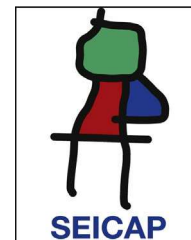




Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica,
Alergología y Asma Pediátrica

www.elsevier.es/ai



ORIGINAL ARTICLE

Component-resolved diagnosis in vespid venom-allergic individuals



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Received 5 February 2014; accepted 25 May 2014

Available online 2 September 2014

KEYWORDS

Wasp venom allergy;
Component-resolved
diagnosis;
Ves v 5;
Pol d 5;
Hymenoptera

Abstract

Background: Hymenoptera venom-allergic patients frequently present multiple sensitisations. **Objectives:** To define the allergic profile by components in wasp allergic patients. To study the usefulness of specific IgE to components in cases of double sensitisation.

Materials and methods: Wasp allergic patients who needed *Polistes* and/or *Vespula* venom immunotherapy were included. Before immunotherapy and after two years of treatment the following specific IgE (sIgE) levels were measured: *Apis mellifera*, *Vespula* spp. *Polistes* spp., rVes v 5, rPol d 5, nVes v 5, nPol d 5, nVes v 1, nPol d 1, nApi m 1, nApi m 2 and peroxidase. Skin tests with venoms were performed. Based on the sIgE and the skin test results, *Polistes* and/or *Vespula* immunotherapy was administered.

Results: Thirteen patients were included. Double sensitisation to *Polistes/Vespula* was detected in eight patients. Sensitisation to rVes v 5 and rPol d 5 was found in two of eight cases, to nVes v 1 and nPol d 1 in eight of 13 cases, and to nVes v 5 and nPol d 5 in 2 of 13 cases. Three patients received double immunotherapy with both wasps. One patient was treated with *Vespula* and nine with *Polistes*. sIgE levels decreased after two years of treatment. In patients who showed double sensitisation but were treated with only one venom, sIgE to both venoms decreased.

Conclusions: Components analysis can be useful to study double positivity. In case of doubt, double immunotherapy should be administered. Phospholipase was found to be a major allergen in our population.

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Introduction

Patients with hymenoptera venom allergy frequently present multiple sensitisations, which can sometimes complicate the choice of immunotherapy (IT). Double sensitisation to honeybee and wasp venom has been detected in up to 59% of patients.¹

This multiple positivity can have several causes: (a) true multiple sensitisation, (b) sensitivity due to cross-reactivity, or (c) sensitisation to cross-reactive carbohydrate determinants (CCDs).^{1,2}

Cross-reactivity between honeybee and wasp venoms is weak. It may be due to the hyaluronidase components (Api m 2, Ves v 2 and Pol d 2), which present a 50% similarity and contain CCDs.^{3–5}

The high level of cross-reactivity between venoms of the different species of wasps is explained by the similarity of the venoms and of the structure of their allergens.⁶ Cross-reactivity between wasps of the *Vespinae* family (*Vespula*, *Dolichovespula* and *Vespa*) and *Polistes* is generally lower than within the *Vespinae* family.^{4,7} The phospholipases of *Vespula* and *Polistes* (Ves v 1 and Pol d 1) are very similar, but there is less similarity between antigen 5 of each venom 5 (Ves v 5 and Pol d 5)³; for this reason, the Pol d 5 and Ves v 5 allergen components are considered to be markers of primary sensitisation to wasp venoms and, in particular, to *Polistes* and *Vespula* venoms.

There is a considerable volume of research into cross-reactivity between honeybee and wasp venoms,^{1,2,5,8–10} but this is not the case for *Vespula* and *Polistes* venoms.^{11,12} The present study was therefore undertaken with the following objectives: (a) to determine the molecular allergenic profile (by components) of a sample of patients allergic to the venom of wasps present in our region and (b) to investigate the usefulness of the determination of the serum levels of specific immunoglobulin E (sIgE) to the individual allergen components in cases of double positivity to wasp venoms.

Materials and methods

Patients with a diagnosis of allergic systemic reaction following wasp stings, who needed *Polistes* and/or *Vespula* venom immunotherapy, and attended in our outpatient section in 2010 were included in the study.

