

Efficacy and safety of subcutaneous immunotherapy with a biologically standardized extract of *Ambrosia artemisiifolia* pollen: a double-blind, placebo-controlled study

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Summary

Background The allergological relevance of *Ambrosia* in Europe is growing but the efficacy of the injective immunotherapy for this allergen has been documented only in Northern America.

Objective We sought to study the safety and efficacy of injective immunotherapy in European patients sensitized to *Ambrosia artemisiifolia*.

Methods Thirty-two patients (18 M/14 F, mean age 36.78, range 23–60 years) suffering from rhinoconjunctivitis and/or asthma and sensitized to *Ambrosia* were enrolled and randomized in a double-blind, placebo-controlled (DBPC) study lasting 1 year. A maintenance dose corresponding to 7.2 µg of Amb a 1 was administered at 4-week intervals after the build-up. During the second and the third year, all patients were under active therapy in an open fashion. Symptom and medication scores, skin reactivity to *Ambrosia* (parallel line biological assay), and pollen counts were assessed throughout the trial.

Results Twenty-three patients completed the trial. No severe adverse event was observed. During the DBPC phase, actively treated patients showed an improvement in asthmatic symptoms ($P = 0.02$) and drug ($P = 0.0068$) scores days with asthmatic symptoms ($P = 0.003$), days with rhinitis symptoms ($P = 0.05$), and days with intake of drugs ($P = 0.0058$), as compared to before therapy. No improvement for any of these parameters was detected in the placebo group. Moreover, the number of days with rhinitis and asthma was significantly higher in the placebo as compared to the active group ($P = 0.048$ and $P < 0.0001$, respectively). Patients who switched from placebo to active therapy improved in rhinoconjunctivitis, asthma, and drug intake. The skin reactivity decreased significantly (12.2-fold, $P = 0.0001$) in the active group whereas a slight increase (1.07-fold, $P = 0.87$) was observed in the placebo group after the DBPC phase. After switching to active therapy, patients previously under placebo showed a significant decrease of this parameter (4.78-fold, $P = 0.002$).

Conclusion Injective immunotherapy is safe and clinically effective in European patients sensitized to *Ambrosia*.

Keywords *Ambrosia artemisiifolia*, asthma, clinical efficacy, injective immunotherapy, monosensitized patients, PLBA, skin reactivity

Submitted 16 July 2003; revised 6 April 2004; accepted 20 April 2004

Introduction

The genus *Ambrosia* (Compositae family) includes several species very relevant from the allergological point of view, namely: *Ambrosia artemisiifolia* (short ragweed), *A. bidentata* (southern ragweed), *A. psyllostachia* (western ragweed), *A. aptera* (western giant ragweed), and *A. trifida* (giant ragweed).

IgE to allergens from these weeds are largely cross reactive, and *Ambrosia artemisiifolia* is considered the reference

species, because of the high number of sensitized individuals in Northern America.

Allergy to *Ambrosia* has long been a phenomenon restricted to Northern America. However, in the early 1980s, an increasing number of sensitization's, soon followed by allergic symptoms started to be reported also in Central Europe [1]

Ambrosia is not a new genus in Europe, since it was firstly observed there as early as 1915, whereas the first report for Italy dates back to 1942 [2]. In the early 1980s, *Ambrosia* showed an exponential growth in Central Europe, with a scattered distribution, and specific concentrations in the Lyon area in Eastern France, in Hungary, and in Northern Italy [1, 3, 4]. This phenomenon may be explained by two factors i.e. the switch from traditional cultures (wheat, maize) to sunflower (*Ambrosia* seeds are often contained in sunflower

¹Deceased

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seeds, since both species belong to the Compositae family), and the general increase in environmental pollution [1].

The prevalence of ragweed allergy in the area where this study was conducted was reported in two epidemiological investigations: in the first one, it ranged between 2.37% and 8.75%, in the general population [5]. In the second one (a prospective study conducted on patients affected with symptoms from respiratory allergy) the prevalence was 2–40% depending on the involved areas [6].

The largest part of the scientific work on allergy to *Ambrosia* was produced in Northern America since the end of the 1950s [7]. Subsequently, several trials dealing with injective therapy with aqueous, absorbed, and modified were conducted in Northern America. Especially, 13 of these studies have been reviewed by the WHO [8], and included in references supporting the efficacy of the injective immunotherapy in patients sensitized to ragweed.

