

LETTER TO THE EDITOR

ORAL DESENSITISATION WITH FOOD IS FOOD-SPECIFIC AND PROTEIN-SPECIFIC

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The avoidance of food(s) is the main therapeutic approach to food allergy. Nevertheless, orally- or sublingually-administered food allergens have gained attention and a number of food-allergic children can tolerate gradually increasing amounts of cow's milk and hen's egg. Our purpose is to show that oral desensitisation with food is an allergen-specific therapeutic approach and for this, we describe 4 illustrative children with IgE-mediated food allergy. The first was allergic to cow's milk and hen's egg, the second to cow's milk, hen's egg and fish. Both underwent oral desensitisation to both cow's milk and hen's egg. The third child was allergic to cow's milk, hen's egg and fish and underwent oral desensitisation with cow's milk. The last child was allergic to raw but not to cooked/boiled hen's egg and underwent the oral desensitisation with hen's egg. The first 2 children reached the clinical tolerance to cow's milk after the cow's milk oral desensitisation, but reached the hen's egg tolerance only after the hen's egg oral desensitisation. Moreover, the second child did not tolerate fish after being desensitised to both cow's milk and hen's egg. The third child tolerated cow's milk, but not hen's egg and fish, at the end of the cow's milk oral desensitisation. The fourth child could tolerate the previously not tolerated raw hen's egg after the oral desensitisation with raw hen's egg. In conclusion, we indicate that oral desensitisation with food is allergen specific. The induction of the clinical tolerance to one food is not followed by the tolerance to the other food(s) that the patient is allergic to. To obtain a double or multiple food tolerance, separate desensitisation protocols, one for each food, have to be carried out. Oral desensitisation with food discriminates between raw and cooked proteins.

Adverse reactions to food due to Immunoglobulin E (IgE) reactivity can range from mild to generalized anaphylactic reactions (1). Although the achievable approaches are the subject of current debate (2), the avoidance of the offending food(s) continues to be the main suggested therapeutic approach, together with prescription of self-injectable epinephrine (3). Due to the likelihood of an unintentional food exposure, the possibility that epinephrine may not be available or not properly administered (1), and due to a significant reduction in health-related quality of life, particularly when potentially life-threatening

reactions exist (4) oral desensitisation (OD) with food allergens has recently gained increasing attention. As a consequence, a substantial number of food allergic patients (especially children) can tolerate gradually increasing amounts of several allergenic foods including cow's milk (CM), hen's egg (HE), peanut and hazelnut (5-10).

One of the most important topics is to define whether OD with a specific food is an allergen-specific therapeutic approach, or whether OD with one food can influence the reactivity to other food allergens. Indeed, the specificity of OD with foods is

Key words: cow's milk allergy, hen's egg allergy, food allergy, oral desensitisation, therapy for food allergy

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0394-6320 (2011)

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generally assumed, but until now not demonstrated.

In this report we describe the responses of 3 children with IgE-mediated food allergy to two or more foods (patients 1, 2 and 3) and 1 child with IgE-mediated food allergy to raw but not to cooked or boiled HE (patient 4) (Table I) in order to evaluate the allergen specificity of OD. We show that in every patient the induction of tolerance to one food did not automatically lead to the tolerance to the another food. Indeed, to obtain the double tolerance, we had to perform two separate desensitisation protocols, one for each food.

MATERIALS AND METHODS

A detailed clinical history was obtained. Patients 1, 2 and 3 had multiple food allergies; in particular, patient 1 was allergic to CM and HE, patients 2 and 3 to CM, HE and fish. Patient 4 was allergic to raw but not to cooked or boiled HE (Table I).

Skin prick tests

Skin prick tests (SPTs) with commercial common inhalant allergens, with commercial food allergens (casein, α -lactalbumin, -lactoglobulin, egg white, yolk, wheat, cod, peanut, soy) (Lofarma, Milano, Italy), directly with the food (raw whole CM, raw and boiled HE-egg white, raw and boiled HE-yolk) and with the casein hydrolysate used to mask foods in the food challenges were performed as previously described (6) and were read at 15 min. The mean of orthogonal wheal diameters measuring at least 3 mm more than the negative control were considered positive.

