

Methodological Aspects of Nasal Allergen Challenges Based on a Three-Year Tree Pollen Immunotherapy Study

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During 3 years of immunotherapy with tree pollen extracts, 31 patients were provoked annually. Changes in nasal reactivity were followed by registration of expiratory nasal peak flow, number of sneezes, and amount of secretion. The reproducibility of the peak flow measurements was studied. The results from all three parameters were used to form a total nasal provocation score which, better than each parameter separately, could demonstrate the variation in sensitivity. Provocation with an allergen concentration of 1 HEP was the most effective means of showing changes in specific sensitivity of nasal mucosa.

Key words: immunotherapy; nasal allergen challenge; nasal allergy; nasal expiratory peak flow; total nasal provocation score.

Accepted for publication 10 March 1986

CLINICAL ASPECTS

The efficacy of immunotherapy can be demonstrated by following the specific sensitivity of the shock organ. In nasal allergy the use of three parameters is recommended: nasal peak flow, sneezes and secretion. The sensitivity can be expressed in one figure as a total nasal provocation score. In the present study, an allergen concentration of 1 HEP was best for demonstrating the effect of immunotherapy.

The effect of immunotherapy has been evaluated by various subjective and objective parameters, as decreasing skin reactivity with the actual allergen, as well as changes in total and specific IgE levels. Evaluation of the patients' symptom scores is the most direct way of studying the effect, but these scores are influenced by several factors, of which variation in allergen exposure is the most important. To circumvent this problem, changes in the sensitivity of the shock organ can be studied under standardized conditions. The aims of this study are to present an analysis of the results obtained with methods for nasal challenge that have been used in clinical trials (2, 4, 10, 14, 15), and to evaluate the optimal allergen concentration for following the de-

creasing sensitivity of the nasal mucosa during hyposensitization.

MATERIAL AND METHODS

In a multicentre study 45 patients with allergic rhinitis caused by tree pollen underwent a 3-year course of immunotherapy with tree pollen extracts. Alum-adsorbed, partly purified standardized allergen extracts of alder, birch and hazel (*Alnus*, *Betula* and *Corylus*), manufactured by Allergologisk Laboratorium-ALK, Copenhagen, Denmark, were used. The clinical results of this treatment will be published separately (5, 6, 14).

Thirty-one of the patients (12 women, 19 men; mean age 33 years) from two of the centres - Copenhagen and Malmö - were pro-

voked out of the pollen season, before, during and after immunotherapy, once annually from 1980 to 1983. After adaptation to the test environment, the nasal patency was measured as nasal expiratory peak flow using a mini-Wright peak flow meter connected to an anaesthetic mask. The patients were initially trained to use the equipment, and when reproducible values were achieved three consecutive measurements were performed and the highest value used to express patency (initial value).

Before provocation the patient inspires maximally and holds his breath to avoid inspiring allergen into the lung. 0.1 ml of the test solution is then sprayed into each nostril by a de Vilbiss spray No. 15. During the following 10 min the patient sits in a bent-forward position, and breathes through the mouth. The number of sneezes was counted and the nasal secretion collected in a funnel connected to a syringe. After 10 min the nose was cleared by sniffing or nose-blowing and another peak flow measurement performed as described above. To reveal unspecific hyperreactivity, the allergen provocations were preceded by a control provocation using saline solution (control value). The allergen extract used was aqueous and contained

a mixture of equal amounts of alder, birch and hazel pollen extracts, partially purified and standardized by the company's (ALK) usual methods. The strength was expressed in HEP units (1), and the same batch was used throughout the study. The provocations started with an allergen concentration estimated from the sensitivity of the actual patient. If the initial challenge was negative a 10-times stronger allergen concentration was used next time, and the procedure was continued to maximal strength, or until a convincingly positive reaction was achieved in at least two of the three parameters: sneezes, secretion and decrease in nasal patency. The conditions for positive reactions were set to: ≥ 5 sneezes, ≥ 0.5 ml of secretion and $\geq 20\%$ decrease in peak flow, respectively. The allergen extract was used in the following strengths: 0.01, 0.1, 1.0, and 10 HEP.

