

Immunotherapy with partially purified and standardized tree pollen extracts

II. Results of skin prick tests and nasal provocation tests from a three-year double-blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel

J. Å. WIHL,¹ H. IPSEN,⁴ B. NÜCHEL PETERSEN,² E. P. MUNCH,³ H. JANNICHE² & H. LØWENSTEIN⁴

¹Dept. of Otorhinolaryngology, University of Lund, Malmö General Hospital, Sweden, ²Dept. of Lung Diseases, Copenhagen County Hospital, Gentofte, ³The Allergy Clinic, Frederiksberg Hospital, Copenhagen, Denmark and ⁴ALK Research, Hørsholm, Denmark

Patients allergic to tree pollen entered a 3-year course of immunotherapy (1980-83) with either birch pollen extracts alone ($n = 26$) or patient-tailored extracts of birch, alder and hazel pollen ($n = 27$). The clinical and immunological results of this study are published elsewhere. This paper contains an evaluation of skin prick test and nasal provocation test results. There were no significant differences between the two treatment groups concerning these two parameters. In both groups the allergen-specific sensitivity in the skin showed seasonal variations but a significant decrease. During the years of treatment there was also a significant decrease in the specific sensitivity of the nasal mucosa. With the present demands for purification and standardization of allergen extracts it is of practical and economic interest to know that tree pollen-allergic patients showing positive reactions to birch, alder and hazel extracts can be effectively treated using birch pollen extract alone.

Key words: immunotherapy; nasal provocation test; skin prick test; tree pollen allergy.

Accepted for publication 11 February 1988

Tree pollen allergy is a common cause of springtime rhinoconjunctivitis and asthma in northern Europe (3, 16). In severe cases, treated with immunotherapy, it has been a tradition to use extracts containing a mixture of pollen from birch, alder and hazel (*Betula*, *Alnus* and *Corylus*). These genera are botanically closely related, and most of the patients with springtime symptoms give positive skin reactions when tested with pollen extracts from the three genera separately. Based on aerobiologic studies of pollen frequencies it is known that the exposition to birch pollen is 10 to 100 times higher than to alder and hazel (11). The symptoms of most patients also

coincide mainly with the appearance of birch pollen in the air.

The aim of this study is to evaluate whether springtime rhinoconjunctivitis and asthma can be treated as effectively with an extract of birch pollen alone as with a mixture of birch and/or alder and hazel pollen. For this purpose a 3-year double-blind immunotherapy study was performed. The patients were allocated into two groups: one treated with any combination of alder, birch and hazel corresponding to the patient's IgE response (Group I (ABC)) and the other with birch pollen extract alone (Group II (B)). The clinical and immunological results are published separately (8, 9). The results of skin

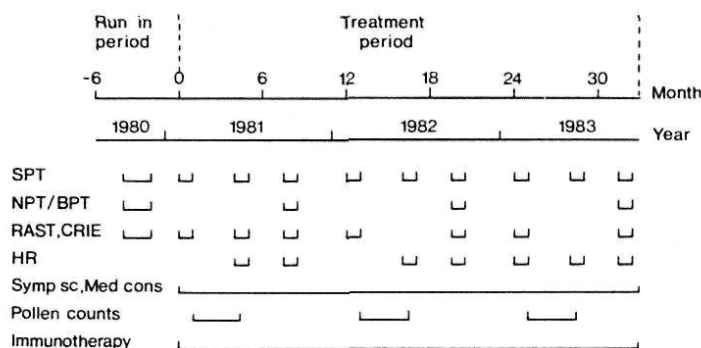


Fig. 1. Study design. SPT: skin prick test; NPT: nasal provocation test; BPT: bronchial provocation test; RAST: radioallergosorbent test; CRIE: crossed radioimmuno-electrophoresis; HR: histamine release; Symp sc: symptom score; Med cons: medicine consumption.

prick tests (SPT) and nasal provocation tests (NPT) are presented here.

