International Archives of Allergy and Immunology

Clinical Allergy – Brief Report

Int Arch Allergy Immunol 2020;181:536–539
DOI: 10.1159/000507217

Received: February 19, 2020
Accepted: March 13, 2020
Published online: May 20, 2020

Disease-Specific Molecular Profiles Highlighted by Radar Graphic Display

Enrico Scala[a] Damiano Abeni[b] Riccardo Asero[c]

[a]Experimental Allergy Unit, IDI-IRCCS, Rome, Italy; [b]Health Services Research Unit, IDI-IRCCS, Rome, Italy; [c]Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano, Milan, Italy

Keywords

Allergic asthma · Food allergy · IgE cross-reactivity · Lipid transfer protein · Microarray · PR-10 · Profilin · Lipocalin · Serum albumin · Polcalcin

Abstract

Background: Management of hundreds of analytes obtained from the molecular multiplex techniques currently available may represent a challenge for clinicians in daily clinical practice. ***Objectives:*** The aim of the study was to describe a comprehensive and simple approach to assess such complex molecular results, to display relevant disease-specific signatures at a glance, and to facilitate their interpretation. ***Method:*** A total of 6,332 consecutive allergic patients, categorized based on clinical symptoms reported at the time of the first visit before IgE testing, were evaluated through ImmunoCAP ISAC112®. ***Results and Conclusions:*** The occurrence of bronchial asthma is associated with polcalcin, serum albumin, or lipocalin reactivity. Higher risk of severe reaction to food is linked to tropomyosin or nonspecific lipid transfer protein reactivity (in the absence of plant pathogenesis-related proteins [PR-10] or profilin sensitization). We used radar graphic display to highlight, at a glance, the molecular reactivity profiles associated with relevant disease-specific patterns.

Introduction

Food and respiratory allergic diseases exert a relevant health and economic impact in Western countries. Their inaccurate or missed diagnosis may cause, therefore, a heavy socioeconomic burden. Novel molecular multiplex techniques are commercially available, allowing for a more comprehensive evaluation of IgE sensitizations. This "precision medicine" approach may revolutionize the allergy diagnostic workup and decrease the aforementioned burden, improving physicians' daily clinical practice [1]. These complex arrays detect simultaneously hundreds of analytes, but the management of this manifold information is difficult.

We describe here a novel, more inclusive, and simple approach to assess such multifaceted molecular results, to display relevant disease-specific signatures at a glance, and to facilitate their interpretation.

Materials and Methods

This observational cross-sectional study was undertaken at the outpatient Allergy Unit of IDI-IRCCS in Rome, Italy, a National Reference Center for Allergic and Dermatological Diseases. Participants

Edited by: O. Palomares, Madrid.

karger@karger.com
www.karger.com/iaa

Karger

Prof. Enrico Scala
Experimental Allergy Unit, Istituto Dermopatico dell'Immacolata – IDI-IRCCS
Via Monti di Creta, 104
IT–00167 Rome (Italy)
e.scala@idi.it

gave their written informed consent and were recruited between 2013 and 2019 (CE: 493-1). An allergen microarray was used to test each participant's specific IgE sensitization (Immuno Solid-phase Allergen Chip – ImmunoCAP ISAC 112; Thermo Fisher Scientific, Uppsala, Sweden). Specific IgE values were expressed in ISAC standard units (ISU). Values ≥0.3 ISU were considered as positive.

Clinical Categories

Study patients were categorized based on clinical symptoms reported at the time of the first visit before IgE testing and were therefore blinded to microarray data. Clinical categorization was as follows: (a) oral allergy syndrome (OAS): symptoms unequivocally suggestive of adverse reactions to food localized to the oral mucosa and characterized by itching, burning sensation, or angioedema in the lips, mouth, and/or pharynx due to the ingestion of milk, hens' egg, fish, crustaceans, fruits, vegetables, nuts, or legumes; (b) food allergy (FA): a history of symptoms suggestive of adverse reaction to a suspected food-derived trigger, including urticaria and external angioedema, laryngeal angioedema, respiratory difficulty, and/or presyncope/syncope in the last 6 months; (c) food-dependent, exercise-induced anaphylaxis (FDEIA): a history of at least 1 episode of anaphylaxis following physical exercise performed within 4 h after a meal, diagnosed according to the clinical criteria proposed by Muraro et al. [2]; (d) respiratory symptoms: participants with symptoms of rhino-conjunctivitis (or rhinitis) (R) or bronchial asthma (BA).

