doi.org/10.1586/1744666X.2014.978761

Novel *in silico* technology in combination with microarrays: a state-of-the-art technology for allergy diagnosis and management?

Expert Rev. Clin. Immunol. 10(12), 1559–1561 (2014)

Giovanni Melioli

Author for correspondence: Allergy and Respiratory Diseases, Department of Internal Medicine, IRCCS S.Martino-IST-University of Genoa, Largo Rosanna Benzi 10,16132, Genova, Italy
Tel.: +39 346 516 8826
giovanimelioli@gmail.com

Giovanni Passalacqua

Allergy and Respiratory Diseases, Department of Internal Medicine, IRCCS S.Martino-IST-University of Genoa, Largo Rosanna Benzi 10,16132, Genova, Italy

Giorgio W Canonica

Allergy and Respiratory Diseases, Department of Internal Medicine, IRCCS S.Martino-IST-University of Genoa, Largo Rosanna Benzi 10,16132, Genova, Italy

'Allergen microarrays, in poly-sensitized allergic patients, represent a real value added in the accurate IgE profiling and in the identification of allergen(s) to administer for an effective allergen immunotherapy.' Allergen microarrays (AMA) were developed in the early 2000s to improve the diagnostic pathway of patients with allergic reactions. Nowadays, AMA are constituted by more than 100 different components (either purified or recombinant), representing genuine and cross-reacting molecules from plants and animals. The cost of the procedure had suggested its use as third-level diagnostics (following *in vivo*– and *in vitro*–specific IgE tests) in poly-sensitized patients. The complexity of the interpretation had inspired the development of *in silico* technologies to help clinicians in their work. Both machine learning techniques and expert systems are now available. In particular, an expert system that has been recently developed not only identifies positive and negative components but also lists dangerous components and classifies patients based on their potential responsiveness to allergen immunotherapy, on the basis of published algorithms. For these characteristics, AMA represents the state-of-the-art technology for allergy diagnosis in poly-sensitized patients.

The large majority of patients with suspected allergic symptoms can be diagnosed by identifying allergen(s) with the standard *in vitro* and *in vivo* methods (which include first-level assays, such as the skin prick test, and second-level assays, such as detection of specific anti-allergen IgE). Nonetheless, in a small but consistent number of complex cases (i.e., multiple sensitizations with the standard tests), a third-level approach is needed. Third-level *in vitro* assays can reliably identify both genuine and cross-reacting components [1]. This information can be obtained by either using single components (both recombinant allergens and purified natural allergen molecules) or a multiplex approach, which is represented by allergen microarrays (AMA). The reasons for a third-level approach are related to improving not only the diagnostic accuracy but also, more importantly, a better definition of the causal allergen(s) involved and, consequently, to decide the allergen(s) to be included in allergen immunotherapy (AIT) prescriptions accurately [2]. This consideration is especially relevant in European countries, where only a few different allergenic extracts are administered, whereas in other countries, such as USA, virtually all the positive allergens are administered and are mixed together [3].

AMAs were developed in early 2000 and immediately represented a very interesting tool for allergy diagnosis. Although the first versions of the assays were considered suitable to support a complete serological IgE sensitization diagnosis, in 2010, the presence of discrepancies between specific IgE on whole extracts (sIgE) and AMA (namely

Keywords: allergen • allergy • artificial intelligence • component • expert systems • laboratory test • microarray

EXPERT REVIEWS

 10.1586/1744666X.2014.978761 ISSN 1744-666X

ImmunoCAP ISAC™, Thermo Fisher Scientific ImmunoDiagnostics Division, Milano, Italy) became evident [4]. For example, the *Phleum pratense* components were well represented in AMAs (and discrepancies are very rare). For other allergens (such as Ragweed, Alternaria, Aspergillus and dog), the agreement between positive sIgE and positive AMA was reported to range between 13 and 30%. The possible explanations for these discrepancies were the sIgE concentration was too low, there was an absence of the relevant component in the AMA panel and the presence of an IgE against the cross-reacting molecules (such as profilins, polcalcins and PR-10) in the whole allergen extract (that resulted in positive standard tests) in the absence of a specific IgE toward genuine allergens (such as Amb a 1, which is present in AMA). These considerations suggested that AMA should be used in already well-studied patients, as a third-level assay [1,5].

The specificity of AMA is extremely high; thus, if an IgE toward an allergenic component is positive, the sIgE to the allergen extract are positive too [4]. Sensitivity is still a matter of debate. Indeed, the correlation analysis of the sIgE and AMA results seems to demonstrate that the sensitivity is not entirely reliable [4]. In this context, other authors have shown that the comparison of single-plexed recombinant molecules with the same components in multiplexed AMA is good [6].

