
The VISYT trial: Venom Immunotherapy Safety and Tolerability with purified vs nonpurified extracts

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Background: Venom immunotherapy (VIT) is a highly effective treatment but can induce systemic adverse effects.

Objective: To evaluate the safety and tolerability of VIT with purified and nonpurified extracts for treating yellow jacket and honeybee allergy.

Methods: Ninety-four patients (mean age, 46 years) with a history of insect venom allergy were randomly allocated to undergo purified extract VIT (group A [44 patients]) or nonpurified extract VIT (group B [50 patients]). Fifty-six patients were allergic to yellow jacket venom (group A: 25; group B: 31) and 38 to honeybee (19 per group). The induction phase was performed using a 2- or 7-day ultrarush scheme. The maintenance phase lasted 11 weeks. Local and systemic reactions were recorded after each injection.

Results: A total of 1,401 VIT injections were performed. Six systemic reactions were observed in 4 patients (honeybee-allergic patients only) (4% of patients; 0.4% of injections): 1 patient in group A (2%) and in 3 in group B (6%) ($P = .57$). Local extensive reactions were recorded after 5 injections in 4 patients (9%) in group A (2 yellow jacket- and 2 honeybee-allergic patients) and after 17 injections in 12 patients (24%) in group B (8 yellow jacket- and 4 honeybee-allergic patients) ($P = .02$). Total reactions (systemic and large local) numbered 6 in group A (0.9% of injections; 11% of patients) and 20 in group B (2.7% of injections; 30% of patients) ($P = .001$).

Conclusion: In patients with honeybee or yellow jacket venom allergy, VIT with purified extracts has a significantly lower propensity toward severe local reactions compared with VIT with nonpurified extracts.

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INTRODUCTION

The efficacy of venom immunotherapy (VIT) is well documented¹ but can be affected by local and systemic adverse effects.^{2,3} Furthermore, the safety and tolerability profiles of VIT differ between patients allergic to honeybee and those allergic to yellow jacket venoms.⁴ In Europe, purified and nonpurified aqueous venom extracts are commercially available for the ultrarush, rush, and clustered and maintenance phases and aluminium hydroxide adsorbed (depot) preparations for the conventional build-up and maintenance phases, with many European specialists switching to depot preparations after updosing.⁵ Purified extracts could be better tolerated than nonpurified products.⁶ In addition, compared with nonpurified extracts, purified products could improve diag-

nostic procedures in patients with suspected insect venom allergies.⁷ However, to our knowledge, no controlled comparative trials have been conducted to evaluate local and systemic reactions in patients allergic to yellow jacket and honeybee venoms treated with VIT with different venom extracts (purified vs nonpurified products).

We performed a multicenter, randomized, prospective, controlled trial (the VISYT trial: Venom Immunotherapy Safety and Tolerability with purified vs nonpurified extracts) to evaluate the safety and tolerability of VIT using purified vs nonpurified products in terms of systemic and local extensive reactions in patients allergic to yellow jacket and honeybee venoms. Six allergy clinics with at least 15 years of specific expertise in VIT participated.

PATIENTS AND METHODS

Patients

All the study centers received approval from their institutional review boards. All the patients provided written informed consent. Between October 1, 2005, and April 30, 2008, 94 patients (mean [SD] age, 46 [18] years; 30 women and 64 men) were enrolled. The key inclusion criteria for enrollment were a history of at least 1 systemic reaction to insect sting and demonstrated specific IgE by means of intradermal skin testing to standardized venom allergen extracts or in vitro testing. Specific IgE antibody levels were mea-

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sured using a fluorescence immunoassay (Pharmacia, Uppsala, Sweden). The exclusion criteria were the general contraindications for VIT, including pregnancy, β -blocker therapy, mastocytosis, and serious medical or surgical conditions. Eligible patients were randomly allocated to undergo VIT with purified aqueous extract product (Aquagen; ALK-Abellø, Hørsholm, Denmark) for the induction phase and purified depot extract (Alutard; ALK-Abellø) for the maintenance dose (group A [44 patients]) or VIT with nonpurified commercially available products (Stallergenes, Antony, France [23 patients], and Anallergo, Firenze, Italy [27 patients]) (group B [50 patients]). The purification process mainly consists of the filtration and elimination of low-molecular-weight compounds ($<1,000$ daltons [D]) contained in insect venom extracts. A randomization list was generated using a computer program specially designed to perform randomized treatment allocation (Arcus Stat, Cheshire, England). Two different lists were generated, 1 for patients allergic to yellow jacket venom and 1 for those allergic to honeybee venom, to achieve a balanced distribution between the 2 treatment groups. Fifty-six patients were allergic to wasp venom (25 in group A and 31 in the group B) and 38 to honeybee venom (19 per group). Forty-six percent, 29%, and 25% of patients had a history of type IV, type III, and type II reactions after insect sting, respectively, according to the classification of Mueller.⁸ The induction phase was conducted using a 2-day (in the case of yellow jacket allergy) or 7-day (in the case of honeybee allergy) ultrarush/rush scheme. The maintenance phase lasted 11 weeks for both groups.