MATERIAL AND METHODS

Fifty-one patients with springtime rhinoconjunctivitis and asthma caused by tree pollen entered the study. Based on sex, age, clinical history, SPT, NPT, RAST and CRIE, the patients were allocated into matched pairs. The treatment alternatives were randomly distributed within the pairs into treatment with a tailor-made extract of birch, alder and hazel pollen ($n = 27$, Group I (ABC)), based on the patient's own sensitivity pattern, and an extract of birch pollen alone ($n = 26$, Group II (B)). Inclusion and exclusion criteria and patient data are published in detail elsewhere (9). During the years of treatment the patients were followed by repeated *in vitro* and *in vivo* tests as shown in Fig. 1.

Allergen extracts

The allergen extracts used for *in vitro* and *in vivo* tests, as well as for treatment, were partly purified and produced by Allergologisk Laboratorium ALK, Copenhagen, Denmark, and standardized in SQ units. They were lyophilised and used in either aqueous glycerinated or alum adsorbed forms. The same

extract was used throughout the study. the specifications of the extracts are given in detail elsewhere (9).

Skin prick test

During the study the patients were tested a total of 10 times, before and after the pollen season and in the winter between the seasons, as can be seen in Fig. 1. The skin prick tests were performed in duplicate and as a titration on both lower forearms using Soluprick SQ® extracts of alder, birch and hazel separately, and with the following concentrations: 0.01, 0.03, 0.1, 0.3, 1.0 and 10 HEP. Glycerine solution served as a negative control and histamine chloride solution in the strength 1 mg/ml as a positive control. Ordinary blood lancets were used for pricking. After an interval of 10 (histamine) to 15 (allergen) min the weals were encircled with a ball-point pen and by transparent tape transferred to mm paper. The results of SPT are given as skin index (SI), calculated as follows:

$$SI = \frac{D(1) \times D(2)}{D(1, his) \times D(2, his)}$$

where D(1) and D(2) are the mutual perpendicular diameters of the weal produced by the allergen preparation measured in millimeters and D(1, his) and D(2, his) are the mutual per-

pendicular diameters of the histamine control, measured in millimeters.

Nasal provocation test

The nasal provocation tests were performed with a mixture of equal parts (in SQ units) of aqueous extracts of alder, birch and hazel. The tests were performed annually and well outside the pollen season, e.g. in October and November, Fig. 1. On each occasion an initial control provocation was performed with saline solution, after which allergen strengths of 0.01, 0.1, 1.0 and 10 HEP were used. The strength of the starting dose was based on the patients's own sensitivity. 0.1 ml of the allergen extract was sprayed into each nostril. Three effect parameters were recorded: nasal patency (as expiratory nasal peak flow), sneezes and secretion. A scoring system was adapted to the outcome of each parameter. One point each was awarded to a 10% decrease in nasal peak flow, 1 sneeze or 0.1 ml of secretion. The scores were added to a total nasal provocation score allowing the NPT results to be given in one figure. A detailed description of the provocation performance and the calculations behind the score are published separately (14).

Statistical methods

The Wilcoxon matched pair rank sum test was used to evaluate differences within the treatment groups and the Mann-Whitney U-test to evaluate differences between the groups (10). All two-sided stochastic probabilities < 0.05 were considered significant.

RESULTS

Skin prick test

Although the patients were skin prick tested with a series of seven allergen strengths ranging from 0.1 to 10 HEP, the statistical calculations are here only presented for 0.3, 1.0 and 10 HEP. The weals elicited by the most diluted extracts were initially small and hence no differences suitable for statistical calculations were

achieved. In Figs. 2-4 the results are given graphically and separately for each allergen and treatment group. The pollen frequencies concerning Copenhagen expressed as number of pollen/m³ air/week are also given in the figures.

All the curves expressing weal size have a somewhat uniform appearance, which is most obvious for allergen strengths of 0.3 and 1 HEP. During 1981 there was a continuous decline in weal size, during 1982 an increase, with maximum after the pollen season in the autumn, and during 1983 there was no pronounced change in weal size. Using the Wilcoxon matched pair rank sum test the size of the weals was compared with the initial weal achieved in January 1981. A significant difference ($P < 0.05$) is expressed in the figures with an asterisk above the actual weal size sign. In the majority of comparisons there was a significant decrease of weal size during treatment with the exception of the tests performed after the 1982 pollen season. Differences in weal size from two consecutive tests were calculated using the same statistical method. A significant difference is expressed in the figures with an asterisk on the line between the two test occasions. A visual comparison of the curves for both treatment groups gives a strong impression that they are very much alike for all three allergens. This has also been tested statistically using the Mann-Whitney U-test. Of 90 comparisons of weal sizes from both treatment groups only three showed a slight significant difference ($P < 0.05$). These differences are estimated incidental, and not an expression of a real difference between the two treatment groups.

