

Preseasonal intranasal immunotherapy in birch-alder allergic rhinitis

A double-blind study

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A double-blind, placebo-controlled study was carried out to test the clinical efficacy and safety of local nasal immunotherapy (LNIT) in powder form. Twenty-two patients suffering from allergic rhinitis strictly associated with early spring symptoms, with positive skin prick tests and RAST for birch-alder, all responders to a specific nasal provocation test (NPT), received randomly active or placebo treatment for 4 months. Immunotherapy consisted of administration of a set of capsules containing progressively increasing amounts of birch (*Betula pendula*) and speckled alder (*Alnus incana*) allergens in powder form with controlled granulometry. The active (birch-alder) and placebo (lactose) group completed the treatment according to a similar schedule. During the pollen season (March–April), the patients who took the active treatment reported less sneezing and rhinorrhea than the placebo group, on the basis of a symptoms score, and the differences were statistically significant; the need for drugs (terfenadine) was also significantly reduced. These findings agreed well with the results of specific NPT after the treatment; only patients in the active group had a higher threshold dose of nasal specific reactivity to birch-alder allergens than in tests before the LNIT.

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Many placebo-controlled clinical trials have demonstrated the efficacy of specific injective immunotherapy in improving the symptoms of allergic rhinitis. However, this treatment can be time-consuming and carries the risk of systemic reactions. Clinical trials with local nasal immunotherapy (LNIT) using aqueous allergen preparations have revealed some problems of frequent local reactions (1, 2); besides, aqueous allergen preparations present some disadvantages, mainly poor stability and difficulty in administering a precise amount of allergen (3, 4).

The introduction of an extract in powder form has achieved better allergen stability and standardization (5). Previous studies have reported the clinical efficacy and safety of powder nasal immunotherapy in *Parietaria* (6), grass (7), and mites (8) allergy.

In the present study, we evaluated the clinical and immunologic efficacy and convenience of LNIT with allergen in powder form in seasonal rhinitis caused by birch and speckled alder.

Material and methods

Subjects

Twenty-two patients suffering from typical early spring hay fever were carefully selected and recruited by four allergy clinics in Lombardy, in northern Italy (Cremona, Abbiategrasso, Lecco, and Casatenovo). These patients had a history of seasonal tree-allergic rhinitis for at least 3 years, positive skin prick tests to birch and speckled alder, positive RAST to birch and alder (at least class 2), and positive response to a specific nasal provocation test.

Criteria for exclusion were perennial rhinitis, asthma requiring daily medication, prior specific immunotherapy, pregnancy, breast-feeding, serious systemic disorders, long-term use of nasal corticosteroids or decongestants, and nasal polyps.

At the first visit, a complete history was recorded, and physical and ENT examinations were done. Patients were questioned about their hay fever symptoms during the previous spring. Prick skin

tests were done not only with birch and speckled alder, but also with *Alternaria*, pellitory, mixed-grass, mugwort, *Dermatophagoides pteronyssinus*, and cat and dog dander extracts (Laboratorio Farmaceutico Lofarma S.r.l., Milan, Italy) to exclude sensitization to other allergens. Histamine and saline controls served as positive and negative controls, respectively.

Each subject gave informed consent before entry into the study.

Allergen preparation

The active product (Allerkin®), supplied by Laboratorio Farmaceutico Lofarma S.r.l. (Milan, Italy), was in powder form. This product consists of a mixture of birch and speckled alder extract incorporated into an inert lactose, contained in hard gelatin capsules (25 mg per capsule). For control of granulometry, the powder was submitted to particle size analysis.

For this analysis, the powder was suspended in silicone oil, sonicated, and analyzed with a laser-light-powdered analyzer (wavelength 632.8, model 715, Cilas Alcatel, Marcoussis, France). The minimal and maximal acceptability size values were as follows: 75% of particles must be 15–80 µm, with a partition value (50/50%) corresponding to 45 ± 5 µm. The following dosages were prepared: 2.5, 5, 10, 20, 40, 60, 80, 120, 160, and 240 AU. The placebo consisted of lactose (25 mg) and looked the same as the active product.

