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POSITION PAPER

In vivo diagnosis of allergic diseases—allergen provocation tests

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Abstract

The allergen challenge test has been the mainstay of diagnosis of allergic diseases for a long time since it offers a direct proof of the clinical relevance of a particular allergen for the allergic disease symptoms and severity. Standardisation and availability for daily practice (including safety issues) are still to be refined but most of the challenge tests have safely crossed the border from research tools to diagnostic tests available for daily practice for a well trained clinical staff.

In most of the cases, the standard diagnosis of an allergic disease is based on a detailed clinical history and demonstration of sensitization (either by skin test or *in vitro* testing) to a relevant allergen concordant with the history. The allergen provocation test is rarely required. However, the history of allergy diagnosis started with the patch test (1894), which is essentially a provocation test. Systematically applied allergen challenges followed, with the introduction of conjunctival (1907), nasal (1914), and bronchial provocation (1925). Besides being a diagnostic tool, the allergen provocation is a long-standing model for studying the mechanisms of allergic diseases or the efficacy of anti-allergic drugs. Due to safety issues and lack of standardization in conjunction with increased availability of high-quality reagents for skin tests or *in vitro* diagnosis, the allergen challenge was almost abandoned from daily practice and reserved for research purposes.

In this position article, we provide a critical appraisal of allergen challenge methods, together with recommendations on indications, procedure, and safety. A separate task force report will focus on skin testing.

Nasal allergen provocation test

Rationale and indications

When atopic sensitization confirmed by serum-specific IgE antibodies (sIgE) or skin prick test (SPT) is compatible with the patient's history, the diagnosis of allergic rhinitis (AR) can be easily made (1). However, sensitization and clinical history may be discordant or systemic atopy can be absent, while history suggests AR (the so-called local allergic rhinitis) (2). In this case, a nasal allergen provocation test (NAPT) is indicated (Table 1). Several nonallergic conditions can mimic AR, and as management is different, it is of importance to differentiate from local allergic rhinitis (3). Occupational allergy (OA) is another indication for NAPT, due to the lack of standardized allergens available in a regular SPT kit or for

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Table 1 Indications and contraindications for nasal allergen provocation test (3)

Indications	Clinical setting	Discrepancy between history and results of skin prick test (SPT) or sIgE measurement
		To confirm a diagnosis in a patient who has difficulty accepting the consequences of disease, such as avoiding pets or changing jobs
		To confirm the diagnosis of occupational allergic rhinitis or LAR
		Before AIT for AR to confirm the burden of allergen-induced symptoms and select an allergen in polysensitized patients
	Research setting	To study pathophysiological mechanisms in allergic rhinitis and efficacy of medication or of AIT extracts and routes
		To identify the threshold of the maximum dose of allergen needed in clinical research to reproduce nasal symptoms
Contra-indications		
Advanced nasal polyposis		
Recent history of nasal surgery (<3 months)		
Respiratory tract infection in the last 2 weeks		

sIgE measurement. The decision for allergen-specific immunotherapy (AIT) in a patient with AR depends on the demonstration of the burden of AR symptoms induced by an allergen, which might not be always deducted from history. Selection of the allergen in a polysensitized patient might also require a NAPT (Table 1).

Procedure

Different methods for NAPT are used, each with advantages and drawbacks. For clinical daily use, qualitative measurements via a symptom scale are appropriate, while for research, quantitative measurements with high reproducibility are essential (Table 2).

Nasal allergen provocation test can induce an immediate response (10–20 min), a late response (after 6–8 h), a dual immediate and late response, or a delayed response (beginning 24–32 h, reaching maximum at 32–36 h, and resolving within 56 h) (2–4). An associated conjunctival response is observed in most of the patients (3, 5).

The recommended washout periods are at least 1 month for pollen sensitization, 1 week for OA, >72 h for oral and >24 h for topical antihistamines, 7 days for topical corticosteroids, and >6 h for cromoglycates (3).

During provocation, a doctor should be present and an emergency kit available for potential bronchoconstriction or anaphylactic event. Before the challenge, the patient acclimatizes for at least 30 min in the room where the NAPT is performed. At first step, the control solution is applied, and after 10 min, the reaction is scored. Subsequently, every 10 min, increasing concentrations of allergen are administered and the reaction is registered (3, 6). At the end of the challenge, the patient can use a local decongestive spray and/or an antihistamine to decrease symptoms. Several methods for allergen application are used: paper disks, sprays, or nebulized solutions. Nasal spray is the preferred method as an exact dose can be applied. Temperature, pH, and osmolarity of the solution should be checked carefully.