In spite of the increasing importance of *Ambrosia* in Europe, no double-blind studies with injective immunotherapy were conducted in European patients. On the other hand, it has been shown that the immune responsiveness to Amb a 6 is significantly associated to HLA-DR5 in allergic humans [9], and population-specific IgE reactivity profiles have been reported in European patients sensitized to birch pollen [10].

Here, we performed for the first time a double-blind, placebo-controlled (DBPC) study in a European population with a different environmental exposure to *Ambrosia*, and with a different genetic background, as compared with Northern Americans. This study allowed us to evaluate the safety and efficacy of specific injective immunotherapy to *Ambrosia* in European patients.

Materials and methods

Study design

This study was planned as a multicentre, randomized, double-blind, placebo-controlled trial for 1 year, followed by an open phase with the administration of the active therapy. All patients were submitted to active treatment for at least 2 years. Before the DBPC phase, a baseline assessment (1998 pollen season) was performed. The study design is detailed in Fig. 1. Three centres, one located in Milan, and the two others within 30 km northwest from Milan, took part in the trial. Patients were assigned at random to the placebo or to the active group according to a computer-generated key-code.

The experimental protocol was approved by the Ethics Committee in each centre, and all patients signed an informed consent before starting the trial.

Patients

Thirty-two patients, 18 males and 14 females aged between 23 and 60 years (mean age 36.78 years) and living in the area of each involved centre, were enrolled before the *Ambrosia* pollen season 1998. They had a clinical history of rhinoconjunctivitis and/or asthma in late summer, as typical in subjects sensitized to *Ambrosia*.

All were skin test positive to *Ambrosia artemisiifolia* extract and had specific IgE to the same allergen.

Patients were not included if at least one of the following conditions occurred:

- sensitization to other inhalant allergens possibly interfering with the assessment of the outcomes of the trial;
- previous immunotherapy for *Ambrosia* in the last 3 years;
- pregnancy already in progress or planned;
- severe asthma; and
- any other standard contraindication [11].

Diagnosis

In vivo diagnosis was performed with a biologically standardized (100 BU/mL) extract of *A. artemisiifolia* (ALK-Abellò, Madrid, Spain), containing 18 µg/mL of the major allergen Amb a 1 in glycerosaline solution [12–14].

Weals >3 mm in diameter were considered as positive [15] when skin reactivity to positive, and negative controls was as expected.

In vitro specific IgE determination in sera was performed by means of the CAP system (Pharmacia, Uppsala, Sweden), with a cut-off value of 0.35 KU/L.

Treatments

The active treatment was prepared from a biologically standardized extract of *A. artemisiifolia* pollen, absorbed onto aluminium hydroxide and suspended in phenolated (0.4% w/v) saline solution (ALK-Abellò).

The treatment set was composed of three vials with potencies 10, 100, and 1000 STU/mL, corresponding to contents 0.09, 0.9, and 9 µg/mL of the major allergen Amb a 1, respectively.

The placebo was indistinguishable from the active treatment for appearance, colour, and taste.

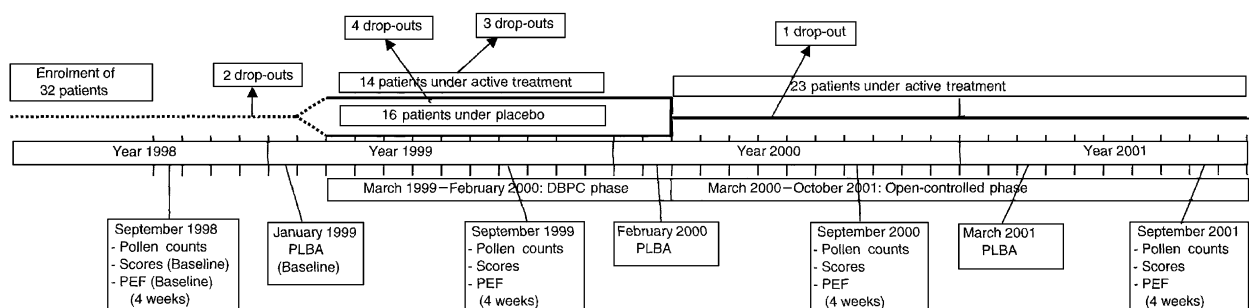


Fig. 1. Demographic data.