The powder was administered into the nostril with a manual nasal insufflator (MIAT, Milan, Italy), consisting of a body made of a nasal adapter, a capsule holder, two spring-activated pins to perforate the capsule, and a rubber pump containing the air reserve necessary to distribute the powder. During administration, the pressure on the rubber pump produces an air stream which passes through the holes at the ends of the capsule and carries the powder into the nasal adapter and onto the nasal mucosa. As regards the standardization of allergen extracts, the preparation was labeled in allergenic units (AU) determined by RAST inhibition (9) in comparison with an in-house reference (IHR) preparation, partially purified by removing molecules smaller than 3.5 kDa. The batch-to-batch variation was $\pm 20\%$, in terms of RAST inhibition activity. The international standard of *Betula* has been tested; nevertheless, a titration by international units of the intranasal product used in this study has not been possible, as it is a mixture of *Betula* and *Alnus* pollen allergens. The comparison of intranasal and subcutaneous treatment sets of the same manufacturer in terms of allergenic potency expressed in AU was calculated in the following terms: a stand-

ard preseasonal treatment schedule consists of about 2950 AU in the case of LNIT, and of about 10 900 AU in the case of subcutaneous immunotherapy.

Treatment schedule

The patients were randomly distributed into two treatment groups, 11 in the placebo group and 11 in the active group. There were no relevant differences between the two groups with regard to the duration and severity of the symptoms, age, or sex (Table 1). The investigation was designed as a double-blind study.

LNIT was self-administered from November 1991 until the end of February 1992. Briefly, patients sprayed the allergen powder directly into the nasal cavities, once a day, on alternate days, in both nostrils. In both groups, each patient self-administered a premedication with cromolyn sodium spray (one puff in each nostril), 20 min before treatment in order to prevent local side-effects. Patients were advised to vocalize during administration to avoid inhaling the allergen into the bronchial tree. The treatment schedule provided for the following increasing steps: 2.5, 5, 10, 20, 40, 60, 80, 120, 160, and 240 AU. Each dose was administered four times before the next dose was given. The calculated total amount of allergen given was about 2950 AU. The study design is reported in Fig. 1.

Before and after treatment and after season, venous blood was collected for total IgE, birch-, and speckled alder-specific IgE by RAST (Sferikit specific-IgE and Sferikit total-IgE, Laboratorio Farmaceutico Lofarma S.r.l., Milan, Italy); specific IgG was determined by ELISA (10). The serum samples were stored at -20°C until analysis. Results were expressed in RAST units for specific IgE, IU/ml for total IgE, and P/N (positive/negative) ratio for specific IgG.

The NPT was done with the nasal insufflator with increasing dosages (every 20 min) of the allergen preparation in powder form, the positive threshold dose being assessed on the onset of four evident symptoms: itching of the nose, sneezing, nasal discharge, and obstruction.

Nasal reactivity was assessed by NPT before and after LNIT.

Table 1. Demographic data

	Active group	Placebo group
Patients (n)	11	11
Mean age (years)	32.5 (20–44)	34.6 (17–43)
Sex	5 M/6 F	5 M/6 F
Duration of symptoms (mean) (years)	4.7	5
Rhinitis during last season (weeks)	6.6	6.8