AMA advantages include the availability of a wide spectrum of components (mainly inhalants and food, which are genuine and cross-reactive), the possibility to obtain specific added values [4,7], an accurate description of the IgE profile by the identification of sensitization to cross-reacting versus genuine components [7–10], the direct identification of an IgE that is specific for potentially harmful components [5] and, finally, the need of only a few microliters of serum (in particular for pediatric patients) to obtain a large screening [11–13]. The disadvantages include the cost of a single test, certain difficulties in the interpretation [14,15], the possibility that unexpected results will need to be interpreted and explained to the patients [5] and the presence of discrepancies between a skin prick test sIgE results and AMA results.

Therefore, after more than 10 years of experience using microarrays in allergy diagnoses, in the authors' opinion, AMA represents a state-of-the-art technology for allergy diagnoses, provided that certain aspects are carefully considered. The authors' opinion is that the large majority of patients with inhalant allergen sensitization can be well studied using *in vivo* skin prick test, sIgE and a small panel of components, both recombinant and highly purified from natural allergen extracts [5]. Only in highly poly-sensitized patients, in which not only the risk of a sensitization to cross-reacting components is present but also a pollen-food syndrome is suspected, AMA can be used [5]. The choice between single or multiplexed diagnostics will be based on the number of tests required for the evaluation of the proper number of components. Specifically, if it is more than 10, an AMA should be indicated. Of course, in very complex cases (e.g., a food allergy, anaphylaxis and young pediatric patients), microarrays should be routinely used.

The introduction of a large panel of molecules, which are characterized by specific features, in allergy diagnostics, can be considered a real revolution. For this reason, an article was published to improve the knowledge of the 'molecular allergy terminology' [16]. The large number of results produced by a microarray analysis allows for additional data to be obtained by the use of a computer learning machine approach [15]. The interpretation of AMA was particularly investigated in an article [14] that described the development of an expert system, which was dedicated to ImmunoCAP ISAC. A number of added values, which provided results that were different from those that were described at the beginning of our experience, were identified. For example, a cluster analysis [13] of the ratio between genuine and cross-reacting components allowed for the identification of different phenotypes that seem to correspond to an increased risk of AIT failure [8]. Another additional value of AMA, following the interpretation proposed by Allergenius, is the explanation of the discrepancies between sIgE test and AMA results. Indeed, any discrepancies should be carefully managed. For example, it should be useful to check whether other allergen sources that are present in AMA have positive results in their cross-reacting components. Thus, this would be a demonstration of the positivity of the whole extract allergen test.

Microarray technology is improving with time, and not only are the number of spotted components increasing but also the strategy for the component choice is also getting better. Future trends include, for example, MeDALL [17], which is a novel approach with 170 different allergens. Of course, the costs and the interpretation difficulties will be proportionally increased; however, such a tool will have a tremendous impact on allergy diagnoses if used by allergists with specific experience in molecular diagnostics. In addition, the molecule-based approach is expected to be of special relevance in the diagnosis of life-threatening diseases, such as hymenoptera venom allergy [18]. Other approaches also seem to be interesting, including flow cytometry [19], which should allow for an accurate serological analysis using very common laboratory instruments (at least, more common than a microarray reader). To improve the study of the fine relationships between components and IgE, plasmon resonance [20] seems to be a very powerful tool. If MeDALL and flow cytometry are available in a short time, the clinical relevance of IgE avidity for allergens is at present largely unknown. For this reason, it is reasonable to consider that surface plasmon resonance will be a research tool in the near future.

On the basis of these considerations, we need to determine which indications could be suggested for the use of microarray allergy tests in 2014.

These tests should mainly be used for an accurate description of the IgE profile and the allergen identification for AIT in poly-sensitized patients. Although there is a large consensus for the former [5], the latter is still a matter of debate. Clear evidence that the use of components, including AMA, may significantly improve the quality of the administered AIT has been already given [7,9]. Indeed, it was also demonstrated that a better result is obtained using AIT in patients who are sensitized against genuine components [8]. In addition, the biochemical nature of both genuine and cross-reacting components should

be considered at the light of AIT identification, for example. Moreover, the role of cross-reactive carbohydrate determinants that are present on components purified from allergen extracts was considered to be relevant for the documented risk of false-positive results [21] and the consequent possible errors in the identification of allergen sources for AIT [10].

AMA technology represents a state-of-the-art method in allergy diagnosis if the patient is characterized by a poly-sensitization, in particular, by a pollen-food syndrome; a molecular strategy for the identification of allergen sources for AIT is used and the user has the scientific culture or specific informatics tools for the interpretation of the AMA results.

