

Short communication

Diagnostic value of nasal provocation challenge with allergens in children

Jean R, Rufin P, Pfister A, Landais P, Waernessyckle S, de Blic J, Scheinmann P. Diagnostic value of nasal provocation challenge with allergens in children.

Allergy 1998; 53: 990–994. © Munksgaard 1998.

A total of 106 children suffering from perennial rhinitis and/or asthma, and all allergic to *Dermatophagoides pteronyssinus* (DP), underwent nasal provocation challenge (NPC) with DP to determine the best method of diagnosis. Posterior rhinomanometry was uninterpretable in 17 patients and gave negative results in 31. Clinical scores for sneezing and rhinorrhea were more effective but did not diagnose the disorder in 11 children. However, nine of the 11 had significant increases in eosinophil count in the late phase. Clinical scores and cytology were also useful for assessing whether NPC with allergens was positive in children. The feasibility and safety of NPC with DP are high for rhinitic and stable asthmatic patients, but mild reactions may occur during the late phase.

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Key words: allergy to *Dermatophagoides pteronyssinus*; children; nasal cytology; nasal provocation challenge; nasal provocation test; rhinomanometry.

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Accepted for publication 4 June 1998

Nasal provocation challenge (NPC) with allergens has been studied in adults (1, 2), but its diagnostic value in allergic children with perennial rhinitis and/or asthma has rarely been assessed (3, 4). The aim of this study was to determine:

- 1) the best method: rhinomanometry (RNM), clinical scores, cytology, or a combination of methods
- 2) the safety and feasibility of an ambulatory method for children.

Material and methods

Patients

A total of 106 children, 77 boys and 29 girls, allergic to *Dermatophagoides pteronyssinus* (DP) and aged 4–16 years (mean 9.3), were included in the study. Forty-nine had asthma and allergic rhinitis (group 1); 28 had asthma without rhinitis (group 2), and 29 had allergic rhinitis without asthma (group 3). All had positive skin prick tests to lyophilized extracts of DP (Laboratoire des Stallergènes, France) and high blood concentrations of specific IgE (RAST $\geq$ class 3).

Twenty nonatopic children, aged 5–16 years

(mean 9.4), suffering from chronic rhinitis with borderline results in prick tests to DP and with specific RAST class 0, were also studied (control group 1). In addition, 19 atopic children, aged 4–15 years (mean 7.6), with allergic rhinitis and/or asthma and positive skin tests and RAST for animal danders but negative tests for DP, were also tested with DP at the explicit request of their pediatricians (control group 2).

Corticosteroid treatment was stopped for 8 days, sodium cromoglycate and antihistaminic treatment for 4 days, and any nasal drops for 24 h before the study. For reasons of safety, theophylline and beta₂-agonists were not stopped. Informed consent was obtained from the parents and children whenever possible.

Nasal provocation challenge with DP

The test was performed at 9 a.m., with patients free of acute exacerbation but sometimes presenting with nasal symptoms and moderate bronchial obstruction ($sRaw < 1.2$ kPa·s⁻¹/l). The same batches of lyophilized allergen were used as for the prick tests. The strength of the DP extracts is

expressed in IR units (IR=index of biologic reactivity) (4, 5). Extracts were dissolved in normal saline immediately before use and transferred to plastic hand-held nebulizers. One dose of the allergen solution (0.1 ml) was instilled into each nostril during apnea or expiration. A nose-clip was applied immediately after each insufflation and kept in place for 20 min to prevent inhalation into the bronchi. The first dose was 0.2 IR. If there was no response according to clinical and/or RNM criteria, a second dose of 1.2 IR was administered, followed by a third dose of 3.2 IR if 1.2 IR gave a negative result. A control test with saline solution preceded the instillation of allergen.

Immediate response

The immediate response was assessed by the following three methods:

- 1) *Clinical scores.*
- 2) • the number of sneezes counted by the child and parents, scored: 0 (no sneeze), 1 (1–3 sneezes), 2 (4–8 sneezes), and 3 (≥ 8 sneezes).
• the volume of nasal secretions, scored: 0 (no secretion), 1 (a little), 2 (profuse), 3 (very profuse, fluid, and sticky secretion).
- 2) *Posterior RNM.* This was performed with an NR3 Mercury Rhinomanometer (Glasgow, UK). At least four tidal volumes were recorded. The response was considered positive when total nasal resistance (NAR) at 75 Pa was double that measured after saline instillation (6). The test was stopped only when the sneeze score was 2 or 3 (7) or the secretion score was 3 or NAR was doubled: two markers were necessary to stop the test.
- 3) *Nasal cytology.* Patients blew their noses onto clean glass slides before the test (4, 8), 20 min after instillation of saline, and 20 min after each instillation of allergen. The smears were stained by the May-Grunwald-Giemsa and Luna methods (9). The mean eosinophil count was determined for 50 fields. The response was considered positive when the percentage of eosinophils was at least 10% higher than that recorded at the start of the experiment (4, 10). The increase in the amount of eosinophils was not significant if eosinophilia was $\geq 50\%$ after saline instillation.

