

European Annals ^{of} Allergy and Clinical Immunology

THE OFFICIAL JOURNAL OF AAITO | ASSOCIAZIONE ITALIANA ALLERGOLOGI IMMUNOLOGI TERRITORIALI E OSPEDALIERI THE OFFICIAL JOURNAL OF SPAIC | SOCIEDADE PORTUGUESA DE ALERGOLOGIA E IMUNOLOGIA CLINICA



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Anaphylactic shock to raspberry

Comments on: "Allergen immunotherapy as a drug: the new deal of grass allergen tablets from clinical trials to current practice"

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JIS32014

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Printing

ProntoStampa Srl Via Praga, 1 24040 Verdellino (BG)

EDRA LSWR SpA

Via G. Spadolini, 7 20141 Milano - Italy Tel. 0039 (0)2-88184.1 Fax 0039 (0)2-88184.301 *www.edizioniedra.it*

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F. Bellini¹, G. Ricci¹, D. Remondini², A. Pession¹

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

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KEY WORDS

Cow's milk proteins allergy; oral food challenge; allergometric tests

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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, β-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 \leq 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 \geq 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 (\geq 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, β-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, β -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 \geq 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 ImmunoCAPTM (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).

	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 (1:1000000)	2 mm	0 mm
$p = 0.03, {}^2p = 0.04$		

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).

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 (1:1000000)	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 intake of cow's	s directly connected to 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)

 ${}^{1}p = 0.003, {}^{2}p = 0.004, {}^{3}p = 0.005$

	≥ 12 mm	6-11 mm	≤ 5 mm
	(32/101) Mean age: 8.7 yrs	(55/101) Mean age: 5.3 yrs	(14/101) Mean age: 5.2 mos
	Mean wheal diameter: 14.2 mm (range: 13-20 mm) N (%)	Mean wheal diameter: 8.12 mm N (%)	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 (1:1000000)	5/32 (16)	17/55 (31)	0

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, ≤ 5 mm). 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).

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 \ge 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 (\geq 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.

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Component Resolved Diagnosis (CRD): how much is it presently used by Italian allergists?

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KEY WORDS

Allergy diagnosis; component resolved diagnosis

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Summary

Component resolved diagnosis (CRD) represents an innovative and revolutionary tool in allergy diagnosis. At the same time, some criticisms can be outlined. The present web survey aimed at investigating the role of CRD in daily clinical practice, according to a sample of Italian specialists who manage allergic patients. 127 physicians, mostly allergists, completed the questionnaire, mainly coming from North and Center of Italy. Most of them (80%) were allergists. One physician out of three regularly takes into consideration CRD, that is currently available about in a half of the hospitals where the specialists work. CRD is mostly prescribed in the diagnostic work-up of suspected food allergy, as it can drive risk assessment, epinephrine prescription and dietary advice. Concerning respiratory allergy, CRD is considered useful in investigating cross-reactivity and in defining the best treatment option, even if only 32% of patients treated with immunotherapy had been previously studied with CRD. The present survey points out the need for the specialists to develop a more practical know-how about CRD. Its diagnostic accuracy and its real impact on the clinical management need to be better defined. The lacking of CRD technology in many hospitals limits the possibility for many allergists to directly experience molecular diagnosis.

Introduction

The best approach for the correct diagnosis of allergy is based on information collected from a well-targeted and detailed medical history and physical examination. Nevertheless, once there are sufficient clinical grounds to suggest a diagnosis of allergy, confirmatory in vivo and in vitro tests are usually indicated. In vitro techniques have rapidly grown up in the last two decades (1). Allergen-specific IgE antibody is the most important serological marker used in the diagnosis of allergic disease to confirm sensitization in an individual who has a positive history of exposure. Thanks to Component resolved diagnosis (CRD), nowadays we are able to collect more detailed information about the sensitization profile of allergic patients (2). Third generation auto-analyzers allow accurate, reproducible and quantitative measurements of the levels of IgE antibody directed to single molecular components (ImmunoCAP) (1). Moreover, also a multiplexed, microarray-based allergy test is available (ISAC) (2). It measures IgE antibodies to multiple allergenic components in one analysis and has a high negative predictive value. Defined panels of aeroallergens and food allergens relevant to different age groups are used (3). The multi-allergen screen is a cost-effective test, especially when more than 10 components have to be tested, but produces only qualitative results (1).

At the same time, the CRD approach still represents a challenge for allergists. In the present survey we investigated allergist's opinions about the use of CRD in daily practice and looked for criticism and unmet needs, that may affect its use in daily routine.

Materials and methods

A web anonymous questionnaire was available on the website of the Association of Italian Allergists (AAITO - www.aaito.it) for 60 days, from 1st January 2012 to 28th February 2012. An invitation to participate to the survey was sent twice by e-mail to all 583 members of the Association, 30 days apart. The 23 multiple-choice questions concerned the following items: specialization and provenance of the physicians involved in the survey, CRD-related know-how, number of allergic patients visited per week, diagnostic in vivo and in vitro tools commonly used, reasons for using CRD (ImmunoCAP or ISAC) and expected information.

Results

127 physicians (21.7% of AAITO members) completed the questionnaire, mainly coming from North and Center of Italy. Most of them (80%) were allergists. Other specialists such as pediatricians (19%), pneumologists (11%) and dermatologists (1%) who manage allergic patients in their clinical practice filled the questionnaire as well. The interviewed physicians report to know and use CRD since 30 months on average. They visit 39 patients per week on average (range 6-60). In 29% of cases specific IgE evaluation is requested, in 12% of cases with molecular components. One physician out of three is used to take into consideration both single ImmunoCAP components and ISAC, depending on diagnostic work-up complexity and on the number of single molecular components needed to be tested. Six molecular components per patient are assayed on average. About half of the specialists reported that neither ImmunoCAP nor ISAC is available in the hospital where they work, and therefore 48% of patients are forced to move to another hospital to have the test done. CRD is mostly prescribed in the diagnostic work-up of suspected food allergy (> 90%). It is included also in latex allergy (61% of cases) and Hymenoptera venom allergy (45% of cases) diagnosis (figure 1). CRD is applied especially when patients are polysensitized to inhalant allergens, food, or both, and when clinical profile is quite severe or complex (i.e. discordance between symptoms and in vivo tests results). Concerning respiratory allergy, specialists consider CRD a useful tool in order to investigate cross-reactivity (86.3%) and to define the best treatment option (73.5%).

