Original Article

Higher frequency of early local side effects with aqueous versus depot immunotherapy for Hymenoptera venom allergy

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Summary: Venom immunotherapy has proven a very effective method for the treatment of allergy to Hymenoptera venom. Aqueous instead of depot extracts are prevalently used for this immunotherapy. The advantage of using aqueous extracts has not been fully investigated.
We made an open, non-controlled study on 45 subjects sensitized to either Apis mellifera or Vespula spp. Patients were assigned to either a depot (N=27) or an aqueous (N=18) immunotherapy regimen, and side effects were monitored during the induction and the 3-year maintenance phase. The effect of naturally occurring stings during the treatment and after its interruption was recorded as well.
Side effects were less frequent with the depot extract both on a "per patient" (22.2% versus 50.0%) and on a "per dose" (2.9% versus 10,2%) basis ($p=0.026$ and $p<0.0001$, respectively). Better tolerance was mainly due to the lower frequency of local side effects occurring at early times after vaccination. The efficacy of vaccination was comparable in the 2 cohorts, as expected.
We conclude that depot immunotherapy to Hymenoptera venom should be preferred to aqueous immunotherapy for the lower occurrence of local side effects. This might influence a better compliance with this potentially life-saving treatment.

Key words: Hymenoptera venom allergy, immunotherapy, aqueous extract, depot extracts.

Introduction

A single Hymenoptera sting can induce an allergic reaction and occasionally fatal anaphylaxis [1]. Venom immunotherapy (IT) is highly effective in the prevention of severe local reactions as well as of their systemic effect [2]. Since two controlled studies were published in the seventies [3, 4], showing that aqueous venom extracts are effective as opposed to a mere placebo effect of whole-body extracts, only aqueous preparations of venom extracts are used. Not surprisingly, aqueous extracts are not devoid of side effects and, indeed, depot extracts for Hymenoptera venom are largely used in German-speaking European countries, where it has been reported that they are well tolerated and effective [5-11]. Allergens adsorbed to insoluble substances (aluminum hydroxide, tyrosine, or calcium phosphate) have long been used in medical practice [12, 13]. The

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Table I. Patients (N = 45): demographic data of patients included in the study.

group	N =	sex (M/F)	age (range)	age (mean)
depot	27	19/8	15-68	39.0
aqueous	18	15/3	19-69	42.6
Total	**45**	**34/11**	**15-69**	

slow release of allergen from the injection site is considered an advantage. Indeed, inhalant subcutaneous IT is nowadays almost exclusively performed in Europe with depot extracts, which allow fewer side effects as well as simpler administration schedules.

There is no doubt about the efficacy and the relative safety of aqueous IT [14]. However, the question of whether either evidence-based medical knowledge or a mere cultural barrier has prevented until now to switch from aqueous to depot extracts in Hymenoptera venom IT has never been addressed directly. In fact, although different protocols of immunization with depot extracts have been reported [5-7, 11], no studies are available which provide definite evidence on the side effects related to the use of depot versus aqueous venom extracts.

We are presenting here the results of an open, uncontrolled study where Hymenoptera venom-sensitized subjects with a history of systemic reactions were treated with commercially available preparations of either aqueous or depot venom extracts and carefully monitored over at least 3 years for side effects and efficacy.

Materials and Methods

Patients

Patients who were enrolled in this study fulfilled the following criteria:

- history of hypersensitivity to *Hymenoptera venom*;
- systemic reactions ≥ grade II (according to Muller, and recently revised by Wüthrich [15 16]) after a bee or wasp sting;
- 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 due to the fear of subsequent stings;
- positive skin tests for Apis mellifera or Vespula spp. (Soluprick and Pharmalgen, for prick test and intradermal reaction, respectively; ALK-Abellò, Milan, Italy);
- positive tests for the detection of serum IgE to Apis mellifera or Vespula spp. (RAST, Pharmacia, Uppsala, Sweden);

Table II. Patients (N = 45): severity of reactions to *Hymenoptera* stings and exposure risk.

N =	Grade	Expos. risk	Exposure risk	Treatment (aqueous/depot)
4	IV	low	previous anaphylactic shock	3/1
1	IV	medium	resident in the countryside	1/0
8	IV	high	professional exposure	5/3
1	IV	unknown	unknown	0/1
2	III	low	severity of reactions to stings	0/2
5	III	high	professional exposure	0/5
2	III	unknown	unknown	0/2
6	II	low	severity of reactions to sting	1/5
5	II	medium	resident in the countryside	6/2
3	II	high	professional exposure	0/3
5	II	unknown	unknown	2/3
Total				**18/27**

Grades of severity are expressed according to Mueller [15].