To express the total result of the provocation in one figure, a scoring system was applied to the absolute values. Every 10% decrease in peak flow value in relation to control value was given +1 point. One sneeze was scored as 1 point and 0.1 ml of nasal secretion 1 point. This scoring system has recently been used in another provocation study (15).

Table 1

Patients reacting on the three parameters to different allergen concentrations during 1980-83

Allergen conc.	Year	No. patients provoked	Peak flow		Sneezes		Secretion	
			Percent reacting	Mean % decrease in reacting patients	Percent reacting	Mean No. in reacting patients	Percent reacting	Mean (ml) in reacting patients
0.1 HEP	80	30	57	13.6	20	4.3	10	0.2
	81	28	50	12.6	18	1.2	3	0.1
	82	25	68	10.6	8	1	4	0.6
	83	16	31	16.2	6	2	6	0.1
1 HEP	80	29	86	25.9	69	5.3	66	0.8
	81	31	71	24.6	71	3.9	39	0.5
	82	30	67	17.4	53	2.6	30	0.6
	83	31	45	14.4	19	2	19	0.5
10 HEP	80	13	100	37.0	92	8	54	0.9
	81	23	83	41.2	83	6.6	61	1.0
	82	25	84	24.5	85	4.4	64	0.9
	83	29	76	30.0	62	6.4	62	0.6

Statistics

The results are presented as mean values $\pm$ SEM. Two-way analysis of variance and the Wilcoxon matched pairs rank sum test were used to calculate differences between values (12). All two-sided stochastic probabilities < 0.05 were considered significant. Relations between the parameters were studied by calculation of Kendall's coefficient of concordance (12).

RESULTS

Based on each patient's expected nasal sensitivity, provocation in the first year (1980) started with 0.01 HEP in 25 of the 31 patients. That year only 13 reached the maximal strength of 10 HEP. In 1983 only one patient started with 0.01 HEP, whereas 29 were tested with 10 HEP, indicating a decreasing sensitivity of the shock organ. In the following analyses concerning all three parameters the results of provocation with allergen strengths of 0.01 HEP are excluded as only a few patients were tested with this strength in the 4 years.

From the 1980 percentage figures shown in Table 1 it seems that peak flow changes appeared with higher frequency than changes in the other two parameters. The percentage of patients with peak flow changes increased from 57 at 0.1 HEP to 100 at 10 HEP. Sneezes showed an increase from 20 to 92% and secretion from 10 to 54%. However, there were also a few patients with obvious reactions in the form of sneezes and secretion, but no change in nasal peak flow. From 1980 to 1983 121

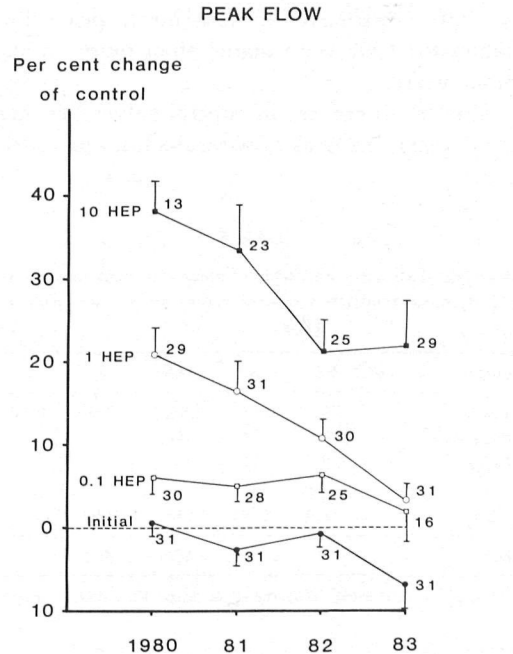


Fig. 1. Mean values $\pm$ SEM of nasal peak flow measurements during 1980-1983 expressed as percentage decrease in control values (dotted line). Number of patients provoked on each occasion is shown on the curves $\square$ = 0.1 HEP, $\circ$ = 1 HEP, $\blacksquare$ = 10 HEP and $\bullet$ = initial values.

provocations were performed using 1.0 HEP, and of these, 13 (10%) reacted only with sneezes and secretion.

