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Patch testing in atopic dermatitis patients

The exposure of atopic dermatitis (AD) patients to aeroallergens or food allergens can exacerbate or maintain the disease. Atopy patch tests (APTs) are able to identify these triggering factors and consist of the epicutaneous application of allergens for 48 hours, with an evaluation of the eczematous lesions induced after 48 and 72 hours, according to the reading criteria of the European Task Force on Atopic Dermatitis (ETFAD). APTs show a higher specificity than skin prick and specific IgE tests, since the pathophysiological mechanism of the reaction induced is very similar to that which occurs in AD lesions. The standardization of APTs to aeroallergens has brought a certain reliability to this method, which is not the case for food APTs, in which the positive predictive value must be improved to avoid any unnecessary dietary restrictions. Thus, optimization of APTs and progress in the knowledge of the pathophysiology of eczemas could help to develop new immunobiological diagnostic methods and specific immunotherapy for AD.

Keywords: atopic dermatitis, patch test, atopy patch test, aeroallergens, food allergens

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Patch tests are used for diagnosing delayed hypersensitivity allergic reactions, like allergic contact dermatitis (ACD). More recently, atopy patch tests (APT) have been developed with aeroallergens and food allergens to diagnose delayed allergic outbreaks of atopic dermatitis (AD) [1, 2]. In fact, exposure of patients suffering from AD to aeroallergens (house dust mites, cat dander, grass pollens) or food allergens can provoke an exacerbation or prolongation of the disease [2-4]. Prick tests and specific IgE can be useful for detecting aggravating factors, but their precise implication in the genesis of the skin lesions should be controlled by carrying out APT, which are more adapted to the pathophysiology of AD [5, 6].

Summary of the pathophysiology of atopic dermatitis

Atopic dermatitis (AD) is a disease caused by specific T cells to allergens, which occurs in the context of a genetic disorder inducing a cutaneous xerosis and an alteration of the epidermal barrier [7]. The AD develops on an atopic field, characterised by an increase in total IgE and in specific IgE to certain aeroallergens or foods [8]. Changes in the skin barrier of patients with AD favour their sensitization to allergenic proteins: the allergen penetrates the epidermis and fixes on the specific IgE present on the surface of Langerhans cells (LC). This binding activates the LC, which migrate to a lymph node and present the allergen to T cells, provoking a cascade of events like those described in allergic contact dermatitis [9]. The appearance of lesions of eczema is not due to IgE but to the cytotoxic activity of T cells, leading to the apoptosis of keratinocytes [10]. The distinctive features of AD compared to ACD are the possibility for the allergen to

penetrate the skin, in particular for house dust mite antigens, and the capacity of LC to fix the IgE, via their FcεRI receptor. This explains why the great majority of AD patients have isolated positive APT tests without positive IgE or positive prick tests.

The influence of age

Sensitisation to allergens is an early phenomenon which coincides with the beginning of AD in childhood. A study has recently shown that atopic children aged from 3 to 12 months have positive APT in 89% of cases, positive prick tests in 16% and positive specific IgE in 30% of cases. These children, tested in the same conditions 2 years later, present positive APT only in 60% of cases, whereas prick tests and the specific IgE is positive in 63% and 73.5% of cases [11].

The hypothesis is then that there is initially a sensitization of T cells (manifesting as the eczema of AD), with only a subsequent sensitization of the IgE responsible for the respiratory symptoms (asthma and allergic rhinitis).

Principles and usage of atopy patch tests

APT are defined as the epicutaneous application of allergens in order to evaluate their ability to reproduce an eczema in the person being tested. With regard to the pathophysiology of AD, the APT can be considered as provocation tests similar to those used for the diagnosis of food or respiratory allergies [12]. Their aim is thus to identify the aeroallergens and/or foods aggravating an AD, and ultimately to propose avoidance measures when possible.

APT are indicated, in adults as well as in children, in the following situations [13]:

- persistent and/or severe AD (SCORAD>40), in the absence of a known trigger of allergic contact dermatitis, after the failure of topical treatments which have been correctly used (including immunomodulators) and phototherapy (for adults);
- suspicion that symptoms are aggravated by aeroallergens and/or food, with negative specific IgE and/or negative prick tests;
- multiple IgE sensitizations without clinical relevance. For Taieb *et al.*, an allergological check-up is also justified [2];
- in a child with moderate AD (SCORAD between 15-40) requiring the use of dermal corticosteroids > 30 g/month.

Beyond the clinical indications, APT also enable the pathophysiology of AD to be studied by reproducing, in an experimental setting, an eczema at the site of application of allergens.

