

Efficacy, safety, and modulation of immunologic markers by immunotherapy with honeybee venom: Comparison of standardized quality depot versus aqueous extract

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ABSTRACT

Venom immunotherapy (IT) is a very effective method for the treatment of Hymenoptera venom allergy. We compared safety, efficacy, and modulation of specific immunologic parameters in 70 patients sensitized to Apis mellifera, treated for ≥ 5 years with standardized quality (SQ) aqueous IT, either with a rush ($n = 20$) or with a cluster ($n = 20$) induction protocol, or with an SQ depot extract and a cluster induction protocol ($n = 30$). We made an open, noncontrolled study. Side effects were monitored and the effects of field stings during the maintenance phase of the treatment and after its interruption were recorded. Skin reactivity to Apis was measured by end point dilution and specific serum immunoglobulin E (IgE) were measured by a solid-phase-based assay. The depot IT was better tolerated than aqueous IT with rush induction. This was caused by mainly the lower frequency in the induction phase of systemic side effects (3.4% versus 36.8% [$p < 0.0041$] on a "per patient" and 0.1% versus 0.9% [$p = 0.0092$] on a "per dose" basis, respectively). The cluster protocol with the aqueous extract tended to be better tolerated than the rush protocol. About one-half of patients from each group were re-stung during the study and all suffered only minor discomfort. Reduction of skin reactivity and of serum-specific IgE was significant in the three groups ($p < 0.02$ in all cases). SQ Depot IT to Apis venom allergy administered with a cluster protocol induces less side effects and is equally effective than IT with SQ aqueous extract administered with a rush protocol. (Allergy Asthma Proc 27:151–158, 2006)

Hymenoptera stings can result in severe and, occasionally, fatal anaphylaxis in sensitized individuals.¹ Moreover, the fear of being stung is understandably associated with a relevant impact on the quality of life.² Venom immunotherapy (IT) is recommended in exposed individuals because it is highly effective in the prevention of both local reactions and of systemic and potentially life-threatening manifestations.³ The striking results of two controlled studies published in the 1970s,^{4,5} showing that aqueous venom extracts were effective, as opposed to a mere placebo effect of Hymenoptera whole-body extracts, generated a wide consensus on the opportunity to treat venom-sensitized individuals with aqueous extracts, although they are not devoid of side effects. In particular, *Apis* as compared with *Vespula* extracts cause a higher number of more severe and often systemic side effects.^{6–8} Thus,

although there is no doubt about the efficacy and the relative safety of aqueous IT,⁹ the compliance to this treatment can be severely impaired by side effects, including the possibility of anaphylactic shock that this treatment is primarily committed to prevent. This problem also implies that only selected allergological centers usually perform venom IT, limiting the opportunity to extend this efficacious treatment to all patients that could benefit from it.

To overcome problems related to the usage of aqueous extract, the absorption of allergens to insoluble substances (aluminum hydroxide, tyrosine, or calcium phosphate) has been proposed since several decades ago.^{10–12} Accordingly, presently, IT for inhalant allergens is, almost exclusively administered with depot extract. Surprisingly, the switch from aqueous to depot formulations in the distinct case of venom IT still has not taken place despite the fact that solid data have accumulated over the years, particularly in German-speaking European countries, indicating that depot extracts were well tolerated and equally effective.^{13–20}

Here, we show the results of an open, noncontrolled study, where 70 *Apis mellifera* venom-sensitized subjects with a history of systemic reactions were treated with commercially available preparations of either

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Table 1 Patients Included in the Study

Group	n	Sex (M/F)	Age (range)	Grade of Reaction			
				1	2	3	4
Aqueous rush	20	16/4	42.1(18–73)	1	5	11	3
Aqueous cluster	20	16/4	43.8(28–76)	0	10	5	5
Depot cluster	30	26/4	37.3(12–68)	1	9	11	9
Total	70	58/12	41.4(12–76)	2	24	27	17

Demographic data and grade of the severity of reaction to *Apis* sting according to Mueller²¹ before IT.

standardized quality (SQ) aqueous or SQ depot venom extracts and were carefully monitored after each injection. The clinical results of three different induction protocols and of two types of extracts were compared in terms of efficacy and safety. In addition, biological end points were considered: the degree of skin test positivity was evaluated carefully by end point dilution and honeybee serum-specific IgE was measured before entering the study and then 5 years later. The results of the study are discussed in the perspective of improving the management of IT in Hymenoptera venom allergic patients.

