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Our Experiences with the Use of Atopy Patch Test in the Diagnosis of Cow's Milk Hypersensitivity

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SUMMARY Atopy patch test has been recognized as a diagnostic tool for the verification of food allergies in infants and small children suffering from atopic dermatitis. The test also has a role in the diagnosis of food allergies characterized by clinical signs associated with the digestive system. Yet, in spite of numerous studies, the test itself has hitherto not been standardized. Our study enlisted 151 children less than two years of age, who exhibited suspect skin and/or gastrointestinal manifestations of food allergy to cow's milk, and in whom tests failed to prove early type of allergic reaction. Atopy patch test was positive in 28% of the children with atopic dermatitis, 43% of the children with suspect gastrointestinal manifestation and 32% of the children with skin and gastrointestinal manifestations of food allergy. In our experience, atopy patch test is an excellent addition to the hitherto used tests for the diagnosis of food allergies. It targets specifically delayed type hypersensitivity reactions, which are difficult to confirm with other diagnostic tools. It is furthermore simple to perform, noninvasive and produces a minimum of undesired side effects. For these reasons, it should become part of the routine diagnostic toolset for food allergies to cow's milk in infants and children, and applied before a food challenge test.

KEY WORDS: cow's milk allergy, children, atopy patch test

INTRODUCTION

Food allergy is an immune reaction to an ingested antigen. Most food allergies appear in childhood and they are typically temporary, whereas in adults they develop less frequently but usually are permanent. The prevalence of food allergies in children is 6%-8% and in adults 1.4%-2.4% (1,2). While we today recognize more than 200 food allergens, a small subset of this group is responsible for 90% of all food allergic reactions. In children, these common allergens include cow's milk, hen's egg, peanuts, soy, flour, nuts, shells and fish. The leading antigen in this group is cow's milk, affecting 2%-3% of formula-fed and 0.3%-0.5% of breastfed infants. In adults, the most common food allergens are shells, peanuts, nuts more generally and fish (1,2).

With respect to the type of immune reaction, food allergies can be divided into IgE-mediated

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(early) and non-IgE-mediated (delayed) responses. The clinical symptoms and signs in food allergies range widely (3) and include digestive (4), skin (5) and respiratory system (6) signs, anaphylactic reaction, and some extremely rare manifestations (3). The most frequent IgE-mediated gastrointestinal manifestations of food allergies are oral allergic syndrome, vomiting, abdominal cramps and diarrhea, whereas non-IgE-mediated responses include chronic diarrhea, hemorrhagic proctitis/proctocolitis, chronic constipation, allergy-mediated gastroesophageal reflux and eosinophilic gastroenteropathy (3,4). IgE-mediated skin manifestations of food allergies include hives, angioedema and atopic dermatitis (AD), whereas non-IgE-mediated responses manifest as AD and vasculitis (3,5). Finally, IgE-mediated respiratory tract symptoms are allergic rhinitis, laryngitis and asthma, and non-IgE-mediated hypersensitivity pneumonitis and Heiner syndrome (6).

In AD, the role of hypersensitivity to food in inducing AD symptoms cannot be denied (7-11). The prevalence of food allergy in children suffering from AD ranges from 20% to 80% in different studies, and is estimated to be around 30% (9-11).

To prove food hypersensitivity in children, we use family history, clinical picture, *in vivo* and *in vitro* tests. *In vivo* tests for the diagnosis of food hypersensitivity include skin prick test (SPT), atopy patch test (APT), skin application food test (SAFT), and food challenge tests (open challenge test, single-blind food challenge test and double-blind placebo-controlled food challenge test (DBPCFC). DBPCFC is today the 'gold standard' in the diagnosis of food hypersensitivity (6,7,12-14). *In vitro* test usually involves specific IgE antibodies (6,7,12-14). While early type of food allergy is more easily confirmed by medical history, clinical picture, SPT and measurement of specific IgE antibodies in serum in combination with a positive food challenge test, the delayed-type allergy reaction to food poses a diagnostic problem (6,7,12,13).

