

Application of different in vitro tests for the precise diagnosis of double sensitization to Vespula-Polistes

Giraldo-Tugores M^{1*}, Vaquero-Rey A^{1,2*}, Santacruz-Santos M^{2,3}, Rodríguez-Martín E⁵, De Andrés A^{3,5}, Ballester-González R⁵, Barra-Castro A^{1,3}, Fernández-Lozano C^{1,2,3}, Martínez-Botas J^{4**}, Antolín-Amérigo D^{1**}

¹Allergology Department, Hospital Universitario Ramón y Cajal, IRYCS, Madrid, Spain

²Biochemistry-Research Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

³Universidad de Alcalá, Madrid, Spain

⁴Biochemistry-Research Department, Hospital Universitario Ramón y Cajal, IRYCIS and CIBEROBN, Madrid, Spain

⁵Immunology Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

*Both authors have equally contributed and should be both considered as first authors.

**Both authors should be considered as senior authors.

Corresponding Author:

Margarita Giraldo Tugores

Carretera Colmenar Viejo, km. 9, 100, 28034 Madrid

E-mail: mgiraltug@gmail.com

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The identification of the culprit insect in double sensitization (DS) to *Vespula-Polistes* is a complex challenge. In Spain, the DS to *Vespula-Polistes* has been found to range from 50.5% to 61.5% [1].

The principal allergenic proteins of *Vespula-Polistes* are phospholipase A1 (Ves v 1/Pol d 1), hyaluronidases (Ves v 2/Pol d 2), dipeptidyl peptidases IV (Ves v 3/Pol d 3), and Antigen 5 (Ves v 5/Pol d 5) [2].

A 68-year-old woman developed repeated and extended local reactions with progressive local extension of the sting while she was at her summer home in Segovia (Spain). Last reaction was in the leg, extending up to the ankle without systemic symptoms. Basal tryptase value (ImmunoCAP, ThermoFisher Scientific®) was 4.67µg/L. DS was based on Intradermal Skin Tests (IST) and specific IgE (sIgE) levels. IST was performed with commercial lyophilized venoms (*Polistes dominula* and *Vespula spp.*, Pharmed®/ALK-Abelló SA), which were diluted with the albumin-based specific solvent according to the manufacturer's instructions (Pharmed®/ALK-Abelló SA), reaching a concentration of 100µg/ml. This venom's preparation was the same in sIgE-INH/CAP-Inhibition (sIgE-INH) and Basophil

Activation Test (BAT). For the rest of the techniques, PBS was used as a reconstituent. IST performed with *Vespula-Polistes* venoms were both positive at 0.1µg/mL; 1 and 3 months after the sting reaction. sIgE levels (ImmunoCAP): Total IgE 984 KU/L, *Vespula spp.* 88.60 KU/L, *Polistes spp.* >100 KU/L, *Apis* 0.31 KU/L, *rPol d 5* 0.86 KU/L, *rVes v 5* 2.86 KU/L and *rVes v 1* >100 KU/L. A 1:2 dilution of patient's serum revealed sIgE *Polistes* 192 KU/L and *Ves v 1* 110 KU/L. In addition, we determined *Polistes spp.* sIgG4 0.86 mgA/L and *Vespula spp.* sIgG4 0.30 mgA/L, after informed consent.

Subsequently, it was decided to carry out sIgE-INH and BAT as complementary tests in order to differentiate the primary sensitizer.

The sIgE-INH was carried out by incubating separately two 50 µl aliquots of patient's serum at room temperature during 1 hour with 100 µl of 100 µg/ml *Vespula spp.* and *Polistes dominula* venom (ALK Pharmed®) [adapted from 3, 4, 5]. A percentage of heterologous inhibition 70-75% is considered strongly suggestive of sequence identity [4, 5]. An inhibition higher than 70% was detected in the sera pre-incubed with the venom of *Polistes dominula*: 98% of homologous inhibition and 97% of heterologous inhibition. In the case of inhibition with *Vespula spp.* venom the patient present homologous and heterologous inhibition of 64% in both cases; this result, less than 70% but very close to it, raises suspicions of possible cross-reactivity between *Vespula* and *Polistes* [5].

BAT was performed with three concentrations of *Vespula-Polistes* venoms. A percentage of CD63-positive basophils was obtained from 5 µg/mL of both venoms [6]. Degranulation was almost double for each concentration after *Polistes* than after *Vespula* stimulation (supplementary figure 1).

As routine techniques were not sufficient for an accurate diagnosis, we performed non-conventional tests: Protein Slot-Blotting (PSB), Western-Blot and Peptide Microarrays Immunoassays (PMI).

To perform PSB 50 μ l of venoms at different concentrations and patient's serum diluted 1:10 was used [7]. It was revealed using an α -hIgE-HRP (Southern Bioch) and visualized by chemiluminescence using the Clarity Western ECL Substrate (Bio-rad). IgE-binding to *Polistes* was higher than *Vespula* in all concentrations (Figure 1A). A slight reduction of *Vespula* recognition was observed when the serum was incubated in the membrane with both venoms simultaneously (Figure 1AI).

