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Oral food challenge in children: an expert review

Position paper of the Section of Pediatrics of the French Society of Allergology and Clinical Immunology (SFAIC) and of the Pediatric Society of Pulmunology and Allergology (SP2A)

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Key words

Oral food challenge, food allergy, child, skin prick test, atopy patch test, specific IgE determination, adrenaline, GRADE evidence

Summary

Oral food challenges are indicated for the diagnosis of food allergy and the double-blind, placebo-controlled oral food challenge is considered the gold standard diagnostic method in children with suspected food allergy. This practice parameter for oral food challenges in children was prepared by a workgroup at the request of the French Society for Allergology and Clinical Immunology (SFAIC) and the French Paediatric Society for Allergology and Pulmonology (SP2A). We aimed to develop practical guidelines for oral food challenges in children for the diagnosis of suspected food allergy or the evaluation of food tolerance. We also considered the safety measures to be implemented during testing and management of the potentially serious allergic reactions that may arise during the test. The strength of the recommendations was established, using the GRADE evidence-based approach. We considered four issues: 1) the selection of children for oral food challenges (indications and contraindications); 2) the procedure used (material, where the test should be carried out, technique and management of reactions); 3) interpretation of the test and 4) consequences of the test.

Abbreviations: FA, food allergy; OFC, oral food challenge; DBPCFC, double-blind placebo-controlled food challenge

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Introduction

The frequency of food allergies (FAs) is currently estimated at around 5% of the paediatric general population (1, 2). FAs in children may be life-threatening (3, 4) and have become both a major public health problem and a source of concern to many healthcare professionals. Treatment is based on avoidance, which may be difficult to achieve given the high frequency of masked allergens (5). Children may grow out of some FAs, whereas others may persist and alter quality of life. FAs have particularly important repercussions for children of school age (6, 7).

The correct diagnosis of FAs on the basis of reliable criteria is therefore essential, together with follow-up of their progression. This requires a combination of skin tests (skin prick tests and atopy patch tests in some cases), specific IgE determinations and oral food challenges (OFCs) (1, 8). There is currently a trend towards the development of screening tests for FA diagnosis, reducing the indications for OFCs. This approach has resulted in the establishment of threshold values for skin tests and specific IgE predicting the likelihood of a clinical reaction (9-18). However, threshold values have not been established for all foods, and they depend on the food considered, the study population, the age of the child at the time of diagnosis and the symptoms (19). Thus, in practice, with the exception of certain well defined situations, OFCs are still frequently indicated, and the double-blind placebo-controlled food challenge (DBPCFC) is the gold standard for FA diagnosis (5, 8).

In OFCs, the subject is asked to ingest the food tested, with the aim of reproducing the symptoms, taking into account the time and the quantity of the food required to generate symptoms. OFCs can be used to evaluate the amount of a food required to trigger symptoms (expressed as a cumulative reactogenic dose, eliciting dose or as the dose triggering symptoms) and the nature of clinical signs related to ingestion of the suspected food. Indications for this test are now better known (20-25). However, no global recommendations developed from a literature review have ever been published concerning the indications, consequences and safety measures relating to OFCs or the management of allergic reactions arising during these tests.

This document is an expert review, prepared by a workgroup at the request of the French Society for Allergology and Clinical Immunology (SFAIC) and the French Paediatric Society for Allergology and Pulmonology (SP2A). We aimed to build a practice parameter and to formulate recommendations specifying the indications, procedure and consequences of OFCs in children. We considered four major issues: 1) the selection of children for OFC (indications and contraindications); 2) the complete procedure which should be followed (material, where the test should be carried out, technique and management of reactions); 3) the interpretation of the OFC and 4) the consequences of the OFC. These recommendations focus in particular on the three major foods most frequently implicated in FA in children: cow's milk, hen's eggs and peanut. OFCs are carried out similarly for other foods and these recommendations could therefore be applied to other foods. These recommendations concern paediatric tests, and are aimed at physicians involved in the management of FA in children.

We carried out a literature review, based on studies published between 1971 and 2007 identified by querying the PubMed® database. The search was limited to studies published in English or French. Some articles were also identified from the bibliographic references cited in the articles identified by the PubMed® query. In this analysis, priority was given to systematic reviews, studies of cohorts of allergic children and recommendations issued by scientific societies. The working draft of this practice parameter was reviewed by a large number of experts on FA. The working draft concerning each issue was published in French (26-31). This document represents an evidence-based and broadly accepted synthesis and consensus viewpoint of the working group on OFC for FA in children. The strength of the recommendations and the quality of the evidence were defined according to the GRADE evidence-based approach (Tab. 1) (32).

I What are the indications and contraindications for OFC?

The main indication for OFC is testing whether a child is allergic to the food suspected (grade 1A). The indications for OFC are: (i) testing whether a child is allergic or tolerant to a particular food and (ii) determining whether a child has grown out of the FA and whether the food can safely be reintroduced into the diet. Indications for OFC should also take into account the food concerned (nutritional value, difficulties with avoidance), signs associated with the FA, the age of the child, the course of the allergy and the constraints imposed by the FA (19-25).

The clinical situations analysed included both immediate (generally within two hours, more rarely within four

Table 1 - Grading recommandations according to the GRADE working group (32)

Grade of recommendation	Benefit vs risk and burdens	Methodological quality of supporting evidence	Implications
1A: strong recommendation, high-quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	RCTs without important limitations or overwhelming evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
1B: strong recommendation, moderate quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
1C: strong recommendation, low-quality or very low-quality evidence	Benefits clearly outweigh risk and burdens, or vice versa	Observational studies or case series	Strong recommendation, but may change when higher quality evidence becomes available
2A: weak recommendation, high-quality evidence	Benefits closely balanced against risk and burdens	RCTs without important limitations or overwhelming evidence from observational studies	Weak recommendation; best action may differ depending on circumstances or patients' or societal values
2B: weak recommendation, moderate-quality evidence	Benefits closely balanced against risk and burdens	RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies	Weak recommnendations; best action may differ depending on circumstances or patients' or societal values
2C: weak recommendation, low-quality or very low-quality evidence	Uncertainty in the estimates of benefits, risk, and burden; benefits, risk, and burden may be closely balanced	Observational studies or case series	Very weak recommendations; other alternatives may be equally reasonable

hours of ingestion) and delayed (atopic eczema, gastrointestinal food-induced allergic disorders) manifestations and sensitisation to a food that the child had never consumed.

I.1 Diagnosis of FA

I.1.1 General aspects

Clinical history, skin tests (skin prick tests and atopy patch tests) and specific IgE (ImmunoCap, Phadia, Uppsala, Sweden) determinations may lead to OFCs (grade 1A). History checks the time at which occur the symptoms, the relationship with any feeding, and clinical features. OFC is not indicated in children with a clinical history suggestive of allergy and positive results in skin tests or specific IgE (5) (grade 1A). The clinical history is considered suggestive of allergy if associated with an IgE-dependent mechanism – if cutaneous signs (eczema, rash, urticaria, angioedema), gastrointestinal signs (nausea, vomiting, diarrhoea, abdominal pain), respiratory signs (rhinoconjunctivitis, cough, respiratory distress, bronchospasm) and/or arterial hypotension occur shortly after ingesting the food. Anaphylaxis is a life-threatening event, but may also be defined as the occurrence of clinical signs affecting at least two organs (3, 4).

OFC is indicated if clinical history is not considered sufficiently convincing – if the symptoms reported are imprecise and/or do not seem to be markedly associated with consumption of the food concerned, particularly in cases of atopic eczema.

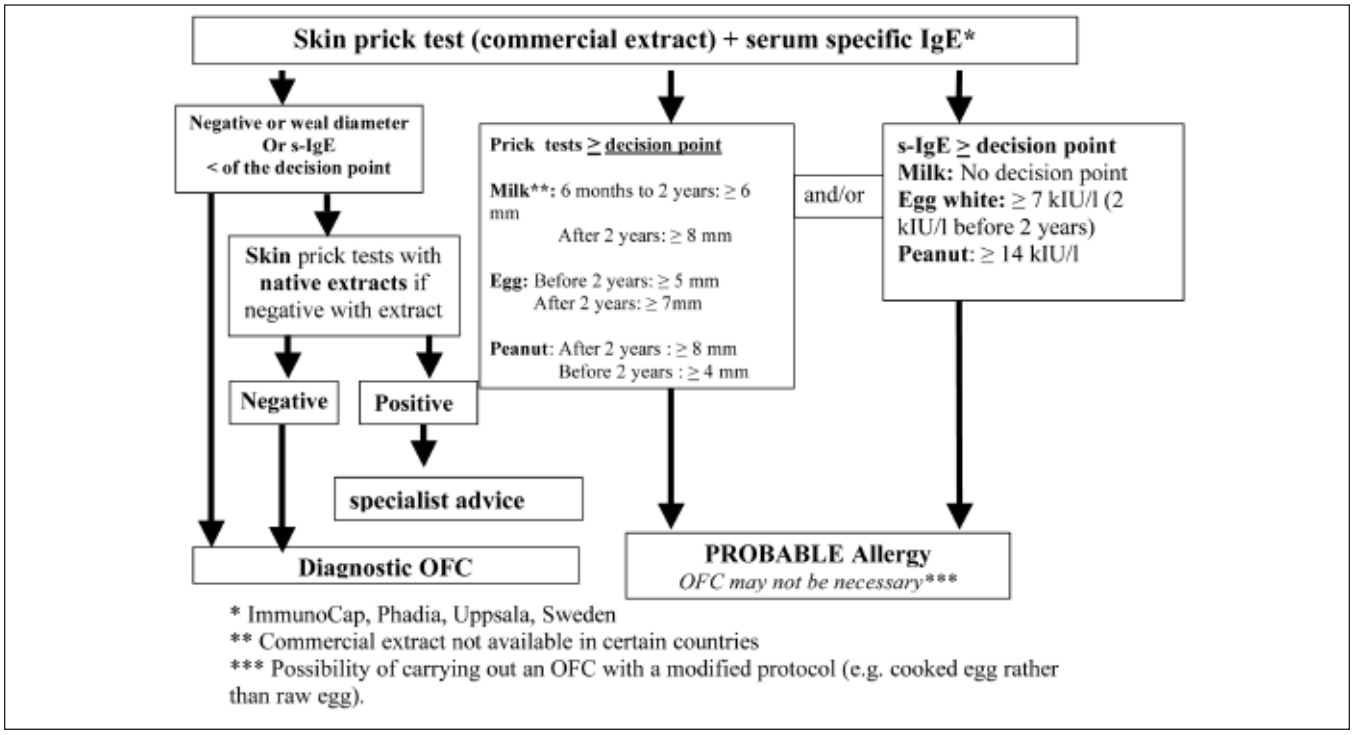
I.1.2 Indication for OFC in cases of suspected IgE-dependent FA

The indication for OFC in cases of suspected IgE-dependent FA is based on decision point values for skin prick tests and food-specific IgE tests (ImmunoCap, Phadia, Uppsala, Sweden), when such values exist (Fig. 1). However, decision points may vary with the method, extract, foods involved, and features of the population, such as age and disorders considered (24, 33, 34). Negative results in prick tests using a commercial extract should lead to control with the natural food (16, 35-37) (grade 1B).