APT is defined as patch test with allergens known to cause IgE-mediated sensitization. The method is based on cutaneous T-cell response following epicutaneous allergen application (15,16). Allergen is applied onto healthy skin, usually on the back or upper arm. The response in the form of an eczematous skin lesion is read after 48 or 72 hours (17,18). In 1996, Isolauri and Turjanmaa first pointed to the importance of APT in the diagnosis of food allergy in small children with AD (19). The role of APT in the diagnosis of food allergies remains unexplained. Interpretation of the test is subjective and has not been standardized (20,21). In this article we will discuss our experiences with APT.

MATERIALS AND METHODS

The study recruited children aged 3 weeks to 24 months (median 13.5 months), who, on the basis of history and clinical picture, have been suffering from clinically suspect food allergy to cow's milk proteins. All children, in the course of 2008, were examined at Zagreb Children's Hospital Department of Children's Diseases, at Referral Center for Pediatric Gastroenterology and Nutrition of the Department of Pediatrics, or at Outpatient Clinic for Child and Adolescent Dermatology and Venereology. The clinically suspect food allergy to cow's milk protein was defined as primary skin manifestation (AD) or primary gastrointestinal manifestation or both. AD was diagnosed using standard diagnostic criteria (22) and the severity of the condition was evaluated with SCORAD index (23). Because severe clinical picture of AD is linked to a higher probability of food allergy, the majority of study patients were diagnosed as suffering from moderate (25-50 points) or severe (over 50 points) AD. Gastrointestinal manifestations of food allergies included chronic diarrhea, hematochezia, abdominal colic and vomiting, chronic constipation and poor weight gain (2).

Study patients were divided into three groups: group 1 included children with primarily skin manifestations (AD); group 2 children with primarily gastrointestinal manifestations; and group 3 children with both skin and gastrointestinal manifestations.

All children were subjected to the standard diagnostic tests for food allergies to cow's milk proteins. These include absolute eosinophil count, total serum IgE, specific serum IgE to cow's milk, stools for occult bleeding, and, if necessary, milk oral challenge test. The study included children in whom IgE-mediated response to cow's milk proteins could not be confirmed (negative SPT to cow's milk and negative specific serum IgE to cow's milk).

APT was performed with an allergen prepared at the Institute of Immunology, Inc., Zagreb, Croatia. The allergen was mixed in a concentration of 20% into the petrolatum vehicle and applied with Curatest Lohmann Rauscher patches. The cream milk powder with the appropriate certificate of quality was bought from Dukat, Inc. Petrolatum was applied as negative control in all subjects (Fig. 1).

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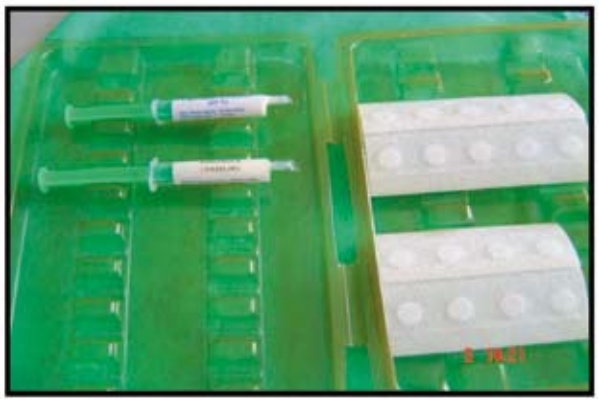

Figure 1. Allergen prepared in injections and plasters for epicutaneous testing.

Samples of the allergen were applied on the skin of the back (Fig. 2) with Curatest Lohmann Rauscher plasters for epicutaneous testing to the standard allergen series with 12-mm chamber diameter. Within the chamber, we applied the allergen in the amount of 5 mL. They were kept under occlusion for 48 hours. The skin of the back had to be free from eczematous lesions and other signs of inflammation. No application of anti-inflammatory medications was allowed for seven days before the test, no emollients for two days before the test, and no exposure to UV rays for four weeks before the test. Children were not allowed to take antihistamines or other anti-inflammatory medications for seven days before the test. Test results were read as follows: (a) a check 20 minutes after the application of the allergen (early reaction); (b) first assessment 48 hours after the test, 20 minutes of allergen removal; and (c) second assessment 72 hours after the test.