Vespula-Polistes extracts (5.6 μ g protein/lane) were resolved in a 10-15% SDS-PAGE gel under denaturing conditions. Western-Blot showed an IgE recognition of a ~33 kDa protein in *Polistes*, probably corresponding to Pol d 1 (Figure 1B). A band was recognized in *Vespula* with much lower intensity, probably corresponding to Ves v 1 [1].

We performed an IgE and IgG4 binding analysis by PMI as described by Martinez-Botas et al [8]. Different concentrations of venom extracts were printed on sciCHIP EPOXY slides, each feature in triplicate. Microarrays were hybridized with 100 μ l of the patient's serum diluted 1:10. Quantification analysis revealed a positive correlation between the fluorescent signal (FS) obtained and the venom's concentration in both IgE and IgG4 (supplementary figure 2). IgE recognition was slightly higher in *Vespula* than in *Polistes* at all concentrations studied. However, for IgG4, higher values were obtained for *Polistes*, being clearly visible even at 0.125 μ g/ μ l.

The sIgE showed DS with the complete extracts of both venoms, being higher for *Polistes*. However, considering that Phospholipase A1 (Ves v 1) is one of the most important allergens

in vespids and with no information available for Pol d 1, it was not possible to accurately determine the most relevant allergen.

By sIgE-INH, *Polistes* seems to be dominant over *Vespula*, but it cannot be definitely established due to the indetermined value of the percentage of inhibition obtained for *Vespula*. This can be explained by the fact that there is only a 52% of sequence identity between Pol d 1 and Ves v 1, suggesting a possible low cross-reactivity between them.

BAT was useful in the identification of the dominant allergen [9]. BAT with complete venom extracts resulted in a high basophil activation to *Polistes* in all concentrations but still positive to *Vespula* with 5 and 10 µg/ml.

Because of the increasingly polarized results to *Polistes*, having a very low Pol d 5 value with respect to the total extract, we performed a PSB and Western-Blot as alternative methods for assessing IgE, which allowed to differentiate higher sIgE-binding to the *Polistes* component Pol d 1.

PMI could not distinguish primary sensitization since there was no significant difference between the signal obtained for sIgE in both extracts, but revealed a greater binding of IgG4 to *Polistes*, possibly due to greater exposure to that species. This is consistent with the sIgG4 ImmunoCAP.

sIgE-INH and BAT suggested *Polistes* as the primary allergen, but PSB and Western-Blot allowed the identification of *Polistes* and Pol d 1 as the primary sensitizer, respectively. We report a complex case of hymenoptera allergy in which conventional techniques cannot identify the clinically relevant allergen. The combined use of unusual *in-vitro* techniques as a possible proposal allowed us to achieve an accurate diagnosis against *Polistes*.

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Conflicts of interest

The authors have no conflict of interest to declare.

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References

1. Ruiz-Leon B, Serrano P, Vidal C, Moreno-Aguilar C. Management of Double Sensitization to Vespids in Europe. *Toxins*. 2022;14:126.
2. Alergia_Himenopteros_SEAIC_20_04.pdf.
Available:https://docs.google.com/viewerng/viewer?url=https://www.seaic.org/wp-content/uploads/2021/05/Alergia_Himenopteros_SEAIC_20_04.pdf&hl=es.
Accessed July 12, 2022.
3. Hemmings O, Du Toit G, Radulovic S, Lack G, Santos AF. Ara h 2 is the dominant peanut allergen despite similarities with Ara h 6. *J Allergy Clin Immunol*. 2020;146:621-30.
4. Caruso B, Bonadonna P, Severino MG, Manfredi 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.
5. Straumann F, Bucher C, Wüthrich G. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Value of FEIA inhibition for the identification of the cross-reacting IgE antibodies in double-sensitized patients to honeybee and wasp venom. *Int Arch Allergy Immunol*. 2000;123:268-74.
6. Loli-Ausejo D, Vílchez-Sánchez F, Cabañas R, Fiandor A, Lluch-Bernal M, González-Muñoz M, et al. Basophil activation test in the diagnosis of hypersensitivity reactions to quinolones in a real-life setting. *Clin Exp Allergy*. 2021;51:503-7.
7. De Kochko A, Qu R, Marmey P, Rance I, Fauquet C, Beachy R.N. Protein slot blotting: An easy, rapid and reliable technique to identify the expression of a protein in transgenic plants. *Plant Mol Biol Rep*. 1994;12:274-8.

8. Martínez-Botas J, Fernández-Lozano C, Rodríguez-Alonso A, Sánchez-Ruano L, de la Hoz B. Epitope Mapping of Food Allergens Using Noncontact Piezoelectric Microarray Printer. *Methods Mol Biol.* 2021;2344:119-35.
9. Santos AF, Alpan O, Hoffmann HJ. Basophil activation test: Mechanisms and considerations for use in clinical trials and clinical practice. *Allergy.* 2021;76:2420-32.

FIGURE

Figure 1. Recognition of the sIgE.

A) Slot-blot. Patient serum incubation with *Vespula spp.* and *Polistes dominula* (I), only with *Vespula spp.* (II), only with *Polistes dominula* (III), at different concentrations (100, 50 and 25 $\mu\text{g}/\text{mL}$). **B) Western Blot.** Patient serum incubation with the proteins obtained by acrylamide/bisacrylamide gel for *Vespula spp.* and *Polistes dominula*.

