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## Specific nasal provocation tests in patients hypersensitive to mould allergens

Marek Modrzyński<sup>1ABCEFG</sup>, Edward Zawisza<sup>2CDG</sup>

<sup>1</sup> "Euromedica" Regional Outpatient Allergology Clinic, Grudziądz, Poland

<sup>2</sup> Warsaw Branch, Polish Society for Combating Allergic Diseases, Warsaw, Poland

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### Summary

**Background:**

In the case of allergies to outdoor mould spores and plant pollen allergens, which are often concomitant, doubts may arise as to the role of mould allergens in inducing specific clinical manifestations. Establishing their significance is crucial for qualifying the patient for specific immunotherapy. This information also helps to reduce the percentage of therapeutic failures.

**Material/Methods:**

The study population comprised 31 patients with seasonal allergic rhinitis who were allergic to both plant pollen allergens and mould spores. In each patient we performed a skin prick test, sIgE level determination, and a specific nasal provocation test using mould allergens, according to standard allergological diagnostic procedure, and the results were objectified using acoustic rhinometry.

**Results:**

Positive NPT results were obtained in 22 patients. The positive results were more frequent in persons allergic to *Cladosporium herbarum* (92.3%) than in those allergic to *Alternaria alternata* (55.5%); these differences were statistically significant ( $p=0.039$ ). No differences were found between the two subgroups in terms of the sIgE levels ( $p=0.13$ ). In the study group as a whole, a high correlation between the results of acoustic rhinometry and the intensity of clinical manifestations was observed after administration of the NPT (correlation coefficient of 0.9). The correlation between the sIgE levels and the results of the skin prick tests and the NPT was much lower (0.48 and 0.66, respectively).

**Conclusions:**

The specific nasal provocation test is a safe and easy diagnostic modality, and acoustic rhinometry provides a good method of measuring it.

**key words:**

plant pollen allergens • allergic rhinitis • sIgE • acoustic rhinometry

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**Author's address:**

Marek Modrzyński, "Euromedica" Regional Outpatient Allergology Clinic, ul. Chełmińska 74, 86-300 Grudziądz, Poland, e-mail: euromedica@lekarz.net

## BACKGROUND

Mould spores are common, well-known allergens that can be responsible for atopic diseases of the upper and lower respiratory tract. Because of the ubiquity and diversity of the fungi, the clinical manifestations in allergic persons can occur all year long, with possible exacerbations depending on the allergen concentration in the environment. Allergy to fungi is rarely monovalent; most frequently it co-exists with hypersensitivity to inhaled allergens that may appear in the environment on or about the same time. The classic example is the concomitant allergy to plant pollen and so-called outdoor fungi, the greatest concentration of which occurs, in the temperate climate zone, during summertime [1]. Since neither the positive results of skin prick tests nor the presence of relevant sIgE in serum explicates the role of a given allergen in the induction of clinical manifestations, the concomitant allergy may lead to diagnostic problems [2,3]. This is especially important with regard to decisions regarding the application of specific immunotherapy for fungal allergens, especially since the therapy is long and its efficacy, although confirmed [4], is considered to be lower than in the case of desensitization for pollen allergens. Additionally, the cost of therapy is higher, since it is not possible to administer a single vaccine containing both mould and pollen allergens. It is therefore necessary to apply a test that will simulate natural exposure to the allergen. This requirement is largely met by provocation tests. One type, which is particularly useful in allergological outpatient facilities and was used in this study, is the specific nasal provocation test (NPT).

The primary aim of this study was to assess the results of specific nasal provocation tests with mould allergens in patients with signs and symptoms of seasonal allergic rhinitis and positive results in skin prick tests for mould and plant pollen allergens. The ultimate goal is to objectify the need for specific desensitization after adding the mould NPT to the conventional diagnostic modalities.