The administration of the therapy, active or placebo, began between January and March 1999 in all centres according to the treatment schedule, detailed in Table 2, and lasted 12 months. Then, the randomization code was broken, and patients who had undergone active therapy during the DBPC phase of the trial received monthly for 1 year the maintenance dose they had reached. In contrast, patients treated with placebo during the DBPC phase started the active therapy from the induction phase, and continued with the monthly maintenance dose for at least 20 months of active treatment.

During the pollen season, the maintenance dose was reduced by 60–80% in all patients. After the pollen season the maintenance dose was gradually increased again up to the amount reached before.

Early local and systemic side-effects related to the treatment were reported keeping patients under observation for at least 30 min after each administration.

Patients were also asked to report by phone or before each administration any delayed side-effect possibly related to the treatment.

Early or late local reactions larger than 2.5 cm in diameter as well as any systemic reaction were considered as criteria either for modification of the injection (e.g., repetition of the last well-tolerated dose, dose reduction) or for the temporary or definitive interruption of the therapy.

Pollen counts

The daily pollen count for *Ambrosia* of seasons from 1998 to 2001, was performed in two of the centres involved in the trial (Legnano, Rho), and expressed in grains/m³. To this aim, a Burkard pollen trap was used, which had been placed at an height of around 20 m on the roof of a building in the centre of the city. Results from these two centres were similar for amount, and timing (not shown). Because of the limited distance among these, and the other medical centre where patients were recruited, these pollen counts were considered valid for all of them.

Clinical parameters for efficacy assessment

Symptom and medication scores during the pollen season During the *Ambrosia* pollen season 1998, 1999, and 2000 (and also 2001 for patients initially belonging to the placebo group), patients were asked to fill in a diary card for symptoms and medications. The score grading for rhinoconjunctivitis included four steps, from no symptoms (score 0) to severe symptoms (score 3). Five symptoms (sneezing, running nose, nose itching, stuffy nose, eye symptoms) were considered, for a maximum score of 15. The score grading for asthma included five steps, from no symptoms (score 0) to heavy symptoms (score 4, persistent cough plus asthmatic symptoms during day and night).

Only the following rescue drugs were allowed on need: oral antihistamine (cetirizine, score 2 for each tablet), antihistamine eye drops (levocabastine, score 1 per drop), antihistamine nasal spray (levocabastine, score 1 per actuation), β_2 -agonist (salbutamol, score 2 per actuation), and oral cortisone (25 mg/tablet, score 3 per tablet).

During the pollen season in each year patients were regularly seen on a weekly basis to evaluate their clinical situation, and to check the correct filling in of diary cards.

Skin reactivity to the sensitizing allergen The skin reactivity of each patient was assessed according to the parallel line biological assay [16] before starting treatment (January 1999), in February 2000, and in March 2001. In brief, four different allergen strengths (4, 20, 100, 500 BU/mL) were tested in duplicates on the volar surface of the forearm by means of a disposable standard plastic prick lancet (Morrow-Brown, Oakhurst, NJ, USA), allowing a distance of at least 25-mm among tests. The tests were performed by the same operator in each centre according to a fixed pattern, at the same time of the day, and at least 1 month after the last intake of any drug possibly interfering with skin reactivity. Fifteen minutes after testing, the contours of each weal were outlined with a fine-tipped ballpoint pen, and transferred onto paper for the planimetric evaluation of the area.

Statistics The experimental design allowed for several comparative analysis. Firstly, a direct comparison between the active and the placebo group before and after the DBPC phase for all of the experimental parameters was performed.

Secondly, the clinical outcome after 1 year of therapy was evaluated either including only the active group before and after the DBPC phase, or mixing these patients with the patients switching from placebo to active treatment during the second year.

Thirdly, changes in patients who were under active treatment during the DBPC phase, and continued on active therapy for a further year were considered, and compared with those formerly under placebo who underwent 2 years of active therapy during the open phase.

The changes in skin reactivity were analysed by a specific software developed for parallel line biological assay (PLBA) [16] whereas all other statistical analysis were performed with a standard statistical software (BMDP for Windows v. 1.0, BMDP Statistical Software Inc., LA, USA).