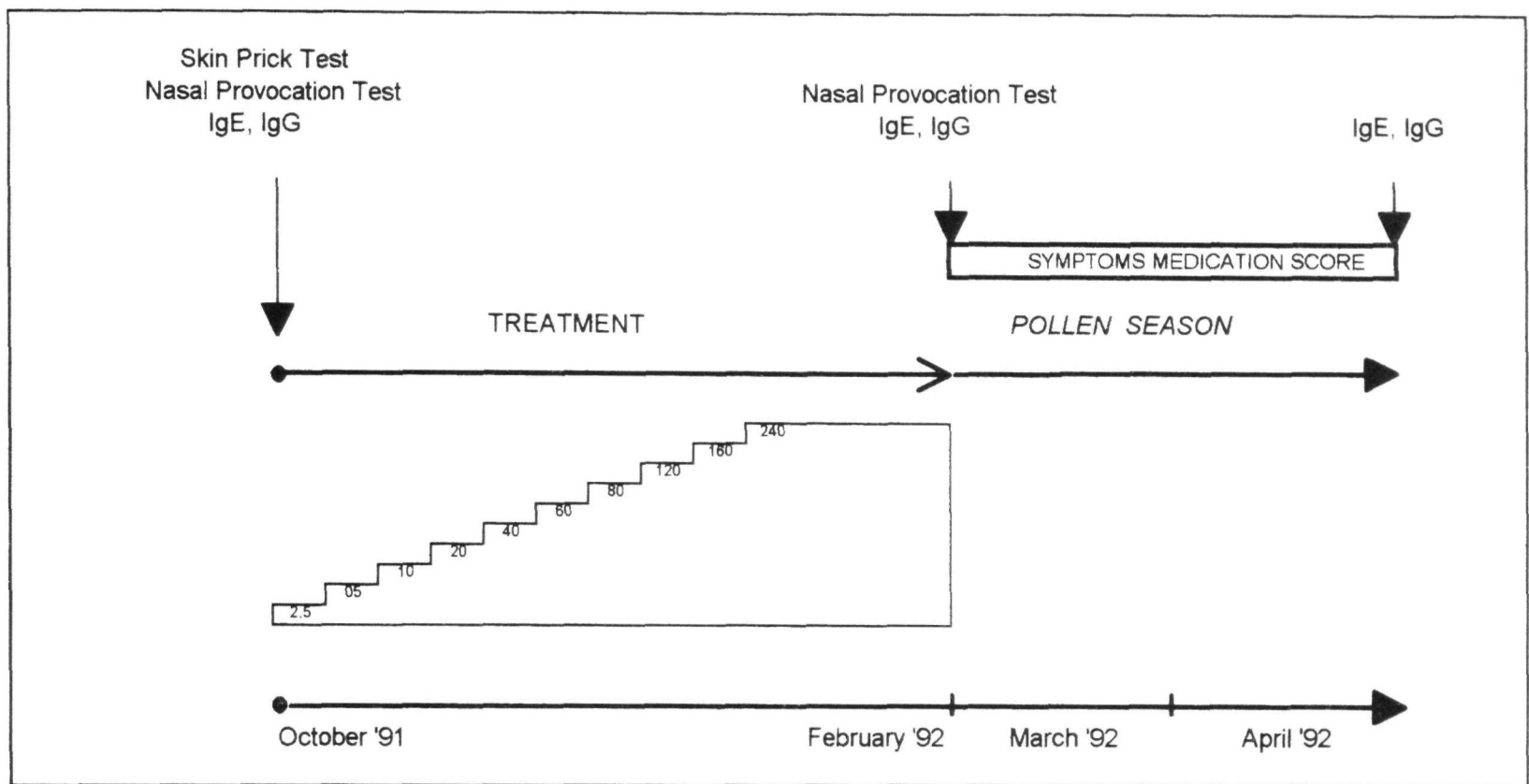


Fig. 1. Study design.

Clinical evaluation

Patients were assessed clinically at the beginning and end of treatment and after the pollen season. Treated and control patients were provided with a diary card covering 2 months of the tree blossoming season on which they recorded their symptoms. The symptoms of sneezing, nasal discharge, nasal itching, nasal obstruction, and watering eyes were graded daily from 0 to 3 (0 = none; 1 = mild; 2 = moderate; 3 = severe).

All patients had access only to an oral antiallergic drug (terfenadine tablets 60 mg) to be used as needed during the 2 critical months of the season. The number of tablets taken was recorded.

Pollen counts

Daily pollen counts were taken with a VPPS 2000 pollen trap (Lanzoni, Bologna, Italy). Pollen counts reported as grains per cubic meter were the mean of the data obtained from the four allergy clinics involved in the trial.