Limitations

Intrinsic nonspecific nasal hyper-reactivity (7) is a potential bias, and allergen application itself may produce nonspecific reactions due to preservatives such as glycerol, benzalkonium chloride, or phenol. Few validated parameters are available to measure response. There is poor standardization and reproducibility of some allergen extracts such as mites and animal dander. In addition, allergen extracts may not represent the native allergen. The amount of allergen applied is far greater than that entering the nose during natural allergen exposure.

Safety

Nasal allergen provocation test is a safe procedure, including for patients with asthma. Incorrect technique or excessive dosing may lead to bronchoconstriction. Pharyngeal itching may occur, while angioedema was rarely reported. No severe anaphylactic reactions were described.

Table 2 Measuring the nasal allergen provocation test

Clinical setting	Symptoms: nasal and nonnasal symptom scores, Visual Analogue Scale (VAS), number of sneezes, or Lebel scoring list
	Evaluation of nasal mucosa aspect by anterior rhinoscopy
	Peak nasal inspiratory flow measurement
Research setting	Collection and measurement of nasal secretion
	Analysis of inflammatory cells, cytokines, and mediators in nasal lavage fluid/tears, nasal brush, or biopsy
	Assessment of nasal airway resistance (anterior/posterior rhinomanometry)
	Acoustic rhinometry
	Nasal NO
	Area under the curve for symptom score

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Bronchial allergen provocation test

Rationale and indications

Demonstration of airway hyper-reactivity (AHR) is mandatory for a proper asthma diagnosis (8). AHR can be measured by challenging the airways with a variety of physical and chemical stimuli. The airway narrowing is measured by changes in FEV1 after gradually increasing the dose of provoking agent. Once a 20% decline in FEV1 is achieved, the inhalation of the provoking agent is stopped. AHR is expressed as the provocative concentration (PC20) or the provocative dose (PD20) of the stimulus that causes a 20% reduction in FEV1.

The provoking stimulus can directly target-specific receptors localized on bronchial smooth muscle (histamine or methacholine) or induce airway constriction indirectly by the release of mediators from inflammatory cells (exercise, inhaled allergens, adenosine monophosphate, hypertonic saline, or mannitol) (9, 10). Allergen-induced airway responses can be reduced or totally inhibited by inhaled corticosteroids (ICS) or other anti-inflammatory drugs; thus, the bronchial allergen provocation test (BAPT) serves as a model for evaluating anti-inflammatory and airway relaxation effects of drugs in asthma (11). In addition, BAPT is useful for understanding the mechanisms of allergic airway inflammation and AHR.

In an experienced setting, BAPT is highly reproducible with small within-subject variability. Its indications have changed over the years (Table 3). Nowadays, BAPT is mostly restricted to specialized centers with demonstrable expertise and experience.

Procedure

Informed consent should be obtained before the BAPT. It is strongly recommended to perform BAPT in a clinical setting.

Bronchial allergen provocation test elicits an early airway response (EAR), which peaks at 30 min and resolves within 3 h. In 50% of the subjects, a late airway response (LAR) follows, usually 4–8 h after provocation.

The recommended washout periods are >4 weeks for topical anti-inflammatory medication, >8 weeks for systemic steroids, >1 week for antihistamines, 6–72 h for bronchodilator agents, and >8 h for caffeine-containing products. BAPT should be ideally performed outside the relevant allergen exposure or season (12, 13).

Diurnal variation of lung function and spirometer-induced bronchoconstriction are assessed during a control day when the patient is tested at the same time points as during the provocation day. Allergen provocation starts early in the morning to allow the measurement of LAR. Ideally patients are observed for at least 10 h after challenge.

Two types of allergen challenges can be applied within the lower respiratory tract: segmental and total lung challenge. Total lung challenge can be performed with high-dose (incremental or bolus) and or with low-dose allergen challenge (13, 14).