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Demographic data on the patients included in the study are shown in Table I. Severity of pre-IT systemic reactions, expressed according to Muller [15], as well as the exposure risk, are specified in Table II.

Patients were recruited at 8 different medical care units in Northern Italy. All patients were informed about this study and provided a written consent.

At each medical Unit participating to this study, patients were assigned to aqueous or depot IT; these two regimens were alternated in consecutive subjects.

Hymenoptera extracts

The allergen-enriched extracts we used in the 2 cohorts for aqueous and depot IT (Pharmalgen and Alutard, respectively) were prepared from the same source by the supplier (ALK Abellò). This accounted for homogeneous immunogenicity. For depot IT, raw venom extracts of Apis mellifera or Vespula spp. 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 (ALK-Abellò).

Immunization schedules

An 8-week, 12-dose induction modified rash schedule was used for aqueous IT, as previously described, with minor modifications[7]. Briefly, in the first and in the second visit at the medical Unit the patients received two doses, at 30 minutes interval, of 0.01 and 0.1 μg and of 1 and 2 μg, respectively. In the third and fourth visits patients received 2 doses, at 60 minutes interval, of 4 and 8 μg, and of 10 and 20 μg, respectively. Forty, 60, 80 and 100 μg were administered subsequently in single doses at weekly intervals. This phase was followed by the monthly administration of 100 μg of venom extract per dose, for at least 3 years.

For depot IT, a progressive schedule was used for induction, consisting of 15 weekly injections of increasing doses of venom extracts (0.02, 0.04, 0.08, 0. 2, 0.4, 0.8, 2, 4, 8, 10, 20, 40, 60, 80 and 100 μg). Subsequently, for maintenance therapy, a monthly administration of 100 μg venom extract per dose per 3 years was used.

No premedication was used.

Evaluation of side effects

Patients were kept on an outpatient regimen on the day of the treatment. They were observed for 6 hours, and special alert was maintained for the first 60 minutes. Systemic and local side effects were recorded according to previously described criteria [14, 15], with minor modifications. Specifically, local erythematous and swelling reactions were recorded if erythema and swelling were > 10 cm diameter.

All patients were instructed to immediately report any delayed reactions to the center, and they were anyhow interviewed during subsequent visits about any reaction or discomfort which occurred within 24 hours that could have been possibly related with it.

Evaluation of efficacy

In Italy it is not allowed to perform intentional challenge sting tests under medical control. Therefore, patients were asked to report of any sting they were

Table III. Side effects (induction phase).

		DEPOT per patient (N = 27)	**per dose (N = 405)**	**AQUEOUS per patient (N = 18)**	**per dose (N = 216)**
SR	Grade I	0	0	0	0
	Grade II	2 (L)	7 (L)	2 (E)	9 (E)
	Grade III and IV	0	0	0	0
Total		*2*	*7*	*2*	*9*
LR	Local pruritus	0	0	1 (L)	1 (L)
	Edema / erythema	4 (1E + 3L)	5 (1E + 4L)	6 (1E + 5L)	12 (1E + 11L)
Total LR		4	5	7	13
Total		**6**	**12**	**9**	**22**

Events per patient and per dose are listed.
SR: systemic reaction, classified according to Mueller [15].
LR: local reaction, E: early event; L: late event

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subjected to during the course of IT or after its discontinuation, as well as of the reactions they observed. This information was used to evaluate the protection they had achieved.