Peanut: The decision points established for peanut are a weal of at least 8 mm diameter in children over the age of two years and of at least 4 mm in children under the age of two years in skin prick tests with commercial extracts, or a specific IgE concentration of at least 14 kIU/l (ImmunoCap, Phadia, Uppsala, Sweden) (13-15, 38, 39) (grade 1C).

Cow milk: The decision points established for cow's milk are a weal of at least 8 mm diameter in children over the age of two years and of at least 6 mm in children under the age of two years, in skin prick tests with commercial extracts, which are no longer available in certain countries (including France) (15) (grade 1C). It is currently not possible to calculate a decision point for specific IgE levels for cow's milk from published data (11-13, 17). The decision point varies according to age group and the prevalence of FA and atopic eczema in the population studied. Garcia-Ara et al. obtained a threshold level of 2.5 kIU/L with a positive predictive value (PPV) of 90% (mean age of 6.5 months, FA prevalence 44%) (11). In the study by Roehr et al., the threshold level was 17.5 kIU/L, with a PPV of 86%, for a population with a mean age of 13 months and an FA prevalence of 55% (12). In the prospective study by Sampson and Ho (13), a threshold level of 32 kIU/L with a PPV of 95% identified in a retrospective study (9) led to an OFC being carried out in 34% of cases. The mean age of the patients was 3.8 years and the prevalence of FA was 66%. Celik-Bilgili et al. reported a threshold level of 88.8 kIU/L, with a PPV of 90%, in a population with a mean age of 13 months and an FA prevalence of 49% (17).

Figure 1 - Diagnostic procedure for children with suspected IgE-dependent food allergy (cow's milk, hen's eggs, peanut



Hen's egg: The decision points established for hen's egg are a weal of at least 7 mm in children over the age of two years and of at least 5 mm in children under the age of two years, in skin prick tests with commercial extracts (egg white) or a specific IgE concentration of at least 7 kIU/l (2 kIU before the age of 2 years) (egg white, ImmunoCap, Phadia, Uppsala, Sweden) (10, 13-16) (grade 1C).

I.1.3: Indication for OFC in cases of suspected delayed reaction

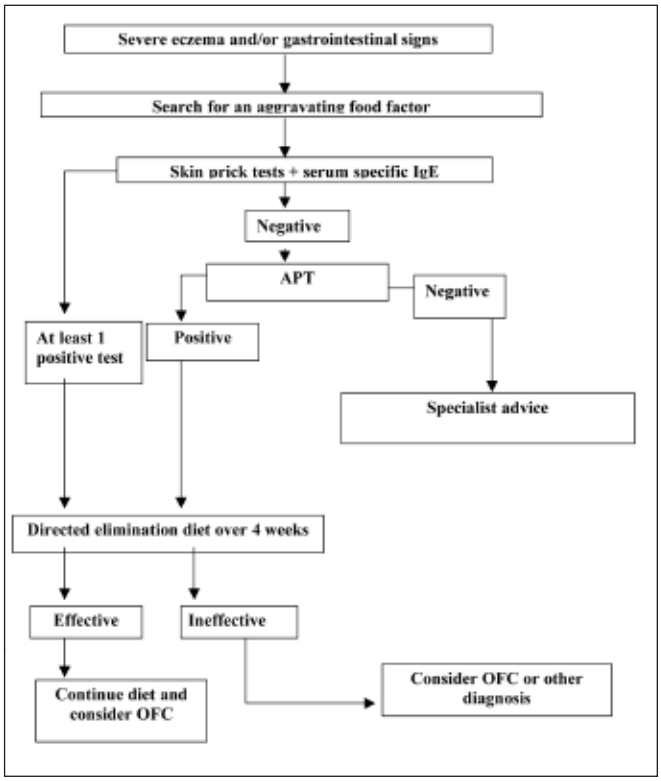
In cases of delayed reactions, eczema or gastrointestinal disorders, OFC is indicated if an avoidance diet for the food identified in allergy testing (skin prick tests, specific IgE , atopy patch test) and/or food diaries – maintained over four weeks, or possibly longer for gastrointestinal symproms, according to the EAACI position paper – proves to be effective, particularly for gastrointestinal symptoms (Fig. 2) (5, 18, 25, 33, 40-44) (grade 1C). If an improvement is observed, the timing of an OFC should be discussed, on a case-by-case basis, in specialist consultations.

Atopy patch tests can be useful as an additional diagnostic tool, following negative prick test and undetectable specific IgE, in cases of delayed reactions, particularly to cow's milk (18, 44-47) (grade 2B). However, a recent evaluation of children with atopic eczema suggested that the need for OFC was not significantly lower in cases of suspected food-induced eczema (18). Additional studies are required to resolve this issue. Standardized atopy patch tests could be useful in the diagnostic work-up for children with gastrointestinal symptoms (41, 42) (grade 2C).

I.1.4 Indication for OFC in cases of sensitisation to foods never consumed

In children sensitised to foods that they have never consumed, and in documented cases of sensitisation or suspected cross-reaction between food allergens (Fig. 3), the indications for an avoidance diet or OFC depend on the food concerned, the age of the child and the results of allergological tests, according to specialist advice (grade 2A). If an avoidance diet is prescribed, allergological assessment should subsequently be repeated (grade 2C). When testing for cross-reactions, a negative skin prick test with the food in its native state rules out allergy to that food. If the skin prick test is positive and the child has never consumed the food, the possibility of carrying out an OFC should be discussed during a specialist consultation (33) (grade 2B).

Figure 2 - Diagnostic procedure for children with delayed signs (eczema, gastrointestinal signs)



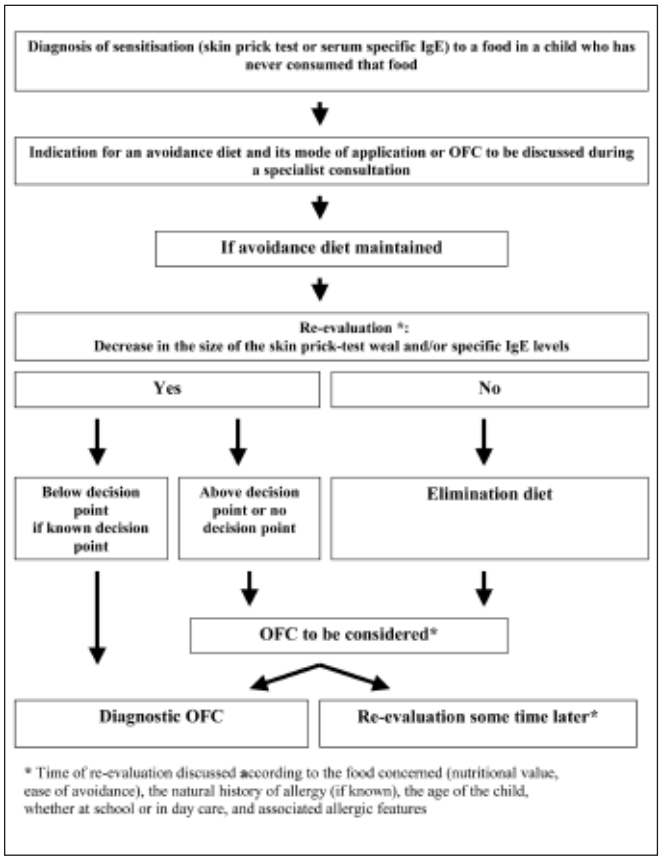
I.2 Evaluation of the eliciting dose

OFC can be used to determine the amount of a food required to trigger symptoms (expressed as the *eliciting dose or cumulative reactogenic dose*). Determination of this dose alone is not an indication for OFC in clinical practice, because it may be affected by several factors such as fat content, may change over time, or may be different in real life (48-51) (grade 1C).

I.3 Evaluation of tolerance to a food

The aim is to define indications for OFC in children with a known FA, when the natural history of this allergy is naturally progressing towards possible resolution. Tolerance to cow's milk and hen's eggs is frequently acquired, but is rarer for peanut (1, 52-56) (grade 1C). Tolerance is rarely acquired after the age of five to seven years and is almost never acquired after the age of 12 years (4, 57-59)

Figure 3 - Diagnostic procedure for children sensitised to a food that they have never consumed



(grade 1C). For this indication, the OFC is sometimes referred to as a *reintroduction test.* Before OFC, it is important to obtain agreement with regular food consumption in case of negative food challenge (60) (grade 1C).
The skin prick test weal diameter and/or specific IgE level values considered significant for the acquisition of oral tolerance depend on the food considered, the age of the child and the nature of the initial clinical reaction. An analysis of intra-individual variations in specific IgE levels may be useful (33, 61-65). In individuals, OFC may be considered when serum food-specific IgE levels decrease to a range at which about 50% of children of the corresponding age tolerate the food concerned, e.g. < 2 kIU/l for hen's eggs (white), < 2 kIU/l for cow's milk, < 5 kIU/l for peanut with an uncertain medical history of allergy or < 2 kIU/l if there is a clear history of allergic reactions (ImmunoCap, Phadia, Uppsala, Sweden) (33, 64).
Finally, the decision to carry out an OFC depends on medical history, current age of the patient, age at which the FA considered is most frequently cured (1 year for cow's milk, 3 years for hen's eggs and 6 years for peanut), and repeated test results (grade 1C).