The pollen curves given in Figs. 2-4 can also be expressed in absolute figures as in Table 1. This gives the total number of relevant tree pollen caught in the spore trap each year. The figures of 54 for 1981, 4259 for 1982 and 1689 in 1983 give a good illustration of the huge natural variation in pollen exposure.

Nasal provocation test

The results of the nasal provocation tests are given graphically in Fig. 5. Only the three

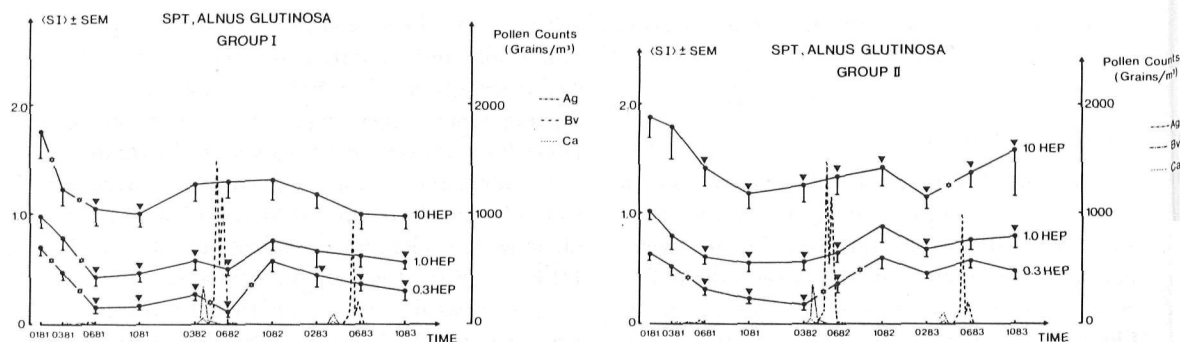


Fig. 2. SPT results for Group I (ABC) and Group II (B) given as SI in both treatment groups using an extract of alder. SI + SEM is given on the y-axis (left) and year and month of the test occasion on the x-axis. Pollen frequencies (number/m³ air/week) are given on the y-axis (right). Ag: alder, Bv: birch, Ca: hazel. * above an SI value indicates a significant difference ($P < 0.05$) between that value and initial value. * on a line indicates a significant difference ($P < 0.05$) between the two SI values that the line connects.

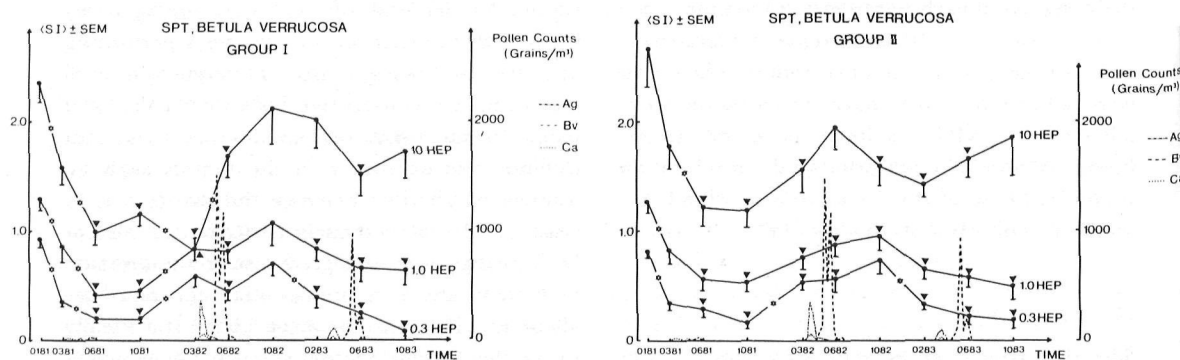


Fig. 3. SPT results given as SI in both treatment groups using extract of birch. Symbols: as Fig. 2.

