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Cow's milk allergy (CMA) in children: identification of allergologic tests predictive of food allergy

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Summary
Oral food challenge (OFC) is still considered the gold standard for diagnosis of food allergy (FA). Skin prick test (SPT) and specific IgE (sIgE) tests are very useful but limited in their predictive accuracy. End point test (EPT) has been recently considered to determine the starting dose to induce oral desensitization. Allergometric tests combined may discriminate children at higher risk of reactions during OFC. We considered 94 children referred to our Allergy and Immunology Pediatric Department between January 2009 and December 2011 with CMA. Cutaneous allergometric skin tests (SPT and EPT) were periodically performed on all 94 children with CMA; sIgE levels against cow's milk proteins (CMP) α-lactalbumin, ß-lactoglobulin and casein were periodically evaluated through blood samples every 6-12 months. During the period of the study, 26/94 (27.6%) children underwent more than once OFC. We collected 135 OFC compared with clinical presentation: 49/135 (36.2%) OFC were performed shortly after the onset of symptoms directly related to spontaneous intake of milk, to confirm suspicion of FA; 86/135 (63.7%) OFC were performed to evaluate the acquisition of tolerance. Of these, 52/86 (60.4%) OFC resulted positive, 34/86 (39.5%) were negative. The 3D EPT has the best ratio sensitivity (SE) / positive predictive value (PPV), SE 83%, specificity (SP) 58.3%, PPV 89.3%, negative predictive value (NPV) 45.1%. EPT 6D and 7D have the best PPV (100%) with a low NPV (respectively 22.2% and 21.2%). We obtained that a mean fresh milk wheal diameter ≥ 12 mm was predictive of 97% OFC, but only 32/101 (31.6%) allergic children presented this value. The tests with a wheal diameter ≤ 5 were performed on younger children, all of which were less than 9 months old; only 5 other tests performed on less than 9 months olds resulted in the others subgroups (1 in ≥ 12 mm wheal and 4 in the group between 6-11 mm).
We also found that 95% of children with 4D EPT wheal diameter < 6 mm resulted tolerant. This cut off could be useful to decide which children have a lower risk of reactions during the OFC. EPT is more useful than SPT especially for children < 1 year of age being a less operator dependent test, and it could be helpful to discriminate between children with the highest risk to develop anaphylaxis following an OFC (≥ 5D positive EPT) and children with lowest risk (> 2D positive EPT), but it can't replace OFC, that currently remains the gold standard in the diagnosis of FA. We also underline that in allergic children younger than 9 months old, the values of SPT with fresh milk is much lower than in older children, so that it's better to separate this group of age when we try to predict the evolution of OFC through the evaluation with EPT. A validation of such results in a prospective study could maybe be useful to confirm the outcome of our data in the predictivity of OFC.

Introduction

The oral food challenge (OFC) is still considered the gold standard for diagnosis of food allergy (FA). Cow's milk allergy (CMA) is the most frequent FA in infants, affecting 2-3% of children under 1 year of age. OFC confirms the suspicion of CMA, it helps monitoring the resolution of CMA and it evaluates the necessity of dietary restriction (1-7). However, OFC is not without risks; in a recent study, about 28% of these tests resulted in systemic and potentially life-threatening reactions (4). The high prevalence of FA in children increased demand for OFC, and this has created a need to identify those patients with the highest risk to develop anaphylaxis following an OFC. Hence, easy-to-follow parameters that could predict severe reaction to the OFC must be determined to better assess the risk-benefit ratio for each patient undergoing OFC. Previous studies examined the relationship between skin-prick tests (SPT) or specific serum immunoglobulin E levels (sIgE) and the outcome of OFC (5-14). Many Authors (10-12) tried, for instance, to correlate SPT wheal diameters with CM to the outcome of OFC, obtaining different cut off values; in particular, Sporik et al. (10) defined a cut off (> 8 mm), and sensitivity was not high enough to prevent allergic reactions during the OFC in allergic children, and moreover wheal diameters measurement in SPT were influenced by the operator. Furthermore, Calvani et al. (11) evaluated the validity of SPT by taking different cut off points for fresh milk and CMP. Using logistic regression, they defined the wheal size diameter predictive of a 95% positive OFC for fresh milk (15 mm) lactalbumin (9 mm), casein (9 mm) and lactoglobulin (10 mm). Verstege et al. (12) calculated that for fresh milk a wheal diameter of 12.5 and 17.3 mm was respectively predictive of 95% and 99% of positive OFC. They were able to define cut off levels for CM by using the SPT, which was not possible using the sIgE. SPT has high sensitivity, but its specificity is rather low so, alone, it is not sufficient to predict the outcome of the OFC.