Nevertheless, among the patients treated with specific immunotherapy only 32% had been previously studied with CRD. In the case of food allergy, almost 90% of specialists consider CRD a useful tool in order to point out cross-reactivity phenomena. According to more than 65% of specialists, CRD also can drive risk assessment, epinephrine prescription and dietary advice (**figure 2**). Almost 90% of physicians state that their CRD-related know-how comes mostly from scientific congresses and literature (**figure 3**). Lack of CRD technology in the hospital where they work seems to explain why many specialists don't use CRD. Most of them would like to improve their knowledge about CRD through practical courses and e-mail updating (**figure 4**).

100% 80% 60% 40% 20% 0% Inhalant allergy Food allergy Venom allergy Latex allergy

Figure 1 - Prescription of components in different allergy suspicions.

Figure 2 - Benefits of CRD in Food Allergy.



Figure 3 - Sources of CRD know-how.





Figure 4 - Tools needed to increase knowledge on CRD.

Discussion

During the last two decades, the major and minor IgE binding proteins of the most prevalent allergenic natural sources have been characterized at a molecular level, and many of them are available as recombinant or highly purified proteins. Diagnostic tests based on single recombinant (or natural) allergens, both in classical and in microarray format, have been developed allowing to better define the sensitization profile of allergic patients. The CRD represents an innovative and revolutionary concept in allergy diagnosis. It allows to discriminate between cross and co-sensitization (4), to help in selecting the most appropriate immunotherapy (5,6,7) and to estimate the risk of severity of the clinical manifestations in food allergy (8). According to our survey, Italian specialists who manage allergic patients show great interest and awareness of CRD, even if it could be defined more as a theoretical knowledge than as a real know-how. In fact, few specialists report to include the use of CRD in their daily clinical practice. On one hand it may reflect one of the limits of new molecular diagnostic tools. In fact, before the CRD tests and in particular the microarray-based tests become a standard diagnostic tool in clinical laboratory, clinicians and pathologists have to better define their diagnostic accuracy and their real impact on the clinical outcome (7). On the other hand, according to our data a fully spread knowledge about CRD is still lacking. Another problem is the lack of CRD technology in many hospitals. It limits the possibility for many allergists to directly experience molecular diagnosis.

In conclusion, the present survey points out the need for allergists and other specialists who treat allergic patients to develop a practical know-how through courses and constant updating concerning the use of molecular tools. Moreover, an easy-access network involving specialists and referral centers for CRD diagnosis should be created. Finally, it has to be stressed that specialists visit 39 patients per week on average. Considering the burden of allergic disease from an epidemiological point of view, it means that less than 15% of allergic patients is visited by a specialist and therefore an easier access to allergists has also to be improved for a better management of allergic diseases.

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Relationship between Methacholine Challenge Testing and exhaled Nitric Oxide in adult patients with suspected bronchial asthma

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KEY WORDS

Asthma; Bronchial Hyperreactivity (BHR); Methacolin Challenge Testing (MCT); Exhaled Nitric Oxide (FeNO)

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Summary

Usually, hyperresponsiveness to inhaled methacholine is considered closely associated with a diagnosis of bronchial asthma. Recently, it has been clearly pointed out that bronchial hyperreactivity (BHR) is not a constant feature of asthma and that this condition is not always related to airways inflammation.

In the present study we evaluated 42 Patients (21 positive and 21 negative for bronchial hyperreactivity, BHR) with the aim to determine the effect of Methacholine Challenge Testing (MCT) on the levels of exhaled nitric oxide ().

Higher FeNO levels were found before methacholine provocation in the group that eventually resulted positive to the challenge, while after the challenge in both groups FeNO decreased in similar way, with no statistical difference.

These data confirm that MCT is a relevant test for asthma diagnosis, but it is not always related to the severity of bronchial inflammation, while FeNO levels in our study have limited clinical significance when evaluated out of asthma exacerbation.

Introduction

Airways hyperresponsiveness is one of the features that may contribute to the diagnosis of asthma. Methacholine Challenge Testing (MCT) is the best established method of assessing airway responsiveness (1,2,3)

When spirometry, performed before and after administration of a bronchodilator, has not confirmed or eliminated the diagnosis but symptoms (wheezing, chronic cough, chest tightness) continue to suggest asthma, MCT is usually performed in patients who are medication free and don't present a recent exacerbation of disease.

The MCT has excellent sensitivity but poor positive predictive value for asthma, while its negative predictive power is high and always useful in differential diagnosis (4,5)

Improvement in the clinical severity of asthma is associated with improvement of airways responsiveness, and clinical studies of asthma therapies often use MCT as an objective outcome measure, but responsiveness to direct bronchoconstrictor stimuli does not indicate presence and severity of airway inflammation (6).

In fact, challenge with indirect stimuli as Adenosine and Mannitol can provide a better correlation with inflammatory markers, with increase of sputum eosinophil count and exhaled nitric oxide (7).

Nitric oxide is synthetized from L-Arginine in both neuronal and not neuronal tissues through the action of NO synthetase. The epithelium of the respiratory tract is an important source of NO, which increases with the presence of inflammatory cells. Production of NO is assessable by measuring the fraction of NO in exhaled air (FeNO) and elevated levels of this diffusible gas have been proposed as a non invasive marker of airways inflammation (8,9,10). Timing of sampling may significantly alter FeNO measurements, and repeated spirometry maneuvres may reduce FeNO levels (11). Studies in asthmatic children reported that FeNO values are reduced after MCT (12), but it is not clear if the change of FeNO values is a consequence of repeated maneuvres and hyperventilation, or it is due to methacholine induced bronchial constriction.

More studies are necessary to define relationship between results of bronchial provocation tests, i.e. MCT, and values of FeNO. With the aim of providing a better definition of a different risk of acute asthma exacerbation, in this study we measured bronchial hyperreactivity and inflammation in a group of Patients with clinical suspect of asthma and with negative or positive MCT, and we determined the effect of MCT on FeNO levels in all Patients.

Materials and methods

Subjects

42 Patients (20 M, 22 F, mean age 37.04 years) with symptoms (chronic cough, nocturnal wheezing, or dyspnea for more than 3 weeks) and visited in our Outpatients Office, have been selected for a Methacoline provocation test after a normal spirometry before and bronchodilator test after with 400 mcg of salbutamol.

21 Patients were smokers, 11 ex smokers, 10 no smoker, 21/42 were atopic (10 mites, 6 grasses, 4 molds, 1 cat), see **table 1**.

Before and after MCT, FeNO levels were measured in all patients, mean of two consecutive test (?).

All Patients received written information on the test and gave their informed consent.

Methacholine Provocation Test

The bronchial provocation test was performed using Master-Screen Body connected with an aerosol provocation system APS PRO. Two different Methacholine Chloride (Lofarma) concentrations (0.2 and 1%) were diluted in sodium phosphate buffer solution. The Patient breathed against a device a single dose of 30, 30, 60, 120, 150, 300, 600, 1200 mcg up to a cumulative dose of 2490 mcg of the substance. After a minute from each concentration, Patient performed control spirometry.