I.4 Exclusion criteria for OFC

The appropriate selection of indications for OFC should limit the risk of severe reactions. The size of the weal in skin prick tests and specific IgE concentrations are not predictive of the severity of the clinical reaction or of the minimal dose required to trigger symptoms (62, 66) (grade 1C). OFC is rarely performed in infants under the age of 6 months. Nevertheless, age is not a contraindication. The pollen production season may modify the outcome of challenge tests for fruits and vegetables associated with an oral allergy syndrome and cross-reaction to pollen. Nevertheless, season is not a contraindication for fruit and vegetable challenge in cases of oral allergy syndrome.
Exclusion criteria for OFC include (20-25) (grade 1C):
- Active chronic disease
- Poorly controlled asthma or FEV_1 below 80% of the predicted value
- Recent anaphylactic reaction to a food, with consistent allergological test results
- Absence of consent
- Relative contraindication: treatment which may mask or delay clinical reactions or may interfere with the treatment of such reactions (beta blockers, aspirin and non-steroidal anti-inflammatory drugs, ACE inhibitors).

II In what environment and what conditions should OFC be carried out?

II.1 Where should the OCT be carried out?

The OFC should be carried out in hospital environment with facilities for managing severe allergic reactions, geographically close to an intensive care unit with medical and paramedical staff experienced in performing the procedure. The test should be carried out in appropriate conditions, with the necessary level of safety, monitoring and evaluation. The prior information of patients and their families and the obtainment of informed consent are essential for OFCs. The nurses involved must therefore have experience of both carrying out the test and monitoring reactions to the test and a doctor must be present on the site (8, 24, 25, 67) (grade 1B). Any site at which OFCs are carried out should have monitoring facilities, and the drugs and mate-

rials required for resuscitation, to ensure that reactions can be treated appropriately, regardless of their severity or the age of the child (8) (grade 1B). Before beginning the test, the doctor responsible for monitoring should write a protocol for the treatment of adverse reactions.
Day hospital admission may be sufficient. However, monitoring for at least four hours after administration of the last dose is recommended, to cover the period in which immediate severe reactions may occur and for the diagnosis of certain delayed reactions. The occurrence of a reaction may lead to hospitalisation for observation. For delayed symptoms, such as eczema, the OFC should be started in a hospital environment, but extended challenges may then be continued outside the hospital. It may be relevant to complete the test at the hospital or to ask the patient to return for evaluation if symptoms occur (or to take pictures or videos).

II.2 Preparation of the foods used for OFCs

The allergenicity of foods may depend on their presentation (48, 49) (grade 1B). In practice, it is recommended to test the food in the form consumed by the patient (roasted peanuts, for example). For foods consumed in several forms, the choice depends on the indication for OFC (e.g. raw egg, cooked egg) (68). The use of lyophilized food in capsules is not recommended in children, because oral allergy syndrome can be overlooked and the dose may not be high enough.
In open testing, the vehicle used should render the food acceptable to the child. In blind testing, the vehicle is used to disguise the taste of the food. There are currently no standardised, validated consensual recommendations. A recent study indicated inherent difficulties in this procedure (69). A non-suspect food can be used to mask the test food and as a placebo. The preparation to be tested and the placebo must have similar tastes, appearances, odours, textures and volumes (69, 70). The food should be present in the vehicle at the highest concentration possible at which it remains undetectable. All ingredients likely to provoke undesirable reactions should be avoided. The vehicle should have a low fat content, particularly for peanut challenges (49). Paste-like vehicles, such as mashed potato and apple compote, are the most frequently used, but liquid vehicles are also possible. Liquid foods can be masked in extensive hydrolysates of cow's milk, or in amino acid-based formulas. A dietician or pharmacist may have a useful input in the development of recipes and reintroduction protocols.

II.3 The patient

II.3.1 Diet

The food tested should, in all cases, have already been eliminated from the child's diet. The main purpose of the diet is to ensure that the patient is symptom-free or as close to symptom-free as possible, for diagnostic OFC and its evaluation. The food concerned should therefore have been avoided for at least seven to 14 days (depending on the food) for immediate reactions and at least four to six weeks for delayed reactions (25, 43) (grade 1C). For children who are still breast-feeding, the suspect food should be eliminated from the mother's diet (23). A dietician may be required to control the nutritional aspects of an avoidance diet.

II.3.2 Clinical state and maintenance treatment

The OFC should be carried out in conditions of clinical stability, in the absence of other signs (e.g. infections) likely to make interpretation difficult. In cases of atopic dermatitis, the OFC should be carried out on patients with minimal treatment or no local treatment (25, 71) (grade 1C). In the case of patients with co-existing or food-induced asthma, short-acting inhaled agonists and inhaled anticholinergics may be continued up to four and six hours before challenge, respectively. Maintenance treatments for asthma should be continued, even on the day of the test (24, 72) (grade 1C).
Some treatments that may modify the result of the test should be stopped at various times before the OFC. Leukotriene receptor antagonists should be withheld for up to one week, antihistamines for a minimum of 48 hours (hydroxyzine should be stopped 72 hours before the test and latest-generation antihistamines should be withheld for at least one week) (22). Other maintenance treatments, such as neuroleptics, oral corticosteroids and immunosuppressors, are not compatible with OFC (24) (grade 1C).

II.3.3 Should the OFC be carried out in the fasting state?

The child should have fasted for at least two hours before the OFC, to prevent any interference with the food tested (immediate reaction) and to prevent the occurrence of clinical signs attributable to fasting and making interpretation of the test difficult. During the OFC, the medical staff may allow the child to eat certain foods with no risk of reaction. Water and apple juice are authorised.

II.3.4 Should an intravenous catheter be implanted?

The insertion of a intravenous catheter before the OFC is recommended, due to the unpredictable and sometimes serious reactions observed and the possible requirement for treatments administered intravenously (24) (grade 1C). However, this measure is not indispensable and should be considered on a case-by-case basis, as a function of clinical history, age, allergic background, the food concerned and the results of allergy testing (73).

II.3.5 Clinical evaluation before the test

A clinical evaluation should be carried out before the OFC. The results and monitoring parameters should be reported on a monitoring sheet, dated and signed by the doctor, authorising or prohibiting the OFC, according to the state of the child (74).

III What procedures should be used when carrying out an OFC?

III.1 Types of challenge

OFCs can be carried out in open, single-blind or double-blind procedures. First-line tests in children are carried out in open conditions, particularly when searching for objective signs in a young child (8) (grade 1C). *For open challenge tests,* the food is given in its natural form. *For single-blind tests,* the food (or placebo) is given in a vehicle that disguises the appearance and the taste of the food. The child is unaware of the nature of the food given (test food or placebo), whereas the doctor, nurses and parents have this information. *For double-blind, placebo-controlled tests,* none of the parties involved (patient, doctor, nurses, family) is aware of the composition of the product delivered to the child. The double-blind method is the preferred method for scientific research protocols. DBPCFC is considered to be the gold standard (24). DBPCFCs are particularly recommended for studies of delayed reactions (e.g. eczema) and in cases of a particular psychological context or of subjective symptoms (e.g. abdominal pain in older children) (33). It is always followed by the ingestion of the food in its usual quantity, in open conditions, with monitoring (75) (grade 1B). The interpretation of DBPCFCs is summarised in table 2. In infants and young children, open controlled OFC are sufficient for FA diagnosis (24).

Table 2 - Interpretation of double-blind, placebo-controlled oral food challenge

Food	Placebo	Recommandation
+	-	Avoidance diet
+	+	Repeat the test
-	-	No diet
-	+	No diet

III.2 Dosing

The food is given in incremental doses, beginning with an initial dose of 1 mg (possibly even less) to 250 mg (protein content), depending on the indications. The lowest doses are used for subjects with a history of severe reactions. Reference to natural foods or food proteins may be used and this choice should be defined in advance. Reference to protein content is preferable, but it should be specified whether the weight of the natural food is used for the challenge. The OFC may be preceded by a labial challenge test (76) (grade 2B). However, some children may have contact reactions during labial challenge despite tolerating oral intake.

Incremental doses are delivered every 15 to 30 minutes (24). In the absence of clinical signs, the highest dose administered should correspond to the normal daily intake for children of the corresponding age (24). There is no standardised protocol. Various incremental protocols are available and the choice depends on the clinical history of the subject. In patients with delayed symptoms, the OFC is carried out over several days, and the monitoring procedure focuses on delayed symptoms (25, 40). Certain clinical situations require particular procedures. This is the case for exercise anaphylaxis, in which the OFC must be combined with physical exercise. There is no validated procedure for tests of this type.

In DBPCFCs, the placebo is given in the same incremental manner, on another day, chosen at random (20, 21, 24, 25) (grade 1B). It is also possible to integrate ingestion of the placebo into the progression (51). Finally, the OFC may be carried out over several days, alternating ingestion of the test food and of the placebo. This procedure is particularly useful for delayed or subjective signs (24).

III.3 Specific features linked to the food

As a rule, the total dose should correspond to a normal daily intake (20-22, 23-25) (grade 1B). For peanut, OFCs

with cumulative doses of at least 8 g (1 peanut weighs about 600 mg) can be carried out. For cow's milk, progressive increases for OFCs are measured in ml (1 to 250 ml). The dose of milk administered should be adapted according to the age of the subject. The milk generally consumed by the child should be used. OFCs for hen's eggs can be carried out with raw or cooked egg, with the total amount corresponding to the equivalent of one egg. For OFCs with raw egg, the use of a low initial dose is recommended. A negative result for OFC with cooked egg shows that the child tolerates cooked egg, but provides no information about the tolerance of raw egg (68).
The progressive increases used for OFCs with other foods are highly variable. The initial dose takes into account the severity of symptoms already presented, or the food (e.g. low initial dose for fish, shellfish, and sesame). The final dose may be high (e.g. for wheat).

IV How should allergic reactions occurring during OFCs be managed?

The clinical symptoms likely to occur during food OFCs range from benign signs (often cutaneous) to more serious respiratory and/or cardiovascular signs. It is important to bear in mind the possibility of anaphylactic reaction. H1 antihistamines may mask the early signs of anaphylaxis. Recognition of the initial symptoms, followed by the treatment of these symptoms, may prevent progression to more serious clinical situations (22) (grade 1C). Patients with persistent asthma have the highest risk of anaphylaxis.