So far, sIgE and SPT have not been found useful for predicting severe reaction when used in isolation. Correlations between milk proteins sIgE levels and the outcome of OFC can be found in many papers (13-17). Anyway, the parameters to predict the challenge outcome vary by children age, by proteic fractions considered and by measuring methodics. In some studies, the age of children seems to be correlated with IgE and SPT cut off levels, particularly for food challenges with egg and milk, with lower cut off levels in infants under 2 years of age (15,16). In a recent study, Wulfert et al. (17) found that CMP sIgE values, in particular sIgE against casein and β-lactoglobulin, could be able to make a discrimination between allergic and non allergic children, without identifying a cut off. Furthermore, they found a direct correlation between sIgE values and age of tolerance, in particular children that acquired milk tolerance at a later age had higher levels of casein or cow's milk sIgE. Another cutaneous test, the end point test (EPT), has been recently considered in FA diagnosis (18-20). Mori et al. (19) have used EPT to determine the starting dose of oral desensitization in allergic children. In our previous study (20), we demonstrated that EPT represents a cheap, economic and useful test, and that it could provide a good prediction of the outcome of OFC. This study is a continuation of the previous one, to assess if increasing the number of subjects and combining the different tests (SPT, sIgE, EPT) improves the performance in the prediction of the outcome of OFC.

Material and Methods

Subjects in the study

We considered 94 children referred to our Allergy and Immunology Pediatric Department between January 2009 and December 2011 with CMA. Of these, 44 patients were involved in our previous study. During the period of the study, 26/94 (27.6%) underwent more than once an open OFC. This retrospective study was approved in July 2012 by the Ethical Committee of University Hospital S. Orsola-Malpighi of Bologna. The mean age at diagnosis of 94 children with CMA was 6 months (4-12 months).

Inclusion criteria

- Specific symptoms after ingestion or contact with milk and / or derivatives: respiratory symptoms (rhinitis, bronchospasm), gastrointestinal (vomiting, diarrhea), skin (hives, eczema exacerbation), generalized (anaphylaxis).
- SPT and sIgE positive for CMP (α-lactalbumin, ß-lactoglobulin and casein).

Exclusion criteria

- Subjects with systemic and chronic diseases (different from allergic diseases) and with other physical or mental retardation, neurological abnormalities, thoracic surgery, tuberculosis.
- Patients with severe medical conditions that, in the opinion of the investigator, contraindicate the patient's participation in the study.

Plan

All 94 children with CMA were periodically performed to cutaneous allergometric skin tests (SPT and EPT); sIgE levels against CMP (α-lactalbumin, ß-lactoglobulin and casein) were periodically evaluated through blood samples every 6-12 months. EPT were performed on the same day of SPT by the

same investigator on the volar surface of the forearm. The investigator was not blind and the outcome of OFC was known.

Skin Prick Test

In all 94 children SPT was performed with fresh cow's milk and commercial milk extract (Lofarma, Italy). The positive control was carried out with a histamine standard (1 mg/ml) and the negative control with a glycerosaline solution. A wheal reaction ≥ 3 mm was required for positivity.

End Point Test

EPT consists of seven progressive dilutions of fresh cow's milk (30 mg/ml) with saline solution (1D: 1/10 = 3 mg/ml, 2D: 1/100 = 0.3 mg/ml, 3D: 1/1.000 = 0.03 mg/ml, 4D: 1/10.000 = 0.003 mg/ml, 5D: 1/100.000 = 0.0003 mg/ml, 6D: 1/1.000.000 = 0.00003 mg/ml, 7D: 1/10.000.000 = 0.000003 mg/ml) in 10 ml plastic tubes. For the dilution 1:10 we added 9 ml of saline solution to 1 ml of fresh milk. To obtain the dilution 1:100 we added 9 ml of saline solution to 1 ml drawn out from the 1:10 dilution and so on. In data analysis we considered wheal diameters start from 2 mm in EPT.