The simplest way to measure the response after a high-dose challenge is to calculate the maximal decrease in FEV1 compared to baseline during the EAR and LAR. More accurate is to compare the FEV1 after allergen inhalation with the FEV1 at the corresponding time point on the control day. A positive response for EAR is defined by a decrease in FEV1 $\geq$ 20% from baseline. A PC15 threshold can be calculated from the airway response curve. The use of area under the curve (AUC) of the percentage decrease in FEV1 *vs* time during the first 2 h after challenge (AUC0-2 h) limits the effect of outliers. The LAR is usually characterized by a decrease in FEV1 of $\geq$15% from baseline. As with the EAR, the LAR can be expressed as the AUC of the percentage decrease in FEV1 *vs* time, usually from 3 h after challenge

Table 3 Indications and contraindications for bronchial allergen provocation test (11–13)

Indications	Clinical setting	Diagnosis of occupational asthma
		Discrepancy between history and routine diagnostic procedures such as sIgE measurement and skin prick test (SPT)
		Demonstrate late airway response
		Confirm the diagnosis in a patient who has difficulty accepting the consequences of disease, such as avoiding pets or changing jobs
	Research setting	Study pathophysiological mechanisms in allergic asthma
		Study the pharmacological efficacy of medication, especially discovery and proof-of-concept for new targeted controller therapies for allergic asthma
Contraindications		
Unstable asthma or not being able to remain controlled after stopping medication for washout purposes		
FEV1 < 70% of predicted and/or baseline PC20 (methacholine) or histamine >16 mg/ml at screening		
Recent (<1 year) hospital admission for asthma or frequent asthma exacerbations requiring hospital admission or oral corticosteroids		
Active or recent (<4 weeks) respiratory tract infection		
Spirometry-induced bronchoconstriction (<2 baseline FEV1 measurements of eight attempts within 5%)		
Recent vaccination (<1 week)		
Use of systemic beta-blockers		
Recent major surgery; history of angina pectoris, myocardial infarction, or compromised left ventricular function; any history of cerebrovascular accident, arterial aneurysm, seizures, untreated hypertension, active hyperthyroidism, active or chronic infection, immunologic disorder, cancer, pregnancy or lactation, severe drug allergy, or history of anaphylaxis		

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onward (AUC3-8 h). At least a 2-week interval should elapse between consecutive challenges (13).

The choice of the allergen extract for inhalation is based on the largest skin wheal elicited by SPT or the highest serum-specific IgE level, in agreement with a history of asthma symptoms occurring after exposure to that allergen. Allergen dilutions are prepared from commercially available standardized extracts, with particular attention to avoid endotoxin contamination. In some countries, skin testing extracts can be inhaled with the approval of local ethics committees, for the European Union, GMP certificate is needed, whereas in the United States, FDA approval for inhaled delivery is required.

Limitations

The magnitude of the bronchoconstriction induced by BAPT may be more difficult to control than that with direct challenges. Moreover, the induced bronchoconstriction is usually prolonged due to the release of proinflammatory mediators. BAPT can result in a transient increase in symptoms and medication use. A limited number of standardized allergen extracts are commercially available. Although BAPT is considered the reference test for occupational asthma, there is lack of procedure consensus and false-positive and false-negative results may occur (15, 16).

Safety

Bronchial allergen provocation test is safe, including the pediatric population, in a dedicated setting with experienced staff (13, 17). Generalized reactions are rare, but asthma exacerbations may occur. Therefore, it is necessary that during the challenge, a doctor is present and rescue medication and a resuscitation set are available. In case of a LAR, 24-h admittance in a general ward is recommended. The patient should not leave the clinic before FEV1 is stable (within approximately 10% of the baseline value), with rescue medication and instructions.

At the end of observation period, combined beta2-agonists and ICS are administered to control both the immediate bronchoconstriction and the inflammatory response. For LAR, oral corticosteroids are usually prescribed for a short period to prevent asthma worsening during the next days.

Conjunctival allergen provocation test

Rationale and indications

The conjunctival allergen provocation test (CAPT) involves the instillation of defined concentrations of an allergen solution in the ocular conjunctiva to elicit an IgE-mediated allergic reaction of the ocular surface mucosa, in a presumed sensitized patient (18).