All subjects gave their written informed consent, and the study protocol was approved by the institute's committee on human research (CE: 493-1).

Statistics

All data were analyzed using the SPSS/PC+ statistical package (SPSS, version 15, Chicago, IL, USA). The relationship between the categorical variables was analyzed using Pearsons' χ^2 or Fisher's exact test when indicated. Odds ratios (ORs) were computed for each disease versus all the others.

Crude OR values have been shown using a radar chart. The radar chart, also known as spider chart, is a graphical approach used to visualize simultaneously multiple observations through a 2-dimensional chart, where several quantitative variables can be shown at the same time on axes starting from the same point. In our case, each spoke represents one of the molecules studied, and the length of the spoke is proportional to the respective OR of the variable, relative to the association with the clinical symptoms studied. A line is drawn connecting each pan-allergen value (i.e., nonspecific lipid transfer protein [nsLTP], profilin, or plant pathogenesis-related proteins [PR-10]) for each spoke [3].

Results

Patients and Clinical History

We examined 6,332 consecutive patients with detectable specific IgE to one or more antigens currently spotted in the ImmunoCAP ISAC, from a total of 42,718 individuals attending the clinic during the study period.

Respiratory Symptoms

Figure 1 and Table E1 (see online suppl. Table E1; see www.karger.com/doi/10.1159/000507217 for all online suppl. material) show the crude ORs and the 95% confidence interval (CI) where significant direct (>1) or inverse (<1) associations were observed between IgE reactivity to single molecules and clinical symptoms. IgE sensitization to grass, olive tree, pellitory pollens, house dust mites, and animal dander was associated both R and BA, while cypress and chenopod pollen reactivities were positively associated with R only (Fig. 1A). Interestingly, IgE sensitization to polcalcins, PR-10, and profilins was positively (OR >1) associated with rhinitis, while IgE sensitization to tropomyosins and nsLTPs was inversely associated (OR <1) with this disease (Fig. 1B, blue line). By contrast, asthma was positively associated with IgE sensitization to polcalcins, lipocalins, and animal serum albumins, but not with profilins or with PR-10 components (Fig. 1B, orange line).