Skin prick tests (SPT) and intradermoreaction were performed before immunotherapy using *Polistes* spp., *Vespula* spp. and *A. mellifera* venom extracts (ALK-Abelló, Madrid, Spain). These tests were considered positive if a concentration $\leq 1 \mu\text{g/l}$ produced a papule $\geq 3 \text{ mm}$ diameter with erythema in the skin prick test or a papule $\geq 5 \text{ mm}$ diameter with erythema in the intradermoreaction.

Before immunotherapy and after two years of treatment the following sIgE were determined: sIgE to complete extracts of honey bee (*Apis mellifera*), *Vespula* (*Vespula* spp.), *Polistes* (*Polistes* spp.) venom and recombinant allergens rVes v 5 and rPol d 5 analysed by ImmunoCAP (Phadia) and sIgE to the natural allergens nVes v 5, nPol d 5, nVes v 1, nPol d 1, nApi m 1 and nApi m 2, as well as to horseradish peroxidase as a marker for CCDs using the ADVIA Centaur® (ADVIA) system (Siemens Medical Solutions Diagnosis, Tarrytown, New York, USA). sIgE values greater than 0.35 kU/l were considered positive.

Based on the sIgE levels and the results of the skin tests, immunotherapy (IT) was initiated with the corresponding venoms (*P. dominulus* and/or *Vespula* spp., Pharmedgen ALK-Abelló). When double IT was required, maintenance treatment was performed with the administration of one venom each month in an alternating regimen. The initiation phase of the IT was always performed in a cluster schedule.

All the patients were informed about the study and consented to take part in it.

Results

13 patients (nine male and four female) aged between 8 and 52 years (mean age, 36.6 years) were included in the study. Based on the Müeller classification of systemic reactions, three patients suffered grade I reactions, six patients grade II, three patients grade III, and one patient grade IV.

The clinical characteristics of the patients are summarised in Table 1.

Skin tests

These tests were performed in 10 patients (not carried out in patients 8, 10 and 13) and were positive in all 10. Double positivity (*Vespula/Polistes*) was detected in four cases (patients 4, 5, 9 and 12).

One patient (patient 12) developed systemic grade II reactions to *Vespula* and *Polistes* (0.01 $\mu\text{g/ml}$ in both cases) (Table 1).

Determination of sIgE (Table 2)

sIgE to nApi m 1 and nApi m 2 (ADVIA) was positive only in patient 9, who had suffered a systemic reaction after a bee sting. This patient also had elevated levels of sIgE to *Apis* (Phadia).

sIgE to horseradish peroxidase CCD was negative in all the patients.

sIgE to *Vespula* (Phadia) was positive in eight of the 13 patients but only two of these eight patients showed positive sIgE to rVes v 5 (Phadia) and a further two to nVes v 5 (ADVIA). However, sIgE to nVes v 1 (ADVIA) was elevated in eight of the 13 patients.

sIgE to *Polistes* (Phadia) was positive in all 13 patients. sIgE to rPol d 5 (Phadia) was elevated in 4 of 8 patients. sIgE to nPol d 5 (ADVIA) was positive in 5 of the 13 patients, and sIgE to nPol d 1 (ADVIA) was elevated in all 13 patients studied.

Double sensitisations evidenced by sIgE levels showed the following profiles: 8 of 13 cases (patients 3–5 and 9–13) were positive to *Polistes* and *Vespula* (Phadia); two of eight cases (patients 5 and 9) were positive to rVes v 5 and rPol d 5 (Phadia); eight of 13 cases (patients 3–5, 7, 9, 11–13) were positive to nVes v 1 and nPol d 1 (ADVIA) and two of 13 cases (patients 5 and 13) to nVes v 5 and nPol d 5 (ADVIA).

Immunotherapy and response of the sIgE levels

Immunotherapy (IT) was prescribed in all the patients. Three patients (patients 4, 5 and 12) received double IT with both

Table 1 Characteristics of the patients, skin tests, immunotherapy prescribed and reactions to stings.