Non-parametric data or parametric data for which the check for normal distribution was not possible, were expressed as the median values with interquartile ranges. Drug and symptom scores during the DBPC phase were analysed with the Mann–Whitney rank-sum test (inter-group comparisons) or the Wilcoxon test (intra-group comparisons). These tests (plus Bonferroni adjustment) were also used for the same parameters for multiple comparisons considering the baseline values and the values after 1 and 2 years of active treatment, as explained before. Days with or without symptoms or drug consumption were analysed with the Wilcoxon test (intra-group comparisons). Because of the different allergenic load in different years, parameters for intra-group comparisons were normalized by dividing each value by the 'area under the curve' (AUC) of the pollen counts for the relevant year as appropriated. The *t*-test was also used for the comparison of the pollen counts in the different years, taking into account the average pollen count per each decade within each pollen season.

The χ^2 test was used for inter-group comparisons of symptom scores before and after the DBPC phase.

P-values of 0.05 and 0.01 were considered as 'statistically significant' and 'highly statistically significant' respectively.

Table 1. Demographic data

	Enrolment (1998)	DBPC phase before drop-outs		Open phase before drop-outs Active
		Active	Placebo	
Total number	32	16*	16†	23‡
Males	18	7	11	14
Females	14	9	5	9
Age range years (mean)	23–60 (36.78)	27–54 (37.25)	23–60 (36.31)	23–60 (37.08)
Rhinitis	7	4	3	7
Rhinoconjunctivitis	22	10	12	16
Asthma	13	6	7	12
n.a.	3	2	1	0

*Three drop-outs.

†Four drop-outs.

‡One drop-out.

n.a., patients who performed the DBPC phase but unable to supply diary cards adequate for analysis; DBPC, double-blind placebo-controlled study.

Table 2. Treatment schedule

Vial (strength and conc. of Amb a 1)	Dose (mL)	Dose (μ g Amb a 1)	Cumulated dose (μ g Amb a 1)
Build-up phase (weekly administrations)			
1 (10 STU/mL, 0.09 μ g/mL Amb a 1)	0.1	0.009	0.009
	0.2	0.018	0.027
	0.4	0.036	0.063
	0.8	0.072	0.135
2 (100 STU/mL, 0.9 μ g/mL Amb a 1)	0.1	0.09	0.225
	0.2	0.18	0.405
	0.4	0.36	0.765
	0.8	0.72	1.485
3 (1000 STU/mL, 9 μ g/mL Amb a 1)	0.1	0.9	2.385
	0.2	1.8	4.185
	0.4	3.6	7.785
	0.6	5.4	13.185
	0.8	7.2	20.385
Maintenance phase (administrations every 4 weeks)			
3 (1000 STU/mL, 9 μ g/mL Amb a 1)	0.8	7.2	~75*
	(0.3 during the pollen season)	(~2.1 during the pollen season)	

*Cumulated doses in 12 months, taking into account the dose reduction during the pollen season

Results

Drop outs

Two patients dropped out before starting the DBPC phase and 23 (11 under active treatment, 12 under placebo) completed such a phase. Three patients under active treatment and four patients of the placebo group dropped out; they left the study for reasons independent of immunotherapy (poor compliance to fill in diary cards, family reasons, etc.).

One more patient belonging to the placebo group dropped out during the open phase (second year) so as 22 patients in total completed the whole protocol (Table 1).

Immunotherapy and adverse events

In 1999, patients under active therapy received on average a cumulative dosage of 50.6 μ g of Amb a 1, and 176.2 μ g of Amb a 1 during the full treatment period (March 1999–October 2001). Patients treated with placebo during 1999, and then switched to the active treatment received a cumulative

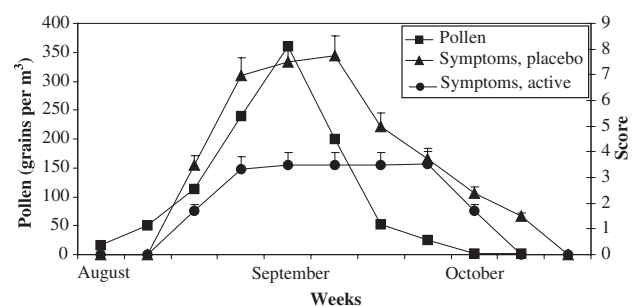


Fig. 2. *Ambrosia* pollen counts, and symptoms scores during the 1999 pollen season. Results of 7 days of follow-up were averaged, and used for plotting. Bars indicate standard deviations.

dosage of 125.6 μ g/mL of Amb a 1 (March 2000–October 2001).

