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ORIGINAL ARTICLE

Microarray based IgE detection in poly-sensitized allergic patients with suspected food allergy — an approach in four clinical cases

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Abstract
Background: Component-resolved diagnosis and microarray technology have been recently introduced into clinical allergy practice, and may be particularly useful in poly-sensitized allergic patients.
Methods: We compare the clinical usefulness of a microarray-based IgE detection assay (ISAC®) with skin tests and specific IgE with standard allergens (sIgE) or their monocomponents in four case reports of patients poly-sensitized to aeroallergens and food.
Results: Case 1: a woman with rhinitis, oral allergy syndrome to several fruits and anaphylaxis to cherry. Diagnostic tests supported non-specific lipid transfer proteins (nsLTPs) primary sensitization.

Case 2: a woman with exercise-induced asthma, rhino-conjunctivitis and oral allergy syndrome to fresh fruits of different families. A diagnosis of primary grass and weed pollen allergy with profilin and pathogenesis-related protein family 10 (PR-10) cross-reactive food allergy was proposed.

Case 3: a man with atopic eczema, asthma, rhinitis, and multiple anaphylactic episodes with cashew nuts and oral allergy syndrome to fruits. The diagnostic workup supported a primary birch pollen allergy with PR-10 and nsLTPs cross-reactive food allergy.

Case 4: a woman with rhino-conjunctivitis, per-operative anaphylaxis due to latex and recent pharyngeal angio-oedema episodes. The diagnosis was a primary grass and weed pollen allergy with equivocal profilin sensitization and no obvious cross-reactivity mediated by nsLTPs sensitization.
Conclusions: The possibility to carry out multiple sIgE measurements with single protein allergens, in particular with the microarray technique, is a useful, simple and non-invasive diagnostic tool in complex poly-sensitized allergic patients.

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Introduction

Component-resolved diagnosis using microarray technology has been recently introduced into clinical allergy practice. The diagnosis of IgE-mediated allergy is based on skin prick tests and supported by standard specific IgE *in vitro* tests. These traditional diagnostic tools use natural allergen extracts, which contain a mixture of allergenic and non-allergenic molecules that are difficult to standardize, mainly defining the source but not uncovering which allergenic molecule(s) elicited the sensitization.

Specific IgE quantitative measurements for allergen monocomponents are nowadays available. This component-resolved diagnosis is based on natural or recombinant allergens with structural and immunobiological properties comparable with their natural sources, establishing a detailed IgE reactivity profile for each patient.[1] With a microarray technique, the sensitivity to multiple allergenic components (purified, recombinant or natural allergens) is evaluated in the same serum sample.[2]

Allergy diagnosis in adult and paediatric poly-sensitized patients needs to be improved in order to clarify the nature and cause of allergic reactions and to promote effective measures of disease management.[2,3] Food allergy in aeroallergen sensitized patients is one example where the most relevant clinical question is the identification of patients with a high risk for systemic reactions. With traditional methods it is sometimes practically impossible to resolve such diagnostic enigmas.

We report four cases of patients poly-sensitized to aeroallergens and suspected food allergy. Our aim was to compare the clinical usefulness of a microarray-based IgE detection assay (ISAC® – Phadia, Sweden) with established methods of allergen-specific IgE detection – skin tests and specific IgE with standard allergens or their monocomponents.

Methods

Patients

Four adult patients with sensitization to multiple aeroallergens and suspected food allergy (three female; ages of 31, 23, 29, and 51 years), followed up at a clinical allergy setting were included.