Patient	Sex/age	Allergic reaction (Müeller grade)	Skin test to Pol ($\mu\text{g/ml}$)	Skin test to Ves ($\mu\text{g/ml}$)	Immunotherapy	Reaction after sting
1	F/42	II	1	Negative	P	No stings
2	F/50	II	0.1	Negative	P	No stings
3	M/50	III	0.1	Negative	P	No stings
4	M/24	II	1	1	P + V	Several stings with no reaction
5	M/48	III	0.1	0.1	P + V	No stings
6	M/42	I	0.1	Negative	P	Sting with noreaction
7	M/52	III	0.1	Negative	P	2 wasp stings with no reaction
8	M/26	I	ND	ND	P	No stings
9	M/34	II (Wasp) III (Bee)	0.1	0.1	Bee (5 y) then P	No stings
10	F/8	II	ND	ND	P	Only a local reaction
11	F/42	I	0.1	Negative	P	No stings
12	M/42	IV	Grade II reaction (0.01)	Grade II reaction (0.01)	P + V	No stings
13	M/16	II	ND	ND	V	4 or 5 wasp stings with no reaction

Abbreviations: F, female; M, male; P, *Polistes*; V, *Vespula*; ND, not done.

wasp venoms. One patient (patient 9) had been previously treated with *Apis* immunotherapy for five years and begun *Polistes* immunotherapy. One patient was treated only with *Vespula* extract and eight only with *Polistes*.

After two years of IT, the sIgE levels decreased in all cases (Table 2). In patient 12, who received double IT, the sIgE levels to *Polistes* and to *Vespula* (Phadia) increased but the sIgE levels to nVes v 1 and to nPol d 1 (ADVIA) fell.

Patients treated with *Polistes* immunotherapy but who had elevated sIgE to *Vespula* (patients 3, 7, 9 and 11) also presented a decrease in the sIgE levels to *Vespula*. In one patient (patient 13) who received *Vespula* IT the sIgE levels to *Polistes* also fell.

No re-sting tests were performed. Five patients suffered stings while receiving the IT. Only one had a local reaction, the others had no reaction at all (Table 1).

Discussion

The diagnostic study of patients who have presented allergic reactions to hymenoptera stings frequently reveals multiple sensitisations, which complicates the choice of the appropriate venom for IT.^{2,5,8-13} Several methods have been used to determine the responsible venom in these cases, including the study of sIgE to allergen components, the IgE-inhibition test, and the basophile activation test. It is currently agreed that determination of serum levels of sIgE to allergen components is the most useful test in patients with multiple sensitisation.^{1,2,5,8,9,12,13}

All our patients presented sIgE to the complete venom of *Polistes* spp. (Phadia) and to nPol d 1 (ADVIA); Pol d 1 was therefore found to be a major allergen (100% of patients). However sIgE to Pol d 5 was only detected in half of the patients (Phadia, four of eight) or less than half

(ADVIA, 5 of 13), making antigen 5 a minor antigen in our population.

Eight of the 13 patients had sIgE to *Vespula* (Phadia). Only two of these patients had sIgE to rVes v 5 (Phadia) and another two to nVes v 5 (ADVIA), meaning that Ves v 5 was also a minor antigen. Ves v 1 was positive in eight of 13 patients, indicating it to be a major allergen.

In the study published by the Hymenoptera Committee of the Spanish Society of Allergy and Clinical Immunology (SEAIC),¹² elevated serum levels of sIgE to the recombinant allergen components rVes v 5 and rPol d 5 were detected in 52% of patients; however the majority of patients (21/25) had sIgE to the natural allergens nVes v 5 and nPol d 5. That group concluded that the diagnostic yield was higher when natural allergen components are used. In our study we found no differences between the recombinant (Phadia) and the natural (ADVIA) allergen components.

Müller et al.,¹ in Switzerland, found that 96% of subjects allergic to *Vespula* had high levels of sIgE to rVes v 5 using the ADVIA system. In another study, those same authors found that study of the sensitivity to rVes v 5 (Phadia) was optimal (100% of patients sensitive only to *Vespula*), whereas only 33% of their patients presented sIgE to rVes v 1 (Phadia).² However, Seismann et al.,⁵ in Germany, found a higher level of positivity to rVes v 1 than to rVes v 5.