Allergic response at the site of APT application was interpreted as negative, uncertain (erythema only) or positive. A positive reaction could be (+) weakly positive: erythema and slight infiltration; (++) strongly positive: erythema, infiltration and papules; or (+++) very strongly positive: erythema, infiltration, papules, vesicles, with 'crescendo' phenomenon (24). 'Crescendo' is defined as an intensification of the response in the patch test between 48 and 72 hours. Irritation or 'decrescendo' type is defined as a decreased intensity of the reaction between the 48- and 72-h evaluations. The result of the skin test is recorded in the forms.

The test was approved by the Department of Children's Diseases Ethics Committee. Parents were informed in detail on the study and the methods used, and they signed the informed consent form.

RESULTS

The study recruited 151 children (65 female and 81 male) aged 3 weeks to 24 months, mean age 13.5 months. In all children, cow's milk allergy was suspected on the basis of history and clinical picture, yet no test succeeded in proving early-type allergy reaction (SPT and specific IgE test results were normal). Group 1 included 50 children with primarily skin manifestations, group 2 included 67 children with primarily gastrointestinal manifestations, and group 3 included 34 children with combined skin and gastrointestinal manifestations. Positive reaction on APT was recorded in 14 (28%) of 50 children with AD suspected of cow's milk allergy, 29 (43%) of 67 children with gastrointestinal manifestation, and 11 (32%) of 34 children with both skin and gastrointestinal manifestations. The test was declared unsuccessful in two patients with AD, whose pronounced nervousness and irritability had led their parents to remove the plaster. One child with AD had diarrhea during the test. No other undesired reactions were recorded. Positive results of APT to cow's milk are shown in Figures 3 and 4.

DISCUSSION

The role of APT in the diagnosis of food allergies remains unresolved (25). The test itself and reagents are not standardized, so the time of occlusion, the concentration of the allergen (fresh milk, powdered milk) and the medium vary (26-28). In recent years, APT has been recognized as a diagnostic method of choice for the confirmation of food allergies in infants and small children with AD (18). It also has a role in the diagnosis of food allergies where the clinical picture is associated with

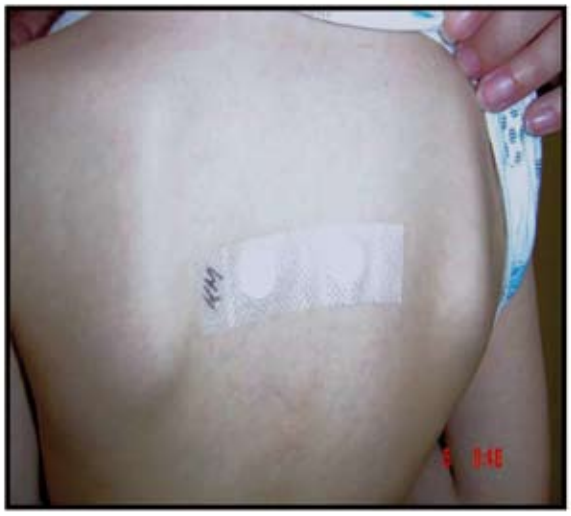

Figure 2. Allergen on the skin of the back.

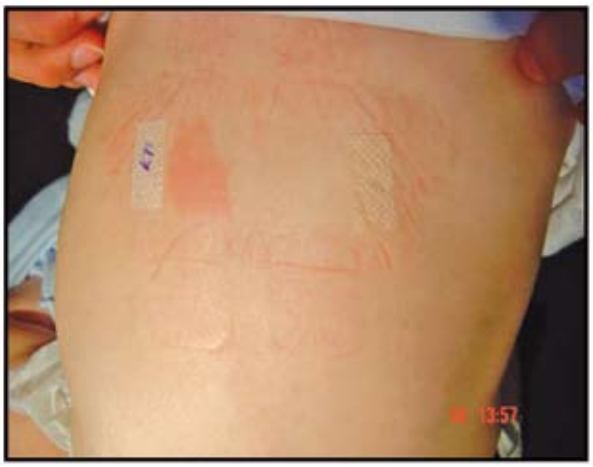

Figure 3. Positive result of APT to cow's milk.