Conjunctival allergen provocation tests have been used both as a practical tool to determine which allergen(s) triggers symptoms in IgE-mediated ocular allergy, especially in polysensitized patients (18, 19), and as a research tool to evaluate the mechanisms and biomarkers of allergic inflammation at the ocular surface, or the response to topical treatments in pharmacological studies (20–22). The CAPT can be used as an alternative to NAPT even if the patients with AR do not report ocular symptoms (23). More recently, CAPTs have been used to investigate the ocular surface as a surrogate of the mucosal sensitivity/tolerance to an allergen, in other IgE-mediated disorders—food, latex or OA, and AIT (19, 24) (Table 4).

Procedure

Conjunctival allergen provocation test involves the instillation of 20–30 μl (to avoid overflow) of defined concentrations of an allergen solution in the inferior external quadrant of the ocular conjunctiva. The contra-lateral eye is used as control with the diluent. Within 5–20 min, a typical IgE-mast cell-dependent immediate reaction occurs with itching, tearing, redness, and conjunctival edema (chemosis). The reaction usually subsides gradually within 30 min (19), but a late-phase reaction may occasionally occur 6–10 and up to 24 h after provocation, especially with larger allergen doses and in highly sensitive patients (20).

To perform a CAPT, the patient should be asymptomatic and outside exposure periods. Contact lenses must be removed 72 h before. Drugs that might influence the allergen response should be interrupted for an adequate washout time (19).

Only lyophilized and standardized allergen extracts should be used. The extracts are reconstituted at room temperature and diluted in sterile phosphate-buffered saline for testing. The solution is stable for 6 h. Phenolic and glycerinated

Table 4 Indications and contraindications for conjunctival allergen provocation test

Indications	Clinical setting	Demonstrate the causal role of a suspected allergen, particularly in persistent forms of allergic conjunctivitis and/or if AIT is considered
		Define clinically relevant allergen(s) in cases with multiple sensitizations
		Clarify cases with discrepancies between a suggestive clinical history and inconclusive skin prick tests and/or specific IgE results
		Diagnosis of occupational disease
	Research setting	Quantify the anti-allergic properties of topical drugs for ocular allergy
		Investigate, at the mucosal surface, allergen tolerance (e.g., AIT outcomes) or the allergic inflammation dynamics and mediators
Contraindications		
Recent eye surgical procedures (3–6 months)		
History of retinal detachment, diabetic retinopathy, or progressive retinal disease		

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solutes should be avoided. The recommended interval between two allergen instillations is at least 15 min. Usually, a scale of dilutions from 2–10 times increments is used. A slower progression is recommended for severe forms or high sensitization (19).

Conjunctival allergen provocation tests are commonly evaluated by the total ocular signs and symptoms score (TOSS): 0 = absent to 3 = severe, with four used for itching. The test is positive if a score of 5 or more is obtained and the challenge is stopped at that point, as there is no need to induce severe symptoms to prove the test positive (19, 21). Several objective measurements can be used, mainly for research purposes such as measurement of cellular, mediators, and cytokine profiles in ocular secretions; assessment of conjunctival erythema using a spectroradiometer, a colorimeter or digital image analysis (25, 26); and quantification of edema of the eyelids and conjunctiva using a fractional millimeter reticule in the eyepiece of a slit lamp microscope (25). Ocular surface sensation can be evaluated using the aesthesiometer of Cochet and Bonnet, or using a 'sensation index,' a measurement that takes into account the intensity and duration of itching (25).

Limitations

Conjunctival nonspecific hyper-reactivity is a potential confounding factor. Instillation in the eye might prove difficult in noncooperating patients. The lack of standardized allergens relevant for a geographical area is an important limitation, which might be overcome in the future by the use of recombinant allergens.

Safety

In experienced hands, CAPT is a safe procedure, including for daily practice. After a positive CAPT, the patient should be observed for at least 2 h, and topical and/or systemic anti-allergic treatment should be provided. Systemic side-effects (generalized urticaria, asthma, and anaphylaxis) are rare. In these cases, the patient should be monitored for 24 h.

Conjunctival allergen provocation test can be used in patients with dry eye and with caution in severe forms of allergic eye disease such as atopic or vernal keratoconjunctivitis.