The test was interrupted if FEV1 decreased more than 20% from the value found after buffer solution, before the first dose of the drug. In this condition, patient was invited to inhale 400 mcg of salbutamol and spirometry was repeated after 20 minutes.

FeNO Measurement

FeNO was measured using HypAir FeNO (Medisoft S.A., Belgium). Each Patient was asked to inhale deeply against the machine via a filtered breathing mouthpiece; after a whole inspiration with a pressure maintained between 4 and 10 cm/ H_2O the subject had to exhale continuously, maintaining an optimal exhalation pressure with a flow at 50 ml/s; at this point the instrument started sampling automatically. Double measurements of NO were averaged and expressed as parts per billion (ppb).

Study design

FeNO levels were measured before MCT as a baseline, then immediately after completion of the provocation test. When test was positive for hyperreactivity (cumulative dose of methacholine reduced FEV1 more than 20% when compared with baseline value after isotonic saline) FeNO was performed before inhalation of the bronchodilator.

Statistical Analysis

All data are expressed as means +/- SE, χ^2 test was performed for Patients with pre-Methacholine FeNO > or < 30 ppb, ANOVA test for FeNO pre/post MCT.

Statistical Analysis was performed by courtesy of Valentina Mirisola, Engineer - Mediservice, Genova.

Results

Table 1 describes general characteristics of the 42 Patients, divided for analysis in 21 positive and 21 negative to MCT. No statistical difference was shown within the groups for age, gender, smoke and allergy. Most part of positive MCT were classified as Relevant Hyperreactivity (PD20 FEV1 induced by dose less than 400 mcg of Methacholine in 85.7% of 21 positive patients).

FeNO before MCT shows no statistical difference between patients positive or negative for bronchial hyperreactivity (ANO-VA p-value 0234) (**table 2**).

In **table 3** we divided Patients with FeNO above or below 30 ppb, in patients with less than 30 ppb there was not significant difference between positive and negative challenge for bronchial hyperresponsiveness (p = 0.072); all three patients with FeNO > 30 ppb were positive to MCT.

In **table 4** we can see that FeNO significantly decreased in positive and negative group after challenge (p = 0.006) but (**table 5**) the mean decrease of FeNO post MCT was not significantly different between positive and negative patients (ANOVA p value = 0.374).

In two well balanced groups no differences were found in FeNO levels in a statistical comparing of smoker/not smoker and allergic/not allergic subgroup of patients.

Variable	Neg	ative	Pos	itive	p-value	Te	otal
	N	%	Ν	%		N	%
Age, years Mean (± SD)	37.52 (±	13.618)	38.81 (±	17.005)	0.788	38.17 (:	± 15.230)
Gender							
Female	9	42.9	13	61.9	0.217	22	52.4
Male	12	57.1	8	38.1		20	47.6
Smoke							
Ex smoker	5	23.8	6	28.6	0.728	11	26.2
No smoker	11	52.4	12	57.1		23	54.8
Smoker	5	23.8	3	14.3		8	19.0
Allergy							
Yes					0.355		
No	12	57.1	9	42.9		21	50.0
	9	42.9	12	57.1		21	50.0
PD20, mcg Mean (± SD)		-	296.88 (±	309.073)	-		-
Bronchial							
Hyperreactivity							
Slight	-	-	1	4.8	-	-	-
Moderate	-	-	2	9.5		-	-
Relevant	-	-	18	85.7		-	-

Table 1 - General characte	eristics of the patients.
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Table 2 - FeNO mean pre-Methacholine Challenge.

	Bl	HR	77 . 1	ANOVA
	Positive	Negative	Iotal	p-value
Mean	18.33	12.47	15.40	
FeNO				0.234
SD	5.336	21.567	15.799	

Table 3 - Percentage of patients with FeNO pre-MCT < or > 30 ppb.

		BHR		75 - 1	X2
		Negative	Positive	Iotal	p-value
< 30 ppb	N	21	18	39	
	%	100.0	85.7	92.9	
FeNO					
pre-MCT ≥ 30 ppb	Ν	0	3	3	0.072
	%	-	14.3	7.1	
Ν	Total	21	21	42	
	%	50.0	50.0	100.0	

		BHR	
	Pre	Post	p-value
Mean	15.40	12.21	
FeNO			0.006
SD	15.799	9.907	

Table 4 - FeNO pre- and post- Methacholine Challenge.

Table 5 - Delta FeNO pre-post- Methacholine Challenge.

	BHR		ANOVA
	Negative	Positive	p-value
Mean	-2.19	-4.18	
Delta FeNO			0.374
SD	3.243	9.655	

Discussion

MCT is usually performed when spirometry is in normal range but symptoms leave a suspect of bronchial asthma. This test can confirm the presence of bronchial hyperreactivity, but is not able to define whether symptoms are related to bronchial inflammation. By trying to relate these two parameters, we were looking for a better definition of risk for asthmatic acute exacerbations. The data show that FeNO values were within normal range (< 30 ppb) in 39/42 patients admitted to the study, and mean value of positive patients was higher than in negative group after MCT (18.33 vs. 12.47 ppb; ANOVA p-value 0234). These basal values were expected, because of the choice of investigating a population without acute exacerbation and with aspecific symptoms, only 3 patients had basal values of FeNO more than 30 ppb and all three had a rapid onset of bronchoconstriction (with 62.4, 122.4, 143.1 mcg of the product); we can assume that a greater number of patients with more than 30 ppb FeNO before test should give a significant statistical difference between the two groups (chi-square p value 0.072 with not balanced population); in other studies (13) FeNO > 34 has high predictive value for PD20 MHC < 16 mmol. MCT confirms its role for the diagnosis of asthma, with 21/42 Patients positive to the challenge. FeNO decreases significantly in Patients with positive and negative challenge (15.4 vs. 12.21 pp, paired samples p-values 0.006) but FeNO post MCT was not significantly different between the positive and negative patients after MCT (ANOVA p-value 0.374).

The reduction of FeNO may be associated with bronchial constriction during the provocation test, but also the group with MCT negative shows a similar reduction, therefore we can assume that the reduction is a consequence of bronchoconstriction and repeated spirometry maneuvers, but not related to bronchial inflammation.