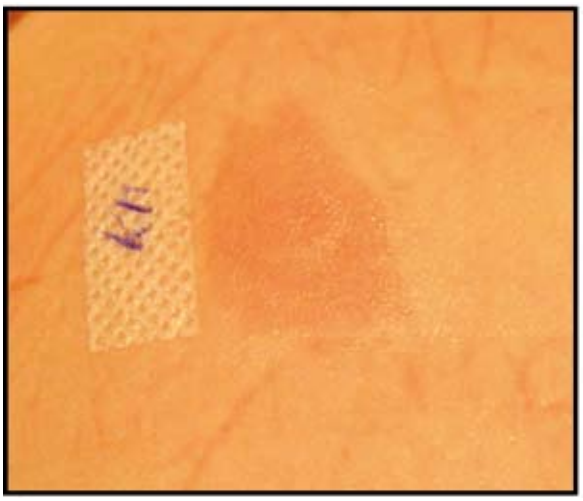

Figure 4. Positive result of APT to cow's milk.

the gastrointestinal system (29-31). All of these are delayed, non-IgE-mediated types of allergic reaction. DBPCFC remains the 'gold standard' in the diagnosis of food allergy (6,7,12-14). However, the food challenge tests must be conducted in a hospital environment and by well-trained personnel capable of swift and successful response should an anaphylactic reaction develop. The food challenge test is furthermore often difficult to interpret, especially in children with AD and polysensitized patients (32). Roehr *et al*. argue that APT, SPT and assessment of specific IgE antibodies in serum of AD patients reduce the need to conduct a food challenge test (27).

The interpretation of APT to food allergens remains subjective and not standardized (33,34). Studies have confirmed that the optimal time of allergen occlusion is 48 hours (17). Niggemann *et al*. demonstrated the 12-mm chambers (Finn Chamber-Herman, Reinbeck, Germany) for allergen application to be superior to 6-mm chambers, even in infants and small children (35). Although some authors found good correlation of APT results when using chambers with a diameter under 12 mm and the food challenge test (19,36,37), in our study we used the Curatest Lohmann Rauscher plasters for epicutaneous tests to the standard allergen series, with 12-mm chamber diameter. Within the chamber, we applied the allergen in a quantity of 5 mL. Because of the patients' young age, we decided to apply the allergen onto the skin of their back only. Because anti-inflammatory medications and antihistamines may modify APT results, the study included children that had not been treated with anti-inflammatory medications for at least seven days prior to the test and with emollients for two days before the test (38-40). The children were not allowed to take antihistamines for seven days before the test. Although some authors suggest prepping the skin before the test, for instance by 'tape stripping the skin 10 times as pretreatment' (41), we decided to skip this step because the thin skin of infants and children under two years of age might have exhibited irritation.

There are few data on the optimal allergen concentrations. Most authors use a drop of undiluted fresh cow's milk (20,24). To exclude false-positive reactions, they also test allergens in lower concentrations, for instance 1:10 (24). Some authors use diluted milk or powdered milk diluted in petrolatum or solution (28,30). The method that we applied, where petrolatum is used as a medium, is easier to conduct, there is no need for skin pretreatment, and the reaction is macroscopically more intense than in the tests where liquid is used as a medium (34). We agreed with the Institute of Immunology, Inc. to produce the allergen in a concentration of 20% in petrolatum as a medium. The allergen was prepared in injections, a form easy to administer and store. The study recruited children under 24 months of age, whose skin is thin, permeable and thus allows for easy penetration of the allergen.