Statistical analysis

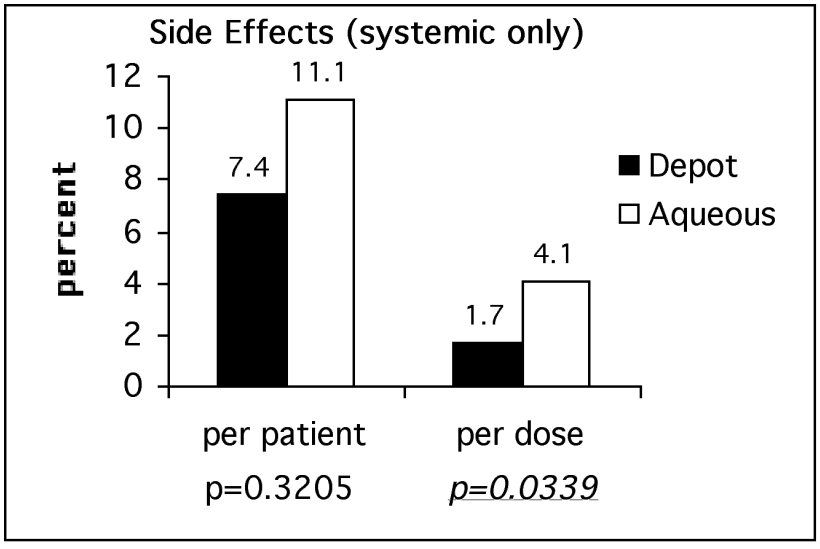
The one-tailed probability of the chi-squared distribution was used to compare the number of side effects (considered both on a "per patient" and on a "per dose" basis) in the cohort subjected to aqueous venom IT versus the cohort receiving depot IT. The confidence interval [17] was used to evaluate the overall proportion of individuals who were protected after vaccination, as it could be extrapolated on the basis of the number of individual who were re-stung.

All analysis was done with standard statistical software (GraphPad software Inc., San Diego, CA). Values of $p < 0.05$ were considered statistically significant.

Results

Side effects: induction phase

A total of 6 patients receiving the depot IT (22.2%) suffered side effects in the induction phase. One patient had an early local reaction, and 5 patients had different late (local or systemic) reactions (Table III). A total 9 patients receiving the aqueous extract (50%) had side effects in the induction phase. Three and 6 patients had early and late effects, respectively (Table III).



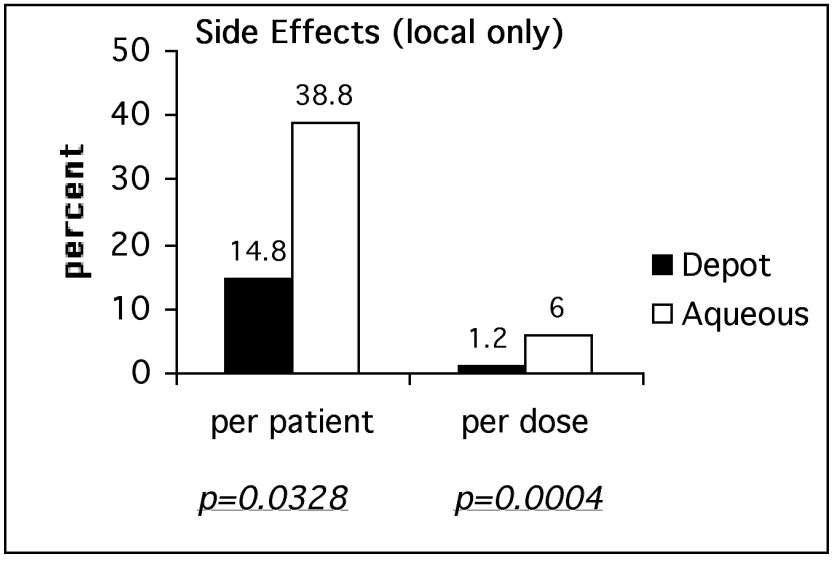


Figure 1. Frequency of side effects in subjects who received either depot or aqueous IT. Results are grouped in "systemic" (top panel) *versus* "local" (bottom panel) and shown as percent both on a "per patient" as well as on a "per dose" basis. Results of the chi square analysis are shown.