Food Allergy

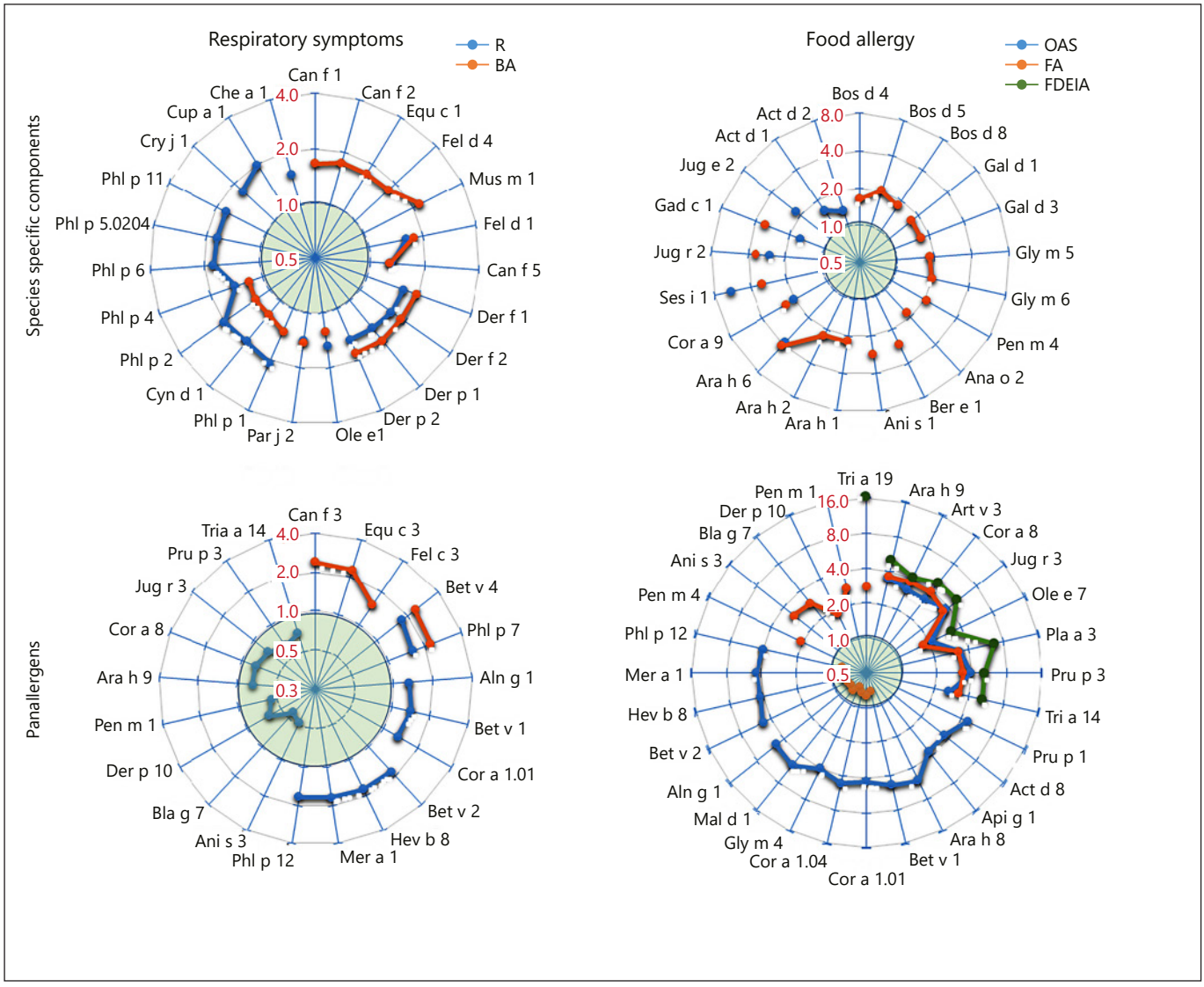
We found a strong positive association between a history of FDEIA and IgE sensitization to both Tri a 19 and nsLTP allergens (Fig. 1D, green line). IgE sensitization to all the main vegetable pan-allergens (profilin, PR-10, and nsLTP) was strongly associated with OAS in our population (Fig. 1D, blue line). On the other hand, severe reactions to food were directly associated with IgE sensitization to nsLTP and tropomyosins but were inversely associated with both PR-10s and profilins (Fig. 1D, orange line). Taken together, nsLTP reactivity was positively associated with OAS, FA, and FDEIA, whereas the presence of PR-10 and profilin was inversely associated with FA occurrence and directly linked to OAS, as presented in the radar chart (Fig. 1D). IgE sensitization to milk (Bos d 4, Bos d 5, and Bos d 8), hen's egg (Gal d 1 and Gal d 3), soybean (Gly m 5 and Gly m 6), crustacean (Pen m 4), seed storage protein (Ana o 2, Ber e 1, Ara h 1, Ara h 2, Ara h 6, Cor a 9, Ses i 1, and Jug r 1), *Anisakis simplex* (Ani s 1), and fish (Gad c 1) allergens was all significantly associated with a severe adverse reaction to foods (Fig. 1C).

Discussion

We studied a large population of ISAC-tested allergic patients to provide a real-life picture of what is actually seen in an allergy outpatient clinic in daily clinical practice.

We found that the main environmental species-specific markers of IgE sensitization (tree pollen such as those

Radar Graphic Display Disease-Specific Pattern



Color version available online

Fig. 1. Radar charts displaying the OR obtained comparing clinical symptoms versus molecular sensitization in 6,332 allergic patients. Only molecules reaching a significant association are shown. OR >1 indicates the increased occurrence of an event, OR <1 (inside the light green circle) indicates decreased occurrence of an event (protective effect). Association of species-specific components with R (blue line) or BA (orange line) (**A**). Pan-allergens directly or inversely associated with R or BA (**B**). **C** Association of species-specific components with OAS (blue line) and severe reaction to food (FA; orange line). **D** Pan-allergens directly or inversely associated with FDEIA (green line), OAS, or FA. OR, odds ratio; R, rhinitis; OAS, oral allergy syndrome; BA, bronchial asthma; FA, food allergy; FDEIA, food-dependent, exercise-induced anaphylaxis.

of Fagales, Cupressaceae and olive trees; grasses; pellitory; dust mite; and animal dander) were associated with R and that all of them but cypress pollen, chenopod, and some molecules from grasses were positively associated with BA. Polcalcin reactivity was associated with both R and BA (with higher OR values in the latter case), while lipocalin and serum albumin sensitization was selectively associated with BA.