Most studies did not use negative control (21). Darsow *et al*. found positive reaction to the medium in 3 (0.99%) of 302 patients (42). To allow for easy interpretation of the test, we used pure petrolatum as negative control. The cases where the reaction to the negative control was similar to the response to the allergen as late as 72 hours after administration were interpreted as irritations. Test results were interpreted in accordance with the interpretations in the literature (24).

No undesired reactions to cow's milk APT may be found in the available literature. In our study, in two AD patients the parents removed the patch

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in the course of the first night (after 12 hours) because of the pronounced irritability and nervousness of the children. Both children suffered from a severe form of AD, so it is possible that they were irritated by the plasters. One child with AD developed diarrhea during the test, but it could not be associated with the test. We recorded no other undesired reactions. APT is a simple and safe test. The literature mentions local reactions to APT to hen's egg, such as contact hives and localized itch around the site of application 5-15 minutes after allergen application (18). It is precisely for that reason that we allowed for the possibility of an early-type reaction and introduced a check 20 minutes following the application of the allergen.

We recruited into the study children under the age of two years whose clinical picture raised suspicion of cow's milk allergy, yet their results of SPT and specific IgE test failed to corroborate it. It is well known that positive SPT and specific IgE in older children do not necessarily confirm cow's milk allergy because of false-positive reactions. The main reason probably lies in the fact that the test results remain positive even after the affected person has developed tolerance. For this reason, we must always perform a food challenge test, to avoid the danger of keeping children on unnecessary and often dangerous diet regimes. Yet in children younger than two years – and especially under twelve months – the situation is slightly different. Infants whose immune system is undeveloped usually react with a negative SPT and show no specific serum IgE, so a finding of positive SPT to cow's milk and specific IgE confirm positive reaction to cow's milk. Yet negative tests do not exclude reaction to cow's milk, because these children may exhibit a delayed type of allergic reaction, which cannot be confirmed using these tests. This especially applies to food allergies manifested with AD, hemorrhagic proctitis or proctocolitis, chronic diarrhea and constipation (43,44). We accept this hypothesis as well as the thesis that APT confirms delayed type allergic reactions, so among the children who had positive clinical picture yet no laboratory proof of allergy we isolated a group in whom allergy could be demonstrated with positive APT result. The largest number of positive results came from the group of children with suspect gastrointestinal manifestation of food allergy to cow's milk (43% of study patients). Fewer positive results were obtained in the group in which patients had both skin and gastrointestinal manifestations (32%), and the lowest number came from the children with AD (28%). The flaw of our study was that we did not perform food challenge test in all children, so we could not evaluate the test reliability.

Children with positive reaction were prescribed a dietary regime that excluded cow's milk over a period of four to six weeks. We then looked for the evidence of improvement of the clinical picture. The patients that exhibited clinical response to dietary regime were then, with approval of their parents, subjected to the food challenge test, which confirmed the allergy. This test was, however, not performed in all children with positive APT and clinical response, mostly because the parents, having seen clinical improvement, were reluctant to expose their child to the allergen again.

Although the food challenge test remains the "gold standard" in diagnosing food allergy, APT may have a value in the process of decision making when reintroduction of cow's milk into the child's diet is considered. Namely, retesting and negativization of the test in those previously positive may be a screening method to establish the moment when a food challenge test with cow's milk may be reintroduced, following a period of elimination dietary regime. Our daily practice has confirmed APT as an excellent addition to the allergy tests to cow's milk routinely used at our Department.

CONCLUSION

In spite of the earlier clinical studies, many questions about the efficiency of APT in the diagnosis of food allergies as well as about standardization of the reagents and procedures remain unanswered. In our experience, APT is an excellent addition to the currently used tests for the diagnosis of food allergies because it targets delayed type allergic reactions that other tests fail to detect. It is furthermore easy to conduct, noninvasive, and produces few undesired reactions. Yet its reliability remains insufficiently investigated. It is thus the task of future clinical studies to determine more precisely the reliability of the test (the share of false-positive and false-negative results), in the first place by consistently conducting a food challenge test, which remains the only fully reliable method.

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