Specific IgE

The determination of cow's milk sIgE was performed by ImmunoCAP™ (Thermo Fisher, Sweden). Values greater than 0.35 kUa/L were considered as positive.

Oral Food Challenge

We started the challenge with 1 drop of cow's milk, then we progressively increased every 20 minutes the amount of milk administered according to this scheme: 1 ml, 5 ml, 10 ml, 20 ml, 40 ml, 50 ml, 100 ml. OFC was considered positive and stopped in the presence of a clear and objective clinical reaction (visible, measurable or even better quantifiable clinical symptoms) especially if occurred in a short time after ingestion. In presence of vomiting, cramping, abdominal pain, diarrhoea, generalized urticaria, cough with bronchospasm after ingestion of food, OFC was stopped. The occurrence of subjective symptoms like itching in the mouth or mild local urticaria around the mouth was followed by the next dose of food (21). The severity of clinical symptoms was graded following a five-level grading system for food-induced anaphylaxis (22). After the last dose, children without reactions were observed for 2 hrs. During OFC, children were completely free from any treatment with antihistamines. Children that did not experienced clinical reactions during the challenge were defined tolerant, whereas those who presented clinical reactions were defined allergic. On the basis of the outcome of the OFC, allergic patients maintained an exclusion diet, contrarily to tolerant patients who were allowed to include milk in their diet.

Statistical analysis

Statistical analysis was performed by means of SPSS 15 for Windows, SPSS Inc., Chicago, Ill. Student's t-test was used for the comparison of mean values. Probability values of less than 0.05 were considered as statistically significant. Two by two tables were used to calculate sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV). SE was defined as the proportion of true positives detected, specificity as the proportion of true negatives detected. PPV describes the proportion of the true positives among the apparent positives, while NPV shows the proportion of true negatives among apparent negatives. Candlestick charts were used to compare the same parameters in different groups of patients. The Geometric Mean of sIgE levels was calculated considering the average of the logarithmic values converted back to a base 10 number. Quadratic discriminant analysis was used to calculate the best parameters. Quadratic discriminant analysis was used for the classification of a sample as Positive or Negative. A Leave-One-Out cross-validation method was applied onto the dataset for testing the classification performance: all samples but one were used for training the method, which was eventually applied to the left sample for classification. The overall performance of the test was obtained by looping this procedure over all the samples. The optimal signature for classification was obtained by considering all the couples of parameters, and selecting the best performing combination of these couples.

Results

We have collected 135 OFC compared with clinical presentation: 49/135 (36.2%) OFC were performed shortly after the onset of symptoms directly related to the spontaneous intake of milk, to confirm suspicion of FA; 86/135 (63.7%) OFC were performed to evaluate the acquisition of tolerance. Of these, 52/86 (60.4%) OFC resulted positive because children showed clinical reactions, 34/86 (39.5%) were negative. Comparing the mean wheal diameter of every EPT's dilution between the group that presented allergic symptoms after intake of milk or derivates and the group without symptoms, we obtained a significant difference ($p < 0.05$) for the first 3 dilutions (**table 1**). No significant differences with commercial extract between two groups were found. Furthermore, we calculated accuracy of EPT and we obtained that 3D has the best ratio SE/PPV (SE 83%, SP 58.3%, PPV 89.3%, NPP 45.1%), EPT 6D and 7D have the best PPV (100%) with a low NPV (respectively 22.2% and 21.2%) (**table 2**).