IV.1 Drugs

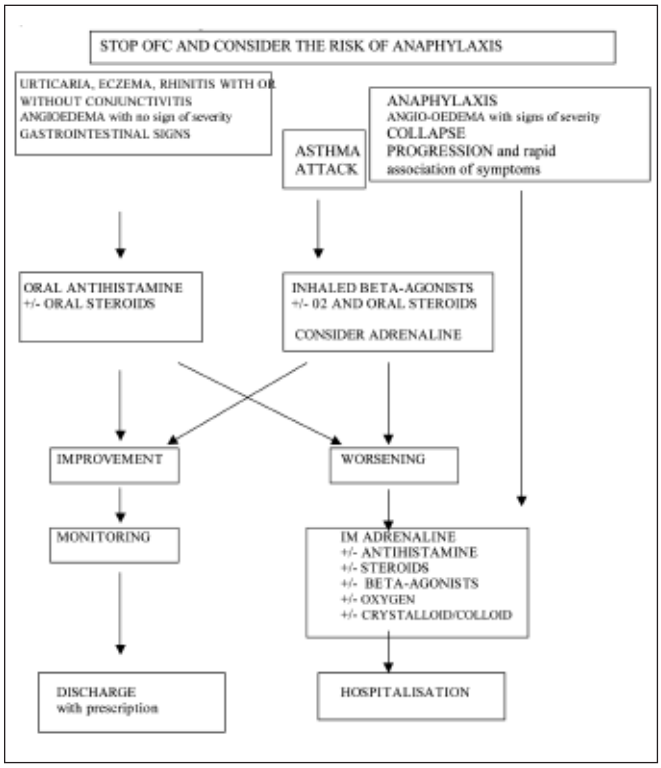
Adrenaline is the first-line treatment for anaphylaxis (3, 4, 67) (grade 1A). There is no contraindication for its use in paediatrics. Delayed injection is associated with a poor prognosis. Adrenaline should be administered by intramuscular injection into the thigh (lateral flank), rather than subcutaneously or intravenously (risk of arrhythmia). The dose is 0.01 mg/kg (maximum of 0.5 mg by injection) (77). Injections may be repeated every five to 10 minutes, or even more frequently, if the symptoms persist or worsen. Intravenous injection should be carried out only in cases of cardiac arrest and requires monitoring in an intensive care unit (3, 4, 67) (grade 1B).
The others options for treating acute allergic symptoms are based on H1 antihistamines, beta-agonist bronchodilators, and corticosteroids (3, 4, 67).
H1 antihistamines are indicated for the treatment of benign allergic manifestations, such as urticaria, angio-oedema, rhinoconjunctivitis and isolated abdominal pain (78, 79). They are not sufficiently effective to control severe allergic reactions (anaphylaxis, laryngeal oedema) and should not delay adrenaline injection (3, 4, 67) (grade 1A).
Short-acting beta-agonists are administered by inhalation and are indicated in cases of isolated asthma attacks provoked by testing (80) (grade 1A). They are administered via a spacer device or nebuliser, depending on the severity of the symptoms. The dose is four to 15 puffs with a spacer device, or one to two puffs/kg (maximum 20 puffs), to be repeated, if necessary, every 10 to 20 minutes (81). The dose used with a nebuliser is 2.5 mg for children weighing less than 16 kg and 5 mg for children weighing more than 16 kg, in salbutamol equivalents, to be repeated every 20 minutes if necessary (82).
Steroids have little or no immediate effect, generally exerting their effects about four to six hours after administration (grade 1B). They should not be used as a first-line treatment for anaphylaxis. These drugs may be indicated in patients with a history of asthma (3, 4, 67) (grade 2C). Oral steroids are administered at a dose of 1 to 2 mg/kg prednisone or prednisolone (maximum 60 mg), and intravenous steroids at a dose equivalent to 1 to 2 mg/kg methylprednisolone.
Other measures include oxygen treatment in cases of asthma or shock (3, 4, 67) (grade 1A). The patient should be placed in a recumbent position with the lower extremities elevated. The administration of a crystalloid-containing (normal saline) or colloid-containing solution at a dose of 20 ml/kg over 10 to 15 minutes, repeating this treatment as necessary, is indicated in cases of hypotension or collapse (grade 1B). In cases of hypotension not responding to adrenaline or crystalloid solution (volume > 40 ml/kg), vasopressor treatment (noradrenaline, vasopressin) should also be given (67) (grade 1B).

IV.2 Indications

Therapeutic management depends on the severity of the clinical symptoms (Fig. 4).

1) Intramuscular adrenaline injection is the first-line treatment for anaphylaxis, laryngeal oedema, collapse or a combination of these symptoms, and for rapid progression of symptoms (2) (grade 1A). The prognosis depends on the rapidity of diagnosis and adrenaline administration (2) (grade 1B).
2) Adrenaline is indicated in cases of asthma attack resistant to short-acting beta-agonists (2) (grade 1A).

Figure 4 - Treatment of allergic reactions occurring during OFC in children



3) In cases of benign or mild reactions, a history of asthma or severe reaction to the food should lead to the administration of antihistamines and oral corticosteroids (grade 2C).
4) All severe reactions, particularly those requiring adrenaline injection, should be followed by monitoring at the hospital, taking into account descriptions of the biphasic reactions that may occur over a 24-hour period (3, 4) (grade 1C).

V How should the OFC be interpreted?

V.1 Criteria

The criteria for OFC interpretation take into account the characteristics of the child (medical history, selection criteria for OFC, food tested, duration of monitoring) and the OFC technique used (open or blind) (24) (grade 2B). A single objective criterion is sufficient to define an OFC as positive, confirming FA. The identification of an isolated subjective sign leads to continuation of the test, with intensive monitoring, in the hope of identifying a secondary objective sign, a switch to blind OFC, or to the test being stopped.
A negative OFC result may be used to confirm the absence or development of tolerance to FA (24) (grade 1A). The OFC may be considered negative if no immediate or delayed reactions are observed (25). In cases of uncompleted OFCs, a lack of reaction provides conclusive information concerning only the dose and form of the food tolerated.
In some cases, the OFC cannot be interpreted and must be repeated in a different manner.
Finally, "false negative" outcomes of OFC are reported. This may be the result of the loss of relevant allergen proteins during preparation of challenge material (OFC with vegetables for example), of a "matrix effect", of the association with facilitating factors (food-dependent exercise-induced anaphylaxis, treatment with anti-ulcer medication for example), or other unexplained causes (49,75, 82-84). Further, DBPCFC is considered as the gold standard for the diagnosis of FA. However, events with placebo may occur. Refuting "false positive" challenges may justify repeated challenges in selected cases (85).
The time between last intake and the appearance of symptoms distinguishes between immediate reactions, which occur within two hours (rarely within four hours), delayed reactions and combined reactions (86). Immediate reactions are most frequent. They may be isolated or associated (5, 23, 87 - 89).

V.2 Signs of reactivity

Symptoms may be cutaneous/mucous, gastrointestinal, respiratory or systemic (Tab. 3) (87, 88). They may also be described as subjective or objective. All signs should be carefully noted on a monitoring form, specifying their time of occurrence and the trigger dose.
Cutaneous signs are the most frequent. Subjects may display rashes, urticaria or angio-oedema (22, 88, 89). These symptoms should be quantified in terms of the percentage of the skin area affected. Eczemateous reactions should be scored using an eczema score established at the start of the OFC and a score established at least 24 hours after OFC. For the SCORAD, a difference of at least 10 points is usually considered to indicate a positive reaction (89). Isolated pruritus is usually considered a subjective sign, unless generalised, extensive or observed in certain areas (the extremities).
Gastrointestinal signs are also frequent: oral allergy syndrome, crampy abdominal pain, nausea, repeated vomit-

Table 3 - Classification of symptoms observed during an OFC

Symptoms	Subjective symptoms	Objective symptoms
Cutaneous		Pruritus
	Isolated or localised	With behavioural evidence of pruritus (e.g. generalised with persistent scratching)
		Erythema Maculo-papular rash Urticaria Angio-oedema Eczema
Gastrointestinal	Isolated pruritus: labial, oral, velo-palantine, pharyngeal Oral allergy syndrome Dysphagia	Enanthema Oedema of the uvula
		Abdominal pain
	Isolated	With behavioural evidence of abdominal pain (e.g. refusing to move, abdominal pain repeated or associated)
	Nausea	Repeated vomiting Diarrhoea
Respiratory	*Nasal/ conjonctival:*	
		Nasal congestion Repeated sneezing, Aqueous rhinorrhoea, Rhinoconjunctivitis
		Laryngeal: Vocal changes
	Chest: Shortness of breath Chest tightness	Stridor Laryngospasm Laryngeal dyspnoea
		Chest: Cough, wheezing, Dyspnoea Asthma attack Decrease in FEV_1 >15% Decrease in peak flow >20%
General	Tiredness, Changes in behaviour, Prostration, Headaches, Apprehension, refusal to take the next dose	Abnormal pallor Increase in pulse >20% Decrease in blood pressure >20 mmHg Decrease in SaO_2 Collapse Anaphylaxis

ing, diarrhoea (22, 88). Abdominal pain may be a precursor of other signs. Diarrhoea may occur rapidly, or some time after the occurrence of abdominal pain and may be acute (protein-losing enteropathy) or chronic

(eosinophilic gastroenteropathy). Following the reintroduction of the food into the daily diet, objective signs of malabsorption may constitute evidence in favour of FA (33).

Respiratory signs may concern the upper or lower airways: rhinitis, rhinoconjunctivitis, cough, wheeze/bronchospasm, dyspnea (24, 88). Such signs also include changes in the variables monitored (decrease of at least 20% in peak flow, decrease of at least 15% in FEV_1 or decrease in oxygen saturation). Isolated asthma attacks are rare (90).

Systemic reactions rarely occur during OFCs (88). Compliance with good practice and the respect of contraindications limit the most severe reactions. Severe reactions may occur with any type of food. Anaphylaxis is a severe, life-threatening, systemic syndrome involving cardiorespiratory symptoms and/or signs such as stridor, wheeze and/or hypotension (3, 4). In the absence of specific treatment, the reaction may progress rapidly, with increasingly severe respiratory and cardiovascular symptoms. Based on the observation that the involvement of skin and other systems, such as the gastrointestinal tract, is generally present initially and may predict the progression of a reaction, a working clinical definition of anaphylaxis has now been proposed by a North American task force and the EAACI (3, 4).