A clear-cut history of oral allergy syndrome (OAS) (defined as immediate itching of the oral mucosa and lips), of urticaria (with or without angio-oedema) and/or of severe gastrointestinal/respiratory or cardiovascular symptoms following the ingestion of food was considered compatible with suspected food allergy.[4]

Skin tests

All study patients underwent skin prick tests (SPT) with commercial extracts of mites, dander, pollens and moulds included in our Immunoallergology Department aeroallergens standard battery (ALK-Abelló, Denmark): *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*, *Thyrophagus putrescentiae*, *Acarus siro*, *Glycyphagus domesticus*, *Felis domesticus* (cat), *Canis familiaris* (dog), *Platanus acerifolia* (London plane tree), *Betula verrucosa* (birch), *Olea europaea* (olive), *Secale cereale* (cultivated rye), *Triticum aestivum* (cultivated wheat), grass mixture – *Dactylis glomerata* (cocksfoot), *Festuca elatior* (meadow fescue), *Lolium perenne* (rye grass), *Phleum pratense* (timothy) *and Poa pratensis* (meadow grass), weed mixture – *Artemisia vulgaris* (mugwort), *Chenopodium album* (goosefoot), *Parietaria judaica* (wall pellitory), *Plantago lanceolata* (ribwort) and *Taraxacum vulgare* (dandelion), *Parietaria judaica*, *Plantago lanceolata*, *Artemisia vulgaris*, *Alternaria tenuis*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Penicillium notatum*.

Skin prick tests with commercial extracts of purified natural date palm profilin (ALK-Abelló, Denmark) and peach, containing uniquely lipid transfer protein (Pru p 3, 30 mg/mL; ALK-Abelló, Denmark), were performed when relevant.

Furthermore, in patients reporting specific food reactions, in order to confirm sensitization, SPT were performed with commercial extracts (ALK-Abelló, Denmark), and/or with fresh foods, using the skin prick-prick technique (SPPT), which included chick pea, nut, hazelnut, peanut, almond, tomato, spinach, pea, kiwi fruit, apple, strawberry, peach, orange, banana, cacao, soy, oat, corn, rye, cow milk, egg yolk, egg white, chicken, pork, rabbit, sheep, cod, tuna, white fish, blue fish, sardine, hake, shrimp, lobster, oyster, anisakis and garlic (Table 1).

All SPT and SPPT were performed using disposable 1 mm tip lancets (Leti, Spain). Readings were taken at 20 minutes and a mean wheal diameter of 3 mm or more compared with the negative control was considered positive.[5] Skin prick test with histamine (10 mg/mL) and saline solution were carried out as positive and negative control, respectively.

Food challenges

Open oral food challenges were performed when pertinent, according to the position paper from the European Academy of Allergology and Clinical Immunology.[6]

Specific IgE

According to aeroallergens and food positive skin test results, specific IgE was measured both to standard allergens (sIgE) and to recombinant or native single allergen components (sIgE comp) using the ImmunoCAP® (Phadia, Sweden) test. IgE values of 0.1 kUA/L or greater were considered as positive (subject sensitized).

A multiple allergen component analysis was also performed for each patient by microarray technique (ISAC® – Phadia, Sweden). Briefly, this test is a solid phase multiple immunoassay in which the proteins (purified recombinant or natural allergens) are immobilized. The antibodies present in the serum are captured by the different allergens, and detected by means of a second fluorescent-labelled anti-IgE antibody. The results are analyzed on a semiquantitative basis. Each of the 103 allergens is bound to the solid phase in triplicate, to ensure reproducibility of the test. The IgE values are presented in arbitrary units called ISAC Standardized Units (from 0.3 to 100 ISU).[2]

Table 1 Results of positive skin prick tests, skin prick-to-prick tests and specific IgE to standard allergens or single allergen components (kUA/L).

