

# Follow-up of Venom Immunotherapy (VIT) Based on Conventional Techniques and Monitoring of Immunoglobulin E to Individual Venom Allergens

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## ■ Abstract

**Objectives:** To assess the efficacy of venom immunotherapy (VIT) and monitor changes in in vivo and in vitro test results after 5 years of treatment and subsequent follow-up. To study the profile of immunoglobulin (Ig) E to individual allergens prior to treatment and 1 year afterwards.

**Methods:** We studied 562 patients with hymenoptera venom allergy (438 to bee, 124 to wasp), all of whom underwent immunotherapy with *Apis* or *Vespula* extract. The patients were followed up using conventional in vivo and in vitro tests, and in 51 cases, specific IgE against the main hymenoptera allergens was measured before starting and after 1 year of treatment.

**Results:** Of the 387 patients who completed VIT, 130 sensitized to *Apis* and 68 to *Vespula* suffered spontaneous re-stings during treatment. Of these, 123 (94.6%) did not suffer any reaction and 64 (94.1%) suffered only a local reaction. Sixty-two patients sensitized to *Apis* and 14 sensitized to *Vespula* suffered spontaneous re-stings after stopping treatment. Only 3 patients suffered a systemic reaction (grade I Müller). At the end of treatment, the results of skin tests and specific IgE to whole extract improved significantly. Reductions in IgE to the main allergens were observed after 1 year of treatment (median differences in Ves v 5,  $-238.0$ ,  $P=.0425$ ; and in Api m 1,  $-183.0$ ,  $P=.0024$ ).

**Conclusion:** The high rate of spontaneous re-stings shows that efficacy is maintained for years after completing treatment in a real-world setting. Determination of IgE to individual venom allergens may offer new perspectives in the diagnosis and follow-up of these patients.

**Key words:** *Apis*. *Vespula*. Hymenoptera venom allergy. Venom immunotherapy. Component-resolved diagnosis.

## ■ Resumen

**Antecedentes:** La inmunoterapia con veneno de himenópteros (ITV) es un tratamiento eficaz. Existen diferentes herramientas para monitorización durante y tras el tratamiento. El objetivo de este estudio es valorar la efectividad del tratamiento y monitorizar cambios *in vivo* e *in vitro* durante y tras 5 años de tratamiento, y estudiar el perfil de la IgE a los alérgenos individuales de venenos antes y al año de tratamiento.

**Métodos:** Se han estudiado 562 pacientes con alergia a veneno de himenópteros (438 a abeja, 124 a avispa). Todos iniciaron IVH con extracto de *Apis* o *Vespula*. Los pacientes fueron seguidos mediante pruebas cutáneas e IgE específica y en 51 se valoró la IgE a alérgenos individuales.

**Resultados:** De los 387 pacientes que completaron el ITV, 130 sensibilizados a *Apis* y 68 a *Vespula* sufrieron repicaduras espontáneas durante el tratamiento, de los que 123 (94.6%) no sufrieron reacción alguna y 64 (94.1%) respectivamente sólo sufrieron reacción local. Además, 62 pacientes sensibilizados a *Apis* y 14 a *Vespula* sufrieron picaduras una vez finalizada la ITV, de los que sólo 3 presentaron una reacción sistémica grado I de Mueller. Al final del tratamiento, las pruebas cutáneas e IgE específica a extracto completo mejoraron significativamente. Asimismo, la IgE a alérgenos individuales disminuyó tras 1 año de tratamiento (diferencias en mediana para Ves v 5:  $-238.0$ ,  $p=0.425$  y para Api m 1:  $-183.0$ ,  $p=0.0024$ ).

**Conclusión:** La eficacia persiste años después de finalizado el tratamiento. La IgE a los alérgenos individuales de venenos puede ofrecer nuevas perspectivas, por su especificidad, en el seguimiento de estos pacientes.