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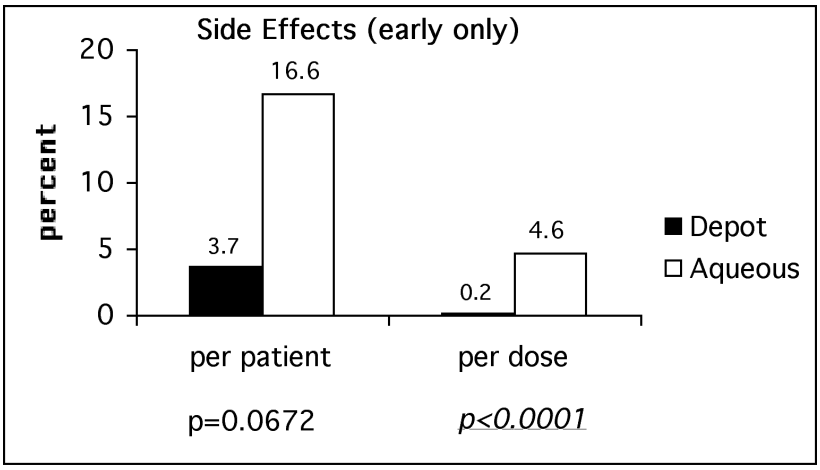
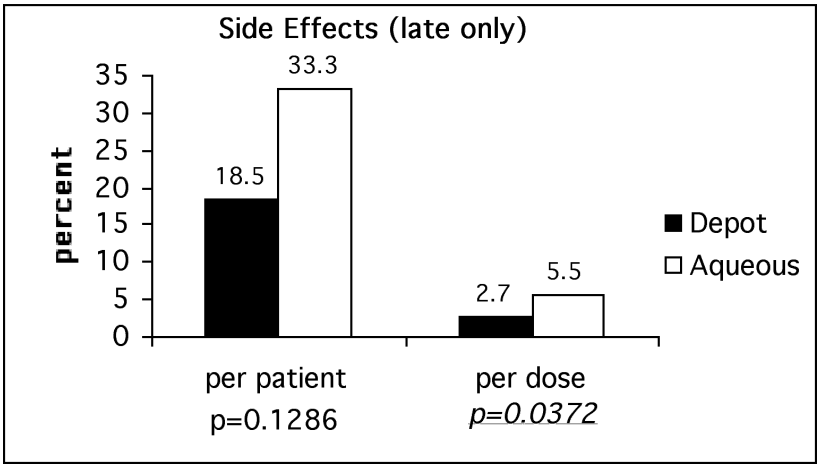


Figure 2. Frequency of side effects in subjects who received either depot or aqueous IT. Results are grouped in "early" (i.e., occurring within 60 minutes from injection) (top panel) *versus* "late" (i.e. occurring in the 6-24 hours following injection) (bottom panel). Results are shown as percent both on a "per patient" as well as on a "per dose" basis. Results of the chi square analysis are shown.



When these results were considered on a "per dose" basis, injection of the depot extract in the induction phase was associated with a total 12 side effects (2.9%), of which 1 and 11 occurred as early and late effects, respectively. Injection of the aqueous extracts was associated with a total 22 side effects (10.1%), of which 10 were early and 12 were late (Table III).

Thus, side effects were significantly less frequent with depot IT on a "per patient" as well as on a "per dose" basis (p=0.0264 and p<0.0001, respectively).

The ratio of doses yielding side effects per number of patients with side effects was 2 and 2.44 for the depot and aqueous extract, respectively.

When separately considered, systemic side effects occurred in 2 patients in each cohort (7.4% for depot and 11.11% for aqueous venom IT, respectively; not-significant). On a "per dose" basis, systemic effects occurred 7 and 9 times in the depot and aqueous IT group, respectively (1,7 and 4.1%, respectively; p=0.0339)(Figure 1, top panel). Differently, local side effects occurred in 4 and 7 patients in the depot and aqueous IT group, respectively (14.8 % and 38.8%, respectively; p=0.0328). On a "per dose" basis, local effects occurred 5 and 13 times in the depot and aqueous IT group, respectively (1.2 and 6,0%, respectively; p=0,0004))(Figure 1, bottom panel).

Lastly, early and late effects were separately analyzed. Early side effects occurred in 1 patient in the depot group, and in 3 patients in the aqueous group (3.7 and 16.6%, respectively; not-significant). On a "per dose" basis, early effects occurred 1 and 10 times in the depot and aqueous IT group, respectively (0.2 and 4.6%, respectively; p<0,0001)(Figure 2, top panel). Differently, late side effects occurred in 5 and 6 patients in the depot and aqueous IT group, respectively (18.5 % and 33.3%, respectively; not significant); on a per dose basis, late effects occurred 11 and 12 times in the depot and aqueous IT group, respectively (2.7 and 5.5%, respectively; p=0.0372) (Figure 2, bottom panel).

Side effects: maintenance phase

In the maintenance phase a single side effect occurred in one patient treated with the aqueous extract. He

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Table IV. Efficacy of venom IT at three years.
All patients who were naturally re-stung after 3 years from the beginning of venom IT suffered only a mild local reaction. Results of efficacy of depot versus aqueous IT are listed according to the known grade of severity of the reaction before the beginning of IT

	Depot	Aqueous
grade IV	2	2
grade III	2	2
grade II	2	1

suffered a systemic reaction characterized by malaise, sweating and dry-mouth. A 20-mg cetirizine dose per os was administered and he recovered within one hour.