MATERIALS AND METHODS

Patients

Patients who were enrolled in this study fulfilled the following criteria: (a) history of hypersensitivity to *A. mellifera* venom; (b) systemic reactions $\geq$ grade 2 (according to Mueller and, more recently revised by Wuthrich^{21,22} after a honeybee sting; (c) significant risks of subsequent exposure to the allergen, either in terms of actual physical risk for severe reactions or of socially relevant impairment of the quality of life because of the fear for subsequent stings; (d) positive skin tests for *A. mellifera* at the intracutaneous reaction performed with the end point technique¹⁵; (e) detection of serum IgE to *A. mellifera* (Table 1).

Two patients who had grade 1 reaction and a class 5 specific IgE level (according to Pharmacia, Uppsala, Sweden) were included also, because their quality of life was severely impaired by the risk of being re-stung, in agreement with current recommendations. Pregnancy and treatment with β -blockers were adopted as standard exclusion criteria.⁶ Demographic data of the patients included in the study and severity of pre-IT systemic reactions, expressed according to Mueller,²¹ are shown in Table 1. All patients were informed about this study and were provided a written consent.

The first nine patients were assigned to depot IT with a cluster induction protocol. Subsequently, the following three regimens were alternated in consecutive subjects: (a) aqueous IT with a rush induction protocol,

(b) aqueous IT with a cluster induction protocol, and (c) depot IT with a cluster induction protocol.

Hymenoptera Extracts

The allergen-enriched extracts we used in the two cohorts for aqueous and depot IT (Aquagen SQ and Alutard SQ, respectively) were prepared from the same source by the supplier (ALK Abell , Milan, Italy). This accounted for homogeneous immunogenicity. For depot IT, raw venom extracts of *A. mellifera* were submitted to purification on Sephadex with recovery of the allergen-containing fraction only and, subsequently, adsorbed onto aluminum hydroxide, as specified by the manufacturer.

Immunization Schedules

Details of the immunization schedules followed in the induction phase of the three IT regimens are described in Tables 2, 3, and 4, respectively. Notably, the number of visits in the induction phase of the aqueous rush, aqueous cluster, and depot cluster regimens were kept homogeneous (13, 12, and 12, respectively) to facilitate the comparison between the single regimens. In the maintenance phase, a 100- μ g/mL dose per visit was reached. This dose was administered every 4 weeks in the first 3 years. Then, the interval between doses was progressively increased up to 6 and 8 weeks in the 4th and 5th year, respectively.

Evaluation of Side Effects

Patients on the rush protocol were hospitalized for 4 consecutive days in the initial phase of the therapy. Patients who underwent the aqueous and depot cluster protocol were kept on an outpatient regimen, which required hospitalization on the days when a visit was scheduled (Tables 3 and 4). Patients were observed for 60 minutes after each injection with aqueous extract and for 6 hours after depot therapy. Both local and systemic reactions were recorded according to previously described criteria.^{9,21} All patients were instructed to report delayed reactions to the center immediately, and they were interviewed during subsequent visits

Table 2 Induction Phase (Aqueous Extract and Rush Schedule)

Day	Visit	Vial	Dose	$\mu\text{g/mL}$	Time	mL Administered	μg Administered
1	1	1	1	0.1	0 hr 0 min	0.1	0.01
1	1	2	2	1.0	1 hr 0 min	0.1	0.1
1	1	3	3	10.0	2 hr 0 min	0.1	1.0
1	1	3	4	10.0	3 hr 0 min	0.2	2.0
2	2	3	5	10.0	0 hr 0 min	0.4	4.0
2	2	3	6	10.0	1 hr 0 min	0.6	6.0
2	2	4	7	100.0	2 hr 0 min	0.1	10.0
2	2	4	8	100.0	3 hr 30 min	0.2	20.0
3	3	4	9	100.0	0 hr 0 min	0.4	40.0
3	3	4	10	100.0	1 hr 0 min	0.4	40.0
3	3	4	11	100.0	2 hr 0 min	0.6	60.0
4	4	4	12	100.0	1 hr 0 min	0.5	50.0
4	4	4	13	100.0	2 hrs 0 min	0.5	50.0

Doses given to hospitalized patients.