Three patients under active treatment showed a systemic reaction (13.0% of patients). Generalized urticaria was observed in one case after the administration of 0.1 mL from vial 3 (corresponding to 0.9 μ g of Amb a 1), and rhinoconjunctivitis in two cases, after the administration of 0.3 and

Table 3. Clinical outcomes of the DBPC phase (placebo: $n = 12$; SIT: $n = 11$)

Parameter	Treatment	At baseline* (1998)	After treatment* (1999)	P value
Asthma symptom scores	Placebo	0.5 (0.1–0.9)	0.8 (0.1–1.9)	NS
	Active	1.2 (0–2.6)	0.4 (0–1.3)	0.02
Days with asthmatic symptoms	Placebo	7.5 (1.5–17.5)	13 (2.5–22)	NS
	Active	21 (2–31)	5 (0–15)	0.003
Scores for rhinitis symptoms	Placebo	5.8 (2.8–14.7)	9.3 (4.1–12.9)	NS
	Active	7.5 (3.6–9.9)	4.5 (3.6–9.1)	NS
Days with rhinitis symptoms	Placebo	20.5 (11–27.5)	30.5 (21.5–35)	NS
	Active	29 (28–36)	24 (20–28)	0.05
Scores for rescue drugs	Placebo	1.9 (0.4–3.3)	2.8 (0.3–4.4)	NS
	Active	3 (2.2–5)	0.6 (0–2.4)	0.0068
Days with drug intake	Placebo	10 (3.8–21.3)	15.5 (2.3–26)	NS
	Active	19 (14–28)	7 (0–13)	0.0058

*Median and (inter-quartile range).

DBPC, double-blind placebo-controlled study; NS, not significant.

0.4 mL from vial 3, respectively (corresponding to 2.7, and 3.6 µg/mL of Amb a 1) (three out of 950 doses, 0.31%). All reactions resolved spontaneously with no need of pharmacological or medical intervention, and with no need of adjustments of the schedule. These figures are in the range of those reported in similar trials of subcutaneous immunotherapy with allergen extracts [17, 18].

Pollen counts

The AUC for pollen counts during the pollen season for *Ambrosia* (late July–middle of October) was estimated in 1998, 1999, 2000, and 2001. The value for 2000 (104.1) was significantly higher as compared with 1998 (61.7, $P = 0.043$) and to 1999 (58.5, $P = 0.049$), whereas no difference was detected when comparing 1998 and 1999. The value for 2001 (72.0) showed no statistical difference as compared with other years.

Symptom and medication scores

Scores for asthmatic symptoms ($P = 0.02$) and drugs ($P = 0.0068$) decreased significantly in the active group after the first year of SIT (DBPC phase) as compared to before, with no change for the placebo group. A significant decrease was also seen in the active group in the same period for days with asthmatic symptoms ($P = 0.003$), days with rhinitis symptoms ($P = 0.05$), and days with intake of drugs ($P = 0.0058$), whereas again no difference was seen in the placebo group (Table 3). In Fig. 3 it is shown that pollen count parallels the symptom score, as expected when symptoms are properly recorded. After the DBPC phase, the number of days with rhinitis, and with asthma was significantly higher in the placebo group as compared with the active group ($P = 0.048$, and $P < 0.0001$, respectively).

When considering the outcome after 1 or 2 years of SIT, as compared with the assessment before SIT in the same patients, scores for rhinoconjunctivitis, asthma, and drugs decreased significantly after the first year ($P = 0.008$; 0.0014; 0.0008, respectively). These outcomes were further improved or confirmed after the second year of treatment ($P = 0.0006$; 0.0028; 0.0004, respectively). Days with a score > 0 were also significantly decreased after the first year of specific im-