Table 1 *- Mean wheal diameter (mm) of EPT at different dilutions (1st dilution = 1:10 [1D], 2nd dilution = 1:100 [2D]…). Comparison between EPT performed in presence of allergic symptoms (n. 101) or in absence of allergic symptoms (n. 34).*

	EPT performed in presence of allergic symptoms (101)	**EPT performed in absence of allergic symptoms (34)**
Fresh milk[1]	9.3 mm	5.1 mm
1D (1:10)[1]	7.1 mm	3.5 mm
2D (1:100)[2]	5.6 mm	2.8 mm
3D (1:1000)[2]	4.5 mm	2.8 mm
4D (1:10000)	3.4 mm	2.2 mm
5D (1:100000)	2.7 mm	2 mm
6D (1:1000000)	2.4 mm	0 mm
7D (1:10000000)	2 mm	0 mm

[1]p = 0.03, [2]p = 0.04

Table 2 *- End point test (EPT): sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) of each dilution obtained by 101 tests performed in presence of allergic symptoms or in absence of allergic symptoms (34).*

EPT	**SE**	**SP**	**PPV**	**NPV**
1D (1:10)	100%	4%	81.4%	100%
2D (1:100)	93%	20.8%	83.1%	41.6%
3D (1:1000)	83%	58.3%	89.3%	45.1%
4D (1:10000)	60%	79.1%	92.4%	32.2%
5D (1:100000)	36%	95.8%	97.2%	26.1%
6D (1:1000000)	17%	100%	100%	22.2%
7D (1:10000000)	12%	100%	100%	21.2%

Table 3 *- The determination of sIgE was carried out or in presence of symptoms directly connected to intake of cow's milk or to OFC: comparison between OFC performed in presence or absence of allergic symptoms.*

Determination of sIgE	**Presence of symptoms directly connected to intake of cow's milk or to OFC**		**Absence of symptoms directly connected to intake of cow's milk or to OFC**	
	geometric mean	**range**	**geometric mean**	**range**
Casein[1]	18.6 kU/L	(0.4-100 kU/L)	0.41 kU/L	(0.35-13.4 kU/L)
α-lactoalbumin[2]	10.3 kU/L	(0.35-100 kU/L)	0.36 kU/L	(0.35-3.6 kU/L)
ß-lactoglobulin[3]	5.4 kU/L	(0.35-38.3 kU/L)	0.43 kU/L	(0.35-11.1 kU/L)

[1]p = 0.003, [2]p = 0.004, [3]p = 0.005

Table 4 *- Percentage of positivity of the wheal at different dilutions of EPT (1st dilution = 1:10 [1D], 2nd dilution = 1:100 [2D]…) of cow's milk, divided following the fresh milk wheal diameter (≥ 12 mm, 6-11mm, ≤ 5mm). In 101 cases, EPT were performed before OFC, strictly after the appearance of symptoms directly related to spontaneous intake of cow's milk proteins. Mean age 5 yrs (range 3 mos-14 yrs).*

	≥ 12 mm	**6-11 mm**	**≤ 5 mm**
	(32/101) **Mean age: 8.7 yrs** **Mean wheal diameter: 14.2 mm** **(range: 13-20 mm)** **N (%)**	**(55/101)** **Mean age: 5.3 yrs** **Mean wheal diameter: 8.12 mm** **N (%)**	**(14/101)** **Mean age: 5.2 mos** **Mean wheal diameter: 4.7 mm** **N (%)**
1D (1:10)	32 (100)	55 (100)	14 (100)
2D (1:100)	32 (100)	55 (100)	14 (100)
3D (1:1000)	32 (100)	49/55 (89)	11/14 (79)
4D (1:10000)	32 (100)	37/55 (67)	2/14 (14)
5D (1:100000)	17/32 (53)	25/55 (45)	2/14 (14)
6D (1:1000000)	8/32 (25)	21/55 (38)	0
7D (1:10000000)	5/32 (16)	17/55 (31)	0

SIgE levels against milk's proteins both in the group of allergic reactions and in the group without symptoms have been reported in **table 3**; it has to be emphasized that only two patients with severe symptoms and a large SPT wheal presented very low levels of sIgE against milk's proteins (below 1.5 kU/L). Using the discriminant analysis previously described, we also evaluated the best parameter signature, a combination of STP with fresh milk, 3D (1:1000) and 4D (1:10000) that increases the accuracy of this allergometric test (PPV 85.1%, NPV 61.8%). We divided skin tests related to allergic symptoms according to fresh milk wheal diameter in 3 groups (**table 4**). We obtained that a mean fresh milk wheal diameter ≥ 12 mm was predictive of 97% OFC, but only 32/101 (31.6%) allergic children presented this value. EPT with a wheal diameter ≤ 5 were performed on younger children, all of which were less than 9 months of age; only 5 other EPT performed on less than 9 months olds resulted in the others subgroups (1 in ≥ 12 mm of wheal and 4 in the group between 6-11 mm). Furthermore, we obtained that 95% of children with 4D EPT wheal diameter < 6 mm were tolerant. OFC remains the gold standard in the diagnosis of FA, moreover this predictive test could discriminate with a high precision those children with the highest risk to develop anaphylaxis following an OFC.