VI What are the consequences of an OFC?

Whatever the result obtained in the OFC, this test has an effect on diet and, more generally, on the daily life of the child.

VI.1 In cases of a negative OFC

If a negative OFC result is obtained, the food can be reintroduced, in the form tested, into the daily diet of the child (4, 24, 33) (grade 1B). However, it is important to ensure that the quantity tolerated by the child on the day of the test corresponds to the amount the child is likely to eat during a normal meal. There is a risk of recurrence, as reported for egg and peanut (55, 91). It would therefore appear sensible to recommend regular consumption of the food in the form tested and tolerated on the day of the OFC (60) (grade 2B). At least during the first few months after the OFC, and regardless of the food concerned, we advise the maintenance of an emergency kit (55) (grade 2C).

VI.2 In cases of a positive OFC

Continuation of the avoidance diet is recommended; together with food education measures and the prevention of any deficiencies likely to be caused by the diet (33) (grade 1B). These objectives often lead to the intervention of a dietician. The maintenance of an emergency kit is recommended in cases of positive OFC (51) (grade 1B). Its composition may be changed, particularly as concerns the need for adrenaline, according to the reaction observed, the trigger dose, allergic background and the allergen concerned (92,93) (grade 2B). A written action plan should be produced and educational measures should be targeted at the patients and their families (67, 85, 92-95) (grade 1B).

In cases in which the food allergy is likely to resolve over time, the patient should undergo clinical follow-up, with control prick tests and specific IgE determinations to define possible indications for a new OFC. OFC remains indispensable for confirmation of the development of tolerance (23, 58, 64) (grade 1C).

VII Conclusion

This practice parameter for OFC in children with FA is an updated review based on an evidence-based approach. It aims to provide guidelines and support for physicians and to improve the quality of care received by children with FA. However, for several items, data remain conflicting, sparse or entirely absent. The recommendations for these items correspond to a consensus statement from the working group experts.

Several questions remain unanswered:

How sensitisation to a food the child does consumes without signs of allergy change over time?

What is the natural history of sensitisation and allergy to the nuts of other trees in a child with confirmed peanut allergy?

What is the natural history of non-IgE-based FA?

For these items, cohort studies, with follow-up from childhood to adulthood are required. The development of new methods should lead to progress in diagnostic work-up and provide information about the allergy and its natural history.

Several questions concerning OFCs also remain unanswered:

Why do false-positive or false-negative OFCs occurred?
Can an OFC lead to tolerance induction?
Which procedure is the most appropriate for evaluating non-IgE features, particularly as concerns gastrointestinal hypersensitivity to the food?
Should the procedure for food tolerance evaluation differ from that for diagnosis?
What are the specific indications for low-dose OFC?
What conclusions should be drawn about an incomplete OFC without reaction?
How does the patient consume the food in the real life after a negative OFC?
Further studies and evaluations are required to answer these questions. The proposed practice parameter is subject to subsequent changes and updates, taking into account advances in our knowledge concerning FA diagnosis and OFCs.

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Multiple drug hypersensivity: insight into the underlying mechanism and correlation with autoimmune diseases

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Key words

Autoimmune diseases, chronic idiopatic urticaria, diagnosis, management, multiple drug hypersensitivity

Summary

Background: *Subjects with drug hypersensitivity are sometimes simultaneously reactive to several drugs. This nosological entity is defined as multiple drug hypersensivity (MDH). Urticaria and angioedema are the commonest clinical manifestations of hypersensitivity drug reactions (HDR). These clinical signs are also pathognomonic of chronic idiopathic urticaria (CIU), whose pathogenetic mechanisms are still largely unknown. The diagnosis of CIU includes autologous serum skin test (ASST) and autologous plasma skin test (APST), which demonstrated a high positive and negative predictive value, in multiple nonsteroidal anti-inflammatory drugs (NSAIDs) intolerance.* ***Objectiv****e: to explore the underlying mechanism of MDH and to assess the correlation between such tests and autoimmune diseases (AD).* ***Methods:*** *Twenty eight subjects with MDH referred to our Allergy/Immunology Unit were enrolled from May 2006 to May 2007. Eight healthy subjects served as controls. In addition to common diagnostic tools used in the diagnostic algorithm of MDH, enrolled subjects also underwent ASST and APST.* ***Results:*** *Patients were predominantly female (23 female vs 5 male; mean age 52.2 years). In 61% of cases MDH was associated with either CIU or AD. NSAID and antibiotics were the major causes of HDR, both implied in 54% of subjects. The proportions of MDH-subjects with positive ASST and APST were 46.4% and 28.6%, respectively. All patients with MDH+AD+CIU (4/4) presented a positive ASST.* ***Conclusions:*** *In patients with MDH, ASST proved to be frequently positive, as previously described for multiple NSAID intolerance. In ASST-positive subjects, the activity of several drugs appears to add up FceRI-specific autoantibodies in the induction of the release of allergic mediators.*

Introduction

Subjects who experience an adverse reaction to a single drug sometimes display similar reactions to several others. Non steroidal anti-inflammatory drugs (NSAID) and antibiotics are those more commonly implicated. This nosological entity is defined as multiple drug hypersensitivity (MDH). In MDH the pathogenetic mechanism involved in degranulation of mastocytes and basophils does not depend on drugs molecular structures, which often are widely different, but on poorly characterized host's intrinsic factors (1). Urticaria and angioedema are among the commonest clinical manifestations of adverse reaction to drugs. These clinical signs are also pathognomonic of another clinical entity, namely chronic idiopathic urticaria (CIU), whose pathogenesis remains unknown.

Autologous serum skin test (ASST) is a recognized tool in the diagnostic pathway of chronic urticaria (2). This test identifies subjects with serum factors which cause histamine-release from mastocytes, an event clinically associated with urticaria. An additional and recently proposed diagnostic tool for CIU is the autologous plasma

skin test (APST) (3). This test allows the identification of subjects with a high level of a factor (F1+2), generated following thrombin formation starting from prothrombin. It has been demonstrated that thrombin may induce rat mast cell degranulation and has consequently a histamine-releasing activity (4).

The available tools for the diagnosis of drug allergy are presently limited to clinical history, prick test, specific IgE dosage and basophils activation test (BAT). However IgE dosage is available only for a few drugs, and BAT is offered only in a few specialized centers. Moreover sensitivity of specific IgE dosage and BAT is affected by the latency time since the hypersensitivity reaction occurred. The gold standard for the management of patients with MDH consists in tolerance tests with alternative drugs or provocative challenge with the incriminated drug, if irreplaceable.

The aim of our study is to evaluate potential additional value of ASST and APST in the diagnosis of MDH, and to assess the correlation between MDH, CIU and autoimmune diseases (AD).

Materials and methods

Twenty-eight consecutive adult subjects (male/female: 5/28; mean age: 53.4 years range: 18-80 years) suffering of systemic MDH were enrolled at our Allergy and Immunology Unit from May 2006 to May 2007. This group represents 23.3% (28/120) of subjects who referred to our Unit in the same period with a single HDR clinical history, to undergo

Table 1 - Main characteristics of the study population

Patients	Sex	Age	AD	CIU	Symptoms	ASST positive	APST positive
1	F	80	NO	NO	U/A	NO	NO
2	F	48	NO	NO	U	NO	NO
3	M	22	NO	NO	U/A	YES	NO
4	F	65	NO	NO	U/A	YES	NO
5	M	71	NO	NO	U	NO	NO
6	F	43	NO	NO	A/AS	NO	NO
7	F	59	NO	NO	U/A	YES	YES
8	M	38	NO	NO	U	YES	YES
9	F	63	NO	NO	U/A	NO	NO
10	F	18	NO	NO	U	NO	NO
11	F	36	NO	NO	U/A	NO	NO
12	F	42	HT	NO	U/A	NO	YES
13	F	68	Sjogren	NO	U/A	NO	NO
14	F	44	HT	NO	A/AS	NO	NO
15	F	57	HT	NO	U/A	NO	NO
16	F	68	HT	NO	U/A	YES	YES
17	F	61	HT	NO	U/A/AS	NO	NO
18	F	57	HT	NO	U/AS	NO	NO
19	F	45	HT	NO	U/A	YES	NO
20	F	27	HT	YES	U	YES	NO
21	F	40	NO	YES	U	NO	NO
22	M	53	NO	YES	U	YES	YES
23	F	58	NO	YES	U/A	YES	YES
24	F	63	NO	YES	U/A	NO	NO
25	F	49	HT	YES	U/A	YES	NO
26	M	58	HT	YES	U/A	YES	YES
27	F	59	HT	YES	U/AS	YES	NO
28	F	69	UCTD	YES	A/AS	YES	YES

AD: autoimmune diseases; CIU: chronic idiopathic urticaria; AS: anaphylactic shock; ASST: autologous serum skin test; APST: autologous plasma skin test, UCTD: undifferentiated connective tissue disease

a tolerance challenge with an alternative drug. All subjects underwent a screening for autoimmune diseases (thyroid autoantibodies and ANA dosage). An accurate anamnesis regarding allergic diseases was acquired and the diagnosis of MDH was made when patients reported hypersensitivity reactions to two or more drugs with different molecular structure. A group of 8 subjects with only AD, a group of 8 subjects with only CIU, and a group of 8 healthy subjects (without MDH nor AD nor CIU), were also included in the study. Antihistamines and steroidal treatment were withdrawn at least 5 days prior to skin tests. Other exclusion criteria were: food allergy or additive intolerance, history of neoplasia (solid or hematologic), physical urticaria and infections. According to the concomitant occurrence of AD and/or CIU the MDH included subjects were classified into two groups: group A=patients with isolated MDH; group B=patients with MDH and/or AD and/or CIU. The diagnosis of CIU was made in subjects with continuous or recurrent urticaria since more than 6 weeks, after having excluded other causes of CU (5).