Outcomes Variables

The primary outcome of the study was the cumulative percentage, calculated per injection and per patient, of systemic and large local reactions. Local and systemic reactions (early and delayed) were recorded after each injection. Local erythema and swelling reactions were recorded if the diameter was greater than 10 cm. Systemic reactions were classified according to the system of Mueller.⁸

Statistical Analysis

Statistical analysis was performed using a statistical software program (SPSS version 13; SPSS Inc, Chicago, Illinois). Differences between the 2 groups in outcome variables (ie, frequency of occurrence of systemic and large local reactions) were evaluated using the Fisher exact test (whenever an expected cell value was <5) and the χ^2 test. The number needed to harm was calculated according to the method of McQuay and Moore.⁹ The unpaired *t* test and the Mann-Whitney test, when appropriate, were used to compare quantitative variables. Two-sided $P < .05$ was considered statistically significant for all analyses. Analysis was performed on the basis of the intention-to-treat principle. Statistical analysis was performed by an independent statistical center (GB Pharma, Pavia, Italy). The study protocol was designed by an independent steering committee, which also surveyed all

phases of the trial. Site monitoring and data collection were performed by a designated contract research organization (GB Pharma Services, Pavia).

Sample Size Calculation

According to previously published studies,¹⁰ we hypothesized that patients treated with purified extracts would have fewer total reactions (systemic plus large local reactions) compared with patients treated with nonpurified extracts. In studies by Rueff et al⁵ and Quercia et al⁶ comparing aqueous and depot preparations, the percentage of patients with large local reactions or systemic reactions treated with nonpurified extracts was 50% to 70%. We hypothesized that treatment with purified extracts could reduce this percentage by at least 50% (from 60% to 30%). Therefore, with a power of 80% and an $\alpha = .05$, at least 42 patients per treatment arm (84 total) should be enrolled in the trial. Sample size was calculated using the GPower Statistical Package (v. 3.03). This study was not powered to detect significant differences between the purified and nonpurified extract groups regarding frequencies of systemic reactions only.

RESULTS

Figure 1 shows the study flow. All 94 enrolled patients concluded the trial evaluation period. Table 1 summarizes the characteristics of the patients at enrollment and randomization. The 2 groups were well balanced according to the main demographic and clinical variables considered. However, patients in group A were older compared with those in group B (mean age, 48 vs 42 years; $P = .06$). A total of 1,401 VIT injections (871 during the induction phase and 530 during the maintenance phase) were performed during the study. Patients in group A received 654 VIT injections and those in group B received 747. Table 2 summarizes the main results of the study. Systemic reactions (urticaria, epigastric pain, or pelvic pain) were observed in 4 patients (honeybee-allergic patients only) (4% of patients; 0.4% of injections): 3 in group B (6% of patients; 0.8% of injections) and 1 in group A (2% of patients; 0.3% of injections) ($P = .57$, Fisher exact test). Local extensive reactions were recorded after 17 injections in 12 patients in group B (2.3% of injections) (8 yellow jacket- and 4 honeybee-allergic patients) and after 5 injections in 4 patients in group A (0.7% of injections) (2 yellow jacket- and 2 honeybee-allergic patients) ($P = .02$, χ^2 test). Total reactions, defined as both systemic and large local reactions, numbered 20 in group B (2.7% of injections; 30% of patients) and 6 in group A (0.9% of injections; 11% of patients) ($P = .001$, χ^2 test). These data allow us to calculate the number needed to harm. For every 5 patients treated with purified extracts instead of nonpurified extracts, 1 reaction (large local or systemic) could be avoided.