The allergen challenge chamber

Rationale and indications

The allergen challenge chamber (ACC) is a specially designed facility that enables exposure to a controlled environment in which subjects are challenged with a defined concentration of airborne allergen. In contrast to other challenges, ACC targets concomitant several locations where a type I allergic reaction occurs (eyes, upper and lower respiratory tract, and even skin) (27). The use of a stable and reproducible allergen load ensures an even symptom score. During a challenge session, patients record their symptoms several times an hour with good compliance. Challenges are possible all over the year, with no regard to seasons (27). In contrast to field or park studies, objective assessments can be added, such as slit lamp investigation, rhinomanometry, spirometry, and endoscopy, with the opportunity to cross-check the validity of the subjective scoring. Furthermore, it is possible to take blood samples, lavages, and scrapings at several time points for pharmacodynamics and pharmacokinetic investigation (27, 28). All ACCs are validated for pollen challenges (grass, birch, ragweed, Japanese cedar), and some are validated for house dust mite and cat.

Allergen challenge chamber is not usually used for individual diagnosis. Instead, it is indicated for assessing the efficacy of anti-allergic treatments, for studies in OA and basic research (29, 30). The ACC model is approved and accepted by the FDA and EMA for phase II trials (proof of concept or dose finding) and for further supporting phase III trials. As they require significantly less time and smaller numbers of individuals, the ACC trials have the potential to reduce the cost of drug development substantially.

Procedure

The target of the ACC is to ensure a defined allergen concentration in the air. The concentration should be high enough to induce moderate allergic symptoms and must be kept stable over several hours. Additional climatic factors such as temperature, humidity, and CO2 are also tightly controlled. All exposure parameters should be highly reproducible.

The use of rescue medication is not permitted, as it mitigates the interpretation of the symptom score.

A challenge session lasts at least 2 h because it takes about 90–120 min until the patients reach the maximum of symptoms. Usually ocular, nasal, and bronchial symptoms are scored using a 4-point scale (0–3). After 2 h, a plateau is reached, which would not vary if the allergen load keeps stable. This plateau reaction is usually monitored for 2–6 more hours.

Limitations

Although there is a broad conformity in between the ACCs around the world (Vienna, Hannover, San Antonio, Toronto, Kingston, Tokyo, Wakayama), individual differences exist and there is no 'between-unit' reproducibility of the outcome of allergen exposure under standardized condition. Other disadvantages are to be mentioned (Table 5).

Safety

Usually, mild/moderate symptoms of allergic rhino-conjunctivitis and/or asthma are reported that either resolve spontaneously or are easily reversed with anti-allergic and bronchodilator medication. Systemic or delayed reactions are rare but can occur, so a 24-h observation after the challenge is recommended, as is the availability of a resuscitation set and trained personnel.

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Table 5 Advantages and disadvantages of using an allergen challenge chamber (ACC) in drug development

Advantages	Disadvantages
Exposure to a defined, controlled level of native allergen	Low number of operational ACCs worldwide
Out-of-season use for seasonal inhalant allergens and the ability to save up to 12 months in the development program	Potential effects of claustrophobia or the influence of other subjects on subjective symptom scores
Rescue medication use can be prohibited or suspended during the challenge	Short exposure times (no more than several hours)
Ability to collect biological samples from participants	A potentially abrupt challenge
Investigation of dose-ranging and onset of action	Lack of seasonal priming in out-of-season challenges
Overall cost and ethical benefits due to use of lower number of subjects compared to natural exposure trials	Lack of standardization of exposure conditions and decision to stop exposure in between the ACCs

Food Allergen Provocation Tests

Rationale and indications

The food allergen provocation test (FAPT) provides a gold-standard diagnostic for food-related adverse reactions leading to appropriate food avoidance (31). The test is also indicated for follow-up of previously diagnosed food sensitivities. There is also a positive effect of the food challenge on quality of life of patients with food allergy (32).

Previously, to the procedure, a predictive analysis of the test result should be undertaken. A severe anaphylactic reaction to a given food with highly positive IgE tests, and a history of several reactions to the same food, will in most cases not need a FAPT. Commonly, such decisions are dictated by specific IgE cutoff values (33), but more recently also by prediction algorithms including other variables than allergy testing (34). Grading of the risk of a positive reaction, including an anaphylactic reaction, is possible by measuring sIgE to recombinant allergens such as Ara h2 for peanut allergy, or Cor a9/Cor a14 for hazelnut allergy (35, 36). The sIgE–total IgE ratio is more accurate than sIgE alone in predicting outcomes of challenges performed (37).