Efficacy

Six (22.2 %) and 5 (27.7%) patients who had received the depot and the aqueous extracts, respectively, were stung one or more times 3 years since the beginning of IT. In all these patients the reaction to each subsequent sting consisted of a limited local reaction which did not require any treatment. The confidence interval of a proportion for 6 and 5 events is similar (56.2 to 100% and 51.6 to 100%, respectively). The patients who were re-stung were differently graded in terms of severity of their systemic reactions before IT (Table IV). Five patients were also stung before completion of the IT (3 who were treated with depot extracts and 1 with aqueous extracts); none of them suffered any systemic reaction.

Discussion

For most people, Hymenoptera stings produce a transient, local inflammatory reaction (pain, redness, and swelling). However, for those who are allergic to components of this venom, stings are associated with significant morbidity and mortality. Indeed, the prevalence of systemic reaction to stings in the general population in Europe and in the United States is around 3% [18].

Complete protection against new stings has been achieved with specific IT in more than 90% of cases for Vespids and 75% for Apis [19]. However, systemic and local reactions to venom IT have been described in a variable proportion of patients, ranging from 4 to 40% [19], with an average rate of systemic reactions of 12% [14]. The incidence of side effects depends on factors such as the age of the patients, the administration schedule and the nature of the extracts (more side effects are registered with Apis then with Vespula extracts). In contrast, the grade of the reaction to natural stings cannot predict the severity of reaction to the IT injections [14]. Notably, the vast majority of side effects occurred during the induction phase, and only a very limited number during the maintenance. On this basis, studies have been published comparing different administration schedules, for instance rush versus cluster IT with aqueous extracts [6, 7, 11]. The rush IT has long been known as a suitable approach for the management of the Hymenoptera sting allergic patients, since it implies little hospitalization. However, severe side effects have long been known, and suggested the usage of prophylaxis for allergen injections, such as passive immunization with IgG from hyperimmune patients [20] or premedication with anti histamine drugs [21, 22]. In partial contrast, in a retrospective study on large cohorts of subjects treated with aqueous extracts in protocols ranging from 9 to 2 days, the 2-day ultra-rush protocol was associated with less side effects[9]. Several variables are related to the evaluation of side effects in different protocols, in particular systemic versus local side effects represent an important discriminatory criterion, since most authors pay attention only to the former in the evaluation of safety.

In the present work we focussed on the direct comparison of aqueous versus depot extracts and set up a careful follow up of all side effects observed in the induction as well as during the maintenance phase of IT, including both local and systemic side effects. Side effects were also monitored with respect to the time of onset and classified as early (those occurring within 60 minutes) or late (those occurring within 24 hours).

We found that the number of patients who suffered side effects was significantly lower in the cohort of patients treated with the depot versus the aqueous extract (6 out of 27 versus 9 out of 18, $p = 0.0264$). This held true at a higher significance level when considering the results on the basis of the number of doses of administered vaccine (12 out of 405 versus 22 out of 216, $p < 0.0001$) . The ratio of the number of doses, which gave effects versus the number of patients with side effects, was 2 with the depot extract and 2.44 with the aqueous extract. Thus, with both IT regimens, single patients suffered several side effects, while most of them had none, which agrees with a previous report [14].

The efficacy of the two-vaccination protocol was similar, since all the patients who were re-stung were protected by the occurrence of effects different from the mere toxic reaction to the stings. Although we could not test protection with a formal challenge test, this result is largely supported by evidence of protection previously reported with depot and aqueous extracts [5, 10, 11].

It is noteworthy that in the comparison of side effects grouped as systemic versus local or as early versus late, significance is close to trend, while other values clearly segregate in favor of the depot IT. In particular, local effects and effects of early onset are dramatically less frequent with the depot as compared to the aqueous extract IT (Figure 1 and 2).

Taken together, our results indicate that the use of a

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depot extract for Hymenoptera venom vaccination is associated with a lower occurrence of side effects, which are occurring mainly at the site of injection and take place prevalently at early times following each vaccination shot. This is in agreement with the faster release of allergen at the injection site in the case of aqueous extracts.

Aqueous and depot extract have similar efficacy, and systemic side effects are not dramatically less frequent with the 2 extracts. However, the lower prevalence of annoying local side effects should be considered a factor in favor of using the depot extract since it could improve the patient's compliance in receiving this potentially life-saving therapy. This aspect awaits further studies on larger cohorts and with careful monitoring of both local and systemic effects. The comparison of efficacy and side effects of both vaccination methods awaits prospective studies on larger cohorts.