Patient	SPT	SPPT	sIgE	sIgE comp
1	London plane tree	Nut	London plane tree = 3.21	Pru p 3 = 35.20
	Mugwort	Hazelnut	Mugwort = 0.84	Cor a 8 = 14.00
	LTP	Peach	Peach = 21.50	Pru p 4 < 0.10
	Nut	Chestnut	Peanut = 4.31	Pru p 1 < 0.10
	Hazelnut	Wine	Nut = 9.52	Ara h 1 < 0.10
	Peanut	Grape	Grape = 1.09	
	Almond	Cooked pea	Pea = 2.84	
	Tomato		Tomato = 4.84	
	Kiwi		Soy = 1.31	
	Peach			
	Banana			
	Soy			
	Oat			
	Corn			
	Garlic			
2	*Dp*	Apple	Dog epithelium = 2.05	rBet v 2 = 9.15
	Df	Pear	Tree mixture (tx7) = 6.75	rPhl p 12 and 7 = 7.07
	Dog	Cherry	Grass mixture (gx1) = 71.00	rHev b 8 = 9.37
	Birch	Plum	Grass mixture (gx2) = 40.20	rBet v 1 = 14.70
	London plane tree	Orange	Apple = 1.55	rHev b 6.02 < 0.10
	Olive	Mandarin	Orange = 2.66	
	Cultivated rye	Grape	Grape = 1.29	
	Cultivated wheat	Kiwi	Kiwi = 2.45	
	Grass mixture	Banana	Banana = 1.84	
	Weed mixture			
	Mugwort			
	Wall pellitory			
	Ribwort			
	Alternaria tenuis			
	Aspergillus fumigatus			
	Profilin			
3	*Dp*	*Dp* > 100.00	Cow milk = 1.14	rBet v 1 = 92.80
	Df	*Df* > 100.00	Egg white = 0.42	Pru p 1 = 72.20
	Ld	*Ld* = 2.75	Shrimp = 0.81	Ara h 8 = 56.90
	Dog	Dog epithelium = 85.30	Fish mixture (fx2) = 0.42	rBet v 2 = 0.43
	London plane tree	London plane tree = 34.50	Cereals mixture (fx3) = 8.43	Ara h 2 = 0.28
	Cultivated rye	Birch = 91.20	Nuts mixture (fx1) = 46.60	Pen a 1 = 0.34
	Cultivated wheat	Olive = 4.22	Peanut = 7.13	
		Cultivated rye = 57.20	Nut = 20.00	
		Cultivated wheat = 48.00	Cashew nut = 1.23	
		Grass mixture = 87.10	Peach = 46.10	
		Cocksfoot = 97.00	Apple = 23.40	
		Timothy = 62.20		
		Wall pellitory = 4.48		
		Latex = 1.37		
4	*Dp*	*Dp* = 5.80	rPhl p 5b = 4.88	
	Grass mixture	*Df* = 4.40	rPhl p 12 and 7 = 0.22	
	Latex	Cocksfoot = 40.10	Hev b 8 = 0.25	
		Timothy = 39.40	Hev b 1 < 0.10	
		Grass mixture (gx1) = 38.10	Hev b 5 < 0.10	
		Latex = 2.42	Hev b 3 < 0.10	

SPT – skin prick tests; SPPT – skin prick-to-prick tests; sIgE – specific IgE; sIgE comp – specific IgE to monocomponents; *Dp – Dermatophagoides pteronyssinus; Df – Dermatophagoides farinae*; *Ld – Lepidoglyphus destructor*; *Tp – Thyrophagus putrescentiae*; tx7 – *Olea europaea*, *Salix caprea*, *Pinus strobus*, *Eucalyptus spp.*, *Acacia longifolia* and *Melaleuca leucadendron*; gx1 – *Dactylis glomerata*, *Festuca elatior*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis*; gx2 – *Cynodon dactylon*, *Lolium perenne*, *Phleum pretense*, *Poa pratensis*, *Sorghum halepense* and *Paspalum notatum*; fx2 – tuna, cod, shrimp, salmon, blue mussel; fx3 – oat, corn, wheat, buckwheat, sesame seed; fx1 – coconut, almond, peanut, hazelnut, brazil nut.

Cases

Case 1 – a 31-year-old Caucasian woman, dentist, referring intermittent rhinitis symptoms, an OAS to peach, and an episode of documented anaphylaxis to cherry, twenty years ago. Since 2007 she started to have OAS to peanuts, nuts, grapes, red wine and quince jam. The SPT and/or sIgE to aeroallergens revealed only sensitization to plane tree, and mugwort pollens. The SPPT and/or sIgE to relevant foods were positive to peach, peanut, nut, and grape. The profilin and the non-specific lipid transfer proteins (nsLTP) SPT, were negative and positive, respectively (Table 1). An open oral food challenge with nuts was positive (development of OAS after contact with oral mucosa). The first diagnostic hypothesis was primary sensitization to nsLTP within plant food cross-reactivity. According to anamnesis and ST results, the sIgE comp performed were positive to Pru p 3 and Cor a 8 (nsLTPs) and negative to Pru p 4 (profilin), Pru p 1 (pathogenesis-related protein family 10 or PR-10 protein) and Ara h 1 (storage protein) (Table 1).