Table 3 Induction Phase (Aqueous Extract and Cluster Schedule)

Week	Visit	Vial	Dose	$\mu\text{g/mL}$	Time	mL Administered	μg Administered
1	1	1	1	0.1	0 hr 0 min	0.1	0.01
1	1	2	2	1.0	1 hr 0 min	0.1	0.1
1	1	3	3	10.0	2 hr 0 min	0.1	1.0
1	1	3	4	10.0	3 hr 0 min	0.3	3.0
1	1	3	5	10.0	4 hr 0 min	0.6	6.0
2	2	4	6	100.0	0 hr 0 min	0.2	20.0
3	3	4	7	100.0	0 hr 0 min	0.4	40.0
4	3	4	8	100.0	0 hr 0 min	0.6	60.0
5	4	4	9	100.0	0 hr 0 min	0.4	40.0
5	4	4	10	100.0	1 hr 0 min	0.4	40.0
6	6	4	11	100.0	0 hr 0 min	0.5	50.0
6	6	4	12	100.0	1 hr 0 min	0.5	50.0

Doses administered on an outpatients regimen.

about any reaction or discomfort that occurred within 24 hours from injection.

Evaluation of Efficacy

In Italy, it is not allowed to perform intentional challenge sting tests under medical control. However, patients were asked to report any sting they were subjected to during the course of IT or after its discontinuation, as well as of the reactions they suffered from.

Evaluation of Immunologic Markers

Specific IgE was measured by a fluorescence immunoassay (Pharmacia). The end point intracutaneous test was performed with 0.01-, 0.1-, and 1- $\mu\text{g/mL}$ concentrations of *Apis* extract (Pharmalgen

ALK-Abellò) by injecting 0.02 mL of solution into the volar face of the forearm. The reaction was read after 20 minutes and considered positive when the diameter of the elicited was ≥ 5 mm. The lowest concentration yielding a positive reaction was considered the end point result.

Statistical Analysis

A two-tailed *t*-test for paired samples was used to compare both the specific IgE values and the score of the skin-prick test before and 5 years after the beginning of IT. The one-tailed probability of the chi-squared distribution was used to compare the number of side effects (considered both on a "per patient" and on a "per dose" basis) in the different cohorts subjected to IT.

Table 4 Induction Phase (Depot Extract, and Cluster Schedule)

Week	Visit	Vial	Dose	$\mu\text{g/mL}$	Time	mL Administered	μg Administered
1	1	1	1	0.1	0 hr 0 min	0.3	0.03
1	1	1	2	1.0	1 hr 0 min	0.1	0.1
1	1	2	3	1.0	2 hr 0 min	0.3	0.3
1	1	2	4	10.0	3 hr 0 min	0.1	1.0
2	2	3	5	10.0	0 hr 0 min	0.2	2.0
2	2	3	6	10.0	1 hr 0 min	0.4	4.0
3	3	3	7	100.0	0 hr 0 min	0.1	10.0
3	3	3	8	100.0	1 hr 0 min	0.2	20.0
4	4	4	9	100.0	0 hr 0 min	0.4	40.0
4	4	4	10	100.0	1 hr 0 min	0.4	40.0
5	5	4	11	100.0	0 hr 0 min	0.5	50.0
5	5	4	12	100.0	1 hr 0 min	0.5	50.0

Doses administered on an outpatients regimen.

The confidence interval on a proportion²³ was used to evaluate the actual proportion of individuals who were protected after vaccination, because it could be extrapolated based on the number of individuals who were truly re-stung. The Wald method modified according to Agresti and Coull was used for the calculation of the 95% confidence interval.²⁴ All analysis was done with standard statistical software (GraphPad Software, Inc., San Diego, CA). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Patients

Demographic characteristic (age and sex) and severity of reaction to *Apis* sting were not significantly different in the three groups assigned to the different IT regimens (Table 1). Two patients (one from the aqueous rush and one from the depot cluster group) dropped out of the study after 1 year for personal reasons, not related to IT.