Double blind placebo-controlled food challenges

Double blind placebo-controlled food challenges (DBPCFCs) were performed in hospital as proposed by Niggemann et al. (11), with some modifications. In the case of CM, the verum meal consisted of 125 ml of raw whole CM + 125 ml of casein hydrolysate + 1 g of banana flavouring (total 250 ml). In the case of HE, the verum meal consisted of 25 ml of raw HE (egg white and yolk) + 100 ml of casein hydrolysate + 1 g of banana flavouring (total 125 ml). The placebo meals consisted only of hydrolysate + 1 g of banana flavouring.

The children were in the fasting state and, in accordance with a random, computerized schedule, were given the verum or the placebo as previously described (6). When symptoms occurred, the test was considered terminated. After 4 hours' observation following the last administration of the CM or HE (or the placebo) the children were discharged.

CM and HE oral desensitisation protocols

Patients 1, 2 and 3 had a history of IgE-mediated CMA, confirmed by positive DBPCFC, and were admitted to the CM-OD. The schedule of the CM-OD was the same as that previously used (6).

Patients 1, 2 and 4 had a history of IgE-mediated HEA, confirmed by positive DBPCFC, and were admitted to the HE-OD. The schedule of the HE-OD consisted of administering increasing amounts of HE starting from one drop of raw HE (egg-white and yolk) with water in the proportion of 1:100, providing approximately 0.06 mg of HE proteins. HE doses were doubled every 8 days until day 80. Subsequently, the HE doses were doubled every 18 days in order to achieve a total daily intake of 25 ml in about 6 months. Dose doubling was carried out gradually by increasing the amount of HE from one dose to the next.

When allergic symptoms or illness intervened during the OD, the daily dose was not increased and instead, the previous dose was repeated. During the 6-month OD, the children were given 0.25 mg/kg/day of cetirizine per os. This drug was suspended 2 weeks after the end of the OD (while children continued to assume CM or HE), when the child reached 200ml/day of CM or 25ml/day (about 3 eggs a week) of HE or the maximum tolerated dose.

Blood samples

Peripheral venous blood samples were collected at the beginning and at the end of the OD protocol and were stored at -20°C.

Fluorescence enzyme immunoassays

Determination of specific IgE antibodies against CM, HE and other common inhalant and food allergens was performed with a fluorescence enzyme immunoassay (ImmunoCAP®) in accordance with the product manual with a lower detection limit of 0.35 kU/l and a maximum limit of 100 kU/l. When necessary a serum dilution 1:10 was performed in order to dose higher specific IgE concentrations.

RESULTS

Case report 1

Patient 1 is a girl, 10 years 10 months old. She was exclusively breast fed up to the age of 6 months and promptly developed diffuse erythema, angioedema of the face, upper arms and trunk and acute dyspnoea after the first introduction of 5 mL of formula milk.

Due to SPT reactivity to CM and HE, a strict diet without these two foods and with soymilk as CM substitute was recommended. At 3 years 2

months of age she inadvertently consumed HE and presented oral pruritus and lip oedema after a few minutes, and vomit after about 20 minutes. Due to her mother's fear to try CM and HE re-introduction, she was maintained on a CM and HE-free diet until her mother took the decision agreeing to desensitise her child to CM and HE. During the elimination diet period the child underwent unintentional introduction of CM several times presenting immediately various combinations of erythema, lip angioedema, and severe asthma.

At 7 years 4 months of age she underwent a DBPCFC with CM presenting immediate symptoms, as shown in Table I. She then began CM-OD that lasted 267 days and was only able to tolerate a maximum dosage of 45ml/day of CM instead of the 200ml/day foreseen by the protocol. During the CM-OD period she presented several allergic symptoms (Table I) within 2 hours of CM administration whenever the dosage was increased.

At the age of 9 years 11 months she underwent a DBPCFC with raw HE and immediately after the administration of a total dose of 0.5 ml of HE presented symptoms (Table I). She then began the HE-OD, which lasted 252 days. Apart from some isolated episodes of pruritus of the tongue and tickling throat, she was able to tolerate increased dosages of HE without major problems up to 25 ml of raw HE/day (Table I). She is currently assuming HE freely and 45ml/day of CM.

As shown in Table II, at the outset SPTs were positive both in the case of CM (casein, α -lactalbumin, raw whole CM, but not to β -lactoglobulin) and HE (all the tested proteins except boiled yolk). About 7 months after the end of the CM-OD, the SPTs to CM molecules were negative but CM-OD did not affect the SPTs to HE that became negative only after the HE-OD.