## MATERIAL AND METHODS

The study was conducted in a group of 31 patients (24 women and 7 men) between 19 and 41 years of age (median age 31.2) with seasonal allergic rhinitis induced by grass pollen (and motherwort in 6 cases), in whom skin prick tests and sIgE determinations (ELISA immunoenzymatic method, RV Mini System Allergopharma, mx903 discs) revealed hypersensitivity to allergens of either *Alternaria alternata* or *Cladosporium herbarum*.

In evaluating the results from skin prick tests (PST), a semi-quantitative positive scale was used to compare the size of the blister following allergen administration and the size of the blister induced by administration of histamine [5,6]:

- 0° – Allergen-induced reaction was equal to the negative control reaction;
- 1° – Mean diameter of the allergen-induced blister was greater than the size of the reaction to the negative control fluid and smaller than half of the diameter of the histamine-induced blister;
- 2° – Mean diameter of the allergen-induced blister was equal to or greater than half of the mean diameter of the histamine-induced blister;

- 3° – Mean diameter of the allergen-induced blister was equal to the mean diameter of the histamine-induced blister;
- 4° – Mean diameter of the allergen-induced blister was at least twice the mean diameter of the histamine-induced blister, or a pseudopodia reaction occurred.

Following the commonly recognized rule, no reaction smaller than 3 mm was taken into consideration.

In order to determine the clinical significance of mould allergy in all the patients, specific nasal provocation tests were applied at the same period in late March and early April, using the allergens of *Alternaria alternata* or *Cladosporium herbarum*, depending on the results of the skin prick test.

A ready-made nasal provocation test manufactured by the Allergopharma company (400 – *Alternaria alternata*; 405 – *Cladosporium herbarum*) was used in this study. The test was administered using a single dose. The allergen was sprayed into both nasal cavities in the region of the anterior part of the inferior nasal concha following the administration of the control solution provided by the manufacturer (0.9% saline solution, applied about 15 min. prior to the application of the allergen). Before the allergen was administered the patient inhaled deeply and held his/her breath.

Only the early-phase reaction (EPR) was taken into consideration in this study, by means of the combination of subjective and objective assessments of the test.

Monitoring of clinical manifestations during NPT was done by a very simple grade scale, proposed by Beli et al. in the 1980s, in which the presence (1 point) or absence (0 points) of one of the nine following manifestations was taken into consideration [7]:

1. Sneezing;
2. Itching nose;
3. Nasal obstruction;
4. Watery nasal discharge;
5. Nasal mucosal edema;
6. Nasal mucosal congestion;
7. Congestion/pruritus of the conjunctivae;
8. Congestion/pruritus of the throat;
9. Wheeze, dyspnea.

According to the authors' suggestions, we considered the test result to be positive if the score was equal to or greater than four. For sneezing the threshold value was assumed to be at least three consecutive sneezes, which reflects data available in the literature.

In order to evaluate test results, acoustic rhinometry (AR) was performed using the RhinoScan 2000 acoustic rhinometer (Rhinometrics, Denmark). The test was performed in accordance with the current consensus [8], an average of 15 minutes following the administration of the allergen, that is, at the time of the most pronounced nasal mucosal edema after positive the provocation test [9,10]. During each test three curves for each nasal cavity were recorded, and the results were subsequently averaged.

In order to prevent the results from being affected by the nasal cycle, on each occasion we summarized the results ob-

**Table 1.** Summary of the test results. A1 – mould allergen, A2 – pollen allergen, sIgE – level of specific IgE in serum (moulds), T – prick skin test result (moulds), NPT – nasal provocation test, Pkt – clinical score in NPT, % $\Delta V_{AR}$  – changes in nasal volume in acoustic rhinometry in course of NPT, A – *Alternaria alternata*, C – *Cladosporium herbarum*, G – grass, M – motherwort (*Artemisia*).