Safety

The occurrence of side effects were significantly lower in the induction phase of the depot cluster group as compared with the aqueous rush group, both on a per patient basis (13.7% versus 57.9%, $p = 0.0032$) and on a per dose basis (1.7% versus 4.9%, $p = 0.049$; Fig. 1). Side effects also tended to be less frequent in the aqueous cluster group as compared with the aqueous rush group, particularly on a per patient basis (25.0% versus 57.9%, $p = 0.054$; Fig. 1 and Table 5).

Side effects in the maintenance phase of the study were lower in the depot cluster group as compared with the aqueous rush group, and this difference was

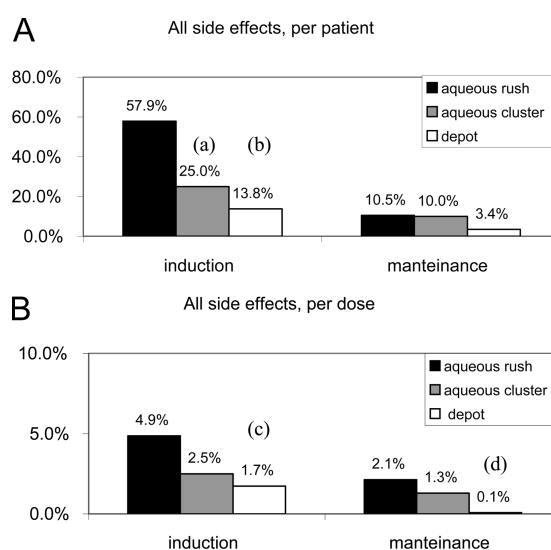


Figure 1. Frequency of side effects expressed as percent on the y-axis in subjects who received either aqueous rush, aqueous cluster, or depot cluster IT with Apis extract. Results are grouped as (A) per patient and (B) per dose and are considered separately when occurring in the induction or in the maintenance phase (as indicated on the x-axis). Pearson's chi-squared analysis applied to the comparison of the proportion of patients with side effects in the considered group versus the aqueous rush group (a) $p = 0.0536$, (b) $p = 0.0032$, (c) $p = 0.0489$, and (d) $p = 0.0001$.

significant on a per dose basis (0.1% versus 2.1%, $p < 0.0001$; Fig. 1 and Table 5).

All side effects observed in the maintenance phase were systemic (grades 2 or 3). In contrast, side effects occurring in the induction phase were either local or systemic (Table 5). Frequency of local side effects was not different in the three groups, whereas systemic side effects (grades 2 or 3) were significantly less frequent

Table 5 Number of Side Effects in Patients Who Underwent Different Treatment Schedules

	Aqueous Rush Per		Aqueous Cluster Per		Depot Cluster Per	
	Patient	Dose	Patient	Dose	Patient	Dose
Induction Phase						
<i>n</i>	19	247	20	240	29	348
Systemic	7	9	1	2	1	2
Local	4	3	4	4	3	4
Total	11	12	5	6	4	6
Ratios	1.1		1.2		1.5	
Maintenance Phase						
<i>n</i>	19	988	20	1080	29	1537
Systemic	2	21	2	14	1	1
Local	0	0	0	0	0	0
Total	2	21	2	14	1	1
Ratios	10.5		7		1	