Similarly, a fall in casein, α -lactalbumin and β -lactoglobulin serum specific IgEs after CM-OD, was observed at the end of the CM-OD while a more evident drop of specific IgEs to egg white and yolk was observed at the end of the HE-OD (Table III).

Case report 2

Patient 2 is a boy aged 7 years 5 months. He had suffered from severe atopic dermatitis since he was 1 month old, while breast-feeding. He was exclusively

breast fed until the age of 9 months. During this time the mother avoided CM, HE and fish because she recorded a worsening of her son's atopic dermatitis after eating those foods. At the age of 9 months the child was weaned and was put on homemade meat-based formula (lamb, rice, olive oil, Calcium, salt - Rezza-Cardi diet) (12) to which wheat was added. Atopic dermatitis worsened and an improvement was noted only when wheat was avoided. CM (and dairy products), HE, wheat and fish exclusion from the diet was prescribed until he reached the age of 1 year and 6 months when a first attempt to reintroduce wheat induced diffuse pruritus, palpebral and face oedema after about 20 minutes. At 3 years, contact with fish (cooked sole) induced pruritus, and oedema of the face and the eyelids. Six months later, a moderate episode of asthma was induced by the inhalation of fumes produced by fried fish. At the age of 3 years and 8 months the child underwent an open food challenge with CM and presented mouth pruritus, tickly throat and lip oedema. One month later, an inadvertent introduction of cooked HE induced diffuse pruritus and erythema. At the age of 4 years he was able to tolerate wheat.

At the age of 5 years the child was under our observation and underwent a DBPCFC with CM. Five minutes after the administration of a total dose of 5 ml symptoms intervened (Table I). He then began the CM-OD that lasted 287 days. At the end of the protocol he was able to tolerate a dosage of 200ml/day of whole CM. During the CM-OD period he presented mild symptoms within 2 hours of CM administration when the dosage was increased (Table I).

At 5 years 11 months, while tolerating CM, he underwent a DBPCFC with raw HE. Within 15 minutes of administration of a total dose of 0.5 ml of HE, he presented symptoms (Table I). He then began the HE-OD, which lasted 252 days. Despite some isolated episodes of tickling throat, he could increase the HE dosage without experiencing major problems up to the maintenance dose of 25 ml of raw HE/day (Table I).

During the HE-OD period the child presented lip oedema, diffuse pruritus and moderate urticaria as a consequence of inadvertent ingestion of cod used in a sauce. At present, the child is on a fish-free diet and is assuming HE and CM freely.

As shown in Table II, at the beginning of the study SPTs gave a positive result to casein, α -lactalbumin, whole raw CM (but not to β -lactoglobulin), HE (all the tested proteins) and cod. A CM SPT reactivity decrease was observed by the end of CM-OD, whereas HE SPT reactivity decreased only after the end of the HE-OD (with the exception of boiled and raw yolk). Cod SPT positivity persisted throughout the study period.

While serum-specific IgEs to casein remained substantially unaffected, specific IgEs to α -lactalbumin and β -lactoglobulin decreased after the CM-OD (Table III). Egg white and yolk IgEs decreased after the end of CM-OD, while a further drop was observed at the end of HE-OD. Specific IgEs to cod were unaffected by CM- and HE-OD.

Case report 3

Patient 3 is a 14 year 9 month-old boy. He had suffered from severe atopic dermatitis since he was 2 months old while he was breast-feeding. Breast-feeding was interrupted and the child was put on a Rezza-Cardi (12) diet until he was 5 months old. A rice-based formula was then introduced as CM substitute, until he was 12. At 1 year of age, the child ate cod and suddenly presented lip and face oedema and his allergologist put the child on a strict diet (without CM, HE, fish) on the basis of the persistent high skin reactivity to these foods.

At the age of 12 years 2 months the child underwent a DBPCFC with CM. Twenty minutes after the administration of a total dose of 10 ml of CM he presented symptoms. He then began CM-OD that lasted 207 days, at the end of which he was able to tolerate 200ml/day of whole CM (Table I).

At 12 years 10 months he underwent a DBPCFC with raw HE. Fifteen minutes after the administration of a total dose of 1 ml of HE, he presented symptoms (Table I).