Statistical analysis

In view of the independence of the sequences of data, distribution-free statistics were employed to compare scores: the Mann-Whitney paired test for symptoms and tablet consumption; Wilcoxon's rank test for NPT findings. Other data, such as serologic results, were verified by Student's *t*-test for paired samples.

Results

All 22 patients completed the study. No systemic reactions were recorded during treatment. Only nine subjects experienced mild local adverse reactions to the LNIT limited to the upper airways. Immediate symptoms consisted of sneezing (one or two sneezes) and nasal itching, mainly at the first dose of new steps; a local burning sensation of the nose and throat was claimed by someone as a late symptom, rarely lasting more than 1 h. Five patients in the treated group and four in the control group reported these reactions, widely sprayed according to scheduled doses and not cumulative according to frequency. All patients regularly reached the maximal dose (240 AU) within February.

For each seasonal symptom and each subject, a total score was calculated by summing the daily scores during the 2 main pollen months. The monthly patterns of birch and alder pollen counts are reported in Fig. 2 in relation to the daily mean airborne concentrations for March and April. This pattern did not differ in severity from the records of previous years, because normally the tree pollen season in northern Italy is characterized by a sudden blossoming and several spikes typical of the Italian spring. A significant difference was observed between the active and placebo groups for nasal itching ($P < 0.01$), rhinorrhea ($P < 0.01$), and sneezing ($P < 0.05$), but there was no difference for nasal obstruction and watering eyes (Fig. 3). Daily consumption of terfenadine was greater in the group treated with placebo than in the group treated with