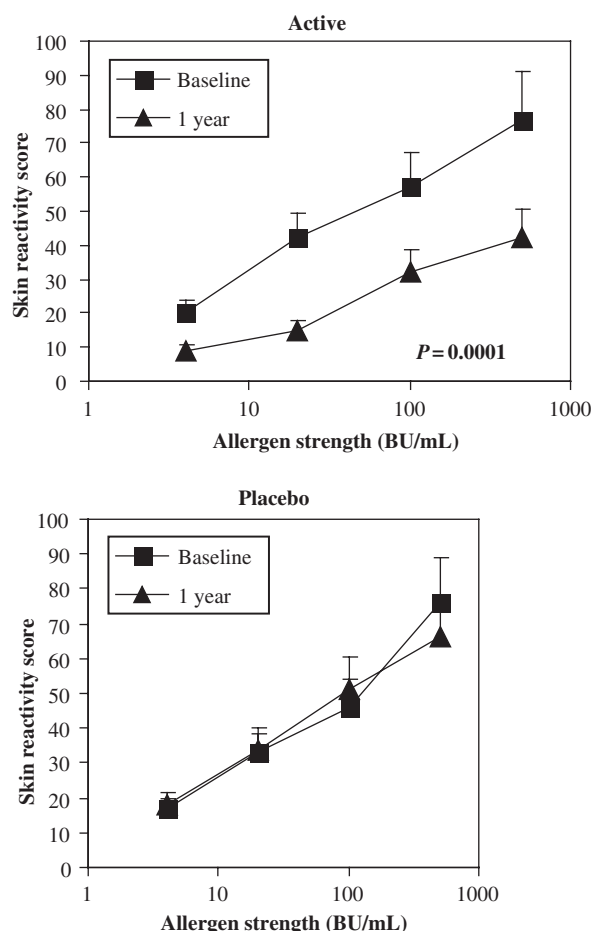


Fig. 3. Skin reactivity to the sensitizing allergen in the placebo, and in the active group. Scores for reactivity, on the y-axis, are according to the parallel line biological assay (see Materials and methods). Allergen strength is indicated on the x-axis. Evaluations were done on patients entering the study (baseline) and at the end of the double-blind placebo-controlled study phase (1 year later). Bars indicate standard deviations.

munotherapy (SIT) for asthma, and for drugs ($P = 0.0034$, and 0.02, respectively) and after the second year of SIT ($P = 0.049$, and 0.0006, respectively), whereas no significant change could be detected in the number of days with rhinoconjunctivitis symptoms (Table 4).

Table 4. Clinical outcomes after 1 and 2 years of active SIT as compared with the baseline assessment (before SIT)

Score	Baseline*	After 1 year* (N = 22)	P-value	After 2 years* (N = 22)	P-value
Rhinoconjunctivitis	8.4 (3.8–11)	3.9 (2.5–5.8)	0.008	3.8 (2.1–6.6)	0.0006
Asthma	1 (0.1–1.9)	0.2 (0–1)	0.0014	0.1 (0–1)	0.0028
Drugs	3 (0.5–4.5)	0.6 (0–1.9)	0.0008	0.2 (0–1.4)	0.0004
Days with a score >0					
Rhinoconjunctivitis	29 (23–36)	27 (21–34)	NS	28 (22–36)	NS
Asthma	16 (2–28)	3 (0–16)	0.0034	7 (0–27)	0.049
Drugs	18 (11–27)	7 (0–21)	0.02	2 (1–14)	0.0006

*Median and (inter-quartile range); N = number of patients considered for the evaluation.

NS, not significant.

Changes in skin reactivity (PLBA)

Skin reactivity was slightly different at baseline between groups, the active group being 1.83-fold more reactive than placebo group, but the difference had no statistical significance ($P = 0.21$).

The active group after the DBPC phase showed a significant decrease in the skin reactivity (12.2-fold with reference to baseline, $P = 0.0001$). This outcome was further improved after 2 years of active treatment (13.44-fold in reference to baseline, $P < 0.0001$), with no difference between the first, and the second year of therapy ($P = 0.47$).

In contrast, a slight increase by 1.07-fold ($P = 0.87$) in the skin reactivity was assessed in the placebo group after the DBPC phase, followed by a significant decrease of this parameter (4.78-fold, $P = 0.0022$) after 12 months of active therapy. In Fig. 3 the raw data of skin reactivity of the placebo, and active groups in the DBPC phase are shown.

Discussion

Specific injective immunotherapy is, nowadays, a recognized therapeutic option in allergic patients with respiratory symptoms because of inhalant allergens [11, 19]. Here, we report that injective immunotherapy is safe and clinically effective in European patients sensitized to *Ambrosia*.