Sensitisation to the allergen components of wasp venom varies depending on the geographic region studied, the predominant wasp in the region, and the extract used for the study (recombinant or natural).

In our study we detected double positivity (skin tests and/or sIgE) to *Vespula/Polistes* (Phadia) in eight patients. In four of these patients, the skin tests were positive with both wasp venoms (patients 4, 5, 9 and 12) and all four also showed double positivity to phospholipases; double positivity to antigen 5 was only observed in patient 5. Double

Table 2 Specific IgE levels.

Patient	nApi m 1 (ADVI)	nApi m 2 (ADVI)	Peroxidase CCD (ADVI)	nVes v 1 (ADVI)	nVes v 5 (ADVI)	nPol d 1 (ADVI)	nPol d 5 (ADVI)	Apis (Phad)	Polist (Phad)	Vesp (Phad)	rVes v 5 (Phad)	rPol d 5 (Phad)
1	0.02 (0)	0 (0)	0.11 (0.15)	0.02 (0)	0 (0)	4.54 (1.29)	0.18 (0)	<0.35 (ND)	0.87 (ND)	<0.35 (ND)	ND (ND)	ND (ND)
2	0 (0)	0 (0)	0.04 (0)	0 (0)	0 (0)	25.57 (2.92)	0.12 (0)	<0.35 (ND)	9.14 (4.75)	<0.35 (ND)	<0.35 (ND)	<0.35 (ND)
3	0 (0)	0 (0)	0.01 (0)	3.57 (0.02)	0 (0.04)	101.23 (5.11)	0.31 (0)	<0.35 (ND)	24 (ND)	14.7 (ND)	<0.35 (ND)	<0.35 (ND)
4	0.01 (0)	0 (0)	0 (0)	17.31 (2.94)	0 (0)	14.66 (1.25)	0.14 (0)	<0.35 (ND)	14.3 (ND)	8.04 (ND)	ND (ND)	ND (ND)
5	0 (0)	0 (0)	0.03 (0)	10.87 (1.29)	3.09 (0.69)	11.72 (1.61)	5.37 (0.69)	<0.35 (ND)	4.58 (ND)	4.66 (ND)	9.5 (ND)	15.5 (ND)
6	0.02 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.73 (1.40)	0.48 (0.40)	<0.35 (ND)	3.09 (4.6)	<0.35 (<0.35)	<0.35 (ND)	0.73 (ND)
7	0 (0)	0 (0)	0.09 (0)	0.43 (0.12)	0 (0)	17.14 (2.38)	1.09 (0.34)	<0.35 (ND)	7.81 (3.82)	<0.35 (ND)	0.35 (ND)	1.64 (ND)
8	0.04 (0)	0 (ND)	0.01 (0)	0 (0)	0 (ND)	3.98 (0.31)	0.48 (0.18)	<0.35 (ND)	5.37 (4.36)	<0.35 (ND)	ND (ND)	ND (ND)
9	1.76 (0.63)	1.27 (0)	0.05 (ND)	5.72 (0.88)	0 (0)	25.67 (3.46)	0.75 (0.19)	1.82 (ND)	14.2 (ND)	4.45 (ND)	0.51 (ND)	1.4 (ND)
10	0 (0)	0 (ND)	0.01 (0)	0.28 (0)	0.03 (0.04)	151.49 (6.55)	0.2 (0)	<0.35 (ND)	82.6 (ND)	2.01 (ND)	ND (ND)	ND (ND)
11	0 (0)	0 (0)	0.04 (0)	0.36 (0.03)	0 (0)	24.76 (2.16)	0.13 (0.01)	<0.35 (ND)	11.3 (ND)	2.4 (ND)	<0.35 (ND)	<0.35 (ND)
12	0.04 (0.07)	0.01 (6.01)	0 (0.20)	6.3 (4.39)	0 (0.39)	6.88 (3.78)	0.16 (0.09)	<0.35 (ND)	3.48 (7.97)	6.39 (10.90)	<0.35 (ND)	<0.35 (ND)
13	0.02 (0)	0 (0)	0.01 (0)	9.11 (3.65)	22.65 (10.41)	8.08 (2.70)	0.62 (0.34)	<0.35 (ND)	5.75 (ND)	7.76 (ND)	ND (ND)	ND (ND)

Abbreviations: CCD, cross-reactive carbohydrate determinants; Polist, *Polistes*; Vesp, *Vespula*; ND, not done.