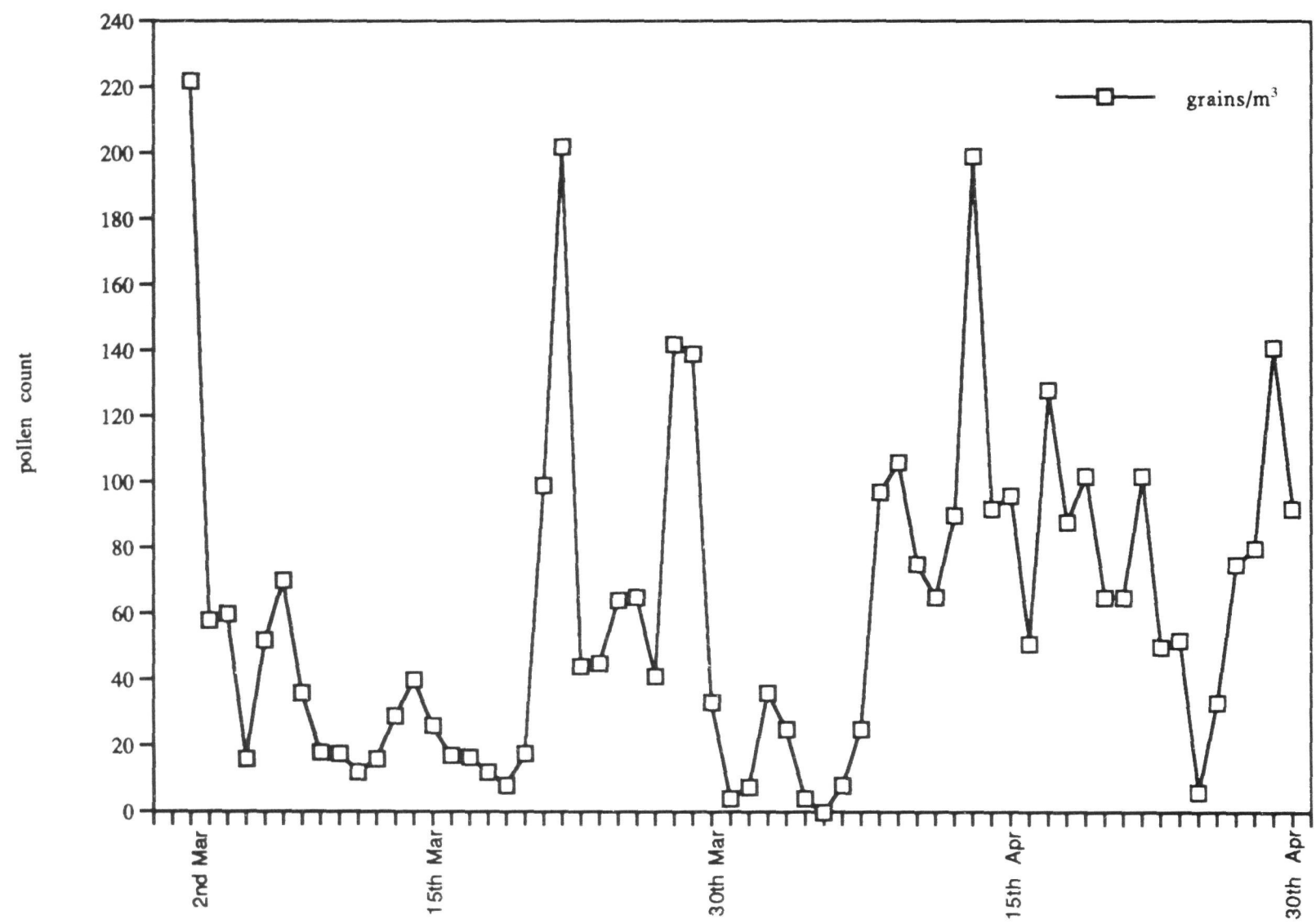


Fig. 2. Daily birch pollen count during pollination period (2 March–30 April 1992).