DISCUSSION

Immunotherapy with Hymenoptera venoms is highly effective but causes allergic adverse effects frequently, especially when honeybee venom is used.¹¹ In this randomized trial, we

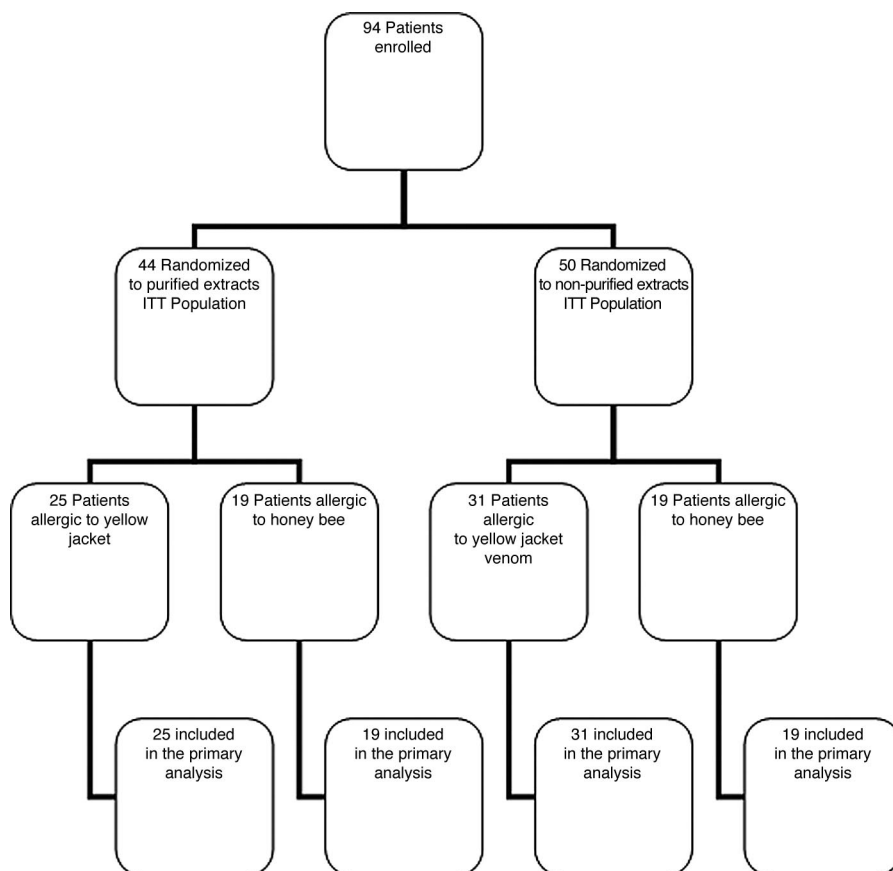


Figure 1. Study flow. ITT indicates intention to treat.

Table 1. Patient Characteristics at Randomization

Characteristics	Group A (purified extracts) (n = 44)	Group B (nonpurified extract) (n = 50)	P value
Age, mean (SD), y	48 (14)	42 (17)	.06
Male sex, %	64	64	.8
Yellow jacket-allergic patients, No. (%)	25 (57)	31 (62)	.6
Honeybee-allergic patients, No. (%)	19 (43)	19 (38)	.5
Type of Mueller reaction after insect sting, No. (%)			.4
IV	22 (50)	21 (42)	
III	13 (30)	14 (28)	
II	9 (20)	15 (30)	
Serum specific IgE, mean (SD), kU/L	6.1 (4.9)	6.5 (4.4)	.8

demonstrated that VIT using purified extracts reduced large local reactions during honeybee or yellow jacket immunotherapy according to an ultrarush protocol compared with nonpurified products. To our knowledge, this is the first randomized trial to address the safety and tolerability profiles of purified vs nonpurified extracts in yellow jacket- and honeybee-allergic patients. Mosbech et al¹² compared the feasibility of 3 different extracts in 32 yellow jacket venom-allergic patients only, demonstrating that systemic reactions

were milder with purified aqueous vs nonpurified aqueous preparations. Also, Alessandrini et al¹³ showed in an open trial that purified depot extracts induce fewer early local adverse reactions compared with aqueous products in patients sensitized to *Vespula*. The lower frequency of reactions observed in the present study after injections of purified aqueous preparations compared with nonpurified aqueous extracts may be due to the former's purification of low-molecular-weight compounds, with recovery of the allergen-containing

Table 2. Outcomes of the Study: Local, Systemic, and Total Reactions

Reactions	Group A (purified extracts) (n = 44)	Group B (nonpurified extract) (n = 50)	P value
Planned injections per patient, total No.	15	15	NA
Actual injections per patient, mean, No.	14.8	14.9	NA
Total reactions (large local + systemic)			
Absolute No.	6	20	
Patients with ≥ 1 reaction, No.	5	15	.04 ^a
Patients with reactions, %	11	30	.001 ^b
Injections with reactions, %	0.9	2.7	<.001 ^b
Large local reactions			
Absolute No.	5	17	
Patients with ≥ 1 reaction, No.	4	13	.06 ^a
Patients with reactions, %	9	26	.02 ^b
Injections with reactions, %	0.5	2.3	.001 ^b
Systemic reactions			
Absolute No.	1	3	
Patients with ≥ 1 reaction, No.	1	3	.5 ^b
Patients with reactions, %	2	6	.57 ^b
Injections with reactions, %	0.3	0.8	.3 ^b

Abbreviation: NA, not applicable.