Procedure

During the test, the patient is exposed to a given food in a safe place, under a standardized protocol (Table 6) (31). FAPT is stopped if objective reactions are observed or the last dose is consumed without symptoms. Immediate reactions usually appear within 2 h after the last food intake, while atopic eczema may worsen several hours or days following the challenge. Urticaria and angioedema are the most common objective signs, with gastrointestinal, respiratory, or cardiovascular system involvement also common. Results of FAPT are easy to interpret when reactions appear rapidly after challenge and correspond to expected symptoms. Recent guidelines provide advice for interpretation of FAPT (31, 38).

Limitations

In recent years, the FAPT has been standardized to provide as accurate results as possible. However, several unmet needs have been identified such as the matrix effect of various preparations and the impact of cooking or of the maturation stage of foods or vegetables on the allergenicity of food. Interpretation of tests might be difficult, in particular for subjective symptoms. Various attempts to provide an objective measurement, such as facial thermography, have been studied (39). Nevertheless, more appropriate objective measurement devices are still needed.

Safety

Vital signs should be closely monitored during FAPT, and equipment and appropriately trained staff should be in place to deal with allergic reactions, including anaphylaxis (31). The FAPT is a safe procedure following the predictive analysis, if the appropriate dosing schedule is chosen and if the challenge is performed by a well-trained staff.

Drug provocation tests

Rationale and indications

A drug provocation test (DPT), or drug challenge, consists in the gradual administration of a drug to a patient who reported a previous reaction to that drug or a similar one. A DPT is mainly a diagnostic tool allowing the identification of drug hypersensitivity (DHS) and differs from a desensitization procedure that has a therapeutic goal and usually involves more steps (40–42). A DPT should be carried out if no other safer procedures (such as skin or *in vitro* tests) are available or cannot support the diagnosis.

The general indications/contraindications of DPTs have first been addressed in an ENDA consensus article (40) and recently accepted worldwide in an ICON document (42) (Fig. 1). DPTs are considered the gold standard for diagnosing DHS to NSAIDs, local anesthetics, non-beta-lactams antibiotics, and other drugs for which safer tests are inexistent or not standardized. DPTs are also the last diagnostic step for drugs such as beta-lactam antibiotics if skin tests and in some cases *in vitro* tests yield inconclusive results. DPTs may also be important to reassure patients who fear a future reaction or suffer from anxiety due to an unconfirmed diagnosis (43).

When the clinical history strongly supports the diagnosis of DHS (two or more reproducible previous reactions) and

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Table 6 Principles for food allergen challenge

Setting	For patients with a very low risk of a systemic reaction, food allergen provocation test (FAPT) can be carried out at home, while for a very high risk of an anaphylactic reaction, an intensive care unit is recommended. Most often, tests are undertaken in an ambulatory setting of an allergy referral centre. Most importantly, the staff needs to be trained to recognize and treat early symptoms of allergic reactions
Allergen doses	The gradual amounts of food and the interval between the doses are dictated by the history of the patient. For immediate type reactions, intervals of 20–30 min are sufficient (61). The safety of a 30-min interval to avoid severe reactions to accumulated doses has been demonstrated (62). For delayed-type reactions (e.g., atopic eczema), longer intervals might be necessary to ensure an accurate assessment (63).
Design of the challenge	Food allergen provocation test (FAPT) can be 'open' or 'double-blind placebo-controlled' (61). The double-blind procedure allows a more objective interpretation and is used in most experimental protocols, as well as in patients with subjective symptoms, important anxiety or delayed-type symptoms. The food should be blinded for taste, smell, texture, and appearance (consistency, color, and shape), with the placebo indistinguishable from active food (31, 61)
Preparation of the food allergen	Reactions to several foods, such as eggs, fruits, or vegetables, are dependent on the degree of cooking (64). In addition, a matrix effect has been observed for various food allergies. For example, the allergenicity of egg in baked foods also containing wheat is diminished (65).