Separate analyses of the three parameters

Peak flow. The peak flow values before (initial values) and after control provocation are not supposed to be influenced by immunotherapy and can thus be used to study the reproducibility of the method. The total mean initial peak flow values for all 4 years was 325 l/min ($n = 124$) and control provocation values 320 ($n = 124$). In no case did control provocation elicit sneezes or secretion, suggesting absence of unspecific hyperreactivity.

Table 2 gives the mean values for each of the 4 years. Two-way analysis of variance showed significant differences between the 4 years for both initial and control values ($P < 0.05$ and P

Table 2
Expiratory peak flow values (l/min)

	Initial values (mean)	Control values (mean)
1980	312.6	314.2
1981	331.0	321.6
1982	342.0	340.6
1983	314.2	303.2
Total	325.1	319.9

< 0.01, respectively). The mean peak flow values for 1982 were higher than those for the other years.

Due to differences in control values between the 4 years, the peak flow results from measure-

Table 3

Analysis of variance and table of means for peak flow values (% decrease relative to control value) for 4 years with 0.1 HEP. (n = 14)

Factor	SS	df	MS	F	P
Years	795.7	3	265.2	2.6	0.06
Patients	1258	13	96.8		
Error	3923	39	100.6		
Year	1980	1981	1982	1983	
Mean	5.0	3.7	10.4	-0.1	

SS: sum of squares, MS: mean square, F: variance ratio.

Table 4

Analysis of variance and table of means for peak flow values (% decrease relative to control value) for 4 years with 1 HEP. (n = 28)

Factor	SS	df	MS	F	P
Years	4688	3	1563	7.6	0.00014
linear trend	4635	1	4635	22.7	8×10^{-6}
deviation	53	2	26.5	0.1	0.88
Patients	9289	27	344.0		
Error	16510	81	203.8		
Year	1980	1981	1982	1983	
Mean	20.4	15.7	10.3	3.0	

SS, MS, F: see Table 3.

Table 5

Analysis of variance and table of means for peak flow values (% decrease relative to control value) for 4 years with 10 HEP. (n = 10)

Factor	SS	df	MS	F	P
Years	4809	3	1603	2.3	0.010
Patients	6004	9	667.1		
Error	18860	27	698.6		
Year	1980	1981	1982	1983	
Mean	38.2	28.6	16.8	9.5	

SS, MS, F: see Table 3.

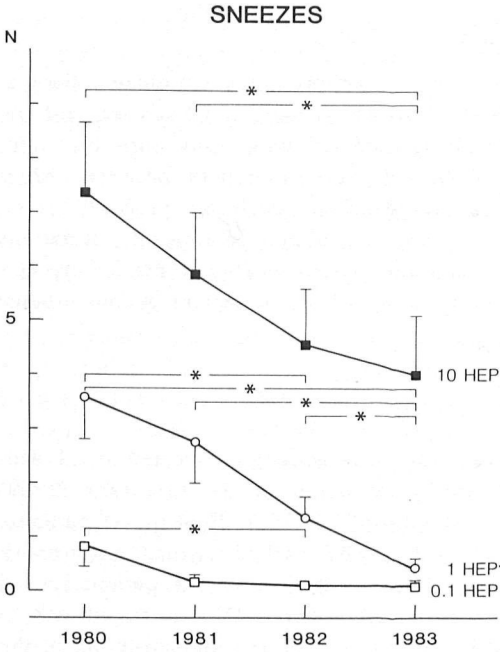


Fig. 2. Mean number of sneezes $\pm$ SEM during 1980–1983 after provocation with 0.1 HEP = $\square$, 1 HEP = $\circ$ and 10 HEP = $\blacksquare$. Significant ($P < 0.05$) differences calculated with the Wilcoxon matched pairs rank sum test are indicated with *.