**Palabras clave:** *Apis*. *Vespula*. Alergia a veneno de himenópteros. Inmunoterapia con veneno de himenópteros.

## Introduction

Hymenoptera venom allergy (HVA) is an important health problem. In Spain, 2 epidemiology surveys on the prevalence of allergic diseases conducted in 1992 and 2005 (3905 and 4991 allergic patients, respectively) revealed that the prevalence of HVA increased from 0.7% to 1.5% during this period [1,2].

Hymenoptera venom immunotherapy (VIT) has proven efficacious since pure venom extracts have been used [3,4], and its efficacy persists years after treatment has been completed [5,6]. Although there are no doubts about the efficacy of treatment, further information is necessary on optimal follow-up and monitoring, not just during the treatment phase but also afterwards. Essential issues include reaching an optimal dose in the shortest time possible, individualizing treatment regimens, adjusting the number of doses required to obtain the lowest possible rate of adverse reactions, providing an immediate record of what happens when a patient suffers a re-sting, and analyzing new tools that could result in more effective patient monitoring. We studied the molecular profile of sensitization to different Hymenoptera venom allergens in order to improve monitoring.

A few years ago, we published an interim analysis of half the sample included in this study [7]. In the present analysis, we studied whether the efficacy of VIT continues beyond the end of treatment by means of field re-sting monitoring, as well as the correlation between efficacy and in vivo (skin tests) and in vitro tests (immunoglobulin [Ig] E). We also studied the sensitization profile of our patients to the main hymenoptera allergens and determined how this profile changes with immunotherapy.

## Material and Methods

### Patients

We evaluated 562 patients, 163 (29.0%) of whom were professional or amateur beekeepers. Mean (SD) age was 45.7

(16.5) years (range, 4-82 y). Of these patients, 438 (77.9%) were sensitized to *Apis* and 124 (22.1%) were sensitized to *Vespula*. The demographic characteristics of the patients are summarized in Table 1. Eight patients (1.4%) were allergic to both *Apis* and *Vespula*. Only patients with anaphylaxis to hymenoptera venom and who required VIT were included in the database. The ethics committee of the hospital approved the study and all patients gave their informed consent.

### Diagnostic Tests and Follow-up

Diagnosis was based on the clinical history, positive results for intradermal tests, and specific IgE determination to each type of venom. We used Müller's criteria to assess the severity of the reaction that caused referral to the Allergy Unit [8].

### Skin Tests

Skin tests were performed in accordance with European Committee Guidelines [9] by means of intradermal injection on the volar surface of the forearm of 0.02 mL of solution containing 0.01, 0.1, and 1 µg/mL of venom protein (Pharmalgen, ALK-ABELLÓ, S.A., Madrid, Spain). Histamine 10 mg/mL was used as the positive control and saline solution was used as the negative control (ALK-ABELLÓ, S.A., Madrid, Spain). The response was assessed 20 minutes after the test started and a wheal diameter of ≥5 mm with erythema was considered a positive result.

### Determination of Specific IgE

Specific IgE was determined for the total venom extract and measured in kU<sub>A</sub>/L, following the CAP method (Phadia, Uppsala, Sweden).

In addition, 1 year ago, we started to determine patients' molecular profile by measuring IgE to the following hymenoptera allergens: phospholipases (Api m 1, Ves v 1, Pol d 1), hyaluronidases (Api m 2, Ves v 2), and antigen 5

Table 1. Patient Characteristics<sup>a</sup>

		Total (n=562)	<i>Apis</i> (n=438)	<i>Vespula</i> (n=124)
Environment	Urban	187 (33.3)		
	Rural	375 (66.7)		
Sex	Female	188 (33.5)		
	Male	374 (66.5)		
Age	≤14 y	14 (2.5)		
	15-17 y	9 (1.6)		
	≥18 y	539 (95.9)		
SRD	Grade I <sup>b</sup>	43 (7.7)	32 (7.3)	11 (8.9)
	Grade II <sup>b</sup>	142 (25.3)	114 (26.0)	28 (22.6)
	Grade III <sup>b</sup>	272 (48.4)	212 (48.4)	60 (48.4)
	Grade IV <sup>b</sup>	105 (18.7)	80 (18.3)	25 (20.2)