| No | A1  | A2 | sIgE | T | NPT | Pkt | % $\Delta V_{AR}$ |
|----|-----|----|------|---|-----|-----|-------------------|
| 1  | G/M | A  | 0.41 | 2 | +   | 4   | 37.3              |
| 2  | G   | A  | 2.16 | 3 | +   | 5   | 48.2              |
| 3  | G   | A  | 1.25 | 2 | +   | 4   | 39.7              |
| 4  | G/M | C  | 5.41 | 3 | +   | 5   | 60.3              |
| 5  | G/M | C  | 1.08 | 2 | +   | 5   | 31.9              |
| 6  | G   | C  | 1.32 | 2 | –   | 1   | 1.2               |
| 7  | G   | A  | 3.23 | 1 | –   | 2   | 8.7               |
| 8  | G   | A  | 0.67 | 2 | +   | 4   | 30.9              |
| 9  | G   | A  | 0.98 | 3 | +   | 6   | 47.5              |
| 10 | G   | C  | 1.76 | 3 | +   | 6   | 55.2              |
| 11 | G   | A  | 3.05 | 2 | –   | 6   | 68.3              |
| 12 | G   | C  | 0.7  | 2 | +   | 5   | 34.1              |
| 13 | G/M | A  | 0.36 | 1 | –   | 0   | 3.3               |
| 14 | G   | A  | 0.92 | 2 | –   | 2   | 6.9               |
| 15 | G   | A  | 2.11 | 3 | –   | 3   | 20.2              |
| 16 | G   | C  | 1.33 | 3 | +   | 6   | 44.2              |
| 17 | G   | C  | 2.12 | 2 | +   | 5   | 49.7              |
| 18 | G   | A  | 1.89 | 2 | +   | 5   | 33.4              |
| 19 | G   | A  | 3.01 | 4 | +   | 7   | 75.9              |
| 20 | G/M | A  | 2.56 | 1 | +   | 5   | 33.7              |
| 21 | G   | C  | 1.12 | 2 | +   | 5   | 41.1              |
| 22 | G   | A  | 3.01 | 2 | +   | 5   | 55.6              |
| 23 | G   | C  | 2.56 | 2 | +   | 4   | 44.4              |
| 24 | G   | A  | 1.12 | 1 | –   | 0   | 2.7               |
| 25 | G   | C  | 12.4 | 4 | +   | 8   | 92.7              |
| 26 | G   | A  | 0.65 | 2 | –   | 1   | 7.7               |
| 27 | G/M | A  | 1.01 | 3 | +   | 6   | 30.3              |
| 28 | G   | C  | 0.86 | 2 | +   | 4   | 40.6              |
| 29 | G   | C  | 4.7  | 3 | +   | 6   | 73.5              |
| 30 | G   | C  | 3.2  | 2 | +   | 5   | 50.1              |
| 31 | G   | A  | 0.73 | 2 | –   | 0   | 0.7               |

tained for the right and the left sides, a commonly recognized method [11]. In accordance with the available liter-

ature, the distance between 2 and 7 cm, as measured from the anterior nostrils, was used as the most representative area [12]. Decrease in the volume of the nasal cavities in the above-mentioned area by at least 30% compared with the baseline value was established as the criterion for a positive result of NPT in AR [13].

The statistical calculations were done using the STATISTICA 5 software. Results with a p value less than 0.05 were considered statistically significant.

## RESULTS

The NPT was very well tolerated by all the patients. None of them showed any systemic adverse effects. The most frequent manifestations we observed were nasal symptoms (pruritus, sneezing, obstruction and discharge). Only two patients manifested pruritus and reddening of the throat, and one had similar signs and symptoms in the conjunctivae. None of the patients showed dyspnea or abnormal PEF (peak expiratory flow) values. A summary of the results obtained is presented in the table below.

As can be seen from the Table 1, 18 patients (58.1%) were allergic to *Alternaria alternata* (subgroup A) and 13 (41.9%) to *Cladosporium herbarum* (subgroup C). Positive results were obtained with the specific nasal provocation test in 22 cases (70.9%): 10 patients in subgroup A (55.5%) and 12 persons in subgroup C (92.3%). The differences between the results in the subgroups were statistically significant ( $\chi^2$  test,  $p=0.039$ ). The NPT results correlated with both clinical manifestations and rhinometry. An example is presented in Figure 1.