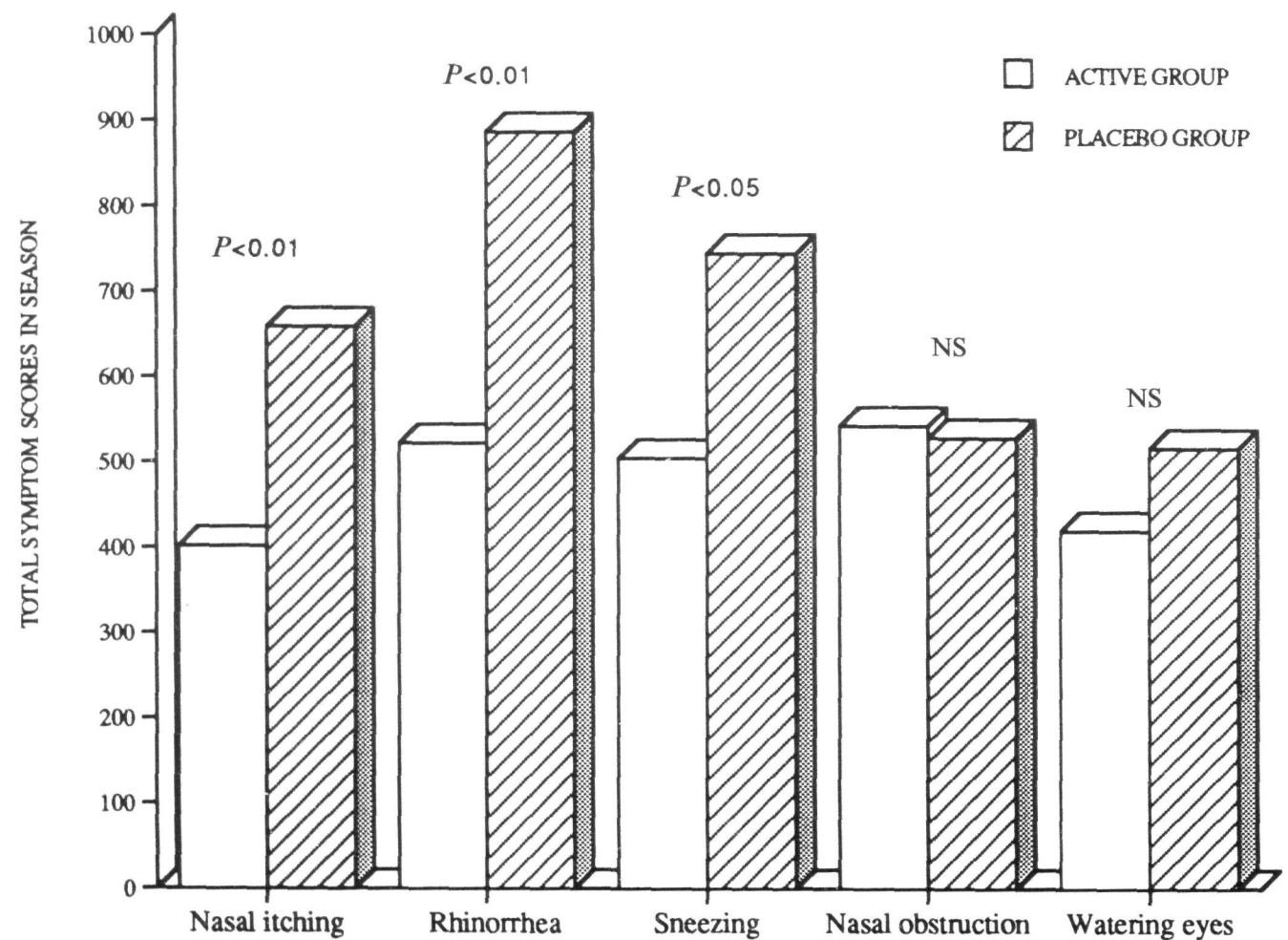


Fig. 3. Total symptom scores during pollination period (□ active group; ▨ placebo group).

allergen (total 299 tablets versus 128), this difference being significant ($P<0.01$). Individual symptoms and medication scores are reported in Table 2.

There was no difference between the two groups in the NPT before treatment. However, the NPT threshold dose observed before and after treatment rose only in patients of the active group (Fig. 4), and the statistical comparison was significant.

No noteworthy findings were obtained from immunologic examinations (total IgE, specific IgE, specific IgG), and the differences between pre- and post-treatment or after the season, within or between groups, were not significant, nor suggestive of any trend. An overall assessment, again double blind, was requested of 22 patients when they delivered the diary cards. The treatment was judged

Table 2. Individual scores for five symptoms and medication consumption covering tree pollen season (2 months)

Active patients	Nasal itching	Rhinorrhea	Sneezing	Nasal obstruction	Watering eyes	Medication consumption
A	18	58	80	74	38	21
B	36	68	59	68	36	2
C	11	28	68	76	20	8
D	39	24	71	79	26	3
E	29	26	28	38	28	16
F	36	27	22	28	29	8
G	43	95	72	72	48	23
H	88	65	28	36	73	2
I	41	48	38	42	45	22
L	24	38	24	16	38	20
M	36	45	14	13	37	3
Total	401	522	504	542	418	128
Placebo patients						
N	105	127	108	108	99	61
O	82	90	87	39	53	57
P	56	72	80	75	48	14
Q	40	69	52	22	28	20
R	23	79	58	36	32	23
S	25	48	54	39	33	17
T	58	57	49	51	44	28
U	28	98	78	37	39	8
V	99	97	98	48	29	15
Z	55	47	39	43	48	24
W	87	102	41	30	63	32
Total	658	886	744	528	516	299