Acknowledgements

The article was revised by American Journal Experts only for English grammar, editing and typing.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the article. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Melioli G, Compalati E, Bonini S, Canonica GW. The added value of allergen microarray technique to the management of poly-sensitized allergic patients. Curr Opin Allergy Clin Immunol 2012;12(4):434-9
2. Sastre J. Molecular diagnosis and immunotherapy. Curr Opin Allergy Clin Immunol 2013;13:646-51
3. Cox L, Jacobsen L. Comparison of immunotherapy practice patterns in the US and Europe. Ann Allergy Asthma Immunol 2009;103:451-9
4. Melioli G, Bonifazi F, Bonini S, et al. The ImmunoCAP ISAC molecular allergology approach in adult multi-sensitized Italian patients with respiratory symptoms. Clin Biochem 2011;44(12):1005-11
5. Canonica GW, Ansotegui IJ, Pawankar R, et al. A WAO - ARIA - GA^{2}LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J 2013; 6(1):17

•• **This is a fundamental consensus that defined how molecular allergy diagnostics should be used in clinics.**

6. Gadisseur R, Chapelle JP, Cavalier E. A new tool in the field of in-vitro diagnosis of allergy: preliminary results in the comparison of ImmunoCAP© 250 with the ImmunoCAP© ISAC. Clin Chem Lab Med 2011;49(2):277-80
7. Passalacqua G, Melioli G, Bonifazi F, et al. The additional values of microarray allergen assay in the management of polysensitized patients with respiratory allergy. Allergy 2013;68(8):1029-33
8. Schmid-Grendelmeier P. Recombinant allergens. For routine use or still only science? Hautarzt 2010;61(11):946-53

• **In this work is shown the first evidence of a correlation between the molecular characteristics of a sensitization and the immunotherapy outcome.**

9. Sastre J, Landivar ME, Ruiz-García M, et al. How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. Allergy 2012; 67(5):709-11

•• **One of the first demonstrations of the role of Molecular Allergy and allergen microarrays (AMA) efficacy in the definition of a AIT in poly-sensitised allergic.**

10. Douladiris N, Savvatianos S, Roumpedaki I, et al. A molecular diagnostic algorithm to guide pollen immunotherapy in southern Europe: towards component-resolved management of allergic diseases. Int Arch Allergy Immunol 2013;162(2):163-72
11. Melioli G, Marcomini L, Agazzi A, et al. The IgE repertoire in children and adolescents resolved at component level: a cross-sectional study. Pediatr Allergy Immunol 2012;23(5):433-40
12. Onell A, Hjälle L, Borres MP. Exploring the temporal development of childhood IgE profiles to allergen components. Clin Transl Allergy 2012;2(1):24
13. Melioli G, Passalacqua G, Canonica GW, et al. Component-resolved diagnosis in pediatric allergic rhinoconjunctivitis and asthma. Curr Opin Allergy Clin Immunol 2013;13(4):446-51
14. Melioli G, Spenser C, Reggiardo G, et al. Allergenius, an expert system for the interpretation of allergen microarray results. World Allergy Organ J 2014;7(1):15

• **This is an attempt to link expert systems to allergy diagnosis.**

15. Prosperi MC, Belgrave D, Buchan I, et al. Challenges in interpreting allergen microarrays in relation to clinical symptoms: a machine learning approach. Pediatr Allergy Immunol 2014;25(1):71-9

• **This is another attempt to use computer analysis and machine learning to analyse data from AMA results.**

16. Melioli G, Canonica GW. Molecular allergy diagnosis: we need to become more knowledgeable. Ann Allergy Asthma Immunol 2012;108(6):387
17. Lupinek C, Wollmann E, Baar A, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. Methods 2014; 66(1):106-19

•• **This manuscript should be a real quantum leap in the evolution of molecular allergy and AMA technology.**

18. Kohler J, Blank S, Muller S, et al. Component resolution reveals additional major allergens in patients with honeybee venom allergy. JACI 2014;133:1383-9
19. Pomponi D, Bernardi ML, Liso M, et al. Allergen micro-bead array for IgE detection: a feasibility study using allergenic molecules tested on a flexible multiplex flow cytometric immunoassay. PLoS One 2012; 7(4):e35697
20. Chardin H, Mercier K, Frydman C, Vollmer N. Surface Plasmon Resonance imaging: a method to measure the affinity of the antibodies in allergy diagnosis. J Immunol Methods 2014;405:23-8
21. Villalta D, Conte M, Asero R, et al. Isolated IgE reactivity to native walnut vicilin-like protein (nJug r 2) on ISAC™ microarray is due to cross-reactive carbohydrate epitopes. Clin Chem Lab Med 2013;51(10):1991-5