Brackets: Levels of sIgE after two years of immunotherapy.

In bold: high values of sIgE.

IT was administered to three patients but in patient 9 it was decided to perform IT only with *Polistes* because the levels of sIgE to rPol d 1 and rPol d 5 were significantly higher than the levels to *Vespula*. In three of the remaining four patients, IT was performed with *Polistes* (patients 3, 10 and 11) and in patient 13 IT was performed with *Vespula*.

In the five patients with single positivity, the determination of sIgE to *Vespula* and *Polistes* (Phadia), with or without skin tests, was sufficient to establish the venom to be used for IT.

Thus, the medical history, the determination of sIgE to *Polistes* and *Vespula* (Phadia) and the skin tests with both wasp venoms would have been sufficient to reach a diagnosis in the majority of our patients and to determine the venom with which to perform vaccination. Study by allergen components would only be useful in case of doubt caused by double positivity with similar sIgE levels. We believe that when there is doubt about the responsible wasp, a good approach is to administer double IT.

In other published studies, the determination of sIgE to allergen components has also been found to be more useful than other techniques in cases of double positivity to hymenoptera, although the majority of those studies involved patients with *Apis/Vespula* sensitisation.² The majority of cases of double positivity to honey bee/wasps are due to sensitisation to CCDs.^{3,5,13} Neis MM,⁹ in a study of patients sensitised to *Apis* and/or *Vespula*, obtained best results on measuring the levels of sIgE to allergen components in the patients with double positivity. Seismann et al.⁵ came to the same conclusion in their study, finding that the use of rVes v 1 and rVes v 5 identified the majority of subjects allergic to *Vespula*.

In cases of double sensitisation to wasps (*Vespula/Polistes*) Monsalve et al.¹² measured sIgE to major natural allergen components (nVes v 1, nVes v 2, nVes v 5, nPol d 1 and nPol d 5); these measurements enabled them to demonstrate sensitisation to only a single wasp in 69% of the 45 cases—to *Vespula* in nine patients and to *Polistes* in 22. Double positivity was confirmed in 14 (31%) of cases. There are no other studies that demonstrate the usefulness of the measurement of sIgE to allergen components in cases of double positivity to *Polistes/Vespula*. In a study published by Caruso B et al.,¹¹ 45 patients with double positivity to *Vespula* and *P. dominulus* were studied by CAP and skin tests. IgE-inhibition tests were performed in all patients. In 25 patients *P. dominulus* inhibited *Vespula vulgaris* and those patients received IT with *P. dominulus*. In six patients *V. vulgaris* inhibited *P. dominulus* and those patients were vaccinated with *V. vulgaris*. The results did not show differences between the venoms in the remaining 14 patients, and those patients received double IT.

In our study, the choice of the IT would appear to have been correct in all patients, as those patients who suffered stings thereafter (five patients) presented only a local reaction or no reaction and, after two years of IT, the levels of sIgE had fallen in all cases. However, in patient 12, who received double IT, the levels of sIgE to *Polistes* and to *Vespula* (Phadia) were higher after two years of IT, although the levels of sIgE to nVes v 1 and to nPol d 1 (ADVI) had fallen. In patients vaccinated with *P. dominulus* but who had elevated sIgE to *Vespula* (patients 3, 7, 9, 11), the levels of

slgE to *Vespula* also fell. In one patient (patient 13) who was vaccinated with *Vespula*, the levels of slgE to *Polistes* also fell at two years.