The pollen from *A. artemisiifolia* (short ragweed) is a very important inhalant allergen in Northern America, and this explains the relevant number of DBPC studies with injective immunotherapy conducted in patients sensitized to it. In total, the first WHO Position Paper [11] considers 13 DBPC studies in patients suffering from rhinitis because of this pollen. Eleven studies showed a positive and statistically significant outcome in a total of 306 actively treated patients as compared with 194 treated with placebo whereas two studies failed to show any advantage of the active therapy (93 patients) as compared with placebo (86 patients). However, the two latter studies were conducted according to the so-called Rinkel method, i.e. with very low amounts of allergen in the range 0.019–0.15 µg of Amb a 1 (formerly called Ag E) as maintenance dose [20, 21]. Other investigators have shown in other trials that significant changes in mediator release after specific nasal challenge begins when a dose of Amb a 1 in the range 0.22–0.6 µg had been reached [22, 23], whereas a significant clinical improvement took place with a maintenance dose of 1.94 µg of Amb a 1 [24].

Two DBPC studies investigating the efficacy of SIT in adults or children suffering from asthma caused by *Ambrosia*

showed poor [18] or no improvement [25]. It must be underlined, however, that the trial in children raised strong criticisms [26, 27], because patients were suffering from perennial asthma, and were treated with mixtures of up to seven different allergens because of symptoms because of multiple sensitizations.

With the aim of avoiding as far as possible any recruitment bias, we have selected for our trial only subjects sensitized to *Ambrosia* and suffering only from seasonal rhinoconjunctivitis and/or asthma during the *Ambrosia* pollen season. Furthermore, we administered as monthly maintenance dose an amount of 7.2 µg of Amb a 1 of a purified and biologically standardized allergen extract. This dose is in the range 5–20 µg of major allergen recommended by the WHO as monthly maintenance dose [8] and in good agreement with the findings by other investigators [22, 28, 29]. The allergenic load was different in the different years of the trial, but we have adjusted the data taking into account the AUC value of the pollen season in each year. In these conditions, we have been able to show a significant improvement in both subjective (score for drugs and asthma; days with asthmatic and rhinitis symptoms and with drug intake) and objective (skin reactivity to the sensitizing allergen, PLBA assessment) parameters during the DBPC phase in the active group as compared with before the treatment. In contrast, the placebo group in the same period showed no benefit for any of the considered parameters. Moreover, in the same period, the number of days with rhinitis, and with asthma was significantly higher in the placebo group as compared with the active group.

The switch to the open phase of the study was requested by the ethics committees of the hospital that coordinated this study, and by those of all other participating Institutions. However, Abramson et al. [17] recently reviewed several randomized controlled trials using various forms of allergen specific immunotherapy, where significant improvement in symptoms was reported since the first year of treatment. Accordingly, the favourable changes in the investigated parameters we obtained after 1 year of active therapy (active group during the DBPC phase, and placebo group 1 year after switching to active therapy) were confirmed or even improved after the second year of active therapy. Taken together, these results strongly support the conclusions of our work, despite the theoretical limits deriving from the switch from DBPC to an open phase.

Only for the number of days with rhinoconjunctivitis there was no significant change during the trial for either group. The discrepancy between the reduction of days with asthma and medication use as compared with the unchanged number

of days with rhinoconjunctivitis might depend on the fact that whereas even light asthma is socially invalidating hayfever symptoms are in certain degree tolerated. In most SIT studies the medication reduction exceeds symptom reduction [30]. Moreover, although rhinoconjunctivitis symptoms did not disappear, their severity was reduced. Finally, it is worth noting that the skin reactivity in the placebo group phase showed a slight increase during the DBPC, followed by a 4.78-fold decrease ($P = 0.0022$) in the same patients after 12 month of active therapy.

Peak expiratory flow values changed significantly for both groups after the DBPC phase, but this change was below 10%, and therefore merely statistical, and likely devoid of any clinical relevance (not shown).

The incidence of non-severe adverse events we observed was quite low, and similar to values reported, for instance, in a recent Cochrane's review by Abramson et al. [17] and in a study by Creticos et al. [18] where similar amounts of Amb a 1 were used. Indeed, the low incidence of adverse events is extremely important when treating non-lethal diseases.

In conclusion, our data confirm that injective immunotherapy in European patients monosensitized to *A. artemisiifolia* with monthly dosages of the major allergen in the range recommended by the WHO, is well tolerated and clinically effective. The positive clinical outcome assessed through subjective parameters is confirmed and supported by the change of an objective parameter such as the skin reactivity to the sensitizing allergen. We have been able to show positive outcomes after 1 year of active therapy, but our data support the notion that further improvements can be achieved with a longer maintenance phase.

Acknowledgements

We wish to thank Dott. Luisa Zanolla from the Department of Cardiology, Verona Hospital, for her support in statistical analysis.

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