All patients gave written informed consent. Blood was drawn by venipuncture in Vacutainer® vials with no additive (for serum) and in vials containing Na citrate as an anti-coagulant reagent (for plasma), Serum and plasma were separated by centrifugation at 2000 rpm for 10 minutes. All subjects underwent ASST and APST. To this aim, aliquots (50 µl) of autologous serum, autologous plasma, and 0.9% sterile saline were separately injected into the volar aspect of the forearm. Skin prick test with histamine 10 mg/ml was carried out as positive control. Areas known to have been involved in spontaneous wheals in the last 24 h were avoided. Wheals and flair responses were measured at 20 minutes. The test was considered positive in case of a wheal response > 1.5 mm in ASST and > 3 mm in APST, compared with negative control (sterile saline solution) developed, as previously described (6, 7).

Statistic

Inter-group comparisons of ASST and APST results in patients with isolated MDH and in those with MDH and/or AD and/or CIU, were performed with the exact Fisher's test for categorical data. A p value < 0.05 was considered statistically significant.

Results

Systemic MDH was diagnosed in 28/120 (23.3%) subjects who were referred to our Allergy and Immunology Unit from May 2006 to May 2007 with a clinical history of HDR. Isolated MDH (group A) was diagnosed in 11/28 patients (39,2%), MDH associated with AD and/or CIU in 17/28 (60.8%). In particular 9/28 patients (32,1%) had MDH associated with AD, 4/28 patients (14,28%) MDH associated with CIU and 4/28 patients (14,28%) MDH associated with both conditions. Hashimoto's thyroiditis resulted the most frequent AD associated with MDH, observed in 11/28 (39.3%).

In our study, NSAID and antibiotics were the more involved drugs in MDH. In particular, 26/28 subjects (92.8%) had allergic reactions after assumption of NSAID, 17/28 (46.4 %) had antibiotics allergy and 14/28 patients (50%) were allergic to both classes of drugs (NSAID and antibiotics). HDR to antibiotics and NSAID was found in 7/11 (63.6%) in group A and in 7/17 (41.2%) in group B (p= 0.4401,n.s.). Detailed information on drugs implied in adverse reactions are reported in table 2. Thirteen out of twenty-eight patients (40.6%) scored positive on the ASST, 4/11 (36.3%) in group A and 9/17 (52.9%) in group B (p=0.4601, n.s.). Eight out of twenty-eight subjects (28.5%) scored positive on the APST, 2/11 (18.1%) in group A and 6/17 (35.2%) in group B (p=0.4188). ASST resulted positive in 1/8 subject (12.5%) in the group with isolated AD, in 1/8 subject (12.5%) with isolated CIU and in 4/11 subjects (36.4%) with isolated MDH. All subjects with MDH+AD+CIU had a positive ASST (p 0.05 versus group with isolated MDH). Both ASST and APST were negative in healthy controls.

Discussion

Our study was based on a previous observation on patients with previous systemic HDR, hospitalized at our Unit to perform a tolerance challenge with an alternative drug (data not published). Twenty out of 121 patients had an associated AD, in particular Hashimoto's thyroiditis, Graves disease, Sjogren syndrome, systemic lupus erythematosus or rheumatoid arthritis. In the AD subgroup of patients, 11 (55%) had a significant clinical history of HDR, mainly represented by urticaria and angioedema, to several drugs with different molecular structures. This nosological entity is defined as multiple drug hypersensivity (MDH). The pathogenetic mechanism of MDH involves the degranulation of mastocytes and basophils induced by several drugs with different molecular structure. The commonest clinical manifestations of MDH are urticaria and/or angioedema, but also anaphylactic shock

might occur. MDH prevalence is still under investigation even if data from the literature show that 2-3% of hospitalized patients present HDR (8), and the patients with AD are more often implied (9-11). In MDH subjects, mastocytes and basophils degranulation might be induced by serum and or plasma host factors rather than by specif-

Table 2 - Category and molecules of drugs implied in hypersensitivity reactions

Groups	Patients	Drugs implied in hypersensitivity
MDH	1	NSAID (acetylsalycilic acid, pyrazolic compounds); antibiotic (amoxicillin)
	2	NSAID (acetylsalycilic acid, nimesulide); antibiotic (clarytromycin)
	3	NSAID (acetylsalycilic acid); cetirizine
	4	NSAID (acetylsalycilic acid, nimesulide); antibiotic (miomycin)
	5	NSAID (acetylsalycilic acid, nimesulide); antibiotic (ciprofloxacin)
	6	NSAID (acetylsalycilic acid, ibuprofen); antibiotic (cotrimoxazole)
	7	NSAID (acetylsalycilic acid, naproxen); antibiotic (roxithromycin,ceftazidime)
	8	NSAID (nimesulide); antibiotics (clarithromycin, amoxicillin)
	9	NSAID (ketoprofen, nimesulide)
	10	NSAID (acetaminophen, nimesulide, acetylsalycilic acid)
	11	Antibiotics (roxithromycin, clindamycin, cefixime)
MDH + CIU and/or AD	12	NSAIDs (acetylsalycilic acid, acetaminophen), codeine
	13	NSAIDs (acetylsalycilic acid), antibiotics (neomycin, sulfathiazole)
	14	NSAIDs (acetylsalycilic acid, nimesulide), penicillin
	15	NSAIDs (diclofenac, nimesulide); antiarrhythmic
	16	Antibiotics (clarytromycin, vancomycin, tinidazole), ranitidine, amiodarone
	17	Antibiotics (amoxicilline, rifamycin, isoniazid, sulfamethoxazole), antitetanic prophylaxis
	18	NSAIDs (diclofenac), antibiotics (amoxicillin, clarithromycin)
	19	NSAIDs (acetylsalycilic acid, acetaminophen, nimesulide)
	20	NSAIDs (nimesulide), sulfonamide
	21	NSAIDs (acetylsalycilic acid, naproxen), antibiotic (amoxicillin)
	22	NSAIDs (acetylsalycilic acid), antibiotic (clarithromycin)
	23	NSAIDs (acetylsalycilic acid, nimesulide), antibiotics (amoxicillin, gentamycin)
	24	NSAIDs (acetylsalycilic acid, nimesulide)
	25	NSAIDs (acetylsalycilic acid, nimesulide), chlorphenamine
	26	NSAIDs (acetylsalycilic acid, ketoprofen)
	27	NSAIDs (acetylsalycilic acid, nimesulide)
	28	Antibiotics (amoxicillin, norfloxacin, nitrofurantoin)

MDH: multiple drug hypersensitivity; AD: autoimmune disease; CIU: chronic idiopathic urticaria; NSAIDs: non steroidal antiinflammatory drugs.

Table 3 - Results of ASST and APST in the different groups of subjects

Patients	M/F	Mean Age (years)	NSAIDS' Allergy	Antibiotics' Allergy	Positive ASST	Positive APST
MDH	3/8	49,3	10/11 (90.9%)	8/11 (72.7%)	4/11 (36.3%)	2/11 (18.1%)
MDH + AUT	0/9	56,1	9/9 (100%)	5/9 (55.5%)	3/9 (33.3%)	2/9 (22.2%)
MDH + CIU	1/3	53,5	3/4 (75%)	3/4 (75%)	2/4 (50%)	2/4 (50%)
MDH + CIU + AUT	1/3	58,8	4/4 (100%)	1/4 (25%)	4/4 (100%)	2/4 (50%)
Total	5/23	53,4	26/28 (92.8%)	17/28 (60.7%)	13/28 (46.4%)	8/28 (28.5%)

ic drug molecules. In this scenery the drug could act as a trigger in a complex chain reaction that involves mastocytes and basophils, leading to "allergic" manifestations. ASST and APST are already included in the algorithm of CIU, and have demonstrated a high positive and negative predictive value in multiple NSAID intolerance (12). The main objective of our work was the clarification of the underlying mechanism of MDH and the correlation between the result of ASST and APST and autoimmune diseases. We therefore performed ASST and APST in all subjects with a clinical history of systemic MDH. The tests were also performed in a subgroup of 8 subjects with isolated CIU, others 8 subjects with isolated AD and in 8 healthy subjects.

The main findings of our preliminary study are that: (1) our study population was selected in a group of 120 subjects with a single (92/120, 76.7%) and a multiple (28/120, 23.3%) drug hypersensitivity. MDH is therefore not so rare, as previously described (13, 14). Twelve MDH subjects (12/28, 42%) presented a hypersensitivity reaction either to NSAID or to antibiotics. No correlation was found between the positivity of APST and ASST the drug class or the severity of the reaction, in agreement with a previous report on APST (4, 15). As a matter of fact, only one patient with a clinical history of drug-induced anaphylactic shock had a weak positivity to ASST; (2) MDH, CIU and AD were frequently associated (17/28, 60.7%), suggesting that MDH might have an autoimmune/autoreactive background. In fact, prevalence of thyroid-targeted autoimmune conditions in the general population is strikingly lower, namely around 0.1-5% and 0.1-0.2% in for Hashimoto thyroiditis and Graves disease, respectively (3, 16). Prevalence of positive ASST among subjects with MDH is relevant (13/28, 46.4%), as previously described by Asero et al. (17). We can therefore assume that in patients with MDH, histamine release could be mediated by a serum factor, as described for autoimmune urticaria; (4) Prevalence of positive ASST among subjects with MDH+AD+CIU is relevant (4/4), and higher than in subjects with isolated MDH (4/11, 36.4%, p: 0.05); (5) prevalence of positive APST among subjects with MDH is lower comparing to that of positive ASST (8/28, 28.5%), and only 1 subject shows a positive APST and a negative ASST (in group B: MDA+AD). ASST and APST positivity was lower than that reported by Asero and collaborators. This discrepancy could be partly explained by the different population selection criteria: Asero performed these tests in patients with different grade of hypersensitivity drug severity, whereas our study population included only subjects with hypersensitivity reactions serious enough to justify an hospitalization. This could have selected a particular population with different intrinsic factors, that could account for an autoreactive background. Taken together all these findings provide a further insight in the mechanism of MDH, suggesting that this condition may be associated to an autoimmune/autoreactive phenotype. We speculate that in MDH subjects several drugs add up their activity to that exerted by FceRI-specific autoantibodies, inducing a non-specific release of allergic mediators. In this context the effectiveness of a prophylactic antihistaminic therapy, taken before the use of any drug, may prevent further HDR. The appropriateness of a similar strategy in patients with MDH needs to be verified. Our preliminary data suggest that patients with AD and positive-ASST had an increased risk to develop HDR. In this perspective, autoimmune antibodies assessment and ASST might be included in the flowchart of patients with MDH; Further studies on larger population are required to enforce our findings.