He is currently assuming CM freely, is on an HE- and fish-free diet, and is waiting to start the HE-OD. We do not know whether the child can tolerate fish because his mother did not give her consent to perform the oral challenge test.

The patient had casein and β -lactoglobulin, negative SPTs at the start of CM-OD but became slightly positive 11 months later, after CM-OD (Table II). Conversely, α -lactalbumin, which was

slightly positive at the beginning, was negative at the end of CM-OD and was still negative 11 months later. Raw whole CM SPTs were positive throughout. As regards HE SPTs, egg white SPTs (using both commercial extracts and whole food) were positive throughout, while yolk showed different results on the basis of the material used (commercial extract, boiled or raw yolk) (Table II). Cod SPTs tested positive throughout the whole period.

As shown in Table III, specific IgEs to casein and β -lactoglobulin slightly decreased at the end of CM-OD while specific IgEs to HE and cod were unaffected by the CM-OD.

Case Report 4

Patient 4 is a girl aged 6 years 8 months. HE was eliminated from her diet after an urticarial reaction at 1 year and 2 months. HE was re-introduced one year later (cooked, boiled or contained in foods like biscuits or cakes) without any problem. At 4 years of age, diffuse pruritus, lip and ocular erythema, eyelid oedema and mild urticaria occurred after raw egg ingestion. When she came under our observation, in order to confirm history we decided to perform two different DBPCFCs with both boiled and raw HE. The former resulted negative, but the latter was positive, with symptoms occurring 8 minutes after the administration of a total dose of 0.5 ml of raw HE (Table I). She then began HE-OD, which lasted 178 days. She could increase the HE dosage without major problems up to 25 ml of raw HE/day. She currently assumes raw and cooked/boiled HE freely.

CM SPTs resulted negative. As regards HE SPTs, those performed with commercial extracts and with boiled egg white and yolk resulted negative, while those performed with raw egg white and yolk, were strongly positive. At the end of HE-OD, SPT positivity to raw egg white slightly diminished, whereas SPT to raw yolk became negative (Table II).

Specific serum IgE for egg white and yolk were positive at the beginning of the HE-OD and remained positive throughout the HE-OD period (Table III).

DISCUSSION

Here we describe 4 representative cases of multiple food allergy: patient 1 being allergic to CM

Table I. Symptoms after DBPCFCs with CM and/or HE and course of CM- and/or HE-OD.

	Sex (Age)	Clinical food allergies	Symptoms after DBPCFC with CM (Eliciting dose)	Time between DBPCFC and symptoms (CM)	Outcome of CM-OD (Duration)	Symptoms during CM-OD	Symptoms after DBPCFC with HE (Eliciting dose)	Time between DBPCFC and symptoms (HE)	Outcome of HE-OD (Duration)	Symptoms during HE-OD
#1	F (7yr 4mo)	CM, HE	Perioral angioedema, urticaria, asthma (2 ml)	5 min	Tolerates 45ml/day of CM (267 days)	Pruritus of tongue, tickly throat, perioral erythema, cough, asthma, abdominal pain	Pruritus of tongue, lip oedema, diarrhoea (0.5 ml)	5 min	Tolerates 25ml/day of raw HE (252 days)	Pruritus of tongue, tickly throat
#2	M (5yr)	CM, HE, fish	Tickling throat, pruritus, perioral angioedema, rhinitis, conjunctivitis (5 ml)	5 min	Tolerates 200ml/day of CM (287 days)	Pruritus of tongue, tickly throat, stomach ache	Abdominal pain, vomit, sneezing and nasal drip (0.5 ml)	15 min	Tolerates 25ml/day of raw HE (280 days)	none
#3	M (12yr 3mo)	CM, HE, fish	Tickling throat, pruritus, profuse vomit, abdominal pain (10 ml)	20 min	Tolerates 200ml/day of CM (207 days)	Pruritus of tongue, tickly throat, stomach ache	Tickling throat, pruritus, lip and face oedema, abdominal pain (1 ml)	15 min	NA	NA
#4	F (4yr 5mo)	Raw HE (Tolerates cooked HE)	NA	NA	NA	NA	Pruritus, lip and face erythema, ocular erythema, eyelid oedema (0.5 ml)	8 min	Tolerates 25ml/day of raw HE (178 days)	none

CM: cow's milk; HE: hen's egg; OD: oral desensitization; NA: not applicable

and HE, patients 2 and 3 to CM, HE and fish and patient 4 to raw but not to cooked or boiled HE. Two patients (1 and 2) successfully underwent both CM and HE OD, patient 3 underwent only CM-OD while patient 4 underwent only HE-OD (Table I).