References

1. Charpin, D., Birnbaum J., Vervloet D. Epidemiology of hymenoptera allergy. Clin Exp Allergy 1994,24:1010-5.
2. Valentine, M.D. Allergy to stinging insects. Ann Allergy 1993,70:427-32.
3. Muller, U., Thurnheer U., Patrizzi R., Spiess J., Hoigne R. Immunotherapy in bee sting hypersensitivity. Bee venom versus wholebody extract. Allergy 1979,34:369-78.
4. Hunt, K.J., Valentine M.D., Sobotka A.K., Benton A.W., Amodio F.J., Lichtenstein L.M. A controlled trial of immunotherapy in insect hypersensitivity. N Engl J Med 1978,299:157-61.
5. Wyss, M., Scheitlin T., Stadler B.M., Wuthrich B. Immunotherapy with aluminum hydroxide adsorbed insect venom extracts (Alutard SQ): immunologic and clinical results of a prospective study over 3 years. Allergy 1993,48:81-6.
6. Mosbech, H., Malling H.J., Biering I., Bowadt H., Soborg M., Weeke B., Lowenstein H. Immunotherapy with yellow jacket venom. A comparative study including three different extracts, one adsorbed to aluminium hydroxide and two unmodified. Allergy 1986,41:95-103.
7. Quercia, O., Rafanelli S., Puccinelli P., Stefanini G.F. The safety of cluster immunotherapy with aluminium hydroxide-adsorbed honey bee venom extract. J Investig Allergol Clin Immunol 2001,11:27-33.
8. Carriere, C., Dell'Andrea M., Jocher C., Mjer-Menapace J., Recchia G., Vigl D., Wall-Nofer W., Wenter F., Zampieri P.F., Puccinelli P., Parmiani S. Specific immunotherapy with purified venoms of Apis mellifera and Vespula spp adsorbed to Al-hydroxide. A ten-year clinical experience in 4 Centers in Trentino-South Tyrol. Ital J Allergy Clin Immunol 1999,9:241-8.
9. Brehler, R., Wolf H., Kutting B., Schnitker J., Luger T. Safety of a two-day ultrarush insect venom immunotherapy protocol in comparison with protocols of longer duration and involving a larger number of injections. J Allergy Clin Immunol 2000,105:1231-5.
10. Elsner, J., Sack A., Petering H., Schaefer T., Korner M., Kapp A. Ultrarush SIT in venom allergy. Allergy 2000,55:582-3.
11. Malling, H.J., Djurup R., Sondergaard I., Weeke B. Clustered immunotherapy with Yellow Jacket venom. Evaluation of the influence of time interval on in vivo and in vitro parameters. Allergy 1985,40:373-83.
12. Miller, A.C., Tees E.C. A metabolizable adjuvant: clinical trial of grass pollen-tyrosine adsorbate. Clin Allergy 1974,4:49-55.
13. Stull, A., Cook R.A., Sherman W.B. Experimental and clinical studies of fresh and modified pollen extracts. Journal of Allergy 1940,11:439.
14. Lockey, R.F., Turkeltaub P.C., Olive E.S., Hubbard J.M., Baird-Warren I.A., Bukantz S.C. The Hymenoptera venom study. III: Safety of venom immunotherapy. J Allergy Clin Immunol 1990,86:775-80.
15. Mueller, H.L. Diagnosis and treatment of insect sensitivity. J Asthma Res 1966,3:331-3.
16. Wuthrich, B. Classification of sting insect sensitivity. J Investig Allergol Clin Immunol 2001,11:132.
17. Newcombe, R.G. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med 1998,17:857-72.
18. Antonicelli, L., Bilo M.B., Bonifazi F. Epidemiology of Hymenoptera allergy. Curr Opin Allergy Clin Immunol 2002,2:341-6.
19. Position, Paper. Immunotherapy with Hymenoptera venoms. Allergy 1993,48:37-46.
20. Bousquet, J., Fontez A., Aznar R., Robinet-Levy M., Michel F.B. Combination of passive and active immunization in honeybee venom immunotherapy. J Allergy Clin Immunol 1987,79:947-54.
21. Muller, U., Hari Y., Berchtold E. Premedication with antihistamines may enhance efficacy of specific- allergen immunotherapy. J Allergy Clin Immunol 2001,107:81-6.
22. Tankersley, M.S., Walker R.L., Butler W.K., Hagan L.L., Napoli D.C., Freeman T.M. Safety and efficacy of an imported fire ant rush immunotherapy protocol with and without prophylactic treatment. J Allergy Clin Immunol 2002,109:556-62.

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