Case 2 – a 23-year-old Caucasian woman, student, referred by OAS to fresh fruits of different families (apple, pear, cherry, orange, mandarin, banana, kiwi fruit, grape, pineapple, and chestnut) for a year. A moderate/severe persistent rhino-conjunctivitis and exercise induced-asthma were diagnosed at the time of patient's appointment. The SPT and sIgE to aeroallergens revealed sensitization to mites, dog, tree, grass and weed pollens, and moulds. The SPPT and/or sIgE to tested fruits were all positive (apple, pear, cherry, plum, orange, mandarin, banana, kiwi fruit, and grape). SPT to profilin and a nsLTP were positive and negative, respectively (Table 1). Considering a OAS with fruits with cross-reactivity to profilins or PR-10 proteins, the sIgE comp performed were positive to Bet v 2, Phl p 12 and Hev b 8 (profilins) and Bet v 1 (PR-10 proteins) and negative to Hev b 6.02 (Table 1).

Case 3 – a 29-year-old Danish man, artist, living in Porto (Portugal) since the age of 20, referred for severe atopic eczema, asthma, rhinitis and several anaphylactic episodes with cashew nuts and OAS to peanut, hazelnut, peach and apple. The SPT and sIgE to aeroallergens revealed sensitization to mites, dog, tree and grass pollens. The sIgE to fruits commercial extracts were positive to peanut, nut, cashew nut, peach, and apple (Table 1). Considering the hypothesis of a natural birch pollen allergy (exceptional in our geographical location, but common in Denmark) and cross-reactivity to PR-10 proteins, profilins and/or storage proteins, sIgE comp were performed to Bet v 1, Pru p 1, Ara h 8 (all PR-10 proteins), Bet v 2 (profilin) and Ara h 2 (2S-albumin), with positive results to PR-10 proteins (Table 1).

Case 4 – a 51-year-old Caucasian woman, secretary, followed for 15 years due to moderate/severe intermittent allergic rhino-conjunctivitis to mites and grass pollens and who completed subcutaneous immunotherapy to grass pollens two years ago, with significant improvement. Six years ago she had a per-operative anaphylaxis (angio-oedema and generalized urticaria during gynaecological surgery) where latex was incriminated (SPT and sIgE positive). Later, she was submitted to further surgery in a latex-free environment, without reported reactions. In 2008 she returned to a medical appointment referring recent acute episodes of pharyngeal angio-oedema without identified trigger factors. The suspected clinical diagnosis was food allergy to an unknown cross-reactive allergen in a pollen and latex sensitized patient. However, cross-reactivity to profilins was barely detectable with residual sensitization to Phl p 12 and Hev b 8 (latex profilin) (Table 1).

Microarray based IgE detection

Table 2 summarizes the IgE results by microarray technique (ISAC® test). In Case 1, sensitization to nsLTPs of *Rosaceae* (Pru p 3), *Betullaceae* (Cor a 8) and *Asteraceae* family (Art v 3) was confirmed. In Case 2, the results showed species specific sensitization to grass pollens and mites and, additionally, to profilins and PR-10 related plant food sensitization involving peanut, hazelnut, apple, and peach. In Case 3, the results confirmed species specific sensitization to mites, cat and dog dander, and timothy pollen. Birch pollen allergy with cross-reactivity to PR-10 proteins of fruits (kiwi, apple, peach) and nuts (peanut and hazelnut) and sensitization to nsLTP from *Betullaceae* (Cor a 8) and *Rosaceae* families (Pru p 3) were also detected. In Case 4, a species specific sensitization to mites and grass pollens was confirmed, additionally detecting weed pollen sensitization to wall pellitory and mugwort mediated by nsLTPs.