Abbreviation: SRD, systemic reaction at the time of diagnosis

<sup>a</sup>All values are expressed as No. (%)

<sup>b</sup>Müller classification (See Reference 8)

(Ves v 5, Pol d 5). Phospholipase A2 (Api m 1) was obtained from Sigma-Aldrich (Madrid, Spain). Recombinant bee venom hyaluronidase (Api m 2) was expressed in baculovirus-infected cells and purified as reported elsewhere [10]. Wasp venom allergens were purified from lyophilized venom (ALK-Abelló Source Material, Spring Mills, Pennsylvania, USA) as indicated [11]. Purified venom allergens were biotin-labelled, and the levels of specific IgE to these allergens were tested using the ADVIA Centaur platform (Bayer HealthCare Diagnostics Division, Tarrytown, New York, USA) and expressed in kU<sub>A</sub>/L, as previously described [12].

### Immunotherapy

All patients were treated with 100% *Apis mellifera* extract or 100% *Vespula* spp. extract (Pharmalgen, ALK-Abelló S.A., Madrid, Spain). The initiation schedule consisted of 9 increasing doses of venom (0.1, 1, 5, 10, 20, 40, 60, 80, 100 µg). Procedure and follow-up have been described elsewhere [7]. All patients on maintenance therapy attained the dose of 100 µg administered at 1-month intervals. A maintenance dose of 200 µg was administered to 6 patients, 4 of whom were professional beekeepers who needed to continue their work; the other 2 experienced systemic reactions from spontaneous re-stings when they were in the maintenance phase (100 µg). Furthermore, 7 patients did not tolerate Pharmalgen. During the initiation phase, 1 patient could not tolerate treatment. In the other 6 cases, initiation and maintenance treatment were well tolerated for more than 1 year; however, unexpected reactions occurred after a maintenance dose. These consisted of facial erythema, generalized pruritus, and mild respiratory distress, some of them requiring adrenaline to control the reaction. Once administration errors, mastocytosis, and concomitant medication were ruled out, and since the reaction occurred again at lower doses, the Pharmalgen extract was changed to Aquagen (ALK-Abelló S.A., Madrid, Spain), which all 7 patients tolerated. Aquagen contains a lower concentration of certain low-molecular-weight substances (eg, melittin) and peptides other than Pharmalgen.

### Statistical Methods

Descriptive statistical techniques were applied. Quantitative variables were described using the mean (SD), 95% confidence interval (CI), size, and minimum and maximum values. The median and interquartile range (IQR) were also described.

The Cochran-Armitage trend test was used to analyze global changes in skin response. A logistic regression model was constructed to analyze changes in concentrations for positive skin test results before and at the end of treatment. A successful outcome was defined as a negative skin test result at the end of treatment or a positive one at a higher concentration than the one used at the start of treatment. The Wilcoxon signed-rank test was used to compare initial and final values of specific IgE.

## Results

To date, 387 patients have completed treatment, with a mean duration of 54.7 (12.2) months. Of all the patients

in the database, 22 were not able to finish treatment for personal reasons, onset of diseases requiring treatment with immunosuppressants, and reasons unrelated to treatment.

### Tolerability to Immunotherapy

No systemic adverse reactions were recorded with the *Vespula* extract. With the *Apis* extract, 100 reactions were recorded in 50 patients (11.4%). Of these, 72 were Müller grade I, 4 were grade II, 10 were grade III, and 1 was grade IV. Patients treated with Aquagen did not suffer any adverse reactions.

### Treatment Monitoring

**Skin tests:** Skin tests were performed before and after immunotherapy in 385 patients. Table 2 shows the results obtained according to whether the patient's condition improved, did not change, or worsened. Two aspects are of particular interest: first, the test became negative in 28 patients (22 with *Apis* and 6 with *Vespula*), and second, a statistically significant higher proportion of patients treated with *Vespula* had better test results than those treated with *Apis* (66.7% and 49.4% respectively,  $P=.0049$  [Cochran-Armitage test]).