NPC with DP was scored as positive if the patient had at least two of the following four markers: sneeze score 2 or 3 (no sneeze before the test), grade 2 or 3 nasal secretions (when initial secretions were scored 0 or 1), NAR doubled, and $\geq 10\%$ increase in eosinophil

count (result available several days after the end of the test).

sRaw was measured in each asthmatic child with a body plethysmograph before the test and 30 min after the last dose of allergen.

Late response

Late response was evaluated by the following two methods 4, 12, 24, and 36 h after the instillation of allergen:

- 1) *Nasal cytology.* The parents were asked to make their children blow their noses on numbered slides. Smears were spread out, air-dried, and sent in a plastic box by mail to the cytology laboratory.
- 2) *A questionnaire on clinical symptoms.* The parents reported the number of sneezes; the volume of nasal secretions graded 0–3; any nasal obstruction graded as 0 (none), 1 (obstruction of one nostril), 2 (of both nostrils), and 3 (nose completely blocked); and other symptoms such as nasal or pharyngeal pruritis, conjunctivitis, cough, and asthma.

The late response was scored as positive if clinical symptoms had appeared or worsened or if there was a significant increase in eosinophil count.

Subsequent NPC

A second NPC with DP was performed 2 weeks later in 23 randomly chosen patients in the same clinical state as before the first test: 14 from group 1; five from group 2; one from group 3, and three from the first control group.

Thirty volunteer patients (22 from group 1 and eight from group 2) were given immunotherapy with DP extracts and underwent NPC with DP every year for 3 or 4 years.

Statistical analysis

Nonparametric tests were used (the Wilcoxon test for paired data). The alpha value was adjusted for the number of tested comparisons by Bonferroni's method. Two-sided tests were performed.

Results

Control groups

The 20 patients of control group 1 showed no significant changes in clinical symptoms or RNM after the final dose of 3.2 IR. Eosinophilia was $2.5 \pm 3.14\%$ after saline, $1 \pm 1.25\%$ immediately