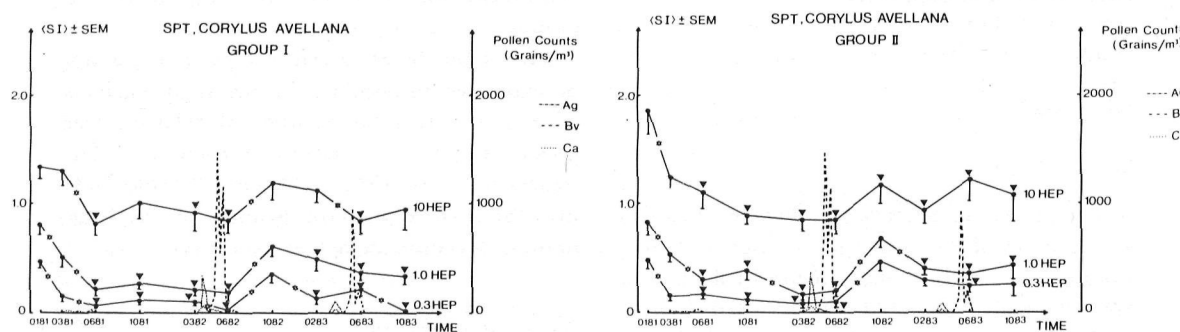


Fig. 4. SPT results given as SI in both treatment groups using extract of hazel. Symbols: as Fig. 2.

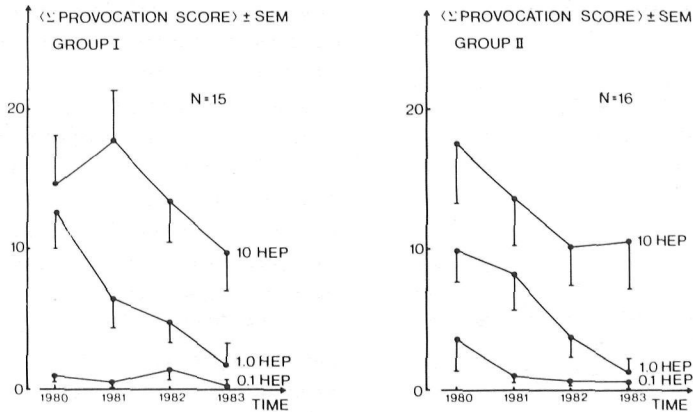


Fig. 5. NPT results in both treatment groups (I (ABC) and II (B)) separately, given as total nasal provocation scores + SEM (y-axis) during the years of treatment (x-axis).

highest allergen concentrations 0.1, 1.0 and 10 HEP are displayed. Complete results from four yearly provocations from 1980–83 were available from 31 patients; 15 in Group I (ABC) and 16 in Group II (B). The results of the provocations are expressed as total nasal provocation scores. As the allergen strength used for provocation was determined by the patient's individual sensitivity the number of patients tested with the different allergen strengths varied during the course of the treatment. Hence, only 13 patients were tested with 10 HEP in 1980 and 16 with 0.1 HEP in 1983 (14). However, almost all patients were tested with 1.0 HEP on each of the four occasions making these tests the most reliable, and the only ones used for statistical evaluations comparing the two groups. A visual comparison of the 1.0 HEP curves for both treatment groups shows a continuous decline in nasal reactivity (Fig. 5). The increase in sen-

sitivity seen in the SPT results from 1982 cannot be traced here.

Comparison of the provocation results using 1.0 HEP for both treatment groups by the Mann-Whitney U-test did not reveal any significant difference, indicating that the two allergen extracts had a similar therapeutic efficacy. Therefore, both groups of 15 and 16 patients were combined when statistical calculations were performed to demonstrate decrease in specific nasal sensitivity during treatment. Using an allergen strength of 1.0 HEP a significant decrease was shown with Wilcoxon matched pair rank sum test in five of six comparisons (Fig. 6).