Table 2. Changes in Skin Test Results Before and After Immunotherapy<sup>a</sup>

	Total	<i>Apis</i>	<i>Vespula</i>
Improvement <sup>b</sup>	203 (52.7)	153 (49.4)	50 (66.7)
No change	177 (46.0)	152 (49.0)	25 (33.3)
Worse	5 (1.3)	5 (1.6)	0
Total	385	310	75

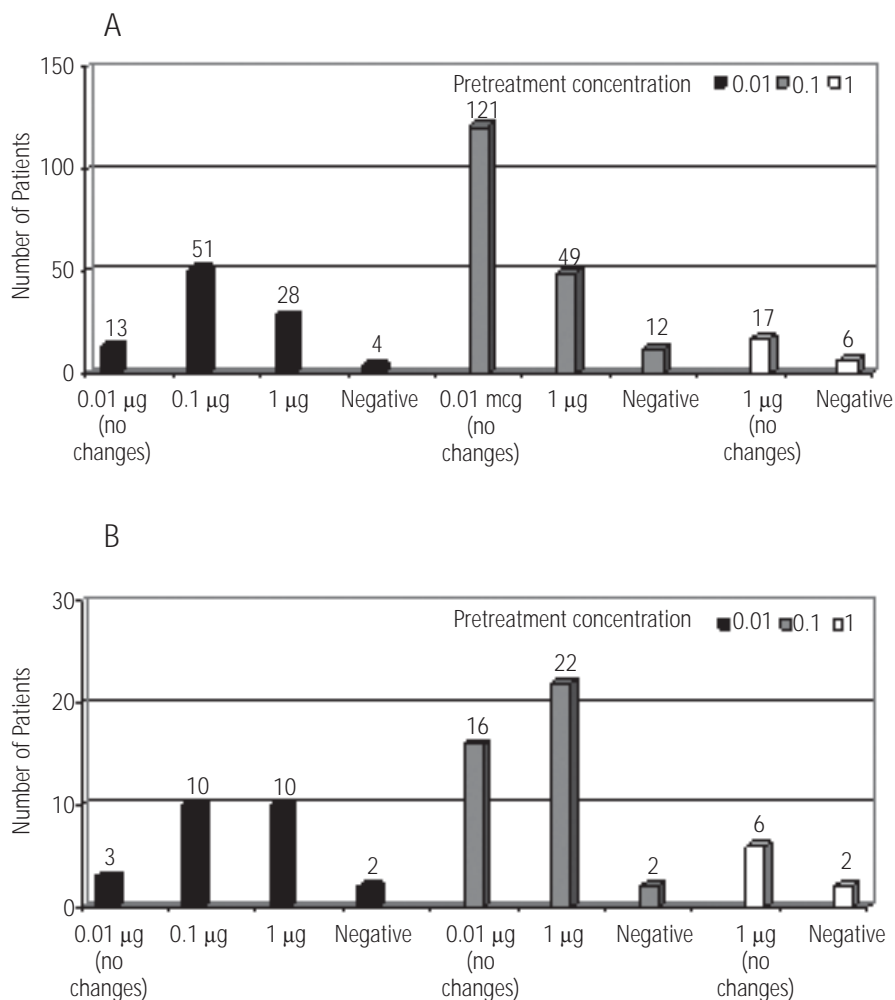
<sup>a</sup>All values are expressed as No. (%)

<sup>b</sup>Improvement is considered as a negative test result or a positive test result with a higher concentration than the pretreatment one.  $P=.0048$  (Cochran-Armitage trend test)

With regard to prognosis, one parameter that may be of interest is the percentage of improvement in the skin test result according to the concentration at which the skin test was positive before treatment. Figures 1A and 1B show these changes in patients sensitized to *Apis* and *Vespula*. Patients treated with a *Vespula* extract had a 2.3-fold higher odds ratio of a successful outcome than patients treated with *Apis*. The statistical significance for testing equality between the 2 patient groups was .0063.