Discussion

In the diagnosis of suspected food allergy, skin tests with conventional extracts have low specificity and low allergenic potency.[5,7] The use of fresh food is an alternative, but with additional reproducibility problems,[8] and increased risk of adverse reactions, including anaphylaxis, especially in young children.[9,10] Food challenges represent the only way to establish or rule out an adverse reaction to a food. However, these challenge procedures are not fully developed and no standardized procedures have so far been agreed upon.[11] Additionally, all these methods should be performed in a safe environment and only by physicians with proper training in the allergy field. Of all the *in vitro* methods, specific IgE determination with whole extracts also presents low specificity.[2,7] Natural or recombinant purified allergens have proven useful, by overcoming the mentioned limitations.[1,12]

ISAC® test has other advantages considering it is a single safe test and available even for allergists not working in a hospital environment, facilitating the diagnosis in children and in patients at risk of systemic symptoms.[3,12] The smaller amount of serum needed also facilitates the use of the technique in paediatric patients. Moreover, the accuracy of ISAC® has been validated in terms of correlation with the routine standard tests and similar potency in terms of mass units of allergen.[13,14] As a limitation, the current number of allergenic components tested does not cover all the relevant allergens. The cost involved in the patients' investigation, in particular performing the ISAC® test can be another putative limitation. However, this information has not been addressed in the available literature, and it would be interesting to make a cost comparative analysis between the performed standard tests (combination of the SPT, SPPT, sIgE and sIgE comp) and the ISAC® test.

Table 2 Results of specific IgE detection by microarray technique (ISAC® test). ISU – ISAC standardised units: <0.3 – negative; 0.3–0.9 – low; 1–14.9 – moderate; ≥15 – high.

Case	Allergenic source specific	Allergen	Protein	ISU
1.	Peach	Pru p 3	LTP	16.0
	Hazelnut	Cor a 8	LTP	4.9
	Artemisia vulgaris/Mugwort	Art v 3	LTP	2.4
2.	*Betula verrucosa*/Birch	Bet v 2	Profilin	15.0
	Olea europaea/Olive	Ole e 2	Profilin	14.0
	Mercurialis annua/Annual mercury	Mer a 1	Profilin	8.3
	Phleum pratense/Timothy	Phl p 12	Profilin	5.8
	Latex	Hev b 8	Profilin	11.0
	Betula verrucosa/Birch	Bet v 1	PR-10 protein	15.0
	Alnus glutinosa/Black alder	Aln g 1	PR-10 protein	4.0
	Hazelnut pollen	Cor a 1.0101	PR-10 protein	3.3
	Hazelnut	Cor a 1.0401	PR-10 protein	4.9
	Peach	Pru p 1	PR-10 protein	2.4
	Apple	Mal d 1	PR-10 protein	1.5
	Peanut	Ara h 8	PR-10 protein	0.5
	Cynodon dactylon/Bermuda grass	Cyn d 1	Grass group 1	11.0
	Phleum pratense/Timothy	Phl p 1	Grass group 1	23.0
		Phl p 5	Grass group 5	13.0
		Phl p 2	Grass group 1	8.8
		Phl p 11	Ole e1-related protein	6.3
		Phl p 4	Berberine bridge enzyme	4.0
		Phl p 6	Grass group 6	2.0
	Ambrosia artemisiifolia/Common ragweed	Amb a 1	Pectate lyase	9.4
	Olea europaea/Olive	Ole e 1	Olive group 1	1.6
	House dust mite	Der f 2	NPC2 family	17.0
	House dust mite	Der p 2	NPC2 family	16.0
	House dust mite	Eur m 2	NPC2 family	1.2
	Dog epidermal	Can f 1	Lipocalin	1.9
	Horse protein	Equ c 3	Serum albumin	0.7
3.	*Betula verrucosa*/Birch	Bet v 1	PR-10 protein	67.0
	Peach	Pru p 1	PR-10 protein	40.0
	Peanut	Ara h 8	PR-10 protein	23.0
	Apple	Mal d 1	PR-10 protein	18.0
	Alnus glutinosa/Black alder	Aln g 1	PR-10 protein	14.0
	Hazelnut	Cor a 1.0401	PR-10 protein	12.0
	Hazelnut pollen	Cor a 1.0101	PR-10 protein	10.0
	Kiwi	Act d 8	PR-10 protein	8.6
	Apium graveolens/Celery	Api g 1	PR-10 protein	8.1
	Soy	Gly m 4	PR-10 protein	1.0
	Peach	Pru p 3	LTP	8.2
	Artemisia vulgaris/Mugwort	Art v 3	LTP	4.4
	Hazelnut	Cor a 8	LTP	3.1
	Phleum pratense/Timothy	Phl p 11	Ole e1 – related protein	36.0
		Phl p 5	Grass group 5	35.0
		Phl p 4	Berberine bridge enzyme	20.0
		Phl p 6	Grass group 6	4.2
	House dust mite	Der p 2	NPC2 family	70.0
		Der f 2	NPC2 family	69.0
		Eur m 2	NPC2 family	33.0
		Der p 1	Cysteine protease	68.0
		Der f 1	Cysteine protease	67.0
	Kiwi	Act d 1	Cysteine protease	0.8
	Dog epidermal	Can f 1	Lipocalin	69.0
		Can f 2	Lipocalin	53.0
	Cat epidermal	Fel d 1	Uteroglobin	57.0