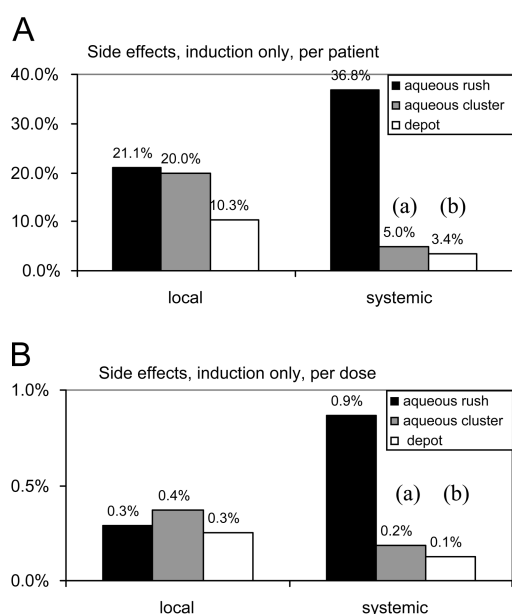


Figure 2. Frequency of side effects occurring in the induction phase and expressed as percent on the y-axis in subjects who received either aqueous rush, aqueous cluster, or depot cluster IT with *Apis* extract. Results are grouped as (A) per patient and (B) per dose. Moreover, local and systemic side effects were considered separately (as indicated on the x-axis). Pearson's chi-squared analysis applied to the comparison of the proportion of patients with side effects in the considered group versus the aqueous rush group (a) $p = 0.0197$ (top) and $p = 0.0349$ (bottom), (b) $p = 0.0041$ (top) and $p = 0.0092$ (bottom).

both in the aqueous cluster group (5.0%) and in the depot cluster group (3.4%) as compared with the aqueous rush group (36.8%; $p = 0.0197$ and $p = 0.0041$, respectively) on a per patient basis (Fig. 2). This result was confirmed when analysis was performed on a per dose basis: systemic side effects were significantly less frequent both in the aqueous cluster group (0.2%) and

in the depot cluster group (0.1%) as compared with the aqueous rush (0.9%; $p = 0.0349$ and $p = 0.0092$, respectively; Fig. 2 and Table 5).

In the maintenance phase, the ratios of the number of doses, which gave effects versus the number of patients with side effects were 10.5, 7.0, and 1.0 in the groups who underwent the aqueous rush and the aqueous cluster protocols and the cluster depot protocol, respectively (Table 5). In the induction phase, the ratio was close to 1 for all three treatment schedules.

Efficacy

More than one-half of the patients of each group who completed the study were naturally re-stung either once or twice (Table 6). No systemic symptoms were reported. The proportion of individuals who were protected after vaccination was 100% (Table 6). The frequency of field stings in the patients we studied was particularly high because of the favorable geographical and economical characteristics of the area where they were living.

Immunologic Parameters

A. mellifera-specific serum IgE was significantly reduced in the three experimental groups (Fig. 3A). Moreover, the dose necessary in the end point intracutaneous *in vivo* assay to elicit a positive skin reaction was significantly reduced in the three groups (Fig. 3B).

DISCUSSION

The main result of this study is that IT of *Apis*-sensitized subjects with a depot extract of *A. mellifera*, when administered with a cluster schedule, is safer and equally effective as compared with *Apis* IT with an aqueous extract administered with a rush schedule. In most people, Hymenoptera stings produce only a tran-

Table 6 Efficacy of IT

Group	n	Re-stung	Proportion	95% Confidence Interval	Re-stung Twice
Aqueous rush	19	11	0.5759	0.3639–0.7706	6
Aqueous cluster	20	10	0.5000	0.3006–0.7029	4
Depot cluster	29	15	0.5172	0.3453–0.6874	6
Total	68	36	0.5254	0.4128–0.6439	16

Numbers of patients who had field stings are shown. The proportions of subjects who were re-stung are indicated for each group, as well as the 95% confidence interval for that proportion. None of the patients who were re-stung suffered any side effects other than local discomfort at the site of the sting. Among re-stung subjects, some were stung twice, confirming the lack of side effects also in this second occurrence. The number of twice re-stung individuals in each group is indicated.