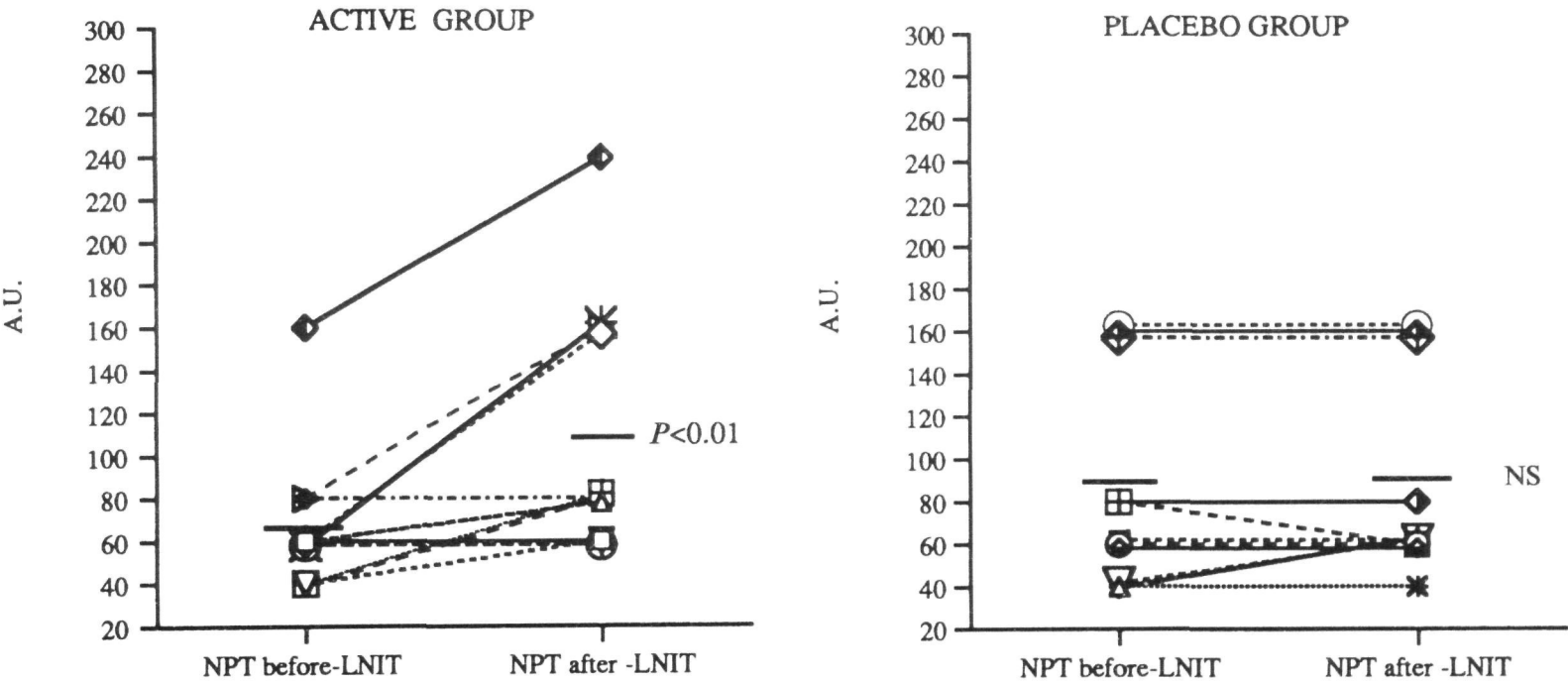


Fig. 4. Threshold doses of each patient before and after LNIT in active and placebo group. Horizontal bars (—) indicate mean threshold dose of each group before and after treatment.

“useful” by 8/11 active treated patients, but only by 1/11 placebo treated ones. However, compliance with the nasal treatment was mostly good, since all patients were carefully assisted.

Discussion

The clinical results of this study confirm the efficacy and safety of LNIT on a preseasonal schedule with a powder product and assessment of the reduction of symptoms in birch- and alder-allergic rhinitic subjects.

Clinical findings in this study agree with other reports on nasal immunotherapy with other allergens, with aqueous and powder form extracts. Taylor & Shivalkar (11) found a significant reduction in the symptom score during the pollen season after immunotherapy with an aqueous grass extract and reduced sensitivity to nonrelated allergens. Metha & Morrison Smith (2) and Cook (1) reported that preseasonal LNIT reduced nasal symptoms during the pollen season. Georgitis et al. (12) and Nickelsen et al. (13) have shown the clinical effectiveness of an aqueous solution of mixed-grass extract, a formaldehyde-modified mixed-grass extract, or a ragweed extract. Mathews et al. could not confirm these clinical benefits (14), and other authors have reported local side-effects with aqueous extracts (15, 16).