Changes in the volume of the nasal cavities, evaluated on the basis of AR results, strongly correlated with the overall extent of clinical manifestations following the test (correlation coefficient =0.9) (Figure 2).

In general, the sIgE levels for the group were relatively low (median of 2.18 IU/ml, SD: 2.26). The differences in this parameter between the two subgroups were not statistically significant (Mann-Whitney test,  $p=0.16$ ). In the study group as a whole, sIgE levels correlated to an average degree with the clinical manifestations observed in the NPT (correlation coefficient =0.485). There was a higher degree of correlation between the NPT and the skin prick tests (correlation coefficient =0.664). Except for one case, the NPT was positive in all the patients, with strongly positive results (+3 and +4). The correlation between the skin prick tests and the sIgE levels was also average (correlation coefficient =0.487).

## DISCUSSION

Although allergy to moulds occurs less frequently than hypersensitivity to plant pollen and dust mites [14], it may be found in about 5% of persons with allergic rhinitis [15]. In the case of concomitant allergy to plant pollen and mould allergens, doubts may arise as to the significance of the latter in the etiology of the disease in individual cases. The clinical picture is not a decisive factor, since the clinical manifestations evoked by the two types of allergens are very similar [16]. Although mould spores, which are smaller in size,

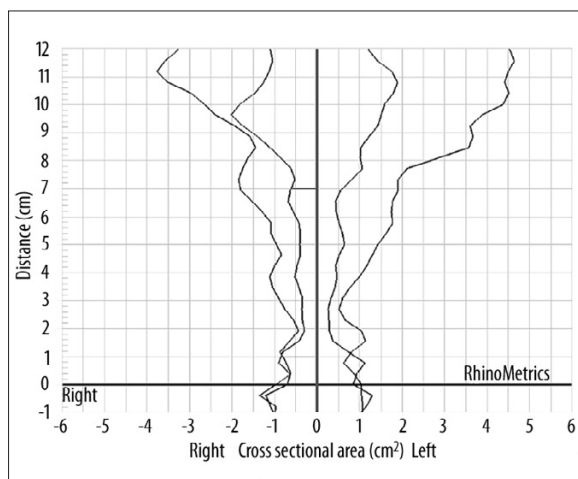


**Figure 1.** Results of acoustic rhinometry in one of the patients from the study group. Outer curves – condition prior to provocation; inner curves – condition following provocation.

penetrate the bronchi more easily, awareness of this fact is not of much use in differential diagnosis. Often the unsatisfactory result of specific immunotherapy directed against a given plant pollen will lead to the assumption that another allergen is largely responsible for the clinical manifestations. Practice shows that in the case of concomitant allergies, allergologists pay more attention to the pollen and apply specific immunotherapy to the allergen alone. Such management can obviously lead to incomplete or no therapeutic effect. On the other hand, one must remember that skin prick tests are positive in as many as 20–30% of the population, and a positive result does not necessarily reflect specific clinical manifestations. False positive results can occur as well. A similar situation occurs in the case of sIgE presence in serum. The value of this test is even lower than that of the skin prick test [17,18]. In this context, it is very important to identify patients with positive skin prick tests and/or the presence of significant levels of sIgE in whom allergy to mould spores may be of considerable importance. This is particularly true in the case of allergy to several allergens.

Of the available diagnostic methods, the nasal provocation test appears to be a good screening tool for determining the role of an allergen in inducing clinical manifestations. The test is quite safe and well tolerated by patients, which was also proved in this study. Despite the fact that it has been used for many years, there is no uniform protocol establishing when and whether it should be performed.