Contrarily to other authors, we can't state that high levels of FeNO were associated with bronchial hyperresponsiveness (few patients > 30 ppb), while we have similar results on reduction of FeNO after the challenge. FeNO < 30 ppb seems to have limited clinical significance for diagnosis in asthma like symptoms, mostly in smokers. So, FeNO has been correctly proposed as a non invasive marker of airway inflammation during asthma attacks, particularly in the inflammatory response to allergens, but at the moment we can't propose the use of the test out of exacerbation of the diseases and we don't suggest the contemporary use during bronchial challenge with methacholine. A different result may be possible during provocation tests with allergens, in this case the inflammation of the bronchial epithelium may be significantly greater and FeNO may grow in a direct relation with allergic inflammation. Further investigations are needed to prove this hypothesis.

Acknowledgments

The Authors would like to thank Rossella Benatti and Maria Ansaloni, technicians of the Respiratory Physiophatology Lab, for their technical support during this study.

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Psychiatric adverse reaction induced by Clarithromycin

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KEY WORDS

Clarithromycin; drug allergy; mania; macrolides; neurotoxicity

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Clarithromycin is an antibiotic of the macrolide family, widely used in respiratory and ENT infections. Immediate hypersensitivity reactions are uncommon. The most frequent side effects are gastrointestinal disturbances, hepatotoxicity and ototoxicity. There are few reports on allergic reactions to macrolides (1-4). We present a case of a 28 years old non-atopic man with a history of irritable bowel syndrome and sulfamide allergy, who was referred to our Allergy Department for an adverse reaction to clarithromycin. He referred the appearance of facial and palmar erythema without pruritus or respiratory symptoms after the first tablet of clarithromycin 500 mg prescribed for pharyngitis. Three hours later, he started with dizziness, anxiety and emotional lability. Symptoms resolved spontaneously over four hours. He had previously taken clarithromycin with good tolerance.

Skin prick test with macrolides (clarithromycin, midecamycin, roxithromycin, azithromycin, erythromycin) were all negative.

Single blind placebo-controlled oral challenges with progressively increasing doses of erythromycin and clarithromycin on separate days were conducted under close medical supervision in our Allergy Department. The patient tolerated up to 500 mg of erythromycin, but 40 minutes after the intake of a cumulative dose of 500 mg of clarithromycin, he experienced dizziness and derealization, perceiving ideas and concepts as running very fast but no perception of rotation. This experience was associated with panic-anxiety and emotional lability, abruptly shifting form elation to unmotivated crying. The episode resolved spontaneously within two hours, and he did not present any type of skin reaction.

Given the psychiatric adverse reaction observed during the drug challenge, further studies were carried out including electroencephalogram (EEG), brain computed tomography (CT), blood cell count, blood biochemistry and immunoglobulins (IgE, IgA, IgM and IgA). All these tests were unaltered. A psy-

chiatric evaluation including clinical interviews by senior psychiatrist and psychological examinations of personality came to the conclusion that the patient suffered from a depersonalization-anxiety syndrome secondary to clarithromycin with no primary underlying psychiatric disorder.

The patient was finally diagnosed of adverse reaction to clarithromycin secondary to neurotoxicity, and he was recommended to avoid it. The psychiatric manifestations induced by clarithromycin seem to be selective in our patient since he tolerated erythromycin, a drug that was allowed as an alternative macrolide.

Psychiatric manifestations induced by clarithromycin have already been reported in adult and pediatric patients, and they include emptiness, depersonalization, paranoid ideation, aggressive behavior, anxiety, confusion, hallucinations, emotional lability, agitation, delusions of grandeur, nervousness and sleep disorders (5,6). The symptoms resolve after drug withdrawal, normally within few days, spontaneously or with antipsychotic drugs (7,8,9). However, in some cases the duration of psychiatric symptoms can last months after withdrawal of clarithromycin (5,6).

The mechanisms involved in macrolide induced psychiatric reactions are not well established. Several theories have been proposed: 1) drug interactions due to the inhibition of cytochrome P450 by clarithromycin; 2) accumulation of the active metabolite 14-OH of clarithromycin in the central nervous system; 3) increased levels of blood cortisol and prostaglandins, hormones that are associated with mania (6,10,11).

In our case, the patient reported in the first reaction mild and transient skin symptoms, suggesting a possible hypersensitivity to the antibiotic, and hours later psychiatric symptoms appeared. The latter were not sufficiently emphasized by the patient at the first visit and therefore overlooked by the allergist who collected the medical history. However, during the drug challenge in our Allergy Department, psychiatric symptoms appeared earlier than in the reported reaction and were of a higher intensity, while signs and symptoms of skin involvement were absent. Fortunately, the reaction was self-limited and resolved spontaneously within hours without anti-psychotic drugs. The patient did not present any residual symptoms at follow up (up to one year) and the EEG performed one week after oral intake was normal.

We present this case to draw attention of allergists to psychiatric manifestations associated with clarithromycin, a type of adverse reaction uncommonly seen in Allergy clinics. Although these reactions usually resolve within hours or a few days, sometimes without the use of psychiatric medications, there are also cases of severe symptoms that persist for months. It is therefore essential that allergists are aware of this type of psychiatric adverse effects when taking the medical history, and avoid diagnostic challenge procedures.

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Adverse reaction to sublingual Parietaria vaccine following an ultra-rush induction

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Key words SLIT; asthma; adverse effects; Pellitory; Parietaria

Summary

In the treatment of respiratory allergies Sublingual Immunotherapy (SLIT) represents a valid alternative to Subcutaneous Immunotherapy (SCIT) for its better safety profile. We describe a case of acute severe asthma following the first maintenance dose of SLIT in a boy allergic to Parietaria pollen. At the initiation of therapy, the patient was in healthy condition and his asthma appeared to be under control. An ultra-rush induction had given no reaction. Despite the good safety profile of SLIT, clinicians should be aware of the risk of adverse effects when prescribing SLIT for respiratory allergies.

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Introduction

Allergen-specific immunotherapy (AIT) represents the only treatment of allergic disorders that is capable of both improving symptoms and modifying the natural course of illness in children. There is evidence that SIT is effective in patients with allergic rhinitis and mild asthma (1). Sublingual allergen-specific immunotherapy (SLIT) has been proven to be clinically effective in children with asthma (2) or rhinitis (3) and has been widely proposed as an alternative to SCIT (4) due to its better safety profile in respect to SIT.

SLIT doses are administered at home following the manufacturer instructions, but there at home several problems can arise. Parents often fear making dosage mistakes. They are aware of possible (but highly unlikely) adverse reactions since they are reported in the instruction books. In order to reduce these problems and to improve adherence to the therapy, some of us have recently experienced an ultra-rush method of induction that has demonstrated to be safe and well tolerated (5). In the described case, a severe adverse event occurred at the beginning of the maintenance period. This is the first time in our experience that we had to stop SLIT therapy because of adverse effects.