Table 2 (*Continued*)

Case	Allergenic source specific	Allergen	Protein	ISU
	Platanus acerifolia/London planetree	Pla a 2	Polygalacturonase	1.3
		Pla a 1	Putative invertase inhibitor	0.7
	Cupressus arizonica/Cypress	Cup a 1	Pectacte lyase	1.3
	Cupressus japonica/Japanese cedar	Cry j 1	Pectacte lyase	0.9
	Olea europaea/Olive	Ole e 1	Olive group 1	0.5
	Milk	Bos d 8	Caseins	0.5
4.	*Phleum pratense*/Timothy	Phl p 1	Grass group 1	6.7
		Phl p 5	Grass group 5	4.2
		Phl p 11	Ole e1 – related protein	2.8
		Phl p 2	Grass group 1	2.4
		Phl p 4	Berberine bridge enzyme	1.8
	Cynodon dactylon/Bermuda grass	Cyn d 1	Grass group 1	1.9
	Parietaria judaica/Wall pellitory	Par j 2	LTP	15.0
	Artemisia	Art v 3	LTP	1.1
	vulgaris/Mugwort	Art v 1	Defensin	4.7
	House dust mite	Der p 2	NPC2 family	15.0
		Der f 2	NPC2 family	13.0
		Eur m 2	NPC2 family	3.3
	Cat epidermal	Fel d 1	Uteroglobin	7.8
	Aspergillus fumigatus	Asp f 3	Peroxysomal protein	0.3

We established a probable association between pollen allergy and food sensitization in all patients. The production of specific IgE directed against common cross-reactive structures (panallergens) shared by pollen and plant-derived food is the most widely supported explanation.[15] Pollen seems to be the primary source of sensitization. The IgE cross-reactivity might be clinically manifested or be irrelevant. The overall sequence identity of surface structures is a major determinant of cross-reactivity and a sequence identity of 35% has been suggested as a cut-off for potential cross-reactivity.[16]

Concerning plant food allergens, the most established and studied protein families with allergenic activity include the non-specific lipid transfer proteins (nsLTPs), the pathogenesis-related protein family 10 (PR-10 proteins), the profilins, the storage proteins (2S albumins, 7S/11S globulins), and the cross-reactive carbohydrate determinants (CCDs).

The first patient had symptoms including anaphylaxis to fruits of the *Rosaceae* family (peach and cherry) and rhinitis symptoms attributed to plane tree and mugwort pollen sensitization. Hypersensitivity to peach is usually not associated with any kind of particular pollinosis in southern Europe,[17] and the main reactions in the Mediterranean area are directed to nsLTPs.Peach nsLTP (Pru p 3) is a major allergen in the South of Europe, being involved in over 60% of patients allergic to peach in the Spanish population.[18] In peach-allergic patients who have experienced systemic reactions, up to 100% were sensitised to nsLPTs.[19] nsLTP is found in amounts approximately seven times greater in peach peel than in pulp.[20] So far, cross-reactivity between nsLTPs from pollen and plant food in Mediterranean patients has only been shown for Art v 3 from mugwort and Pru p 3 from peach,[21] as seen in the case of our patient. The primary nsLTPs sensitization diagnostic hypothesis was confirmed by sIgE comp and ISAC® test.