In her review, Nowak-Węgrzyn states that even if food allergy prevalence appears to be increasing, unlike other allergic diseases, it is "the only disorder for which there is no specific therapy, which is particularly troubling considering the potential for severe reactions" (2). Up to now, the specificity of OD is assumed, but no study has demonstrated it and with these paradigmatic cases we indicated that after

CM-OD, patients 1, 2 and 3 tolerated CM, but could not tolerate HE (patient 1) or HE and fish (patients 2 and 3). Reactivity to HE was overcome only after HE-OD. Patient 2 is still fish-intolerant even though as a result of OD he can now eat both CM and HE freely.

At present, we do not know if CM or HE clinical tolerance is definitive or dependent on the regular administration of the food. In other words, we do not know whether our patients are "responders" or "responders with regular intake" (13). Even though this topic is important from a speculative point of view, it is less noteworthy in clinical practice,

Table II. *Results of SPTs before and after CM- and HE-OD.*

Patient 1	CM-OD (pre)	CM-OD (post)	HE-OD (pre)	HE-OD (post)
Casein	6.0	6.0	4.0	negative
α -lactalbumin	5.0	5.0	3.0	negative
β -lactoglobulin	negative	negative	negative	negative
Raw Whole CM	7.0	6.0	4.0	4.0
Egg-white (commercial extract)	4.0	4.0	5.0	negative
Yolk (commercial extract)	4.0	4.0	4.0	negative
Egg-white (boiled)	6.0	7.0	6.5	4.0
Yolk (boiled)	negative	negative	negative	negative
Egg-white (raw)	9.0	10.0	10.0	6.0
Yolk (raw)	5.0	4.0	5.0	negative
Patient 2				
Casein	5.0	3.0	negative	negative
α -lactalbumin	6.0	5.0	5.0	negative
β -lactoglobulin	negative	negative	negative	negative
Raw Whole CM	8.0	6.5	7.0	5.0
Egg-white (commercial extract)	5.0	7.0	7.0	5.0
Yolk (commercial extract)	4.0	5.0	5.0	3.0
Egg-white (boiled)	6.0	10.0	10.0	negative
Yolk (boiled)	6.0	negative	negative	negative
Egg-white (raw)	7.0	10.0	12.0	6.0
Yolk (raw)	6.0	3.0	3.0	4.0
Cod	8.0	7.0	8.0	8.0
Patient 3				
Casein	negative	negative	3.0	nd
α -lactalbumin	4.0	negative	negative	nd
β -lactoglobulin	negative	negative	4.0	nd
Raw Whole CM	7.0	6.0	6.0	nd
Egg-white (commercial extract)	5.0	4.0	8.0	nd
Yolk (commercial extract)	3.0	3.5	5.0	nd
Egg-white (boiled)	5.0	6.0	4.0	nd
Yolk (boiled)	negative	negative	negative	nd
Egg-white (raw)	9.0	8.0	10.0	nd
Yolk (raw)	4.0	5.0	negative	nd
Cod	8.0	8.0	10.0	nd
Patient 4				
Casein	-	-	negative	negative
α -lactalbumin	-	-	negative	negative
β -lactoglobulin	-	-	negative	negative
Raw Whole CM	-	-	negative	negative
Egg-white (commercial extract)	-	-	negative	negative
Yolk (commercial extract)	-	-	negative	negative
Egg-white (boiled)	-	-	negative	negative
Yolk (boiled)	-	-	negative	negative
Egg-white (raw)	-	-	8.0	6.0
Yolk (raw)	-	-	8.0	negative

Figures indicate mm (mean of orthogonal wheals diameters).

especially for those foods that are normally present in the daily diet, such as CM and HE. On the other hand, the general tendency of specific IgE levels is to become lower for tolerated food (Tables II and III), as already reported (6, 9-10, 13-14).