Conclusion

Our preliminary data indicate that ASST is often positive in MDH patients and that MDH seems to be associated with autoimmune thyroiditis. These findings provide a further insight in the mechanism of MDH, and suggest that MDH might have an autoimmune/autoreactive background.

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Simultaneous occurrence of chronic autoimmune urticaria and non-allergic asthma: a common mechanism?

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Key words

Anti-FcεRI autoantibodies, autologous serum skin test, histamine-releasing factors, chronic urticaria, non-allergic asthma.

Summary

Chronic urticaria is now considered as an autoimmune disorder due to histamine-releasing autoantibodies in 40-50% of cases. These histamine releasing-autoantibodies directed against the high affinity IgE receptor or against IgE can be detected in vivo by autologous serum skin test (ASST) or in vitro by a functional assay employing basophils. ASST positivity has been found also in patients with non-allergic asthma, but its relevance to the disease mechanism remains to be defined. Here, we report two women aged 43 and 75 years who complained simultaneous occurrence of chronic urticaria and asthma. Circulating histamine-releasing factors were detected in both patients by ASST and basophil histamine release assay whereas other possible causes of urticaria and asthma were excluded by clinical and laboratory investigations. The elder woman had associated autoimmune thyroiditis. We suggest that circulating histamine-releasing factors, probably represented by histamine-releasing autoantibodies, might be involved in the pathophysiology of both chronic urticaria and asthma.

Introduction

It is now recognized that chronic urticaria (CU), once considered as a mysterious disorder, has an autoimmune/autoreactive origin in about 40-50% of cases (1). In 1986 Grattan observed that the intradermal injection of autologous serum causes a wheal-and-flare reaction in about half CU patients suggesting the presence of circulating histamine-releasing factors as a possible pathogenic factor (2). Subsequently, skin reactivity to autologous serum in CU patients was found to be associated with functional autoantibodies directed against the α subunit of the high affinity IgE receptor (FcεRI) or against IgE (3, 4). However, histamine-releasing autoantibodies have been detected in about 30% of CU patients, whereas about 50% of the patients show skin reactivity to intradermal injection of autologous serum (5). Furthermore, if Na-citrate autologous plasma is used instead of autologous serum, the proportion of positive patients increases up to 80% (6). It appears indeed that vasoactive and permeability factors other than histamine-releasing autoantibodies are involved in the disease. This view is also supported by the observation that sera from CU patients containing anti-FcεRI autoantibodies retained their ability to induce a wheal-and-flare reaction upon intradermal injection after depletion of IgG (7). Although not all aspects of the CU pathomechanism have been revealed, the recent advances have changed the clinical approach to the

patient, with avoidance of extenuating restriction diets and judicious use of immunosuppressive drugs, namely ciclosporin, in those cases which are not adequately controlled by anti-histamines and steroids. In contrast to CU, "non-allergic" asthma remains nowadays a mysterious disorder. It has been ascertained that allergic and non-allergic asthma share a common background characterized by inflammatory changes of respiratory airways, and the immunopathological differences that can be detected are quite subtle. However, in allergic asthma bronchial inflammation and respiratory symptoms are triggered by allergen exposure and consequent IgE-mediated mast-cell degranulation, followed by recruitment and activation of other inflammatory cells including eosinophils, basophils and T lymphocytes (8). In contrast, the event which provokes bronchial inflammation in "non-allergic asthma" is still elusive. Local expression of epsilon germline gene transcripts and RNA for the epsilon heavy chain of IgE has been found in the bronchial mucosa of allergic and non-allergic asthmatics, but the possible contribution of IgE antibodies to the mechanism of non-allergic asthma has not been elucidated (9). We have recently shown that intradermal injection of autologous serum causes a wheal and flare reaction in about half the patients with non-allergic asthma (10). *In vitro* evidence for histamine-releasing autoantibodies was found only in a minority of patients and so we hypothesized that a hitherto uncharacterized vasoactive factor could account for skin reactivity to autologous serum (10, 11). Here, we report the presence of serum histamine-releasing factors in two patients who complained simultaneous occurrence of urticaria and asthma symptoms.

Case report

Two women aged 43 and 75 years were evaluated at the outpatient Allergy Clinic of the Ospedale Maggiore Policlinico of Milan, Italy, because of CU with angioedema lasting from five years and six months, respectively. Both patients reported that urticaria onset was associated with the simultaneous appearance of asthmatic symptoms. The younger patient had positive skin prick tests for grass pollens and ragweed, but asthmatic symptoms were perennial and apparently unrelated to pollen exposure. After antihistamine treatment (ebastine and levo-cetirizine, respectively) had been stopped for five days, both patients underwent intradermal testing wih 0.05 mL of both sterile autologous serum (ASST) and saline as negative control, as described by Sabroe et al. (12). After coagulation for 30 min at room temperature, blood samples were centrifuged at 500 g for 10 minutes and serum was immediately used for intradermal tests. A skin prick test with histamine 10 mg/mL was used as positive control. Readings were taken at 30 minutes. The diameter of serum-induced wheal was 4 mm in the younger patient and 8 mm in the older patient, in the absence of any wheal induced by injection of saline solution. The diameters of control wheals induced by histamine were 6 mm and 5 mm, respectively. The response to intradermal injection of autologous serum was therefore considered positive in both cases. Sera from both patients were tested for histamine-releasing activity using basophils of a normal donor showing a 30% net histamine release following challenge with an optimal dose of rabbit polyclonal antihuman IgE antiserum (final dilution 1/5000, Sigma Chemical, St. Louis, MO, USA), as described (13). Histamine concentration in the cell supernatant was measured by an automated fluorometric technique and results were expressed as % net histamine release. Histamine release induced by control sera from 20 normal subjects was below 5%, and this value was used as cut-off, also taking into consideration our previous experience (13). Sera from both patients contained significant histamine-releasing activity (21.4% net release in the younger patient and 9.8% in the older patient). Other possible causes of urticaria and angioedema (chronic infections, parasitoses, food allergy, and C1 inhibitor deficiency) were excluded. The older patient had associated hypothyroidism due to autoimmune thyroiditis with a high titre of anti-thyroid peroxidase antibodies, and was being treated with levo-thyroxine since the age of 60. In both patients the diagnosis of asthma was confirmed by respiratory function tests showing mild to moderate obstruction which was reversible after albuterol inhalation. In the younger patient baseline forced expiratory volume in 1 second (FEV1) was 1.32 L (47% of predicted) and increased up to 1.79 L (36% increase) after inhalation of 200 mcg albuterol. In the older patient baseline FEV1 was 1.25 L (58% of predicted) and increased up to 1.5 L (20% increase) after inhalation of 200 mcg albuterol. Clinical features and results of the investigations are summarized in the table 1. Both patients received local treatment with a combination of steroid and bronchodilator (budesonide and formoterol) and oral montelukast (10 mg once a day). Treatment of urticaria was with H1 antihistamines (ebastine and levocetirizine, respectively) and occasionally with short courses of oral prednisone.

Table 1 - Characteristics of the two patients who complained simultaneous onset of urticaria and asthma symptoms

Patient	Age	Sex	Atopy	ASST	BHRA	Anti-TPO antibodies	FEV1 L (% predicted)	Post BD FEV1 increase
1	43	F	Yes	Positive	Positive	Negative	1.32 (47)	36%
2	75	F	No	Positive	Positive	Positive	1.25 (58)	20%

ASST: autologous serum skin test; BD: bronchodilator (200 mcg albuterol); BHRA: basophil histamine release assay; FEV1: forced expiratory volume in 1 second; TPO: thyroid peroxidase

Discussion

The patients reported are peculiar in that they complained simultaneous onset of urticaria and asthma symptoms, an association suggesting that a common mechanism underlies both disorders. ASST and basophil histamine release assay were positive in both patients indicating that CU had an autoimmune/autoreactive origin linked to circulating histamine-releasing factors, probably histamine-releasing autoantibodies. In fact, skin reactivity to autologous serum in CU patients was found to be associated with functional autoantibodies directed against the α subunit of the high affinity IgE receptor (FcεRI) or against IgE (3, 4). Unfortunately, a routine *in vitro* assay able to detect circulating and functionally active anti-FcεRIα and/or anti-IgE autoantibodies is still lacking. ASST has been indeed considered as an *in vivo* screening test for histamine-releasing autoantibodies directed against the high affinity IgE receptor or against IgE (12), and basophil histamine release assay has been used as a confirmatory test showing the presence of functionally active histamine-releasing autoantibodies (14). The results of *in vivo* and *in vitro* tests for circulating histamine-releasing factors suggested that CU and, possibly, asthma had an autoimmune/autoreactive origin in both patients. In addition, the association with autoimmune thyroiditis in the elder patient was another element supporting the theory of an autoimmune aetiology of CU. A high prevalence of autoimmune thyroiditis has been found in patients with CU (15), particularly in those with a positive ASST who presumably have circulating histamine-releasing autoantibodies (16). Conversely, no clear association between autoimmune thyroiditis and asthma has been demonstrated. Data regarding the association of asthma with other autoimmune disorders, such as type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease and multiple sclerosis are rather controversial (17). The association of CU and asthma is not surprising, since it has been observed that bronchial hyperreactivity is common in patients with CU, probably as a result of the chronic activation of mast cells, basophils and eosinophils (18). In fact, it has been demonstrated that sera from CU patients can induce the release of histamine and leukotriene C4 from basophils (19), and both mediators are involved in the mechanism of asthma. In addition, Puccetti et al. have shown that sera from about 80% of CU patients contain autoantibodies directed against CD23, the low-affinity IgE receptor which is located on eosinophils (20). The anti FcεRII/CD23 autoantibodies can activate eosinophils inducing the release of major basic protein which in turn provokes histamine release from mast cells. The eosinophil-mediated activation of mast cells may be relevant to the pathophysiology of CU and asthma. Previously, we investigated the presence of circulating histamine-releasing factors in patients with non-allergic asthma and, in spite of a frequent ASST positivity (about 50% of patients, we found *in vitro* evidence for circulating histamine-releasing factors only in a minority of patients (16%) (11). This may be due to relatively low sensitivity of the basophil histamine release assay, but could also be explained by a low prevalence of histamine-releasing autoantibodies in patients with non-allergic asthma. We suppose indeed that non-allergic asthma is a heterogeneous disorder which may be sustained by different mechanisms. In some patients, like those described in the present report, circulating histamine-releasing factors, probably represented by histamine-releasing autoantibodies, may contribute to the disease pathophysiology. This view is also supported by the recent findings by Sun et al. who detected histamine-releasing autoantibodies directed against the high affinity IgE receptor in about 30% of asthmatic patients (21).