Patients with OAS showed a concurrent IgE sensitization to all vegetable pan-allergens tested, while, as previously reported [4], nsLTP reactivity in the absence of PR-10 or profilin sensitization identified patients at risk of severe reaction to food. Such condition is commonly observed in southern Italy [5] and is a warning signal for affected individuals [6]. FDEIA has been linked to omega-5 gliadin (Tri a 19) reactivity [7, 8], but nsLTPs play a

more relevant role in the Mediterranean area [9]. Taken together, nsLTP reactivity is positively associated with OAS, FA, and FDEIA, and the presence or absence of other pan-allergen sensitizations, as displayed in the radar chart, leads to a better classification of the different patients' subsets.

Eight allergenic food sources also called the "big 8," including milk, hens' eggs, fish, crustacean shellfish, wheat, tree nuts, peanuts, and soybeans, cause about 90% of all food adverse reactions in the USA. Consequently, labelling of the big-8 is mandatory according to the US, EU, Canadian, Japanese, and Australian/New Zealand regulations, following Codex Alimentarius recommendations. Interestingly, all the "big 8" were involved as main offending foods also in our Mediterranean cohort, but other relevant foods (i.e., peach) and *Anisakis simplex* should be included in the list, at least in the EU.

In conclusion, the radar graphic display highlights, at a glance, the molecular reactivity profiles associated with relevant disease-specific patterns in ISAC-tested allergic patients. This novel population approach may be useful in guiding the application of the same graphic technique to individual patients. We provide a tentative example of this in the online suppl. Figure which shows the individual profiles of 4 patients. This novel strategy should be further evaluated to verify whether it can be useful not only in clinical-epidemiological studies but also in everyday clinical practice.

Acknowledgements

We are grateful to Dr. Silvia Cadoni, Dr. Pierpaolo Petasecca, and Mr. Mauro Helmer Citterich, who performed the in vitro tests.

Statement of Ethics

The research was conducted ethically following the World Medical Association Declaration of Helsinki. All subjects gave their written informed consent, and the study protocol was approved by the institute's committee on human research (CE: 493-1).

Disclosure Statement

Enrico Scala has received consultant arrangements and speakers' bureau participation from Stallergenes and Thermo Fisher Scientific. The rest of the authors declare that they have no relevant conflicts of interest.

Funding Sources

This study was funded, in part, by the Italian Ministry of Health, Current Research Program 2018–2020.

References

1 Ferrando M, Bagnasco D, Varricchi G, Bernardi S, Bragantini A, Passalacqua G, et al. Personalized medicine in allergy. Allergy Asthma Immunol Res. 2017;9(1):15–24.
2 Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008–25.
3 von Mayr G. Linien-Diagramme im Kreise: Line charts in circles. In: Die Gesetzmäßigkeit im Gesellschaftsleben. Munich: Oldenbourg; 1877. p. 78.
4 Scala E, Abeni D, Guerra EC, Locanto M, Pirrotta L, Meneguzzi G, et al. Cosensitization to profilin is associated with less severe reactions to foods in nsLTPs and storage proteins reactors and with less severe respiratory allergy. Allergy. 2018;73(9):1921–3.
5 Scala E, Villalta D, Uasuf CG, Pignatti P, Pirrotta L, Guerra EC, et al. An atlas of IgE sensitization patterns in different Italian areas. A multicenter, cross-sectional study. Eur Ann Allergy Clin Immunol. 2018;50(5):217–25.
6 Scala E, Till SJ, Asero R, Abeni D, Guerra EC, Pirrotta L, et al. Lipid transfer protein sensitization: reactivity profiles and clinical risk assessment in an Italian cohort. Allergy. 2015;70(8):933–43.
7 Palosuo K, Varjonen E, Nurkkala J, Kalkkinen N, Harvima R, Reunala T, et al. Transglutaminase-mediated cross-linking of a peptic fraction of omega-5 gliadin enhances IgE reactivity in wheat-dependent, exercise-induced anaphylaxis. J Allergy Clin Immunol. 2003;111(6):1386–92.
8 Matsuo H, Morita E, Tatham AS, Morimoto K, Horikawa T, Osuna H, et al. Identification of the IgE-binding epitope in omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. J Biol Chem. 2004;279(13):12135–40.
9 Romano A, Scala E, Rumi G, Gaeta F, Caruso C, Alonzi C, et al. Lipid transfer proteins: the most frequent sensitizer in Italian subjects with food-dependent exercise-induced anaphylaxis. Clin Exp Allergy. 2012;42(11):1643–53.