Case report

S.L., a seven-year boy, has been affected by allergic asthma since he was three. He was seen in our hospital for the first time in 2009 and on that occasion an IgE-mediated allergy to Parietaria pollen was demonstrated. Prick test (ALK-Abellò) resulted in an 8 mm wheal (mean diameter) and the serum specific IgE was 28 KU/l (CAP FEIA, Phadia, n.v. < 0.35). He was treated with Fluticasone 50 mcg b.i.d. from March to the beginning of July. During the following summer months he was completely free of symptoms. In September, therapy with fluticasone was restarted. Clinical conditions were good and spirometric data were within the normal range for the age. We prescribed SLIT for Parietaria judaica (SLIT-1, ALK-Abellò, Madrid, Spain). Contents of container: 18 sealed aluminium bags, each bag containing a strip of 5 single-dose containers for a total of 90 single-dose containers. Each single dose container contains 0.2 ml (extractable volume) that correspond to 200 STU per dose, which is the maintenance dose to be administered at home three times weekly.

After having obtained informed consent from the patient's parents, we admitted the patient to Day Hospital for routine preliminary exams, after which we began the ultra-rush induction (Day 1). At 40 minutes intervals we administered two 100 S.T.U. doses and a third dose of 200 STU. The patient was kept under observation for four more hours and eventually discharged with written instruction. No adverse events were observed. The patient's prescription called for him to take one 200-STU container every other day.

The following day (Day 2), the patient reported no problems. On Day 3, an hour after taking his first dose at home, the patient presented acute severe bronchial asthma. He was subsequently taken to the emergency room where he was found to have oxygen saturation of 88%. The situation was alleviated with prednisone per os and nebulized albuterol per aerosol. Within two hours, clinical conditions had sufficiently improved, enough so that the boy was sent home with a prescription of albuterol as an "emergency" medication in case of an asthma attack. The day after the episode (Day 4), the patient reported no problems, however on Day 5, after taking a 200 STU dose, the patient again suffered the same acute episode of asthma as he did after the first 200 STU dosage. The following day (Day 6) there were no problems. On Day 7, the patient again took his 200 STU dose, and had another identical acute asthma episode as he had had on Days 3 and 5. At this point, the parents informed us of the events of the previous week during a scheduled check-up, and therapy was discontinued.

At present the patient is still a patient of our outpatient ambulatory and his asthma is well controlled.

Discussion

Asthma has long been recognized as a risk factor for systemic reactions in patients treated with injective immunotherapy (SCIT). A recently published survey found that 15 of 17 patients who had a fatal reaction had preexisting asthma (6), and as such allergens should not be administered to patients with a forced expiratory volume in 1 second (FEV1) under 70% of predicted or to those who have unstable or symptomatic asthma (7).

SLIT, which has been proposed as an alternative to SCIT due to the ease of its administration at home and its better safety profile, has shown a good safety profile concerning severe systemic reactions in children (8). Most of the reactions are localized to the oral mucosa, and very few systemic reactions have been reported, nevertheless severe adverse reactions to SLIT may still occur. The entire topic was recently reviewed by Calderon et al (9). It has been noted that most adverse reactions occur in patients who had already experienced side effects with SCIT. Eleven anaphylactic reactions have so far been reported in the literature, three of which occurred in paediatric age. One of these reactions occurred in an 11 year-old boy, who had asthma as the first and most relevant symptom of adverse reaction to the vaccine (10). Another case of acute asthma as an adverse reaction to the first doses of SLIT was described in an adult woman (11). In our experience, one child presented acute severe short-lasting asthma as an adverse reaction to SLIT for Parietaria. No serious adverse reaction had until then been observed in our Allergy Unit among our patients treated with SLIT. This experience will not change our behaviour going forward, however at the same time it is important to stress the concept that an allergen-specific vaccine, even when taken by means of sublingual drops or tablets, can represent a risk for the allergic patient. Clinicians who prescribe such therapy should be aware of the possibility of serious adverse reactions and should take all the preventive measures in order to ensure the patient's safety.

It is important to highlight the need for a standardization of allergenic extracts. The trend in immunotherapy is toward molecular or even epitopic, peptide therapy. In two large SLIT trials that utilized sublingual tablets and were carried out in paediatric patients, treatment protocol started directly at the target dose (12,13). Results were encouraging and the need for a build-up period in SLIT should likely be reconsidered. Moreover, we advise clinicians to be extremely careful when administering SLIT in patients with a previous history of systemic side effects after SCIT. Asthmatic patients whose disease is less than optimally controlled appear to be at highest risk (6). Finally, we wish to emphasise that the first dose of SLIT should be taken in a doctor's office with an observation period of at least 30 min.

Acknowledgment

The authors wish to thank Adam Atlas for his linguistic support in reviewing the English manuscript.

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Iatrogenic angioedema associated with ACEi, sitagliptin, and deficiency of 3 enzymes catabolizing bradykinin

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KEY WORDS

Iatrogenic angioedema; bradykinin; enzyme deficiency

Summary

New concepts of idiopathic and iatrogenic angioedema underline the role of bradykinin, and the importance of catabolizing enzymes. A case is described of Angiotensin converting enzyme inhibitor (ACEi) and sitagliptin induced angioedema, where AO attacks decreased after the withdrawal of lisinopril but resolved only after the withdrawal of sitagliptin, an inhibitor of dipeptylpeptidase IV. ACE, aminopeptidase P and carboxypeptidase N were decreased down to 17%, 42%, 64% of median references values, and remained low one year after the interruption of these drugs: 56%, 28% and 50%, respectively. The combined deficiency of APP and CPN might enhance the inhibiting effect of the DPP IV inhibitor. The fact that this triple deficiency remained latent before and after the treatment indicates that searching for latent enzyme deficiencies should be carried out when there is intention to treat with a combination of drugs interfering with the bradykinin metabolism.

Introduction

Possible mechanisms of angioedema have greatly benefited from the knowledge of angiotensin-I converting enzyme inhibitors-induced angioedema. The importance of bradykinin and of enzymes catabolizing bradykinin and its active metabolism desArg9-BK has been underlined (1). The ACEi extends the halflife of bradykinin (BK) and kallidin (KD), resulting in vasodilation, vasopermeation and cardioprotection (2). Other substrates of ACEi are desArg9-BK and Substance P. Due to its action on BK degradation, ACE promotes the kininase II pathway that is responsible for 75% of BK and 35% of desArg9BK cleavage (3). The decreased ACE activity and subsequent accumulation of vasoactive kinins is a causative factor for iatrogenic angioedema (AE). In addition to ACE, other metallopeptidases contribute to kinin catabolism: aminopeptidase P (APP), dipeptidylpeptidase-IV (DPP-IV), plasma carboxypeptidase N (CPN) and carboxypeptidase M on endothelium, and Neutral Endopeptidase (NEP). APP metabolizes 21% and 65% of circulating BK and *des*Arg9BK respectively (3,4). Decreased APP activity is associated with more severe AE disease (5). Substance P, BK and *des*Arg9BK are also substrates of DPP-IV (6). The DPP-IV inhibitors (also called gliptins) are a new class of active agents for treatment of type 2 diabetes. Recently, Brown *et al.* demonstrated the increased risk of angioedema in patients taking an ACEi and a DPP-IV inhibitor (7).