DISCUSSION

The efficacy of immunotherapy can be demonstrated by several different methods. Objective methods are of special importance in clinical trials that are not double-blind and without control patients. Change in skin sensitivity is an objective parameter of established value (2, 4, 13, 15) as well as a determination of the sensitivity of the shock organ (1, 5, 12, 15).

In allergic rhinitis change in the sensitivity of the nasal mucous membrane is the most relevant parameter for studying efficacy.

The problems involved in recording symptoms on diary cards are obvious considering the

Table 1

Pollen counts from Copenhagen. Integrated number of pollen/m³/h for the various seasons

	1981	1982	1983
Alder	12	641	188
Birch	36	3495	1477
Hazel	6	123	24
Total	54	4259	1689

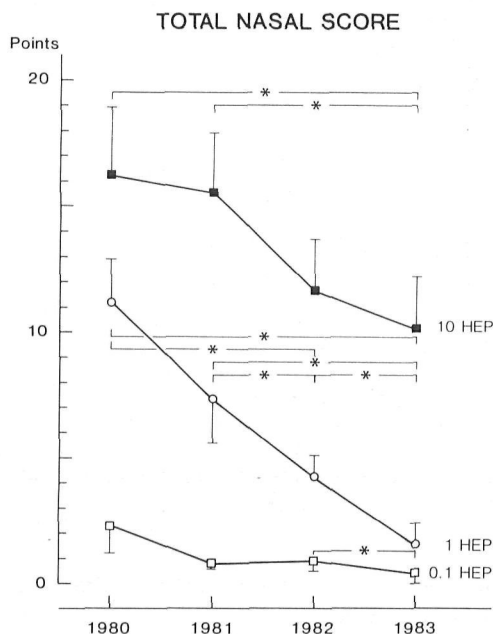


Fig. 6. NPT results of the whole material given as total nasal provocation scores + SEM. Significant differences ($P < 0.05$) calculated with Wilcoxon matched pair rank sum test are indicated with *.

wide variation in natural pollen exposure from year to year. The figures in Table 1 are a good illustration of this problem. However, this can also influence objective parameters, such as skin reactivity which usually increases after the pollen season (6). An effective immunotherapy should eliminate such a seasonal increase in sensitivity. Because of the extremely low pollen counts in 1981 there was probably no natural enhancement of skin sensitivity that year. However, this was clearly detectable in 1982 (Figs. 2-4) in spite of immunotherapy, but not in 1983, perhaps because of immunotherapy.

Only one patient had asthma as the only symptom of allergic disease. In a few other cases asthma was the dominating symptom. Bronchial provocations were performed in these patients, but the total number of tests was too small to allow statistical calculations.

NPT is a rather difficult test as several symptoms need to be recorded. The construction of a total nasal score (14) has probably made the test

more sensitive and able to reveal quantitative changes. Using an allergen strength of 1 HEP, which in the majority of patients gave a reaction of acceptable strength, it was possible to demonstrate successively decreasing nasal sensitivity. Clinically, this strength was the most valuable. The number of observations with 0.1 and 10 HEP were much smaller than with 1.0 HEP, and with these strengths it was only possible to show incidental significant differences during treatment (14) (Fig. 6). In the 4 years 15 patients from one group and 16 from the other were provoked. As no difference in therapeutic effect was demonstrated between the groups they were joined to achieve reliable statistical calculations.

The year to year variation in pollen counts did not appear to influence the NPT in the same way as it did the SPT. This indicates that different mechanisms are probably involved in eliciting skin and nasal reactions. With both SPT and NPT a decreasing specific sensitivity during treatment could be demonstrated. However, it was not possible to show any difference between the two treatment groups. This emphasizes the impression formed by studying the pollen frequencies (11): birch is responsible for most of the symptoms of tree pollen allergy in Scandinavia. All the patients in this study were allergic to birch, and hence birch allergen was included in the treatment extracts of both groups. The close botanical relationship between alder, birch and hazel is also the reason for the antigenic identity demonstrated recently (7).