ments after provocation with the different allergen strengths were expressed as a percentage of the control values. This is shown in Fig. 1, together with the number of patients provoked with the different concentrations. The allergen curves indicate a continuous decrease in shock organ sensitivity during the 3 years of treatment. Analysis of the results from all 4 years using two-way analysis of variance is shown in Tables 3–5. Each analysis corresponds to one allergen concentration, and is based on those patients having complete data for the concentration considered. There are significant differences between the 4 years for 1 HEP only. For this concentration (Table 4), the analysis is pursued by breaking down the sum of squares for the factor years into a component for a linear trend and a component for deviations from a linear trend. The first component is significant ($P < 0.001$), the second is not ($P >$

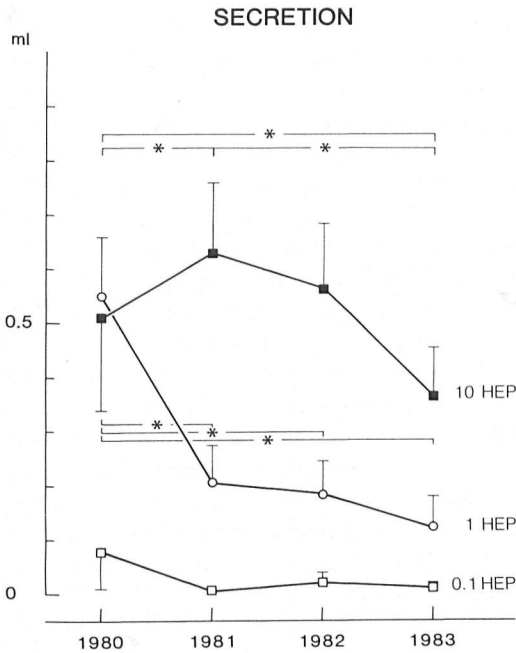


Fig. 3. Mean secretion $\pm$ SEM. Symbols: see Fig. 2.

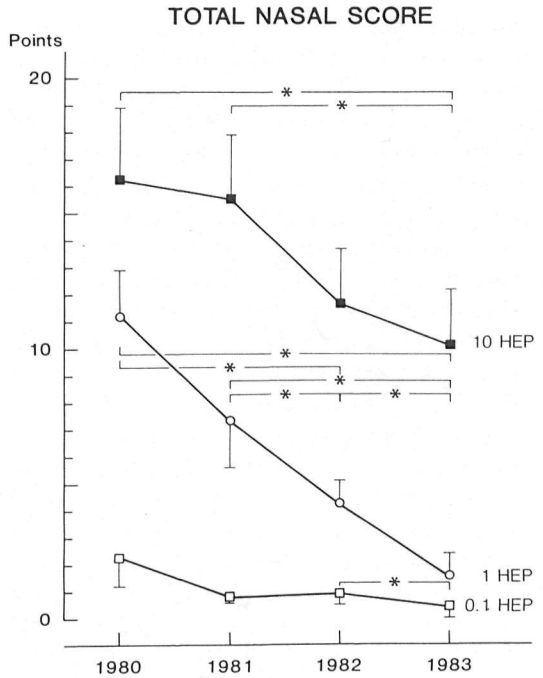


Fig. 4. Mean total nasal provocation score $\pm$ SEM. Symbols: see Fig. 2.

0.05). Differences between years are essentially described by a linear trend.

As a measure of the reproducibility of the method, the residual standard deviation from the analysis of variance was used. For both the initial and the control values the figure was 42 l/min. As the overall mean is approximately 320 l/min (Table 2), the corresponding coefficient of variance is 13 %.

Sneezes. In Fig. 2 the change in nasal sensitivity during the years of treatment is expressed by number of sneezes. Significant differences between the years are calculated with the Wilcoxon matched pairs rank sum test and marked with asterisks in the figure. The concentration of 0.1 HEP gave only a low mean value in the first year, thus a small decrease is difficult to display. The total results are collected in Table 1, where the change in the number of patients reacting during the years is given together with the mean number of sneezes among those reacting.