^a Fisher exact test.

^b χ^2 test.

fraction only. The purification process could reduce the content of histamine and chemical components with histamine-releasing activity. In fact, the purification process mainly consists of filtration and elimination of low-molecular-weight compounds (<1,000 D) contained in insect venom extracts, such as histamine, dopamine, noradrenaline, oligopeptides, and phospholipids.¹⁴

Two relevant limits of this study should be taken into account in evaluating these results. First, this trial was single blinded. However, it is difficult to perform a double-blind design in VIT when comparing different products requiring different modalities of administration (volume, dilution procedures, etc), making necessary a double-dummy design. Furthermore, the major outcomes of this study (large local reactions and systemic reactions) could be considered as objective end points. Another potential limit of the study was that we used the cumulative frequency of large local reactions and systemic reactions as the primary end point. A study comparing only systemic reaction frequency as the primary end point would require at least 900 patients to show a difference, and this would be difficult to perform. In this study, the protocol definition of large local reactions was clearly stated and defined. Of course, the clinical meaning of a systemic reaction is different from the clinical value of a large local reaction. Although large local reactions do not usually necessitate a reduction in dosage, prevent the specialist from reaching full dose, or represent a risk factor for a systemic reaction during VIT, they are frequent and bothersome for patients, sometimes requiring treatment with corticosteroids and antihistamines for several days.¹⁴ In addition, recent data⁷ have shown that the use of purified extracts

compared with nonpurified extracts could increase the sensitivity and specificity of venom allergy diagnosis. In conclusion, this randomized, controlled, multicenter trial provides evidence that patients with honeybee or yellow jacket venom allergy treated with purified extracts have a lower occurrence of severe local reactions compared with those treated with nonpurified extracts.

REFERENCES

1. Bonifazi F, Jutel M, Bilo BM, Birnbaum J, Muller U, Bucher C. Prevention and treatment of Hymenoptera venom allergy: guidelines for clinical practice. *Allergy*. 2005;60:1459–1470.
2. Lockey RF, Turkeltaub PC, Olive ES, et al. The Hymenoptera venom study, III: safety of venom immunotherapy. *J Allergy Clin Immunol*. 1990;86:775–780.
3. Mosbech H, Muller U; European Academy of Allergology and Clinical Immunology. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. *Allergy*. 2000;55:1005–1010.
4. Muller UR, Golden DB, Demarco PJ, et al. Immunotherapy for Hymenoptera venom and biting insect hypersensitivity. *Clin Allergy Immunol*. 2004;18:541–559.
5. Rueff F, Wolf H, Schnitker J, et al. Specific immunotherapy honeybee venom allergy: a comparative study using aqueous and aluminium hydroxide adsorbed preparations. *Allergy*. 2004;59:589–595.
6. Quercia O, Emiliani F, Pecora S, Burastero S, Stefanini G. Efficacy, safety, and modulation of immunologic markers by immunotherapy with honeybee venom: comparison of standardized quality depot versus aqueous extract. *Allergy Asthma Proc*. 2006;27:151–158.
7. Golden DBK, Kelly D. Dialyzed venom skin tests for identifying yellow jacket–allergic patients not detected using standard venom. *Ann Allergy Asthma Immunol*. 2009;102:47–50.
8. Mueller HL. Diagnosis and treatment of insect sensitivity. *J Asthma Res*. 1966;3:331–333.
9. McQuay HJ, Moore RA. Using numerical results from systematic reviews in clinical practice. *Ann Intern Med*. 1997;126:712–720.
10. Cadario G, Marengo F, Raghino E, et al. Higher frequency of early

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- local side effects with aqueous versus depot immunotherapy for Hymenoptera venom allergy. *J Invest Allergol Clin Immunol*. 2004;14:1–7.
11. Birnbaum J, Charpin D, Vervloet D. Rapid Hymenoptera venom immunotherapy: comparative safety of three protocols. *Clin Exp Allergy*. 1993;23:331–333.
12. Mosbech H, Malling HJ, Biering I. 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.
13. Alessandrini A, Berra D, Lodi Rizzini F. Flexible approaches in the design of subcutaneous immunotherapy protocols for hymenoptera venom allergy. *Ann Allergy Asthma Immunol*. 2006;97:92–97.
14. Mueller U. *Insect Sting Allergy*. Jena, Germany: Gustav Fischer Verlag; 1990.
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