The present demands for purification and standardization of allergen extracts involves much work and expense for the manufacturers. It is therefore of practical importance to show that patients with tree pollen allergy and sensitive to alder, birch and hazel can be effectively treated with an extract of birch pollen alone. This conclusion is based on a clinical trial performed in southern Scandinavia where birch is less prevalent than in the north and it is therefore probably valid for the whole of Scandinavia.

ACKNOWLEDGEMENTS

The authors wish to thank Lone Pedersen, Hanne Steensgård, Lise Høi Hansen and Annic Lindebod from the

Department of Lung Diseases, Copenhagen County Hospital, Gentofte, as well as Ingemo Harryson and Astrid Petersen from the Department of Otolaryngology, Malmö General Hospital, for their careful handling of the patients, and the Aerobiological Group of the Danish Asthma and Allergy Association for the pollen data provided.

REFERENCES

1. Aas, K.: Hyposensitization in house dust allergy asthma. A double-blind controlled study with evaluation of the effect on bronchial sensitivity to house dust. *Acta Paediatr. Scand.* 60, 264-268, 1971.
2. Colmes, A.: Serial skin tests as a guide in the treatment of hay fever. Preliminary report. *J. Allergy* 3, 449-458, 1932.
3. Eriksson, N. E. & Jacobsson, A.: Björk allergi vanligt - Björkris i offentliga lokaler en luftförorening. *Läkartidningen* 78, No. 11, 1073-1974, 1981.
4. Frankland, A. W. & Augustin, R.: Prophylaxis of summer hay fever and asthma. A controlled trial comparing crude grass pollen extracts with the isolated main protein component. *Lancet* 1, 1055-1057, 1954.
5. Frostad, A. B., Grimmer, Ø., Sandvik, L. & Aas, K.: Hyposensitization. Comparing a purified (refined) allergen preparation and a crude aqueous extract from timothy pollen. *Allergy* 35, 81-95, 1980.
6. Haahtela, T. & Jokela, H.: Influence of the pollen season on immediate skin test reactivity to common allergens. *Allergy* 35, 15-21, 1980.
7. Ipsen, H., Böwadt, H., Janniche, H., et al.: Immunochemical characterization of reference alder (*Alnus glutinosa*) and hazel (*Corylus avellana*) pollen extracts and the partial immunochemical identity between the major allergens on alder, birch and hazel pollens. *Allergy* 40, 510-518, 1985.
8. Ipsen, H., Schwartz, B., Wihl, J. Å., et al.: Immunotherapy with partially purified and standardized tree pollen extracts. III. Specific IgE response to the major allergens of alder, birch and hazel pollens during immunotherapy. *Allergy* 43, 370-377, 1988.
9. Nüchel Petersen, B., Janniche, H., Munch, E. P., et al.: Immunotherapy with partially purified and standardized tree pollen extracts, I. Clinical results from a three-year double-blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel. *Allergy* 43, 353-362.
10. Siegel, S.: Non-parametric statistics for behavioural sciences. MacGraw-Hill, New York, 1956.
11. Strandhede, S. O., Wihl, J. Å. & Eriksson, N. E.: Tree pollen allergy. I. Feature of plant geography and pollen counts. *Allergy* 39, 602-689, 1984.
12. Taylor, G., Shivalkar, P.-R.: Local nasal desensitization in allergic rhinitis. *Clin. Allergy* 21, 125-136, 1972.
13. Tuft, L. & Heck, C. M.: Ragweed and pollen desensitization in hay fever. Relationship to skin test reaction dosage and clinical results. *J. Allergy* 28, 124-133, 1957.
14. Wihl, J. Å.: Methodological aspects of nasal allergen challenges based on a three-year pollen immunotherapy study. *Allergy* 41, 357-364, 1986.
15. Østerballe, O.: Nasal and skin sensitivity during immunotherapy with two major allergens, 19 & 25, and partially purified extract of timothy grass pollen. *Allergy* 37, 169-177, 1982.
16. Østerballe, O., Dirksen, A., Weeke, B. & Weeke, E. R.: Cutaneous allergy in a Danish multicenter study. *Ugeskr Læger* 143, 3211-3218, 1981.

Address:

J. Å. Wihl

Department of Otorhinolaryngology

Malmö General Hospital

S-214 01 Malmö

Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.