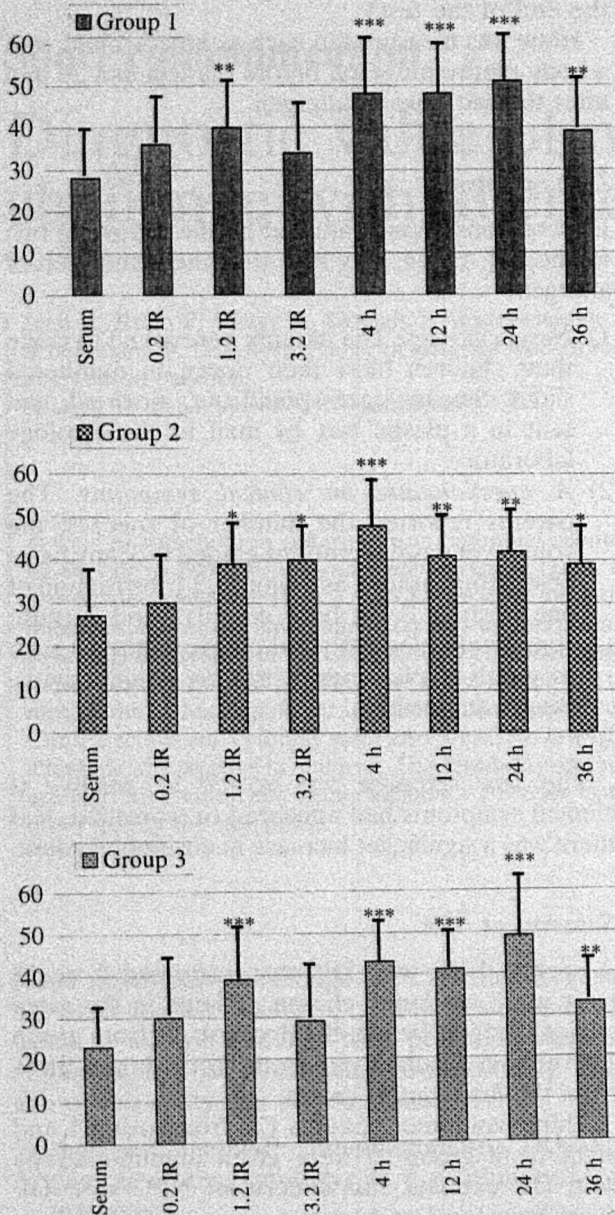


Fig. 1. Eosinophils (percentage of total inflammatory cells) during nasal provocation challenge in 106 children allergic to *Dermatophagoides pteronyssinus* (DP). Group 1: asthma and rhinitis; group 2: asthma without rhinitis; group 3: rhinitis without asthma; IR: index of biologic reactivity. Correlations between responses after saline and after 1.2 IR are marked on figure, as are correlations between response after saline and after 4, 12, 24, and 36 h in three groups of patients. * $0.01 \leq P \leq 0.05$; ** $0.001 < P < 0.01$; *** $P \leq 0.001$.

after 3.2 IR, and $2.6 \pm 2.37\%$ 24 h after 3.2 IR. None of the patients had clinical symptoms of late-phase reaction or an increase in eosinophil count of at least 5%. The 19 patients of control group 2 showed no changes in clinical symptoms or RNM. Eosinophilia was $6.7 \pm 13.3\%$ after saline, $5.7 \pm 11.4\%$ after 3.2 IR, and $8.6 \pm 15\%$, 24 h after 3.2 IR (NS).

Allergic children

All 106 children of groups 1, 2, and 3 (allergic to DP) were given the first dose of 0.2 IR, 93 were given 1.2 IR, and 59 were given 3.2 IR.

Immediate response

- 1) **Clinical scores.** The average number of sneezes was $4 (4.38 \pm 4.16)$: 43 children (40.5%) had a sneeze score of 2, 16 (15%) of 3, and 21 (20%) of 1, and 26 patients (24.5%) did not sneeze. Grade 3 rhinorrhea was observed in 81 of the 106 patients (76.5%), grade 2 in 10 patients (9.5%), and grade 0 or 1 in 15 patients (14%). Obstruction was evaluated by RNM, which is a more objective measure of nasal obstruction than patient perception. A few patients had itching, conjunctivitis, and sore throat.
- 2) **RNM.** NAR was $0.45 \pm 0.25 \text{ kPa} \cdot \text{s}^{-1}/\text{l}$ after saline and $0.81 \pm 0.64 \text{ kPa} \cdot \text{s}^{-1}/\text{l}$ after the last dose of allergen ($P < 0.0001$). NAR was doubled in 58 patients (55%), not doubled in 31 (29%), and uninterpretable in 17 (16%). RNM results were positive in the 15 patients whose secretion score was less than 3, suggesting the presence of nasal obstruction.
- 3) **Nasal cytology.** Eosinophil counts increased significantly after the last dose of allergen (Fig. 1) in the three groups: 19 patients (18%) had initial eosinophilia of $\geq 50\%$ and could not be evaluated; 55 patients (52%) tested positive and 32 (30%) negative.
- 4) **Tolerance.** No cough or dyspnea was reported shortly after the immediate phase. Specific bronchial resistance measured in asthmatic children was unchanged: $0.70 \pm 0.19 \text{ kPa} \cdot \text{s}^{-1}/\text{l}$ before the test and $0.64 \pm 0.20 \text{ kPa} \cdot \text{s}^{-1}/\text{l}$ after the last dose of allergen.

Late response

- 1) **Clinical symptoms.** Fifty-eight of the 106 patients (54.5%) suffered clinical exacerbation of rhinitis and thus had a late-phase response, and 38 (36.5%) had no nasal symptoms; 10 responses to the questionnaire were uninterpretable.
- 2) **Tolerance.** Cough without dyspnea was reported for 34 of the 58 positive patients (14 in group 1, 15 in group 2, and six in group 3) and mild asthma for six (five in group 1 and one in group 2), but standard medication was effective. Eye itching, ear pain, and tiredness were rare.
- 3) **Nasal cytology.** The number of eosinophils greatly increased after 24 h, even in group 2

(Fig. 1). Thirty of the 32 children with no increase in eosinophil count during the immediate phase had a significant increase during the late phase.

Thus, the combination of clinical symptoms, posterior RNM, and immediate and late eosinophilia classed only two of the 106 patients as negative, in contrast to the results of prick tests and RAST.