Discussion

The OFC is currently the gold standard to diagnose FA but it is still a risky test, it is also expensive, and there are no practical parameters neither clear guidelines to discriminate which children should be tested and which shouldn't. Many Authors have tried to correlate cutaneous tests or sIgE levels with the outcome of OFC without significant results. Calvani et al. (11) evaluated the validity of SPT by taking different cut off points. Using logistic regression they defined the wheal size diameter predictive of a 95% positive OFC for fresh milk (15 mm) lactalbumin (9 mm), casein (9 mm) and lactoglobulin (10 mm). Verstege et al. (12) calculated that fresh milk wheal diameters of 12.5 and 17.3 mm were respectively predictive of 95% and 99% positive OFC. Our data show that only 31.6% tests showed a wheal diameter ≥ 12 mm, so that we need other tests in more than 60% cases to have a good prediction. We tried to combine the different allergologic tests to identify the best predictive of FA. We have obtained that 3D has the better ratio between SE/PPV (SE 83%, SP 58.3%, PPV 89.3%, NPV 45.1%); moreover, by combining the different parameters with quadratic discriminant analysis we obtained that fresh milk SPT, 3D and 4D have the best parameters with a PPV of 85.1% and a NPV of 61.8%. The combination of these parameters slightly increases the prediction of the OFC, because about 15% of tests is not predictive of the outcome of OFC. A negative EPT to 3D shows that 45.1% of negative children could present reactions during OFC, this predictive value meaning lower than showed in our previous study. Mori et al. (19) used EPT to determinate the first dose for oral desensitization, considering the dilution immediately below the positive as the starting dose for OFC. They concluded that EPT allows to be more confident with each single child, reducing the risk of reaction at the beginning. In our

previous study (20) we found out that a positive 4D of EPT could be the first step, after a positive SPT to cow's milk to select children who should not try OFC.

Furthermore, 6D and 7D have a PPV of 100%, with a NPV respectively of 22.2% and 21.2%; these results could be useful to select which children are at higher risk to develop anaphylaxis during OFC. We also found that 95% of children with 4D EPT wheal diameter < 6 mm resulted tolerant. This cut off could be useful to decide which children could be undergone by OFC with lower risk of reactions. sIgE against casein were significantly higher in allergic children than in tolerant ones, but it was not possible to define a cut off. EPT is a safe and cheap test, easily performed without risk of adverse reactions. It could be a valid approach to improve the use of the skin test in the diagnosis of FA; EPT is more useful than SPT especially for children < 1 years age, because it is a less operator dependent test; it could be helpful to discriminate between children with the highest risk to develop anaphylaxis following an OFC (≥ 5 D positive EPT), and those with lowest risk (> 2 D positive EPT) but this can't replace OFC, that currently remains the gold standard in the diagnosis of FA. We also underline that in the allergic children younger than 9 months old, the values of SPT with fresh milk are much lower than in older children; so, that it's better to separate this group of age when we try to predict the evolution of OFC through the evaluation with EPT. A validation of such results in a prospective study may be useful to confirm the outcome of our data on the predictivity of OFC.