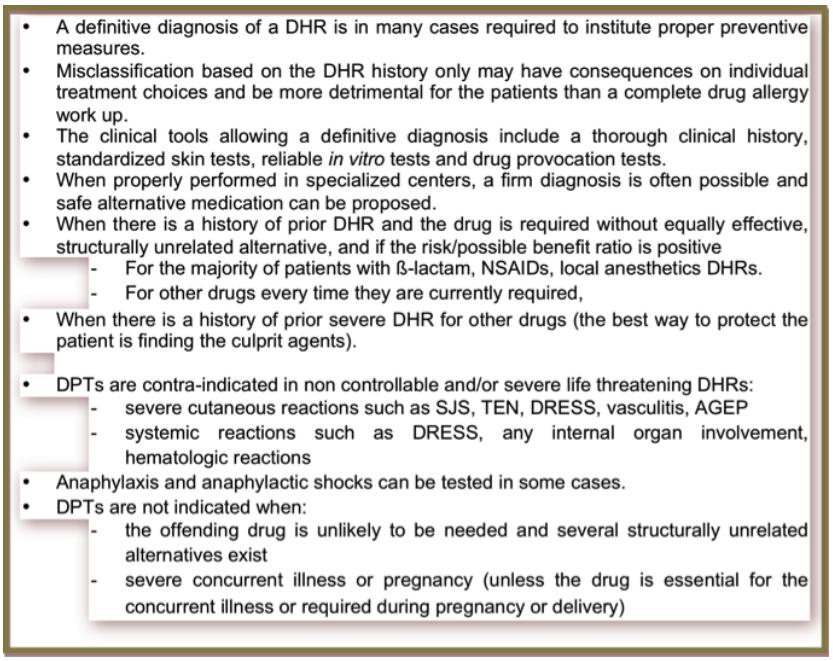

Figure 1 Indications and contraindications for DHR (drug hypersensivity reaction) investigation and drug provocation test (DPTs) (adapted from the ICON document; Demoly et al. 42.

reactions were severe or life threatening, performing a DPT with an alternative drug is recommended (40–42). Anaphylaxis is not an absolute contraindication as long as DPT is carried out in a hospital setting (42). An individualized risk/benefit assessment is always needed: Even for severe reactions if the drug is absolutely needed to treat a life-threatening illness, a DPT can be considered. The coexistence of a severe disease or pregnancy is considered contraindication, although exceptions can apply (40–42). If the suspected drug would not be needed for future treatments of if there are known alternatives with comparable efficacy, DPT is not recommended.

Procedure

The exact DPT procedure varies considerably from one center to another. Guidelines have been proposed, and protocols have been published for most common drugs such as

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beta-lactams (44–46), NSAIDs (47–49), and radiocontrast media (50, 51). A DPT should be carried out ideally with the culprit drug. Theoretically, the route of administration should be the same as the one evoking the reaction, but international guidelines favor the oral route whenever possible and when there is no major chemical difference between the parenteral and the oral forms (40–42). Adjustments of the dose, dose intervals, number of doses, and total dose given are made accordingly to the drug involved, the initial reaction, patient's conditions, and future needs for drug administration. For immediate reactions, a 30- to 60-min dose interval is usually appropriate and a 1-day procedure is sufficient. For nonimmediate reactions, the choice between a 1-day testing *vs* a prolonged DPT to increase diagnostic sensitivity is debated (52).

Whenever possible, the DPT should be carried out with the exact formulation and commercial brand involved in the initial reaction. Multiple molecules present in the drug formulation should be tested separately.

Limitations

Although considered the gold standard for DHS diagnosis, DPT sensitivity is not 100% as unrecognized confounders (infections, underlying disease and its control, concomitant medications, exercise, food intake) might influence the reaction. Specificity is also not 100%, as vague complaints and nonobjective signs are difficult to ascertain and reactions to placebo have been reported. However, the negative predictive value has been shown to be high for the most frequently involved drugs such as beta-lactams (94–98%) (53) and NSAIDs (96%) (54). There are several other limitations (Table 7).

A negative test does not guarantee tolerance to the drug in the future as it rather excludes DHS only at the time when DPT was carried out (55, 56). In addition, DPT does not differentiate between allergic and nonallergic DHS, as the test does not unveil the pathophysiology behind the reactions. Furthermore, desensitization and resensitization may occur during the procedure, but supporting literature is scarce.

Drug provocation test is time consuming and needs a hospital setting with trained personnel. In addition, the patient might refuse to be re-exposed to a drug perceived as harmful.

Safety

The DPT should be carried out under strict surveillance and by trained staff in an appropriate setting equipped to deal with anaphylaxis or other severe reactions. Hospitalization is recommended if a severe reaction is expected (42). The DPT should be carried out at least 1 month after the reported reaction.