**Specific IgE:** In the case of *Apis*, mean IgE values fell  $-8.93$  (12,35 [before treatment]; 4,13 [after treatment]; 95% CI,  $-11.1$  to  $-6.75$ ) at the end of treatment with respect to baseline, while the reduction was  $-5.00$  (8,05 [before treatment]; 3,77 [after treatment]) in the case of *Vespula* (95% CI,  $-9.1$  to  $-0.9$ ).

In the case of the 291 patients sensitized to *Apis* in whom IgE was measured before and after treatment, this parameter became negative ( $<0.35$  kU<sub>A</sub>/L) in 36 patients (12.4%). In the 63 patients sensitized to *Vespula*, IgE was measured before and after treatment and it became negative in 30 (47.6%). In subsequent yearly control visits after the end of treatment, IgE to *Apis* became negative in a further 25 patients (61 patients



Each histogram bar represents the number of patients (figures above the bars) in which skin testing was positive at a specific allergen concentration (post-treatment concentration), classified by the pretreatment concentration (black bars: 0.01 mg, grey bars: 0.1 mg, white bars: 1 mg) that was needed to obtain a positive test. When building the logistic regression model, a successful outcome was considered as a negative skin test at the end of treatment or a positive one at a higher concentration to the one used at baseline.

**Figure 1.** Skin test results after treatment compared with the baseline concentration that gave a positive result. A, *Apis*. B, *Vespula*. The baseline concentration is shown on the horizontal axis.

[21.2%]) and IgE to *Vespula* became negative in another 6 patients (36 patients [57.1%]).

Molecular profiling was performed on 82 patients who were included consecutively over a period of several months. Fifty-one of these were re-assessed after the first year of treatment: 39 had been treated with *Apis* extract and 12 with *Vespula* extract. Figure 2 shows the results at baseline. The most prevalent allergens were phospholipase A2 (Api m 1) in the case of patients diagnosed with *Apis* hypersensitivity and Antigen 5 (Ves v 5) in the case of patients diagnosed with *Vespula* hypersensitivity. It is remarkable that in patients sensitized to *Apis*, almost 50% were sensitized to *Vespula*

hyaluronidase (Ves v 2), which is a very similar percentage to that found in the patients diagnosed with *Vespula* allergy. Among patients sensitized to *Vespula*, 50% were sensitized to bee hyaluronidase (Api m 2), and 33% of these patients were also sensitized to phospholipase A2 (Api m 1).

Many patients were sensitized to allergens from both species. Of these patients, 46.2% who were allergic to *Apis* presented positivity to both Api m 2 and Ves v 2. This figure stood at 41.7% in patients with *Vespula* allergy. In this last group, we observed that the same percentage of patients were jointly sensitized to Pol d 5 and Ves v 5, 33% were jointly sensitized to Pol d 1 and Ves v 1, and 25% to Ves v 1 and

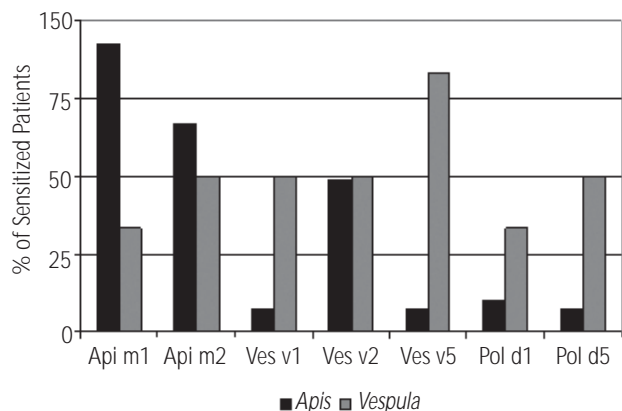


Figure 2. Percentage of patients sensitized to the principal allergens contained in hymenoptera venom (*Apis*, *Vespula*) before treatment, according to the type of clinical sensitization diagnosed in patients.