Secretion. The amount of secretion elicited by provocation is presented graphically in Fig. 3. Significant differences are expressed by the same method as in Fig. 2. From Table 1 it is obvious that 1 and 10 HEP gave measurable amounts of secretion which decreased during the course of treatment. The decrease in the percentage of patients reacting to 1 HEP from 66 to 19 is also a sign of falling sensitivity of the shock organ (Table 1).

Total nasal provocation score

The introduction of a scoring system and the addition of the scores from each provocation parameter to give a total nasal score facilitate a quantitative presentation of the results. The relation between the parameters was studied by calculation of Kendall's coefficient of concordance. A P value ≤ 0.05 is regarded as significant, which means that the calculated parameters are related. Significance was found

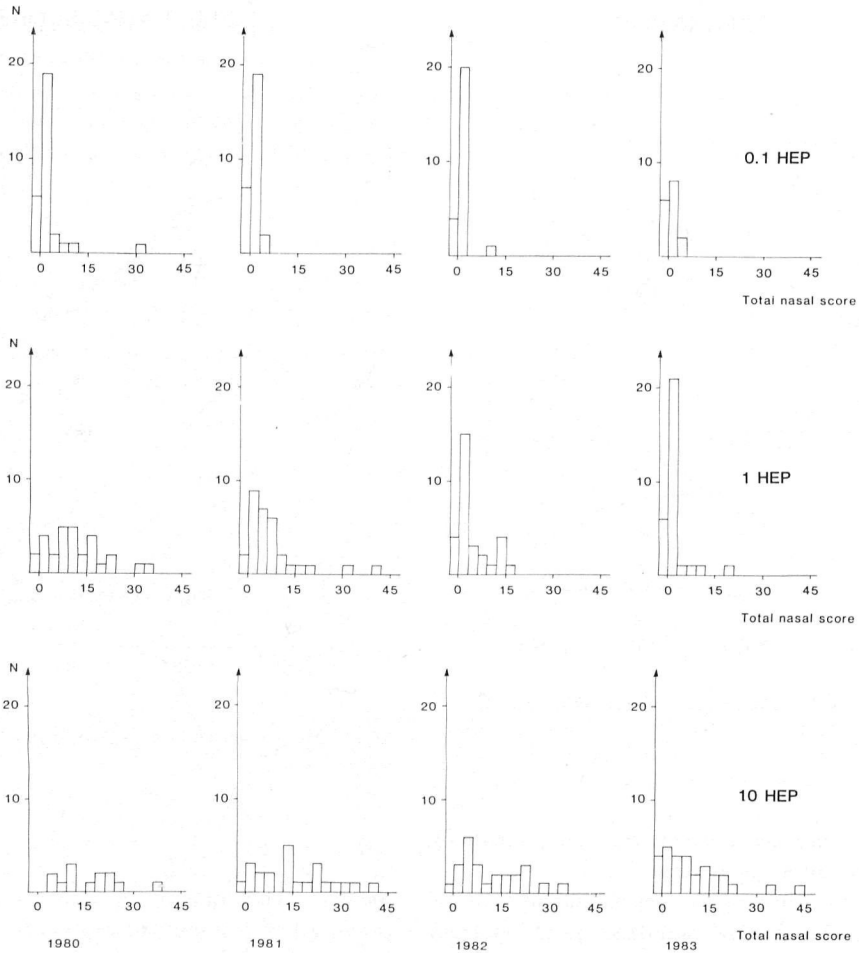


Fig. 5. Histograms showing number of patients reacting (on the y-axis) and strength of reaction to different allergen concentrations during 1980-1983. Reaction strength is given on the x-axis in total nasal scores - range 0-45. Range of each interval is 3 points of total nasal scores.

for 10 of the 12 comparisons between the 4 years and the three highest allergen concentrations, justifying the construction of a total score. In Fig. 4 the results are presented graphically, and show the significant differences between the years. The 1 HEP concentration showed more significant differences than the other concentrations. The results can also be presented as histograms (Fig. 5). This includes the number of patients reacting to the different allergen concentrations, which amplifies the quantitative information. The results with the 1

HEP concentration show an obvious decrease in sensitivity from 1980 to 1983. In 1983 most of the patients gave a negative, or a very weak positive reaction, when provoked with 1 HEP.