Conclusions

- (1) Our population was found to be positive mainly to *Polistes*, the dominant insect in our region.
- (2) Phospholipase was found to be a major allergen component whereas antigen 5 was a minor allergen component.
- (3) In cases of double positivity to both *Polistes* and *Vespula* spp. based on the determination of slgE to complete venoms (Phadia) and/or positive skin tests, the allergic status of the patients can be better defined by measuring slgE to allergen components.
- (4) In case of doubt, double immunotherapy should be administered.

Ethical disclosures

Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Protection of human subjects and animals in research. The authors declare that no experiments were performed on humans or animals for this investigation.

Financial support

No external financial support was provided for the study.

Conflict of interest

Agustín Galán Nieto works in the R&D department of ALK-Abelló, manufacturer of hymenoptera venom immunotherapy. The other authors have no conflicts of interest to declare.

Acknowledgements

The authors thank nurses of our sections, Elena Andrés, M^a José Muñoz, M^a José Ruiz and M^a Dolores Díaz, for their collaboration.

The cases described were presented at Sesiones Interhospitalarias de la Sociedad Madrid-Castilla La Mancha de Alergología e Inmunología Clínica, February 2013, Madrid, Spain.

References

1. Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haerberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespula* venom by estimation of IgE antibodies to species-specific major allergens Api m 1 and Ves v 5. *Allergy*. 2009;64:543–8.
2. Müller U, Schmid-Grendelmeier P, Hausmann O, Helbling A. IgE to recombinant allergens Api m 1, Ves v 1, and Ves v 5 distinguish double sensitization from crossreaction in venom allergy. *Allergy*. 2012;67:1069–73.
3. Hoffman DR. Hymenoptera venom allergens. *Clin Rev Allergy Immunol*. 2006;30:109–28.
4. Biló BM, Fueff F, Mosbech H, Bonifazi F, Oude-Elberink JNG, the EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of hymenoptera venom allergy. *Allergy*. 2005;60:1339–49.
5. Seismann H, Blank S, Cifuentes L, Braren I, Bredehorst R, Grunwald T, et al. Recombinant phospholipase a1 (Ves v1) from yellow jacket venom for improved diagnosis of hymenoptera venom hypersensitivity. *Clin Immunol Mol Allergy*. 2010;8:7.
6. Hoffman DR. Allergens in hymenoptera venom XXV: the amino acid sequences of antigen r molecules and the structural basis of antigenic cross-reactivity. *J Allergy Clin Immunol*. 1993;92:707–16.
7. Pantera B, Hoffman DR, Carresi L, Turillazzi S, Manao G, Severino M, et al. Characterization of the major allergens purified from the venom of the paper wasp *Polistes gallicus*. *Biochim Biophys Acta*. 2003;1623:72–81.
8. Eberlein B, Krischan L, Darso U, Ollert M, Ring J. Double positivity to bee and wasp venom: Improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. *J Allergy Clin Immunol*. 2012;130:155–61.
9. Neis MM, Merk HF. Value of component based diagnostics in IgE-mediated hymenoptera sting reactions. *Cutan Ocul Toxicol*. 2012;31:117–23.
10. Erzen R, Korosec P, Silar M, Music E, Kosnik M. Carbohydrate epitopes as a cause of cross-reactivity in patients allergic to hymenoptera venom. *Wien Klin Wochenschr*. 2009;121:349–52.
11. Caruso B, Bonadonna P, Severino MG, Mandredi M, Dama A, Schiappoli M, et al. Evaluation of the IgE cross-reactions among vespid venoms. A possible approach for the choice of immunotherapy. *Allergy*. 2007;62:561–4.
12. Monsalve RI, Vega A, Marquès L, Miranda A, Fernández J, Soriano V, et al. Component-resolved diagnosis of vespid venom-allergic individuals: phospholipases and antigen 5s are necessary to identify *Vespula* or *Polistes* sensitization. *Allergy*. 2012;67:528–36.
13. Mateo-Borrego B, Borja-Segade J, Castro-Jimenez A, Lara de la Rosa P, Gómez-Torrijos E, Sánchez-Rodríguez N, et al. Component-resolved diagnosis in a case of hymenoptera venom allergy. *Allergy*. 2010;65 Suppl. 92:649.