Api m 1. All these patients were treated with *Vespula* venom, since *Vespula* is the predominant insect in our area. However, determination of IgE to individual allergens performed in the last year has made it possible to detect allergic reactions to *Polistes*, and administration of this extract has been initiated in 2 patients.

The patients' molecular profiles are shown in Table 3. Those values showing significant differences reveal that, in patients treated with *Apis* after 1 year of treatment, sIgE levels for Api m 1 fell in 69.2% of cases and sIgE levels for Ves v 1 fell in 60.5%. In the case of patients treated with a *Vespula* extract, Ves v 5 levels fell in 66.7% of patients. In these patients, the selection of venom for VIT was performed according to the insect responsible for the sting.

It has been hypothesized that immunotherapy can cause new sensitizations to proteins in the extract in patients who have shown no previous sensitization [13]. Thus, when we analyzed the type of patient in which new sensitizations appear

Table 3. Molecular Profile After 1 Year of Immunotherapy

Allergen	Extract	No.	Median	P Value <sup>a</sup>	
Phospholipase	Api m 1	<i>Apis</i>	39	-183	.0024
		<i>Vespula</i>	12	0.0	.2969
	Ves v 1	<i>Apis</i>	38	-1.0	.0017
		<i>Vespula</i>	12	-16.0	.3394
Hyaluronidase	Api m 2	<i>Apis</i>	39	0.0	.1830
		<i>Vespula</i>	12	0.0	.0625
	Ves v 2	<i>Apis</i>	38	0.0	.5190
		<i>Vespula</i>	12	0.0	.9453
Antigen 5	Ves v 5	<i>Apis</i>	39	-2.0	.0051
		<i>Vespula</i>	12	-238.0	.0425

<sup>a</sup>Wilcoxon signed rank test

(patients with negative IgE at baseline that becomes positive after a year) and whether IgE to different allergens becomes negative, we found that, in the case of patients treated with *Apis*, IgE to Api m 1 and Api m 2 became negative in 2 and 1 patients, respectively, while positive sensitization occurred in 1 and 4 patients, respectively.

In the case of Ves v 1, 2, and 5, new sensitizations were observed after the first year of treatment with *Vespula* extract in 3, 1, and 1 patients, respectively. In the same group of patients, Ves v 2 became negative in 6 patients and Ves v 5 became negative in 1.

Re-sting

Although the above factors may have a certain prognostic value, the only way of appropriately assessing treatment response is to observe the reaction to a re-sting, especially if this occurs in the field. Tables 4 (*Apis*) and 5 (*Vespula*) show the severity of the reaction that caused referral for an

Table 4. Severity of Initial Reaction and of Re-sting in the Case of Patients Sensitized to *Apis*

	Re-stung Patients	Reaction to Re-sting, No.				
		No reaction	LR	LLR	Müller grade I	Müller grade III
SRD	Müller grade I (n=32)	9	7	2		
	Müller grade II (n=114)	32	19	12	1	
	Müller grade III (n=212)	58	34	22	2	
	Müller grade IV (n=80)	31	14	13	2	1

Abbreviations: LLR, large local reaction (>10 cm in diameter and lasting for more than 24 h); LR, local reaction (<10 cm in diameter or lasting for more than 24 h); SRD, reaction at the time of diagnosis

Table 5. Severity of Initial Reaction and of Re-sting in the Case of Patients Sensitized to *Vespula*

	Re-stung Patients	Reaction to Re-sting, No.			
		No reaction	LR	LLR	Müller Grade I
SRD	Müller grade I (n=11)	9	4	5	
	Müller grade II (n=28)	14	3	10	1
	Müller grade III (n=60)	30	17	11	1
	Müller grade IV (n=25)	15	9	5	1

Abbreviations: LLR, large local reaction (>10 cm in diameter and lasting for more than 24 h); LR, local reaction (<10 cm in diameter or lasting for more than 24 h); SRD, reaction at the time of diagnosis.