Second NPC: immediate response

The final dose of allergen was the same as for the first test for 16 patients and higher for seven, showing that there was no priming effect (11). Three children tested negative in both tests (control group 1). Only one of the 20 patients who tested positive in the first test tested negative in the second test because of high initial nasal eosinophilia (>50%).

Late response

We studied the clinical scores for 19 patients: 18 had a late response to both tests and one patient had a positive late response in the first test only. Cytologic results were obtained for 15 patients: 11 tested positive in both tests and four tested negative in both tests.

Response after 1–4 years

A total of 79 patients with asthma (groups 1 and 2) were given immunotherapy with DP extracts, and 30 of the 79 agreed to undergo NPC every year. In most patients, the clinical symptoms of asthma improved whereas those of rhinitis did not. After 1 year, all but one of the patients had a positive immediate response. Only 10 patients had a positive late-phase response, whereas 23 had had such a response before immunotherapy. Twenty patients were retested after 4 years: 19 had an immediate response and eight a late response (16 before immunotherapy).

Discussion

We assessed the use of NPC for clinical diagnosis in children suffering from perennial rhinitis and/or asthma, and we used DP because it is the major allergen in children.

RNM is considered to be the most convenient method of diagnosis. An NAR value double that after saline instillation is the standard marker (6). Active posterior RNM is well tolerated by children because the two nostrils are tested simultaneously and the influx of secretions does not affect pressure

recordings. However, results were uninterpretable in 17 cases: this is a major disadvantage of this method compared to the anterior method (1). Thirty-one cases were interpretable but negative, and the anterior method would have been ineffective because there was no clinical obstruction. The increase in nasal secretions and sneezing often began before nasal obstruction due to swelling of the mucous membrane (secretions remaining in the nostrils increased NAR for several minutes and had to be excluded before measurement). Thus, RNM, whatever the method used (active anterior or active posterior), is not appropriate for measuring the response to NPC in children with perennial rhinitis and/or asthma. This suggests that it would have been better to evaluate nasal obstruction in the immediate phase not only objectively by RNM but also by a clinical score, because this would give some idea of the patient's perception of nasal obstruction.

The sneeze score is correlated with the secretion of mediators (7), but it could not be used in 26 patients. The rhinorrhea score was more informative but could not be used for 15 patients with major nasal obstruction. Even when the two clinical scores were combined, they did not detect an immediate response in 11 patients.

For cytology, similar eosinophil counts are obtained as for samples taken by blowing and brushing (12). Cytology is not useful for the immediate phase because results are not known immediately, an initial increase in eosinophil count of at least 50% prevents interpretation of the results, and very fluid (grade 3) nasal secretions cannot be easily stained on glass slides. Cytology is useful for the late phase: an increase in the eosinophil count of $\geq 10\%$ (10) indicated positive NPC results in nine of the 11 patients who had apparently tested negative according to clinical scores during the immediate phase. Nasal cytology is more sensitive than RNM or whole-body plethysmography (13) for detecting the late-phase response and is suitable for children because it is painless and ambulatory.

A major advantage of NPC is its safety. It was studied in 77 asthmatic children: 19 had a second test, and 30 had three or more tests. NPC did not increase bronchial resistance immediately after the test and so may be used in stable asthmatic children. A similar level of safety has been reported for forced expiratory volume and saturation in asthmatic children (3). However, the parents should be warned about the possibility of asthma at home, and appropriate treatment should be planned. Asthma during the late phase may be due either to NPC or to the withdrawal of inhaled corticosteroid therapy for the week before the test.

This ambulatory method is very feasible for

young patients. A composite clinical score including the number of sneezes, the grade of secretions, and the extent of other clinical symptoms, as described by Naclerio et al. (7) and Bousquet et al. (14), could be used. RNM, which requires expensive apparatus, is not essential. Nasal secretions can be collected by blowing the nose 4, 12, and 24 h after the challenge without distressing the child. The parents are valuable coworkers in collecting secretions during the test and completing the questionnaire during the late phase at home.

We could not assess the reproducibility of NPC results because only 23 of the 126 patients were rechallenged after 2 weeks, and only 30 after 1 year. However, our results suggest that a prospective study would be justified, not only for evaluating the reproducibility of NPC with allergens (15) but also for assessing the effects of immunotherapy (5).

In conclusion, NPC in allergic children with asthma and/or perennial rhinitis has high diagnostic value, provided that RNM is not the only method used for evaluation of the response; sneezing and rhinorrhea are important markers for the immediate phase and eosinophil count for the late phase. An ambulatory test could be easily used to diagnose difficult cases in asthmatic children. NPC is safe even if mild reactions may occur during the late phase.

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