In our opinion, the presence of specific IgEs is mandatory for the diagnosis of an IgE-mediated food allergy (3). In general, negativisation of SPTs and a reduction in serum-specific IgE could be considered prognostic indicators of the development

Table III. Results of specific IgEs (kU/l) dosage with immunoenzymatic assay(ImmunoCAP) before and after CM- and HE-OD.

	CM-OD (pre)	CM-OD (post)	HE-OD (pre)	HE-OD (post)
Patient 1				
Casein	257.00	105.00	25.50	37.20
α -lactalbumin	198.00	59.00	20.40	19.25
β -lactoglobulin	310.00	71.20	18.40	21.50
Egg-white	37.00	25.30	99.20	16.30
Yolk	30.02	26.44	28.20	12.32
Patient 2				
Casein	0.99	1.32	0.42	0.59
α -lactalbumin	8.00	4.33	1.95	2.01
β -lactoglobulin	0.40	negative	negative	negative
Egg-white	13.08	9.86	10.14	6.19
Yolk	10.88	2.06	2.63	1.40
Cod	20.42	30.40	29.40	30.70
Patient 3				
Casein	0.79	0.60	-	-
α -lactalbumin	negative	negative	-	-
β -lactoglobulin	0.64	negative	-	-
Egg-white	2.04	3.12	-	-
Yolk	0.48	0.55	-	-
Cod	5.12	8.96	-	-
Patient 4				
Casein	-	-	0.45	0.50
α -lactalbumin	-	-	negative	0.35
β -lactoglobulin	-	-	negative	negative
Egg-white	-	-	5.92	6.37
Yolk	-	-	0.48	0.62

Results over 100 kU/l were obtained diluting the serum 1:10.

of tolerance to CM (14). However, the course of a single molecule could be approximate in describing the prognosis in an individual case. Moreover, in the case of OD, we must look prevalently at the clinical outcome and the fact that a certain food previously not tolerated can be eaten without risk.

Patient 4 is a more peculiar example of the specificity of OD. This girl presented symptoms when she ate raw HE, but not with cooked or boiled HE and this was reflected by SPT reactivity to raw but not to boiled egg white and yolk. It has been described that some children who are allergic to HE are able to tolerate this food cooked but not raw (15). As suggested by Vila et al., during food processing,

heat and/or chemical processes can modify or disrupt many conformational epitopes, exposing linear epitopes (16). Moreover, Cooke & Sampson suggest that the development of IgE antibodies to these linear epitopes can be related to the persistence of HEA (17). The same mechanism seems to work for CM proteins (18) and Nowak-Wegrzyn et al., hypothesizing that some children with CMA would tolerate extensively heated (baked) milk products, surprisingly demonstrated that the majority of their population of CM allergic children tolerate heated CM (19). An allergic individual's antigen-specific IgE binding repertoire can therefore be specific for both sequential and conformational epitopes (16) or

only for sequential epitopes (19).

Alonso-Lebrero et al. described some individuals who were allergic to CM and were successfully subjected to CM-OD but could not tolerate goat's and sheep's milk. They also identified goat casein as the main allergen causing sensitization to goat's milk (20). This phenomenon is easy to understand if we take into account that the homology between two proteins of the same class among different species is not (and could not be otherwise) 100% (21). The α_{s1} - and α_{s2} -caseins from cows, goats, and sheep share 87-98% identical amino acids (21) and therefore 2-13% of these proteins differ from species to species and therefore elicit different IgE responses. We must also consider that 2 individuals may react to the same food developing an IgE response to different proteins of the same food or to two different sites (epitopes) of the same protein (22).

Adverse reactions to foods present a continuous challenge for clinicians. OD appears to be a promising method for treating food allergies, especially for those foods that are frequently present in children's diets.

This series of 4 cases strongly indicates that OD is food-specific, protein specific and is able to discriminate between raw and cooked proteins. On the basis of this reasoning, we should be able to demonstrate that OD is epitope-specific. Moreover, we showed that the induction of the clinical tolerance to one food is not followed by the tolerance to the other foods. To obtain a double tolerance two separate desensitisation protocols, one for each food, have to be performed.

Specific IgE levels decreased overall during the course of OD, but this cannot be used as evidence of the positive outcome of OD. While useful as prognostic indicators of the development of tolerance to CM and HE, the course of single molecules could be approximate in describing the prognosis in individual cases.

Further studies with a larger population of selected patients are required to confirm the suggestions provided by our data.

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