We report the original observation of a patient suffering from ACEi-AE, secondarily worsened by gliptin and persistent after the withdrawal of ACEi. From the biological investigation of kinin metabolism the patient presented with decreased ACE, APP and CPN activities.

Case Report

In February 2010, a 56-year-old man presented to our Allergology Department with recurrent angioedema episodes. The patient's medical history included type 2 diabetes, acute coronary syndrome and dyslipidemia. He suffered from moderate seasonal rhinitis that did not require any treatment. There was no family history of AE. His daily treatment included lisinopril 10 mg/day (since 1995), glibenclamide 5 mg/day, metformin 1000 mg/day (since 2003), sitagliptin 100 mg/day (since 2009), flurbiprofen 50 mg/day, atorvastatin 20 mg/day. For more than 10 years he had experienced unpredictable angioedema of the face, uvula and hands, without pruritus or urticaria, on a yearly basis, which developed over 3-4 days, despite oral corticosteroid therapy with prednisolone 60 mg/day. The swelling attacks sometimes occurred during infectious episodes or stress periods. Since 2009, symptoms had worsened: oedema was associated with abdominal pain and increased frequency of attacks (every 6 weeks).

Biological investigations

C1-esterase inhibitor concentrations and function, enzymatic assays for APP, ACE and CPN were studied as previously described (8,9).

Results/Findings

C1-esterase inhibitor concentrations and function were normal. ACE, APP and CPN activities were decreased down to 17%, 42% and 64% of median reference values, respectively (table 1). Allergy tests indicated sensitization to ragweed and grass pollens. Serum tryptase was found to be within the normal range (2.7 µg/L; N: < 13).

Lisinopril was withdrawn and only three attacks of AE were observed over nine months (2 episodes of facial swelling and 1 episode of foot swelling). Abdominal pain disappeared. Since total recovery was not obtained, sitagliptin was stopped in November 2010 and the patient did not report any AE attack for 19 months following withdrawal.

The retained diagnosis was iatrogenic BK-dependent AE, dependent on ACEi treatment, further worsened by DPP-IV inhibitor, in a patient with latent deficiency of the enzymes involved in bradykinin catabolism, namely ACE, APP and CPN. Results of DPP-IV assay were not available.

Discussion

The incidence of AE in patients taking ACEi was evaluated at 0.5% to 0.68% or even 0.9% (10,11). The diagnosis of ACEi-induced AE is difficult to make because of the high variability of symptom occurrence from the first day of ACEi introduction up to 8 years of therapy (12). The time lag between initiation of ACEi and onset of AE was estimated as 10.2 months; however, about 25% of AE attacks occurred during the first month of treatment and up to 27% of cases occurred after more than 6 months, or even several years, after ACEi initiation. As for the case reported here, the time of symptom occurrence was estimated as 5 years. The clinical manifestations of AE ranged from tumefaction, more or less severe, of the tongue, lips, other area of the face, hands, feet or rarely bowel, to life-threatening airway compromise. The severity and lethality correlated with the involvement of larynx (13-15). Dysphagia and change in voice or dyspnoea should receive primary attention from the

		February 2010	July 2010	July 2012	
Current therapy		Lisinopril, Sitagliptin	Sitagliptin ¹	None ^{1, 2}	
C1-INH	Antigenic C1-INH (RV: 210-345 mg/l)	284	ND	262	
	Functional C1-INH (RV: 17.2-27.4 U/ml)	23,5	ND	21.7	
Aminopeptidase P (RV: 0.21-1.82 nmol/min/ml)		0.30 (42%)	0.36 (50%)	0.20 (28%)	
Carboxyper (RV: 35.7-5	otidase N i5.3 nmol/min/ml)	29.2 (64%)	33.3 (73%)	22.8 (50%)	
Angiotensin Converting Enzyme (RV: 43-95 IU)		10.6 (17%)	40 (64%)	35 (56%)	

The percentages refer to residual activity as compared to median reference value. ¹Lisinopril withdrawn on February 2010. ²Sitagliptin withdrawn on November 2010. C1-INH, C1-Inhibitor. RV, Reference values. ND, not determined.

Table 1 - Successive explorations of patient kinin catabolism

physician. ENT injury might be explained by the overexpression of BK-B1 receptors in ENT tissues, a fact demonstrated by animal experiments in ACEi-treated pigs (16). The clinical features reported here were the hallmark of BK-dependent AE, manifesting as a subcutaneous swelling, sometimes highly distorting, that developed over 3 to 4 days, without pruritus and urticaria, and partially refractory to corticosteroids. Episodes of abdominal pain can be intense, leading to suspected occlusion or surgical disease. Their cause is shown by scanner examination, exhibiting specific aspects of oedema of the intestinal walls and/or intra-abdominal effusion. When pain is less severe, symptoms are considered to be functional, as observed in this patient who had been diagnosed with irritable bowel syndrome. The physiopathology of ACE-induced angioedema postulates a deficiency of the catabolism of bradykinin because ACE is the main enzyme implicated in this catabolism. It was confirmed by the study from Agostoni, showing high levels of plasma bradykinin in ACE-induced angioedema (17). A decrease to the normal level is obtained by the withdrawal of ACE inhibitor (18). In addition, it has been shown that, contrasting with the high level of bradykinin, there is no increase of high molecular weight kininogen catabolic products (17).

Six proteases (kininases) are mainly responsible for kinin catabolism: ACE, APP, CPN, CPM, DPP-IV and NEP. During ACE inhibition, BK, *des*Arg9BK and Substance P are metabolized primarily by APP and DPP-IV, respectively. In hypertensive patients who experienced angioedema while being treated with ACEi, decreased APP activity has been demonstrated (3). Patients' APP activity was found to be defective in three successive investigations: 42%, 50% and 28% respectively of the median value. A single nucleotide polymorphism (SNP), c.-2399C>A, in *XPNPEP2*, the X-linked gene encoding for APP, has been reported to be associated with APP activity. In addition, APP activity is lower in men and SNP is associated with an increased risk of ACEi-induced AE in men (odds ratio, OR: 2.17) (19,20). ACE and CPN activities were also found to be decreased when patients were no longer exposed to ACEi (**table 1**).