References

1. Sánchez-García S, Rodríguez del Río P, Escudero C, García-Fernández C, Ramirez A, Ibáñez M.D. Efficacy of Oral Immunotherapy Protocol for Specific Oral Tolerance Induction in Children with Cow's Milk Allergy. IMAJ. 2012;14:43-7.
2. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS. Adverse Reactions to Food Committee of American Academy of Allergy, Asthma & Immunology. Work Group report: oral food challenge testing. J Allergy Clin Immunol. 2009;123:365-83.
3. Yum HY, Yang HJ, Kim KW, Song TW, Kim WK, Kim JH, et al. Oral food challenges in children. Korean J Pediatr. 2011;54:6-10.
4. Rancé F, Deschildre A, Villard-Truc F, Gomez SA, Paty E, Santos C et al. Oral food challenge in children: an expert review. Eur Ann Allergy Clin Immunol. 2009;41:35-49.
5. Lieberman JA, Sicherer SH. Diagnosis of food allergy: epicutaneous skin tests, in vitro tests, and oral food challenge. Curr Allergy Asthma Rep. 2011;11:58-64.
6. Lins MG, Horowitz MR, Silva GA, Motta ME. Oral food challenge test to confirm the diagnosis of cow's milk allergy. J Pediatr. 2010;86:285-9.
7. Ito K, Urisu A. Diagnosis of food allergy based on oral food challenge test. Allergol Int. 2009;58:467-74.
8. Matricardi PM, Bockelbrink A, Beyer K, Keil T, Nieggemann B, Gruber C. Primary versus secondary immunoglobulin E sensitization to soy and wheat in the multicentre Allergy Study Cohort. Clin Exp Allergy. 2008;38:493-500.
9. Host A, Halken S, Jacobsen HP, Christensen AE, Kerskind AM, Plesner K. Clinical course of cow's milk protein allergy/intolerance and atopic disease in chidhood. Pediatr Allergy Immunol. 2002;13 Suppl15:23-8.
10. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. Clin Exp Allergy. 2000;30:1540-6.
11. Calvani M, Alessandri C, Frediani T, Lucarelli S, Miceli Sopo S, Panetta V et al. Correlation between skin prick test using commercial extract of cow's milk protein and fresh milk and food challenges. Pediatr Allergy Immunol. 2007;18:583-8.
12. Verstege A., Mehl A., Rolinck-Werninghaus C., Staden U., Nocon M., Beyer K and al. The predictive value of the skin prick test wheal size for the outcome of oral food challenge. Clin Exp Allergy. 2005;35:1220-6.
13. Martorell A, García Ara MC, Plaza AM, Boné J, Nevot S, Echeverria L, and al. The predictive value of specific immunoglobulin E levels in serum for the outcome of the development of tolerance in cow's milk allergy. Allergol Immunopathol. 2008;36:325-30.
14. van der Gugten AC, den Otter M, Meijer Y, Pasmans SG, Knulst AC, Hoekstra MO. Usefulness of specific IgE levels in predicting cow's milk allergy. J Allergy Clin Immunol. 2008;121:531-3.
15. Komata T, Soderstrom L, Borres M, Tachimoto H, Ebisawa M. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. J Allergy Clin Immunol. 2007;119:1272-4.
16. Boyano MT, Garcia-Ara C, Diaz-Pena JM, Martin-Esteban M. Prediction of tolerance on the basis of quantification of egg-white specific IgE antibodies in children with egg allergy. J Allergy Clin Immunol. 2002;110:304-9.
17. Wulfert F, Sanyasi G, Tongen L, Watanabe LA, Wang X, Renault NK, et al. Prediction of tolerance in children with IgE mediated cow's milk allergy by microarray profiling and chemometric approach. J Immunol Methods. 2012;382:48-57.
18. Tripodi S, Businco AD, Alessandri C, Panetta V, Restani P, Matricardi PM. Predicting the outcome of oral food challenges with hen's egg through skin test end-point titration. Clin Exp Allergy. 2009; 39:1225-33.
19. Mori F, Pucci N, Rossi ME, De Martino M, Azzari C, Novembre E. Oral desensitization to milk: how to choose the starting dose! Pediatr Allergy Immunol. 2009;21:450-3.
20. Bellini F., Ricci G., Dondi A., Piccinno V., Angelini F. and Pession A. End point prick test: could this new test be used to predict the outcome of oral food challenge in children with cow's milk allergy? Italian Journal of Pediatrics. 2011;37:52.
21. Niggeman B, Beyer K. Diagnosis of food allergy in children: toward a standardization of food challenge. J Pediatr Gastroenterol Nutr. 2007;45:399-404.
22. A. Muraro et al. The management of anaphylaxis in childhood: position paper of the European academy of allergology and clinical immunology. Allergy. 2007;62:857-71.