Georgitis et al. showed that low doses of unmodified aqueous extract were well tolerated but had no clinical efficacy (17). Johansson et al. (18) noted a reduction in symptoms with a glutaraldehyde-modified timothy grass pollen extract in nasal hypsensitization treatment, and local adverse effects were rare (16, 17, 19).

LNIT has only recently been done with an allergen in powder form. Controlled studies have shown good clinical results and safety both in seasonal and in perennial allergic rhinitis induced by grass pollen, *Parietaria*, and *Dermatophagoides* (6, 7, 8, 20, 21).

Experiences with LNIT in tree-allergic rhinitis is missing; however, the significant decrease in nasal reactivity to the allergen in the treated group in the present study is similar to observations with other pollens either with aqueous extracts (11, 12, 18, 22) or, in particular, with extracts in powder form (6, 8, 10, 20). Our patients presented no detectable systemic or local antibody response, and specific IgE and IgG circulating levels were not consistently modified. These findings are in accordance with other studies using either an aqueous extract (15, 23) or a powder form preparation (8). On the other hand, there are reports of significant increases in nasal specific IgA and IgG, though clinical and immunologic findings are not always correlated (18, 19, 24, 25, 26).

Our IgE findings differ from those of other authors (12, 18) who, using aqueous extracts, reported a significant increase in specific IgE antibody levels in serum in pretreatment over preseasonal samples.

Although many studies reported a significant increase of circulating IgG antibody specific for stinging insect allergens as a result of injection therapy (27, 28), a direct correlation with clinical effects is still doubtful; moreover, this finding seems not to be consistent for LNIT (24, 29), as this study confirms.

As for clinical benefit, our results demonstrated a valuable and significant difference in symptoms and rescue medication requirements between patient groups, as has already been observed with subcutaneous birch pollen immunotherapy after one preseasonal conventional treatment. The benefits seemed to have been restricted to nasal symptoms, with no effect on obstruction but a good effect on rhinorrhea, itching, and sneezing.

In previous studies and in the present one, neither active treatment nor placebo presented any worthwhile reduction in eye symptoms. In an earlier study with intranasal administration of ragweed extracts, allergic eye symptoms were reduced (13). It is assumed that relief of eye symptoms is related to the systemic effect of therapy rather than the local response, because the serum antibody response suggested that ragweed extracts were systemically absorbed. However, this did not happen in our study. These differences in the results of various studies may be due to differences in pollen exposure, duration of treatment, total administered dosage, and, mainly, extract formulation.

The immunologic mechanisms by which the LNIT works are not yet clarified. Nevertheless, we can exclude the possibility that it corresponds to the low dose desensitization described in the past, as in the Rinkel or Miller techniques. In fact, the dosage we reached by the described treatment was many times higher than the provocative threshold dose, as shown by the presented data.

We believe that a final answer cannot be made to the question of whether the efficacy consists of a tolerance induced by repeated degranulation. In any case, some clinical and also experimental observations, such as persistence of modifications in the expression of adhesion molecules by nasal endothelial cells after LNIT (30), suggest that the technique of immunotherapy here described is a real local hyposensitization.

One possibility is that allergen deposition in the upper respiratory tract induces an antigen and IgE-isotype-specific suppression (31). A second possibility is that immunotherapy favors the synthesis of local blocking IgG, but this is still under debate. In

any case, it has been demonstrated in rabbits that intranasal administration of pollen allergen (*Parietaria judaica*) leads to systemic absorption (32).

Immunotherapy based on intranasal powder administration in allergic rhinitis may help us to understand better some of the mechanisms involved in successful hyposensitization. Therefore, this therapeutic modality merits further investigation to clarify the mechanism, the ideal length of treatment, and the duration of effect after stopping the treatment.

Safety and time saving for patients, because of home self-administration, are two advantages of this therapy over subcutaneous treatment. It must be stressed that subjects must be appropriately instructed and the treatment must be carefully planned. In such conditions, a simple preseasonal schedule results in a satisfactory clinical response, improving the quality of life of allergic patients.

We conclude that this therapeutic modality offers a valid alternative to the conventional specific immunotherapy in birch-alder tree-allergic rhinitis.

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