Individuals suffering from ACEi-induced AE also show a decrease in DPP-IV activity (6,23) and increased levels of Substance P (6). There is a correlation between the degradation half-life of substance P and the level of DPP-IV activity (6). DPP-IV inhibitors (gliptins) have been marketed because they decrease degradation of incretins. These hormones play an important role in glucose homeostasis, stimulating insulin secretion and suppressing glucagon release. Gliptins were then approved for treatment of type 2 diabetes mellitus in 2006 (21). However, they also decrease the degradation of kinins and Substance P. Although no evidence of AE risk was evidenced during phase III studies, the FDA post marketing surveillance of sitagliptin reported 10 cases of AE reactions that occurred within the first 10 months of marketing (22). In a report by the French National Center of Pharmacovigilance, 10 cases of gliptin-induced angioedema have been reported so far: sitagliptin (6 cases), vildagliptin (3 cases), saxagliptin (1 case) (*Communication by the Nancy Regional Centre of Pharmacovigilance*). Brown and colleagues recently presented the results of premarketing surveillance for AE in clinical trials for the DPP-IV inhibitor vildagliptin: the authors reported no association between vildagliptin use and AE; however, vildagliptin use was associated with an increased risk of AE in individuals taking an ACEi (OR: 4.57). The role of Substance P, associated with BK, as a triggering factor of AE could be put forward.

In our case, the AE attacks persisted after several months of ACEi withdrawal. Some AE episodes occurred during gliptin treatment alone, as already documented. We suggest that the combined deficiency of APP and CPN might enhance the effect of the DPP-IV inhibitor since catabolism of bradykinin and substance P might rely predominantly on DPP-IV. A specific interest of this case is that the triple deficiency of enzymes catabolizing bradykinin had been latent and was only revealed by drugs adding their inhibitory action.

Extensive use of the gliptins for treatment of type 2 diabetes and the common association of a DPP-IV inhibitor and an ACEi in diabetic, hypertensive patients, strengthens the need to be aware of their interaction. Even if DPP-IV inhibitors may have differential impact on DPP-IV, as suggested in a paper, the benefit-risk ratio of the combined prescription of an ACEi and a DPP-IV must be carefully assessed (24). This case suggests to search for the levels of kinin catabolism enzymes (ACE, APP, DPP-IV and CPN) when there is intention to treat with combined drugs at risk to interfere with bradykinin metabolism.

Statement of contribution

E. Beaudouin: is the referent allergist in charge of the patient. He wrote a part of the text.

F. Defendi (French Reference Center for Angioedema): dosage of functional activities of bradykinin catabolizing enzymes. She reviewed the text.

J. Picaud: allergist associated with the first author, in charge of the patient. Contributed to the search of references.

C. Drouet (French Reference Center for Angioedema): he participated to the discussion and reviewed the text.

D. A. Moneret-Vautrin: wrote a part of the text, contributed to the discussion, and reviewed the text.

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Anaphylactic shock to raspberry

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Raspberry (*Rubus idaeus*) is a shrub belonging to the *Rosaceae* family: sub-family *Rosoideae*, gender *Rubus*, and species *Rubus idaeus*. The homonymous fruit is a drupe and is a very enjoyed food. Recently, Marzban and colleagues identified four IgE-reactive proteins in raspberry (1). These authors initially detected two potential allergens Rub i 1 and Rub i 3, using polymerase chain reaction. Rub i 1 and Rub i 3 showed high sequence identity to proteins in Rosaceous species: like Mal d 1 and Mal d 3 from apple. Further, Marzban and colleagues identified a new protein with high sequence homology with class III chitinases. Finally, they detected a raspberry cyclophilin, homologous to Bet v 7. These findings could suggest that raspberry ingestion might cause allergic symptom occurrence, such as IgE-mediated, in sensitized patients.

However, a case alone of allergic reaction to raspberry has been described so far. In fact, a first report concerned a case of anaphylaxis in a milk-allergic child after ingestion of milkcontaminated kosher-pareve-labeled "dairy-free" dessert. The described case occurred after ingestion of "pareve"-labeled raspberry sorbet in a child with milk allergy (2). Actually, anaphylaxis was due to milk-allergy and not to raspberry, as the food was contaminated by milk. The true case of raspberry allergy was occupational. This case concerned a 35-year-old woman who complained of hay fever symptoms, wheezing and breathlessness 2-3 times a month, exclusively in association with inhalation of raspberry powder, used for coating chewing gum (3). Both skin prick test and serum IgE assay were positive for raspberry. Therefore, this was the first and unique description of allergic reaction due to inhalation of raspberry powder.

We report a case of a 52-year-old woman who had an anaphylactic shock immediately after ingestion of raspberry during a quite walking tour in the Alps. She ingested raspberry 4 hours after the last meal, during a pause. She suddenly presented intense itching to palms, dyspnea, and intense flushing, rapidly followed by syncope with sphincters' relaxation. She was assisted and transported by a rescue helicopter to the nearest hospital. After adequate treatment, she recovered without *sequelae*.

During a first allergist visit, she denied any previous allergic reaction. Skin prick test (performed using commercial extracts) was positive only to birch and hazelnut (mean wheal 2 mm; histamine wheal 3 mm). Serum IgE were measured only by ISAC methods, as specific IgE to raspberry is not assayed in the laboratory of the referential hospital. ISAC results showed that rPru p 3 was 1.0 ISU-E and nJug r 3 was 0.6 ISU-E. Then, serum was assayed by ImmunoCAP system: rPru p 3 was 1.94 kUA/L and rCor a 8 was 0.06 kUA/L. The patient was re-evaluated after the results: a more detailed history confirmed that she felt itching after the contact with the peach peel. After 2 months, a prick by prick was performed: raspberry fruit induced an 8 mm wheal. Therefore, the clear relationship between ingestion of raspberry and sudden anaphylaxis (post hoc ergo propter hoc), the positive prick by prick testing, and serum positivity to Rosaceae fruits allows to determine the causality. The explanation of the cross-reactivity between Rosaceae family fruit allergens derives from the matter that fruit proteins with high primary sequence similarity display also homologous tertiary structures, resulting in similar epitopes to IgE molecules (4).

This is the first description (at our best knowledge) of anaphylactic shock after ingestion of raspberry. We would like to emphasize the clinical relevance of history and molecular diagnosis. In fact, to detect positivity to lipid transfer proteins gives important information about the severity of allergic reaction, the prognosis, and mainly the dietary restriction.

The diagnostic workup of severe anaphylaxis should be based on the rigorous demonstration of a cause-effect relationship between suspected food ingestion and documented sensitization, using skin testing and/or serum allergen-specific IgE measurement. In fact, food challenge must be avoided for legal reason. Therefore, consistent history and proved allergy should be sufficient for identifying the causal allergen. However, it should be recommendable also in the routine practice, if available, to use component resolved diagnosis for obtaining more valuable information about risk factors and possible co-sensitizations or co-recognitions.