Live sting insect challenge for hymenoptera venom allergy

Rationale and indications

Skin tests (SPT and intradermal) are 'gold standard' in the diagnosis of hymenoptera venom allergy (57, 58). Because of the risk of a severe reaction and poor negative predictive value, the live-insect sting challenge should not be used as a diagnostic tool in untreated patients (59). The challenge test is, however, indicated to assess tolerance after venom immunotherapy (VIT), especially in patients at high risk of re-exposure to stings or prone to very severe anaphylaxis (59). Absolute contraindications of a sting challenge include pregnancy, acute inflammatory disease, severe or uncontrolled cardiovascular or respiratory disease, and treatment with beta-blockers. The challenge is performed only if the maintenance VIT dose of 100 pg every 4 weeks is tolerated (no systemic symptoms) on repeated occasions without antihistamine prophylaxis before dosing (57–59).

Procedure

The live-insect sting challenge is difficult and unpractical to perform in clinical practice, because live insects are required. The sting challenge differs from other provocation tests in that incremental allergen exposure is not possible and insect biology and several other factors may influence the test result (59). The insect sting challenge has to be performed with an insect of the same species as the one that provoked the reaction. An experienced allergist, with adequate emergency treatment immediately available, performs the procedure. The insect is placed on the extensor side of the patient's upper or lower arm and irritated mechanically until it stings. The sting

Table 7 SWOT analysys of drug provocation test

Strengths	Weakness
Considered the golden standard for drug hypersensitivity (DHS) diagnosis Good performance with good negative predictive value Safe if recommendations are followed	Time consuming Needs trained personnel Does not elucidate the mechanism behind reactions Can induce sensitisation Poor standardization
Opportunities	**Threats**
Improve standardization of protocols for different drugs and adapted to clinical manifestations, patient age, etc. Further compare its performance in multicenter trials	Too many restrictions to its use Widespread use by non trained personnel

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event has to be confirmed by the development of a wheal-and-flare reaction and by the characteristic burning pain. If systemic symptoms occur, the challenge is terminated immediately and therapy is initiated according to the rules of anaphylaxis treatment. If no wheal-and-flare reaction develops, the sting procedure should be repeated. A new bee venom sting challenge performed by a microsyringe instead of a live insect has been proposed (60).

Limitations

The sting challenge has a low predictive value as a patient with a negative test might still react on a field sting. However, the protective VIT effect can be proven in larger groups of patients. In addition, a well-tolerated sting challenge reassures the patient who is frequently anxious about the possible consequences of a resting. The challenge is less reliable with vespids than with honeybees, as the venom amount delivered is much smaller and subject to more variation compared to the honeybee. Sensitization *de novo* or boosting of already existing sensitization is an important risk of the sting challenge test.

Safety

As the aim of the sting challenge test is to identify subjects who develop systemic symptoms, the occurrence of anaphylactic reactions is expected and adequate emergency treatment should be immediately available. The patient must be informed fully of the aims and possible risks and consequences of the procedure, and written informed consent must be obtained. After the sting challenge, all patients have to be observed for at least 2 h in the hospital. Patients with a history of late-onset anaphylactic reactions have to be followed up long enough to detect any delayed reaction (59).

Concluding remarks

Challenge testing is the mainstay of allergologic diagnostics as it directly proves the clinical relevance of the tested allergen. Once a research tool, challenge tests are slowly making their way into daily practice, mainly due to an increased safety profile and better standardization. The use of recombinant allergens and modern techniques to objectively quantify and compare the biological modifications induced by allergen challenge is highly promising. Nevertheless, the distinction between the clinical and the research setting is essential, in terms of indications, protocols, and endpoints used. At present, allergen challenge tests are still resource intensive and impractical in most clinical situations. A cost-benefit analysis is highly needed to asses the value of implementing allergen challenge in daily practice in comparison with the current diagnostic techniques used. In addition, a distinction should be made in-between challenges, with the nasal and ocular provocation more easily implemented into daily practice in comparison with bronchial, food, or insect sting challenge that are reserved for specialized allergy centers. Proper use of clinical judgment in recommending a challenge test, selection of the appropriate allergen for testing, and interpretation of results, as derived of a thorough training as an allergist, are essential to ensure the deserved value of allergen challenge test.

Conflicts of interest

None in relation to the submitted manuscript

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