DISCUSSION

There is a definite need for objective and reliable methods for estimating the efficacy of immunotherapy. This particularly applies when evaluating new modes of treatment. The most logical effect parameter is the sensitivity of the

shock organ. The methods used here have proven useful in earlier clinical studies (2, 4, 10, 14, 15), but, as far as is known, a careful evaluation of their reliability has not been presented before.

Expiratory nasal peak flow is a simple and inexpensive method for following changes in nasal patency. Other possible methods are inspiratory nasal peak flow and rhinomanometry. In a recently performed study the three methods were compared (13): significant correlations were found between the results of all three methods and their advantages and disadvantages were also pointed out. In the present study two-way analyses of variance revealed significant differences between the years for both initial and control values. The values from 1982 deviated markedly from the others. The reason for this could be a change in patients' basal reactivity that year, or a change in laboratory facilities or staff. As the patients were under continuous immunotherapy, the sensitivity of the nasal mucosa was not constant, thus making reproducibility studies of the allergen provocations impossible. The reduction in specific sensitivity of the nasal mucosa was revealed objectively by peak flow measurements. According to the statistical analyses this is best shown by an allergen strength of 1 HEP.

Counting the number of sneezes is the simplest way of objectively presenting changes in nasal reactivity. A significant reduction in the number of sneezes was seen with both 1 and 10 HEP allergen strengths.

There is at present no optimal method for measuring nasal secretion. Filter paper methods as well as nasal washings (7-9) have been used, but both have their drawbacks. The method used here is simple, but needs relatively strong stimulation to produce measurable amounts of secretion. Variations in nasal anatomy, amount and length of vibrissae, and variation in the viscosity of the secretion, can all affect the results. When 1 and 10 HEP concentrations of allergen were used in this study a clear pattern of decreasing sensitivity was seen during the years of immunotherapy (see Fig. 3). Table 1, however, shows no decrease in the percentage of patients reacting to 10 HEP.

For practical reasons it is desirable to be able to express the sensitivity of the shock organ in one single figure. Even though the mean value for each of the three variables used showed significant changes there was wide individual variation in the reactivity pattern. Several patients reacted on only two of the three parameters. For a complete picture of nasal reactivity all three parameters must be evaluated. As the statistical analysis showed a relationship, they were used to form a total score. As seen in Fig. 4, it is more possible to show significant differences between the years of treatment by using the total score rather than the three variables separately. By using a total score it is also possible to give the results of the provocation in relation to other parameters, e.g. as in the histogram in Fig. 5. This figure shows that almost all the 31 patients were provoked with 1 HEP during all 4 years, whereas the results with 0.01 and 10 HEP are much fewer because of too weak or too strong reactions, respectively.

Standardization of allergen extracts has been one of the major subjects of several symposia during the last 10 years. The HEP system has been proposed as the basis for this standardization (1) but has not been totally accepted (11). During the last few years there has also been a trend to increase the allergen strength for both skin testing and provocation (3). Whether this is a good or bad development is difficult to decide at present.

The results of this study, however, clearly indicate: 1) that an allergen strength of 1 HEP was the best for showing a change in specific nasal sensitivity; 2) that nasal peak flow was the most sensitive of the three variables in the sense that most patients gave positive reactions on this parameter; 3) the application of a scoring system to all three variables made it possible to form a total nasal score which considerably simplified the understanding of the results.

ACKNOWLEDGEMENTS

The author wishes to thank Drs H. Janniche and B. Nüchel-Petersen, Pulmonary Department, Lyngby Hospital, Copenhagen, Denmark, for providing patient data, and H.

Ipsen, The Protein Laboratory, University of Copenhagen, Denmark, and C. J. Lamm, Draco, Lund, Sweden, for statistical help and advice.

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