allergy workup and the severity of the reaction on re-sting in the last years of treatment. The results show that of the patients sensitized to both bee and wasp, 94.6% and 94.1%, respectively, did not suffer any reaction at all, or just a local reaction, on re-sting. The results of skin testing in *Apis*-sensitized patients who were re-stung improved at the end of treatment in 47.3% (previously negative in 18%), became worse in 3 patients (2 suffered a Müller grade 1 reaction to re-sting), and in 50.4% there was no change. In the case of patients sensitized to *Vespula*, the skin test result improved in 62.5% (previously negative in 8%) and did not change in 37.5%. When we consider changes in IgE among re-stung patients, in those sensitized to *Apis*, the mean difference between the pretreatment evaluation and that carried out 5 years later was  $-8.23$  (95% CI,  $-11.17$  to  $-5.29$ ,  $P<.0001$ ). In patients sensitized to *Vespula*, these values were  $-4.28$  (95% CI,  $-8.42$  to  $-0.14$ ,  $P<.0001$ ). Moreover, we recorded the reaction to re-sting experienced by some patients once treatment was stopped. The time from the end of immunotherapy to re-sting varied considerably, from 3 months to 11 years. In the case of patients treated with *Apis* extract, we registered these reactions in 62 patients. Forty patients (65.6%) did not have any reaction at all to re-sting, 19 patients (29.5%) had a small local reaction only, and 3 patients suffered a systemic reaction (Müller grade I). In order to assess the long-term effect of VIT, it is remarkable that 10 of the 61 patients sensitized to *Apis* had been re-stung more than 5 years after ending treatment. Eight of these patients did not experience any reaction and 2 had a small local reaction.

In the case of patients sensitized to *Vespula*, 14 were re-stung after treatment. Seven patients (50%) did not have any reaction at all, 4 (28.6%) had a small local reaction, and 3 (21.4%) had a large local reaction. There were no systemic reactions. In 2 cases, re-sting occurred more than 5 years after the end of treatment: one had no reaction and the other suffered a small local reaction.

## Discussion

Hymenoptera venom allergy is a major health problem. It was observed to be more pronounced in our patients, since almost 30% of those who had a systemic reaction to a first sting had a more severe reaction to the following sting, which

led to referral for an allergy workup (data not shown). Our allergy unit covers a largely rural area, and the population is widely dispersed in small villages, with a high risk of re-sting in the field. Therefore, ongoing monitoring is important. Of our patients, 35.2% (198/562) suffered re-stings during the last years of treatment and 13.3% (75) reported further re-stings after the end of treatment.

In the case of the 7 patients with venom allergy who had adverse reactions to Pharmedgen, as described above, the change to Aquagen, an aqueous treatment with a lower concentration of melittin and other low-molecular-weight substances, led to improved tolerance. This extract has been documented previously [14]. Molecular analysis revealed serum specific IgE to melittin in 4 of the 7 patients (data not shown).

Although the efficacy of venom immunotherapy is clear [4,15], it is worth clarifying specific aspects of this treatment.

First, it is agreed that treatment should last at least 3 years. The different risk factors involved—age, insect, severity of reactions prior to treatment, and other [8]—reveal the need for a longer or shorter length of treatment. In a study published in 1998 [6], the percentage of systemic reactions on re-sting was about 5% in patients who received treatment for at least 50 months, increasing to almost 18% in treatments lasting 33–49 months. Therefore, we maintained treatment for 5 years in most patients.

Second, parameters that are useful in monitoring must be identified. In our patients, IgE became negative in 96 patients and skin tests became negative in 28 patients. The overall reduction was significant in both tests at the end of treatment. Although these parameters are not indicative of clinical efficacy, they do provide a means of monitoring changes in sensitization [16]. At our unit, we follow the recommendations of the Subcommittee on Venom Allergy of the European Academy [17], namely, we maintain treatment for 5 years, unless test results become negative at yearly controls. If this is not the case, we discontinue VIT at 5 years regardless of test results, and always if there is no systemic reaction after re-sting in the field.