It was our intention to use the methods of evaluation that were least time-consuming for the examiners, not tiresome for the patients, and yet as reliable as possible. Therefore a very simple clinical assessment scale was used in this study. Additionally, in the provocation test we used the so-called single dose method. The guidelines included in the consensus of the German Society for Allergology and Clinical Immunology were followed, according to which the use of a single concentration of an allergen in NPT is adequate in routine clinical practice [9]. Acoustic rhinometry was used to determine NPT [19]. We decided that in the evaluation

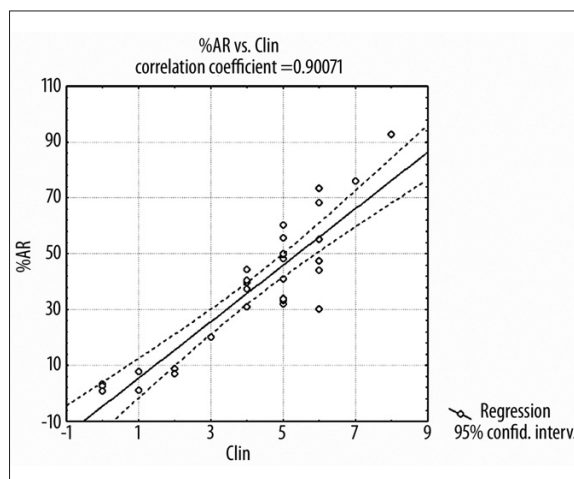


**Figure 2.** Correlation between clinical manifestations and acoustic rhinometry following NPT in the study group.

of test results the measurement of volume change of the nasal cavities along a significant distance was a more reliable and less error-susceptible method, compared to analysis of a cross-section area at a single measurement point (e.g. MCA – minimal cross sectional area). In reports concerning the problem in question, NPIF (Nasal Peak Inspiratory Flow) as well as anterior rhinomanometry (RM) have been used more commonly as objective diagnostic methods; however, this is mainly due to the fact that these methods were introduced in diagnostics much earlier. Compared with RM, AR is a much simpler and quicker examination, although the measurement involves quite different parameters. Whereas in NPIF and RM nasal resistance and flow are assessed as influenced by nasal mucosal edema (functional examination), in AR it is the primary values underlying the results recorded in RM – the cross section and the volume of the nasal cavity (static examination) – which are subject to evaluation.

In recent years an increasing number of authors have used AR to evaluate NPT. The same approach was used in this study. In studies comparing the usefulness of RM and AR, the latter has been found to be more useful, due not only to higher sensitivity, but also to the lack of strict requirements for patient compliance [20–23]. RM is also not reliable in the case of considerable nasal mucosa edema, which makes it largely impossible to perform bilateral provocation.

In this study, the provocation tests yielded positive results in the overwhelming majority of allergic patients. As might be expected, desensitization therapy using only pollen vaccination might not yield desired results in patients of this type. While a positive provocation test result strongly suggests the clinical significance of a given allergen, doubts still remain about a negative result. It should be kept in mind that the test can yield a false negative result. The problem is particularly complex in the case of mould allergens, because the sensitizing properties of fungal spores largely depend on whether their cells are intact or damaged. For example, the minimum number of spores in 1 m<sup>3</sup> of air needed to evoke clinical manifestations in most allergic persons is estimated at 100 for the *Alternaria* genus, but as high as 3,000 for the *Cladosporium* genus [1]. While the concentrations

of *Cladosporium herbarum* spores in the air are very high, allergy to *Alternaria alternata* spores, which have considerably lower concentrations, is much more frequent. It would appear that this phenomenon is associated with the proportion of undamaged spores, which can be as high as 80% in the case of *Alternaria*, compared with only 20–30% in the case of *Cladosporium* [24]. Undoubtedly, the process of preparing the NPT, including lyophilization, is not without effect on their allergenic properties. In our study, the NPT was positive more frequently in persons allergic to *Cladosporium herbarum* than in those allergic to *Alternaria alternata*. The differences were statistically significant, although the subgroups being compared were not large in number. The issue obviously requires further research.

It can safely be concluded that, although the NPT is not an ideal diagnostic method, its wider use might reduce the number of difficult cases, thus making for higher therapeutic efficacy [25].

## CONCLUSIONS

1. The nasal provocation test is a good accessory diagnostic method when used to confirm the clinical significance of allergy to mould spores in persons with positive results in skin prick tests.
2. Acoustic rhinometry is a quick, simple and convenient method of objectifying test results.

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