Technical aspects of atopy patch tests

Materials: allergens, vehicle and controls

The European standardization of APT to aeroallergens has been coordinated by the *European Task Force on Atopic Dermatitis* (ETFAD) and APT are now carried out in a similar manner to conventional patch tests, as used for the diagnosis of allergic contact dermatitis [14]. The skin tests are prepared from lyophilized aeroallergens diluted in petrolatum which gives a more stable solution and reactions which are more frequent and more visible than those of aqueous solutions [15]. The concentrations are higher than those used for prick tests. In adults they consist of between 5,000 and 7,000 PNU/g (protein nitrogen units) or 200 IR/g (biological units) but can be reduced by 50% in children [16]. Several commercial preparations are available: house dust mites (*Dermatophagoides pteronyssinus*-Der p and *Dermatophagoides farinae*-Der f), dog, cat, artemisia, birch, five grasses, dactylis, pheole and plantain (Stallergènes, Antony, France; Allerbio, Courbevoie, France; Chemotechnique, Malmö, Sweden). The concentration of the commercial extract of house dust mites (a mixture of Der p and Der f, Chemotechnique, Malmö, Sweden) diluted to 20% in petrolatum is probably too high, since a dilution of 0.1% of this mixture can be sufficient to identify allergy to house dust mites in AD patients [17].

The APT to food, on the other hand, are not standardized and until validated results are available, fresh foods should be preferred to commercial extracts. In a study by Niggemann *et al.*, the food allergens tested were diluted at 1/10, in order to eliminate false positives induced by irritation. The authors found as many positive reactions with the APT diluted at 10% as for the non-diluted APT, but with stronger reactions for the latter. The raw food item should therefore be placed undiluted on blotting paper: a liquid like milk should be soaked into blotting paper, or it can be in the form of a homogenised paste (wheat flour, peanut...) [18]. More recently, an atopy patch test to cow's milk has been developed in a ready-to-use form and is available in pharmacies (Diallertest®). If its specificity is comparable to that of a

home-made test with a Finn Chamber®, its sensitivity seems to be higher [19]. In the future, the use of recombinant food allergens might be interesting, as is already the case for *Malassezia furfur* allergens [20]. Petrolatum is not an adequate vehicle for recombinant allergens which should be diluted in an aqueous solution for good dispersion.

The preparation of APT should also include a negative control with the vehicle alone (petrolatum), associated with a control for irritation (Sodium Lauryl Sulfate 0.5%). A second negative control using just the chamber can also be applied.

Prior precautions

There is no age limit for carrying out APT and many studies have been undertaken on babies from the age of 3 months [11]. The tests should be made on healthy skin, without any pre-treatment (scratch test, acetone). The prior precautions concerning concomitant treatments are summarised in table 1 [5]. The influence of antihistamines on APT results has not been clearly determined, but stopping their use at least 72 hours before carrying out the tests is recommended [13]. Finally, surprisingly, tacrolimus, the only calcineurin inhibitor available in France, does not modify the APT results, while pimecrolimus, which has a weaker anti-inflammatory and immunomodulatory activity, reduces the severity of the APT reactions [21, 22]. We recommend that an immunomodulatory treatment should not be used on the APT site for at least 48 hours before carrying out the tests.

Procedure and reading of atopy patch tests

The tests are applied on the back using 12mm Finn Chambers®, (Epitest Ltd Oy, Tuusula, Finland), held in place with hypoallergenic adhesive tape. They are left in place for 48 hours and reading of the tests is carried out at 48 and 72 hours. Contrary to standard patch tests, positive reactions can diminish between 48 and 72 hours. Only reactions which are palpable and infiltrated are classed as positive, according to the international criteria for reading patch tests, amended by the *European Task Force on Atopic Dermatitis* (ETFAD) (table 2) [23]. It is essential to clearly distinguish positive reactions from negative or doubtful ones, since only papular or infiltrated reactions are of clinical relevance. Reactions in babies and children are generally more severe than those of adults, their thinner skin encourages the penetration of allergens. New techniques are being developed to ensure a more reproducible reading of patch tests [24].