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A case of protracted hypotension as unique symptom of a biphasic anaphylaxis to amoxicillin

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Key words

Anaphylaxis, biphasic reaction, drug hypersensitivity.

Summary

We are reporting a case of one patient who have experienced itching of palms and soles, thorax erythema, conjunctive injection immediately after oral administration of amoxicillin, and hypotension after 3 hours. In E.D. hypotension was monitored because he was a cardiopatic but it wasn't treated even if it was protracted. A positive result of immediate-reading intradermal test with amoxicillin at 2 mg/ml concentration was found confirming the diagnosis of allergic biphasic anaphylaxis to amoxicillin.

Biphasic anaphylactic reactions have been reported to develop in as many as 20% of anaphylactic reactions (1-4). Late-phase severity varies from mild to severe (rarely fatal). No clinical features on initial presentation identified those likely to have a biphasic response.

We are reporting a case of one patient who have experienced a biphasic anaphylaxis after the administration of amoxicillin. U.Z. is a 68-year-old man who went to Emergency Department (E.D.) for loss of consciousness, thoracic oppression and feeling of tightness of the throat. The patient was affected by ischemic hearth disease, hypertension, diabetes mellitus in therapy with carvedilole, zofenopril, aspirin 160 mg, ticlopidine 250 mg b.i.d., oral hypoglicemic and hypolipidemic agents.

On arrival at the E.D. hypotension was found (BP: 85/55) with no other values out of normal range.

Electrocardiography and assay for cardiac enzymes excluded an acute cardiac event, chest x-ray was negative.

In the history he referred that 3 hours before he had taken one tablet of amoxicillin with onset after 15 minutes of itching of palms and soles, thorax erythema, conjunctive injection. He spontaneously took H1-antihistaminic with resolution of symptoms. After 3 hours he complained weakness, thoracic oppression for which he took sublingual nitroglycerin, feeling of tightness of the throat and finally loss of consciousness, so he was admitted to E.D.

The patient remained in E.D. during 9 hours without a support therapy until blood pressure spontaneously returned to normal value (BP: 120/80). Note that after 6 hours values pressure were still very low (BP: 90/50).

The patient was discharged with diagnosis of thoracic pain and allergic reaction and a therapeutic course with H1-antistaminic and cortisone was prescribed.

The allergological evaluation was made 3 months later in November 2008. He was not affected by symptoms of rhinitis or asthma.

Assays for serum specific IgE to penicilloyl G, penicilloyl V, amoxicilloyl, ampicilloyl resulted negative with a value < 0.35 kU/L.

Patch tests at 5% in petrolatum were performed with benzylpenicillin, ampicillin, amoxicillin, cephalexin, cephalothin, cefazolin, cefradine, cefuroxime and resulted all negative.

The patient was prick and intradermal tested with PPL (final concentration 1.07 x 10 -2 mM/l) and MDM (final concentration 1.5 mM/l) using Diater S.A. (Madrid, Spain) reagents, with aminopenicillins (ampicillin and amoxicillin) at a concentration of 2 and 20 mg/ml 0.9% NaCl, with cephalosporins (cefuroxime and ceftazidime) at 2 mg/ml 0.9% NaCl in order to evaluate cross-reactivity for a possible future use of them as alternative β-lactam antibiotics.

Positive control for prick test was performed with histamine at 10 mg/ml. As negative control for prick and intradermal test 0.9% NaCl was used. Tests were conducted and readings were taken according to the ENDA recommendations (5).

A positive result of immediate-reading intradermal test with amoxicillin at 2 mg/ml was found; so the amoxicillin concentration of 20 mg/ml wasn't tested.

The clinical history was suggestive for allergic biphasic anaphylaxis and the IgE nature of the reaction was confirmed by positive result of immediate-reading skin tests.

No, tryptase levels weren't measured when the patient was admitted at the E.D. No basal tryptase levels weren't measured in our diagnostic approach because the patient never had allergy manifestations before this amoxicillin reaction for which was evaluated in our hospital.

The patient studied in our hospital had not potential risk factors reported in other studies (1-3) but actually there are no reliable predictors of biphasic anaphylaxis.

For this patient the diagnosis of biphasic allergic reaction was under recognized and undertreated, in spite of a protracted and profound hypotension he was not treated but only maintained under observation. To note that these cases are normally poorly responsive to adrenaline (6). Biphasic responses occur with significant frequency and should be taken into consideration when one considers the observation period after the initial event which can be of various grade of severity. Biphasic allergic reactions were reported in 23% of drug/biological reactions (7). In front of a positive anamnesis for recent intake of drugs with referred adverse reactions, even in a patient with cardiovascular disease, we have to be suspicious of an allergic reaction with prompt treatment and sending for following allergological investigation.

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Phenobarbital-induced DiHS and ceftriaxone hypersensitivity reaction: a case of multiple drug allergy

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Key words

Anticonvulsivant drugs, DiHS, Multiple drug hypersensitivity

Summary

Patients with DiHS show an increased risk of sensitization to multiple drugs. We report a case of a young woman who developed cutaneous rash, lymphoadenopathy, malaise and fever after the introduction of phenobarbitale. Because of these symptoms, she was treated with ceftriaxone and she experienced a severe flare-up of the cutaneous and general reaction. Allergological work-up, by cutaneous and lymphocyte transformation test, confirmed a double sensitization to phenobarbital and ceftriaxone. In conclusion, the high risk of DiHS during anticonvulsive therapy should suggest caution in using additional drugs, because of an increased risk of multiple reactions.

Drug-induced Hypersensitivity Syndrome (DiHS) is a life-threatening systemic reaction characterized by cutaneous rash, fever, lymphoadenopaty, internal organ involvement and leukocytosis with eosinophilia. Anticonvulsive drugs are among the most frequent causative agents (1). Patients with DiHS show an increased risk of sensitization to multiple drugs (2,3).

A 30- year-old woman treated with sodium valproate for six years because of a post-traumatic epileptic syndrome, added phenobarbital on therapy. After three weeks she developed cutaneous rash, lymphadenopathy, malaise and fever. The persistence of this clinical picture despite the discontinuation of phenobarbital, induced to start antibiotic therapy with ceftriaxone. After a few doses the patient developed a flare-up of the cutaneous rash, with labial angioedema and a worsening of her general condition, giving to the hospitalization. The laboratory findings showed leucocytosis with eosinophylia and an increase of transaminases (ALT 123 U/l, AST 65 U/l). An allergological consultation suggested the hypothesis of a drug hypersensitivity reaction induced by phenobarbital, with a subsequent sensitization to ceftriaxone. Therefore, antibiotic therapy was stopped. The clinical recovery was very slow.

Four months later, the patient was submitted to the allergological investigations:

- Patch test for anticonvulsive drugs and beta-lactams antibiotics (phenobarbital, carbamazepine, phenytoin, sodium valproate, ceftriaxone, ceftazidime, cefotaxime, penicillin, ampicillin, amoxicillin)
- Cutaneous allergological test for ceftriaxone and phenobarbital (prick and intradermal test – i.d.)
- Lymphocyte Transformation Test (LTT) for phenobarbital, ceftriaxone, cefotaxime, ceftazidime, penicillin G.

The results confirmed a positive late reaction to phenobarbital (positive patch-test, negative prick and intradermal test) and ceftriaxone (positive patch test and i.d. 2 mg/ml at 24 h reading). LTT was positive for both the drugs, at a higher level of Stimulation Index (S.I.) for ceftriaxone (tab. 1). Among the other beta-lactams, LTT was positive for cefotaxime confirming the possible cross-reactivity between these two cephalosporins.

The three diagnostic methods showed a different sensitivity for the drugs investigated. Particularly, intradermal test showed a lower sensitivity than patch test and LTT for phenobarbital, while results were concordant for ceftriaxone.(4,5).

At our knowledge, this case is the first report of multiple drug hypersensitivity with involvement of phenobarbital and ceftriaxone, confirmed by in vivo and in vitro tests. This is an example of sensitization to different drugs administered sequentially, responsible of a paradoxical worsening of clinical symptoms of DiHS, despite the withdrawal of the first causative drug.

The drug-induced massive T-cell activation, occurring in case of DiHS, can increase the risk of hypersensitivity reactions to drugs different from the eliciting one (3). For its clinical features DiHS may be often mistaken for severe infectious diseases and unnecessary antibiotic therapy may be started, with a risk of developing multiple drug reaction. As a practical consequence, we should keep in mind that in case of a clinical picture suggesting a DiHS, particularly frequent in patients on anticonvulsive therapy, empirical treatment with antibiotics should be avoided.

Table 1 - Results of the allergological test

DRUG	PATCH (48/72 h)	LTT - S.I.	I.D (24 h)
Phenobarbitale 30%	+++	11.6	Neg
Carbamazepine 1%	Neg.	n.p.	n.p.
Phenitoin 30%	Neg.	n.p.	n.p.
Sodium valproate 30%	Neg.	n.p.	n.p.
Ceftriaxone 25%	+	46	POS
Ceftazidime 25%	Neg.	0.6	n.p.
Cefotaxime 25%	Neg.	31	n.p.
Penicillin 5%	Neg.	0.8	n.p.
Ampicillin 20%	Neg.	n.p.	n.p.
Amoxicillin 20%	Neg.	n.p.	n.p.

LTT = Lymphocyte Transformation Test S.I. =Stimulation Index
I.D. = intradermal test n.p.= not performed

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Erratum corrige

On the top of the page 90 of the issue n. 3-2008 there was an error: Vol 40, N 2, 90-103, 2008 is wrong and the correct version is the following: **Vol 40, N 3, 90-103, 2008**