Determination of IgE to individual venom allergens is a new technique that may prove useful in the diagnosis and treatment of sensitization to vespids. However, further studies are necessary to confirm the usefulness of this approach and its

role as a monitoring method in immunotherapy. We observed that the predominant allergens before starting immunotherapy were clearly Api m 1 in bee-sensitized patients and Ves v 5 in wasp-sensitized patients. After 1 year of treatment we found significant reductions in IgE levels to these 2 main allergens. As ADVIA-Centaur is not subject to interference from non-IgE antibodies (unlike methods based on solid phase-bound allergens, such as the CAP system), these reductions were real. The titer of non-IgE antibodies is expected to increase during the course of immunotherapy. The percentage of patients with joint sensitization to bee and wasp hyaluronidase ranges from 40% to almost 50%, suggesting significant cross-reactivity between these 2 allergens. It is important to note that both are strongly glycosylated, which could suggest that cross-reactivity is glycan-mediated [18]. We are currently analyzing the possible influence of alcohol intake on cross-reactivity. Joint sensitization was also observed for phospholipases in patients diagnosed with wasp allergy, of whom 25% were sensitized to both Api m 1 and Ves v 1. Finally, a large percentage (41.7%) of *Vespula*-sensitized patients showed joint sensitization to Pol d 5 and Ves v 5. In principle, *Polistes dominula* is not relevant in the area where our patients live, thus suggesting than cross-reactivity between the 2 allergens is not glycan-mediated, since the wasp Antigen 5 allergen is not glycosylated.

After 1 year of immunotherapy, new specific IgE appeared against some allergens. However, the prevalence of these new sensitizations and the level of specific IgE were low (in all but 1 patient the IgE was <1 kU<sub>A</sub>/L). Regarding possible causes of these new sensitizations, other than proper immunotherapy, we cannot rule out a new natural exposure to the allergen or the influence of external factors such as alcohol intake, which could modulate the presence of specific IgE against the glycan moiety of glycosylated allergens.

The most conclusive test of treatment efficacy is a patient's reaction to re-sting. Of all the patients included in our database, 38% experienced re-stings in the field, either during treatment or after completing it. The percentage of patients with no reaction or a small local reaction was 94.6% in those sensitized to bee and 94.1% in those sensitized to wasp. This percentage is maintained even in the case of re-stings several years after the end of treatment, although there are a smaller number of patients in this situation; therefore, we must be careful when drawing conclusions. Some authors report that VIT can be discontinued after 5 to 6 years of treatment with a 5% to 10% residual risk of systemic reaction. Severity of pretreatment reaction is considered to be one such factor [5]. In our patients, 22 out of 105 of those who had a grade IV pretreatment reaction suffered a spontaneous re-sting. Twenty patients (90.9%) had no reaction or a small local reaction. The other 2 patients had a large local reaction and a grade 1 reaction, respectively.

Patients with hymenoptera venom allergy who live in zones of high risk of exposure should be closely monitored, especially in the case of spontaneous re-stings. Monitoring should be a priority objective in allergy units. Taking into account the high rate of spontaneous re-stings, we believe that efficacy is maintained for years after completing treatment in a real-world setting. Other parameters considered when monitoring our patients have different outcomes. A significant decrease in specific IgE to whole venom extract was observed

in the total patient sample and in those who suffered a re-sting alike. A future challenge is to prove whether this decrease in specific IgE titers to whole venom extract is associated with a significant variation in major allergens (Ves v 5 and Api m 1) after stopping VIT, especially in patients who do not have significant reactions to field re-stings. Skin tests have not shown clear value as a monitoring parameter, especially in re-stung patients (bee), despite their indisputable value in the initial diagnosis.

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## Conflicts of Interest

M Lombardero and F de la Torre work for ALK-ABELLÓ, S.A.

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