Table 1. Conditions necessary before carrying out APT [5, 13]

No current outbreak of the disease, no pregnancy
No application of corticosteroids on the APT site for the previous 7 days
No phototherapy treatment on the APT site for the previous 4 weeks
No oral corticosteroids
No cyclosporine or oral tacrolimus
No antihistamine treatments for at least 72 hours (to be adapted to the molecule)

Table 2. International Criteria for reading APT, revised by the ETFAD (European Task Force on Atopic Dermatitis) [23]

-	Negative
?	Erythema alone, doubtful
+	Erythema and infiltration
++	Erythema and a few papules
+++	Erythema and numerous or diffuse papules
++++	Erythema and blisters

Side effects

The occurrence of secondary effects during APT is infrequent (7.9% of cases) [25]. The reactions observed are moderate and most often localised to the site of application of the APT. It mainly concerns a local recurrence of the AD, contact urticaria, irritative contact dermatitis to the adhesive, pruritis or infiltrated erythema lasting several weeks. A generalised relapse of the AD (3 cases have been described) or exacerbation of underlying asthma (2 cases described) have exceptionally been found [26, 27]. There are no cases of active sensitization induced by APT described in the literature.

Interpretation of the results

Sensitivity, specificity and reproducibility of atopy patch tests

The sensitivity and specificity of APT can only be determined by looking at the clinical information (histories of exacerbation due to promoting factors before the tests) and not the results of prick tests or IgE, which do not evaluate the cellular composition of the AD. However, as the clinical history often contributes little and the re-introduction of the allergen is not feasible, the sensitivity and specificity of APT are often weak, even in standardized conditions. The sensitivity and specificity of APT, prick tests and specific IgE in adults are summarized in table 3 [23]. In children, the APT carried out by Niggeman *et al.* had a sensitivity of 76% and a specificity of 95% for diagnosing exacerbations of AD induced by food [18]. The APT had a higher specificity than the prick tests and specific IgE tests regarding their clinical relevance, despite a lower sensitivity.

Concerning allergological investigations of children with AD and suspected food allergy, recent studies have shown that APT only render oral provocation tests unnecessary in 0.5% to 14% of cases and that APT to food have only a weak positive predictive value [28, 29]. The value of APT

Table 3. Sensitivity and specificity of APT, prick tests and specific IgE tests with reference to the clinical history of 253 patients with AD; The allergens studied were house dust (*Der. P*), cat hairs and grass pollens [16]

Test	Sensitivity	Specificity
Prick tests	69%-82%	44%-52%
Specific IgE	65%-94%	42%-64%
APT	42%-56%	69%-92%

in the diagnosis of food allergy, associated or not to AD, seems therefore to be limited, the oral provocation test remaining the gold standard for applying a specific avoidance diet.

Finally, the APT carried out according to the protocol of Darsow and Ring, using non-commercial solutions of aeroallergens, had a reproducibility of 94% over an average observation of 16 months [30]. The results obtained from commercially available solutions are less reproducible: 56% of tests are new positives 4 to 12 weeks after a first test [31].

Clinical relevance of atopy patch tests

During the history, the discovery of a previous exacerbation induced by a specific allergen is a positive predictive factor for the corresponding APT. Although a history of exacerbation by house dust is often difficult to show, house dust mites (*Der p*, *Der f*) are the main aeroallergens detected (36% of cases), followed by cat hairs (22%), and grass pollens (16%) [14]. It should be noted that APT to house dust mites can be positive in healthy individuals in up to 23% of cases, but they are more frequently positive (49%) with a higher positivity, in AD patients [32]. An exception seems to be healthy individuals who have scabies, among whom 70% have positive *Der p* APT, probably due to a cross-reaction with the allergens of *Sarcoptes scabiei* [33].

There are several differences in function of the clinical forms of AD. The frequency of positivity of APT is in fact significantly increased in the case of AD affecting "exposed" parts of the body and when the AD worsens during spring and summer (frequency of APT to grass pollens).

In the event of positive APT, what action should be taken? What should be recommended to the patient? The results of avoidance diets seem poor in practice and there is no specific immunotherapy adapted to AD. For example, two randomised studies on the avoidance of house dust mites, carried out double blind against placebo, showed no significant clinical benefit for patients with AD. These avoidance strategies only seem to be partial as the professional, school and exterior environment are not concerned by the preventive measures applied in the home [34, 35].

Conclusion

APT are able to identify aeroallergens and food allergens implicated in the occurrence or aggravation of AD. The main allergens identified by this method are house dust mites, cat hair and grass pollens. APT enable more specific results to be obtained than prick tests and specific IgE tests because their epicutaneous application induces a reaction in which the pathophysiological mechanism is similar to that of AD. If the standardization of APT to aeroallergens has brought a certain reliability to this method, the same cannot be said for APT to food allergens, where the positive predictive value needs to be improved to avoid unjustified dietary modifications. Improvements in APT and progress in knowledge of the pathophysiology of eczemas will thus allow for new immunobiological diagnostic methods and the possibility of specific immunotherapies adapted to AD. ■

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