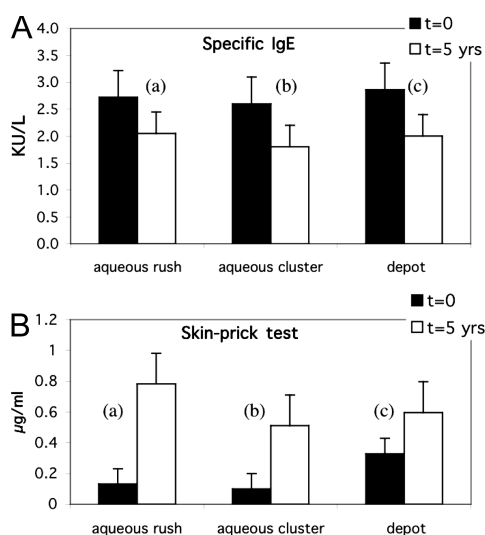


Figure 3. Immunologic parameters in patients treated with venom IT. (A) Mean values of *A. mellifera*-specific serum IgE (in kU/L, on the y-axis) are shown in the top panel for patients who underwent the three induction regimens indicated on the x-axis. Specific IgE was measured before the beginning of IT ($t = 0$ years) and after 5 years of IT ($t = 5$ years). Values of p refer to the results of the two-tailed t -test for paired samples. (B) Mean values of the end point intracutaneous test for *A. mellifera* (in µg/mL, on the y-axis) are shown in the top panel for patients who underwent the three induction regimens indicated on the x-axis. End point values were measured before the beginning of IT ($t = 0$ years) and after 5 years of IT ($t = 5$ years). Values of p of the two-tailed t -test for paired samples. (top) (a) $p = 0.018$, (b) $p = 0.002$, (c) $p = 0.002$. (bottom) (a) $p < 0.0001$, (b) $p = 0.0512$, (c) $p = 0.022$.

sient, local inflammatory reaction characterized by local pain, redness, and swelling. However, for those who develop specific IgE to components of this venom, stings are associated with significant morbidity and mortality. In Europe and in the United States, the prevalence of systemic reaction to stings in the general population is around 3%.^{25,26} Strikingly, a virtually complete protection against new stings has been achieved with specific IT in >90% of cases for *Vespula*

and 75% for *Apis*.⁶ Thus, this treatment is potentially lifesaving. However, unwanted reactions to venom IT can develop in a variable proportion of patients, ranging from 4 to 40%,⁶ which may include up to 12% systemic reactions.⁹ Several factors have been described that influence the incidence of side effects such as the age of the patients, the administration schedule, and the nature of the extracts. The grade of the reaction to natural stings can not predict the severity of reaction to the IT injections⁹ and the vast majority of side effects occurs during the induction phase, whereas only very limited numbers occur during the maintenance. The rush IT requires a 4-day hospitalization period, which can be considered a quite reasonable duration for a potentially lifesaving treatment. However, the rush schedule also implies severe side effects. This has compelled the usage of prophylaxis for allergen injections, e.g., passive immunization with IgG from hyperimmune patients⁷ or premedication with antihistamine drugs.^{27–29}

The reported frequencies of side effects are quite variable for *Apis* rush IT, ranging from 15 to 46%, as compared with 0 to 21% for *Vespula*.^{16,30,31} Slightly lower frequencies of side effects were reported with cluster protocols.^{32,33} In particular, the risk of severe side effects with *Apis* is higher than with *Vespula* IT. Indeed, systemic side effects have been reported in a proportion of patients varying from 5 to 100%,^{6–8} depending on several variables.

On this basis, studies have been published with aqueous extracts, comparing different administration schedules, such as rush versus cluster regimen,^{14,15,18,31} which was found to be better tolerated. Thus, cluster IT with aqueous extract, which can be completed during six weekly outpatient visits, is the usual program for better patient compliance and also for lower medical costs.

The absorption of allergen onto aluminum hydroxide allows slow release of allergen and limits early local and systemic side effects.^{10–12} Besides becoming a common practice for IT with inhalant allergens, depot

extracts were used successfully also in venom IT and were reported to be effective in *Vespula*-sensitized subjects.^{13,14,16-20,34} The lower occurrence of side effects with depot extracts, including nonsevere local early reactions, can improve the compliance for a potentially lifesaving treatment. Here, we directly compared the safety and efficacy of aqueous *Apis* IT, administered either with a rush or with a cluster schedule, with that of a depot extract administered with a 5-week cluster schedule. To our knowledge, this is the first time that this direct comparison was performed. Patients were treated for at least 5 years and specific immunity to *Apis* venom was monitored before beginning IT and then 5 years later. We found that depot IT is safer than the rush schedule for aqueous IT, in agreement with previous results.¹⁵

In addition, in the induction phase, the aqueous cluster schedule tended to be safer than the rush aqueous regimen, although the difference did not reach a significant value. The lower occurrence of systemic side effects in the induction phase with the depot extract was contributing mainly to the favorable safety profile of this regimen.