NsLTPs are small molecules of approximately 9–10 kDa that facilitate the transport of phospholipids and galactolipids across membranes. As panallergens, they have a ubiquitous distribution in tissues of many plant species, resulting in particularly relevant cross-reactivity between fruits and vegetables, including apple, cherry, sweet chestnut, cabbage (with 50% identity to peach nsLTP), walnut, lettuce, and hazelnut.[22] Grape and wine, which also induced OAS in our patient, may contain nsLTP homologous and cross-reactive with peach nsLTP.[23] They demonstrate great stability and are very resistant to pepsin and heat treatment. Allergic symptoms involving nsLTPs are more likely to be systemic and severe, in addition to causing OAS.

Our second patient was sensitized to a wide variety of pollens with clinical asthma and rhino-conjunctivitis. The fact of tolerating cooked and processed fruit; the association with local symptoms such as OAS; and the positive SPT to profilin, were clearly suggestive of sensitization to profilins and/or PR-10 proteins. The sIgE comp were sufficient to confirm this diagnostic hypothesis, not implying additional investigation as oral food challenges, and the ISAC® test results were in accordance with the specific IgE quantification.

Profilins are small (12–15 kDa) proteins acting as actin binding proteins and may play a key role in regulating intracellular transport processes and cell morphogenesis and division. Profilins are minor allergens, highly cross reactive even between distantly related species, including latex, increasing the risk of multiple pollen food sensitivities, not always clinically relevant.[22] Symptoms to citrus fruits, melon, banana and/or tomato have been described as clinical markers of profilin sensitization. Its prevalence in pollen allergic patients in central and southern Europe has been

estimated to be 10–35%, but seems to be rarer in northern Europe.[24] This rate increases to 55% in patient populations with multi-pollen sensitization where grass or weed pollen sensitization is dominating[25] (e.g. our case). When subjected to heat treatment, irradiation or ultrahigh pressure, IgE binding activity was not generally affected. The symptoms ranged from mild to severe, primarily giving rise to OAS as the main clinical manifestation of food allergy.

The first allergen identified from the PR-10 proteins family group was the major birch pollen (*Betula verrucosa*) allergen, Bet v 1. In our region, where birch trees are uncommon or absent, a positive test to birch pollen often reflects sensitization to Bet v 1 homologues (PR-10) in other trees closely related to birch or sensitization to other pollen allergens such as profilins (Bet v 2 homologues) in grass and weed. The food reactions seen in birch pollen allergic patients are primarily explained by IgE antibodies to Bet v 1, induced by birch pollen. Fifty to 90% of birch pollen allergic patients have been reported to have some pollen-related food allergy.[26] PR-10 proteins are primarily localized on the pulp of the fruit. The symptoms are usually mild and restricted to the oral cavity, summed up as the OAS. In general, the PR-10 proteins are labile to pH changes and of intermediate resistance towards heat treatment.

Our third patient was a Danish man sensitized to birch pollen with plant food allergy, reporting anaphylactic episodes with cashew nuts and OAS to peanut, hazelnut, peach and apple. The hypothesis of a primary sensitization to birch pollen in Denmark (rarely seen in north Portugal), with allergy and cross-reactivity to PR-10 proteins and profilins was possible, as discussed above. Cross-reactivity between storage proteins - albumins 2S, involving nuts could not be excluded, considering that they are the dominating allergens and an important risk marker for severe systemic reactions. However, there is no obvious relation between sensitization to pollens and to storage proteins. The performed sIgE comp confirmed only sensitization to PR-10 proteins. The ISAC® test provided further information of sensitization to nsLTPs from *Betullaceae* (Cor a 8) and *Rosaceae* (Pru p 3), risk markers for severe reactions. Storage protein sensitization was excluded by the negative IgE comp to Ara h 2 and storage proteins sensitization was not detected in the ISAC® test (which explores brazil nut, cashew nut, hazelnut, nut, and peanut albumins).