In the present clinical case, history and prick-by-prick were consistent in defining the culprit allergen protein, such as raspberry. Furthermore, CRD, based on findings of the ImmunoCAP and ISAC, allowed to identify co-sensitization with other *Rosaceae* fruits, mainly concerning LTP proteins. This information was useful both for interpreting past reaction to peach and for advising preventive food rules.

In conclusion, also a fruit believed to be harmless may be dangerous, and positivity to LTP should suggest to be cautious with *Rosaceae* fruits.

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O. de Beaumont, T. Yalaoui

Comments on: "Allergen immunotherapy as a drug: the new deal of grass allergen tablets from clinical trials to current practice"

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In their recent article (1) published in *European Annals of Allergy* and Clinical Immunology, Manzotti and Lombardi evaluated the available trials with Grazax[®] and Oralair[®] to support their use in clinical practice.

First, we have noted with particular interest the position of the authors regarding the pre-seasonal and co-seasonal schedule. They consider it to be: "the most suitable schedule for pollens in clinical practice instead of continuous immunotherapy". Though, the efficacy of Grazax[®] has been assessed with a continuous protocol over the 3 years of treatment, its long-term efficacy and safety when administered discontinuously has yet to be assessed. To date, Oralair[®] is the only allergen immunotherapy sublingual tablet with demonstrated efficacy and safety using a pre-seasonal and co-seasonal treatment regimen. Moreover, the authors stated that "Oralair[®] has been shown to be effective and safe in two Phases III double-blind placebo controlled trials"... "and in a trial based in an allergen challenge chamber." In fact, since Oralair[®] has been marketed in 2008, two additional clinical trials (VO53.06 and VO61.08USA) have been completed, bringing the total to four natural field studies including 2012 patients, in addition to the 89 patients in the allergen challenge chamber study (VO56.07A).

Study VO53.06, a multicenter, randomized, controlled trial, evaluated the long-term effect of pre-seasonal and co-seasonal administration of Oralair[®] over a period of three consecutive pollen seasons followed by an observation time. The clinically relevant efficacy shown during the first three years (2) was maintained during the first treatment-free follow-up year, indicating post-treatment long-term efficacy (3).

		Onset of action				
Trial	No. of pts	Type of pts / +Type of the disease of pts included in the study	ARTSS after 1 month	ARTSS after 2 months	ARTSS after 4 months	Oralair [®] Improvement vs. Placebo at 4 months
Horak et al, 2009	89	Adults / Grass pollen-induced rhinoconjunctivitis	$-5.89\pm 2,431$ p = 0.0042	-5.09 ± 2.088 p = 0.200	-4.85 ± 1.995 p = 0.0007	29.3%

Table 2 - Oralair® study in pollen chamber

The VO61.08USA trial (4) conducted in US adult patients with grass pollen-induced allergic rhinoconjunctivitis showed that pre-seasonal and co-seasonal treatment with Oralair[®] demonstrated clinically meaningful efficacy.

With respect to **table 2** - Synopsis of Phase III Oralair[®] studies, we note a number of errors with respect to the results of study VO56.07. We have provided the corrected data. In addition, the correct reference is "*Horak F, Zieglmayer P, Zieglmayer R, Lemell P, Devillier P, et al. Early onset of action of a* 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. J Allergy Clin Immunol. 2009 Sep;124(3):471-7, 477.e1".

Lastly, the authors have noted that "in fact, an extract with only Phleum pratense seems adequate for patients living in Northern Europe but not for patients living in Mediterranean areas." Actually, the 5-grass pollen extract better represents natural exposure conditions encountered by grass pollen-allergic patients, because the 5 species are broadly distributed throughout Europe and North America and their allergen content has been well characterized (5).

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Reply

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It's a great pleasure for us to understand that our colleagues Dr de Beaumont and Dr Yalaoui could find our paper interesting enough to publish their letter (1); it's a honor for us to have the opportunity to answer them on this journal.

As also they reported, the aim of our paper was to evaluate the available trials, at the date of article submission, with Grazax[®] and Oralair[®] to support their use in clinical practice.

Our position regarding the pre-seasonal and co-seasonal schedule is not a personal one, but is coming from international reports in literature. According with this administration schedule, we presented all phase III studies about Grazax[®] and Oralair[®], designed in a very similar way because focused to the same objective: to demonstrate efficacy and safety in order to obtain marketing authorization from European Medicine Agency (EMA). Our purpose was not to define if Grazax® used with a pre-co-seasonal schedule was the "best option" in using that, instead we were looking for evidence from the studies for a possible Grazax® use with a pre-seasonal schedule as we usually prescribe in clinical practice. We concluded with a clear position: "Although no proper pre-seasonal trials with Grazax[®] are today available, we can be optimistic about the pre-seasonal use of this product because it seems to give worthwhile results since the first months of the first year of treatment, in adult, in children and adolescents, but more evidence is required".

We have also reinforced this statement, reporting in **table 1** four studies conducted with Grazax[®] with a range of treatment duration from 5.3 months to 7 months.

We also reported that Oralair[®] is the only allergen immunotherapy sublingual tablet with demonstrated efficacy and safety using a pre-seasonal and co-seasonal treatment regimen.

We apologize for the mistake about **table 2** and we are very grateful to the colleagues for the opportunity to make correction as they did.

Moreover, we would like to thank the colleagues to give us the opportunity to complete our overview about both immunotherapy drugs because the two studies they mentioned have been completed and published after the submission of our article (2,3).

Lastly, we concluded with the statement: "Which patient for which grass pollen drug? We have no definite answer today". At the moment there are not enough studies to define the best grass allergens to put into a grass pollen immunotherapy. Grass pollen allergy is common worldwide, and group 1 and group 5 allergens (Phl p 1 and Phl p 5) are the dominating grass pollen allergens. More than 90% of subjects with sensitization to grass pollen have IgE abs to Phl p 1 and/or Phl p 5 (4,5). The presence of specific components for grass (like Phl p 1 and/or Phl p 5) is fundamental for a better indication for SIT (6). SIT treatments are expensive and prescribed for several years and a correct diagnosis is therefore important.

In conclusion we would like to thank our colleagues for the opportunity to make correction and to add data to an article that can be very useful to clinical allergists that deal with patients and their daily problems all the time.

- 1. Manzotti G, Lombardi C. Allergen immunotherapy as a drug: the new deal of grass allergen tablets from clinical trials to current practice. Eur Ann Allergy Clin Immunol. 2013;45(2):34-42.
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