This is in contrast to what has been reported with IT for *Vespula* spp. extract, where a lower occurrence of side effects with the depot, as compared with the aqueous preparation, was mainly because of fewer local side effects in the induction phase.¹⁹ The different intrinsic characteristics of the two Hymenoptera venoms may be responsible for this difference.

Moreover, here, we show that in the maintenance phase only systemic side effects were observed, which occurred less frequently with the depot cluster schedule, as compared with the aqueous rush regimen. The same tendency was observed with the aqueous cluster versus aqueous rush schedule, but the difference did not reach a significant value.

A large proportion of patients included in this study were re-stung within the 5 years of the study. The frequency of field stings in the patients we studied was particularly high. This can be referred to the geographical and economical characteristics of the area where they were living, as well as to their professional activities (farmers, professional or amateur beekeepers, or forest rangers).

This gave us solid evidence for equal protection generated by IT in patients from the three arms of the study, even if in Italy, intentional rechallenge tests are not ethically acceptable and could not be performed. In fact, none of the re-stung patients suffered anything but local pain and discomfort. This result is in agreement with previous evidence of protection reported with both depot and aqueous extracts.^{13,15,17-19} Notably, almost 25% of patients included in our study were re-stung twice, but, in addition, in this case they all were protected from systemic reactions, regardless of

treatment with the aqueous or the depot extract. The commonly reported rate of protection is around 80% for Hymenoptera venom allergies overall and is lower in the specific case of *Apis* allergy.⁶ Recently, higher venom doses in the maintenance phases have been found to extend the protection to previously unprotected patients who underwent regular 100- μ g doses for maintenance.³⁵ Although we had an apparent protection of 100%, not all subjects were re-stung. Consequently, the 95% confidence interval for the proportion of observed protected individuals takes back the protection rate within reported percentages. However, we also can speculate that the 5-year maintenance therapy we performed could explain the higher protection achieved by the patients we studied, as compared with subjects treated for the usual 3-year period.

The efficacy of *A. mellifera* IT with the three considered protocols, using either SQ aqueous or SQ depot extract, also was supported by the quantitative analysis of *in vivo*- and *in vitro*-specific IgE reactivity, which were performed at study entry and 5 years later. A virtually identical profile of immunomodulation was observed. In fact, specific IgE significantly decreased and the dose eliciting a positive skin reaction significantly increased at a similar extent with the rush aqueous, the cluster aqueous, and the cluster depot IT regimen, respectively. These results suggest that aqueous and depot preparations are immunologically active in a similar fashion on the IgE-specific immune response.

The ratio of the number of doses, which gave effects versus the number of patients with side effects was higher in the groups who underwent the aqueous rush and the aqueous cluster protocols (10.5 and 7.0, respectively) as compared with the depot protocol (1.0). This applied to the maintenance but not to the induction phase. Thus, with both aqueous regimens, single patients suffered several side effects, although most of them did not have any, in agreement with previous reports.^{9,19,36} The recommended interval between injections in the maintenance phase is 4 weeks for the aqueous extracts.³⁷ However, an efficacious protection was reported also with protocols extending the intervals up to 8³⁸ or 12 weeks,³⁹ whereas no data were available on depot formulations. The present work supports the notion that this approach could be useful also with depot extracts.

Our results indicate that the usage of a depot extract for *Apis* venom vaccination is associated with a lower occurrence of systemic side effects and tends to be safer than the aqueous extract administered with the standard 6-week cluster schedule. The depot formulation of the *Apis* venom extract was as much efficacious as the aqueous extract. Tolerability and efficacy of such a potentially lifesaving and effective treatment need to be monitored carefully with additional studies on larger cohorts not only to optimize the treatment but

also to more easily extend it to all people who can benefit from it.

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