Patient number four was sensitized to grass pollens and successfully finished pollen-specific immunotherapy two years ago, raising the hypothesis of plant food allergy mediated by a non-identified panallergen. The history of latex sensitiz ation led to a suspicion of fruit allergy in the context of a latex-fruits syndrome. Between 30% and 50% of individuals who are allergic to latex products are also allergic to specific plant foods.[27,28] Nevertheless, we could not prove nor exclude latex allergy or cross-reactivity between latex and fruits at the present evaluation. It is possible that the time elapsed between the reaction to latex and this study was too long (eight years), decreasing the probability of a positive result, especially in the view of latex avoidance, namely during the second surgery. Sources of latex can be of varying quality and difficult to standardise and the ISAC® test does not assess all latex allergens. In fact, latex allergy is complex and involving around 13 allergens, with most patients being sensitized to more than one allergen.[29] The use of a combination of recombinant latex allergens, Hev b 5, 6 and 7, can diagnose latex allergy with 93% sensitivity and 100% of specificity.[30] In our patient we only confirmed a low sensitization to Hev b8, which is a profilin. The observed frequencies of Hev b 8-specific IgE antibodies in sera of latex-allergic patients in different risk groups range between 6% and 24%,[31,32] but are not associated with clinical reactions. In spite of profilin sensitization being a common finding in at least 50% of South European pollen sensitive patients,[24,25] it was equivocal in our patient (low levels of sIgE comp and a negative result in the microarray assay).

Considering the high frequency of *Parietaria* pollinosis in the Mediterranean area, this sensitization has been described, although rarely, only in association with pistachio nut sensitization.[33,34] In contrast, *Artemisia* sensitization has been implicated in several pollen-food syndromes in variable frequencies, to different food families: celery, carrot, parsley and caraway, fennel and coriander seeds (*Apiaceae*), paprika (*Cruciferaceae*), pepper (*Piperaceae*), mango (*Anacardiaceae*), garlic, onion and leek (Liliaceae), mustard, broccoli and cabbage (*Cruciferae*), peanut (*Leguminosae*), almond and peach (*Rosaceae*) and chamomile infusion (*Asteraceae*).[21] However, Art v 3 can be positive without sensitization to other nsLTPs[35] and there is a low probability of cross-reactivity between Par j 2 and other nsLTPs.[36]

This patient is a probable candidate for an oral provocation with nsLTP cross-reacting foods. Nevertheless, we postponed such procedure, considering she had only plant sensitization on the ISAC test and had no further allergy episodes in the last 12 months of follow-up, coincident with an improved treatment plan (oral anti-H1 and topical steroids), maintained through the pollen season.

Conclusions

In these four clinical cases of poly-sensitized patients with suspected food allergy, the applied *in vivo* and *in vitro* allergy diagnosis approach, complemented by a multiple allergen component analysis, by microarray technique, allowed the identification of sensitization patterns that correlated with the clinical anamnesis (the presence or absence of symptoms and their severity) and conducted to the final diagnosis of:

Case 1 – non-specific Lipid Transfer Proteins (nsLTPs) primary sensitization and food allergy (OAS and anaphylaxis).
Case 2 – primary grass and weed pollen allergy with profilin and Pathogenesis-Related protein family 10 (PR-10) cross-reactive food allergy (OAS).
Case 3 – primary birch pollen allergy with PR-10 and nsLTPs cross-reactive food allergy (OAS and anaphylaxis). Current storage-protein sensitization was excluded.
Case 4 – primary grass and weed pollen allergy with equivocal profilin sensitization and no obvious cross-reactivity mediated by nsLTPs. Current latex and cross-reactive food sensitization was not confirmed.

In conclusion, the possibility to carry out sIgE measurements with single protein allergens, in particular with the

microarray technique, is a useful, simple and non-invasive diagnostic tool in complex poly-sensitized allergic patients.

Conflict of interest

The authors have no conflict of interest to declare.

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