Application of In Vitro Tests to Establish an Accurate Diagnosis of Double Sensitization to Vespula and Polistes Species

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doi: 10.18176/jiaci.0890

Key words: Polistes. Vespula. Slot blot. IgE-immunoblot. Basophil activation test.


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

The principal allergenic proteins of Vespula and Polistes species 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). The last reaction affected the leg, extending to the ankle without systemic symptoms. The basal tryptase value (ImmunoCAP, Thermo Fisher Scientific) was 4.67 μg/L. DS was assessed based on intradermal skin testing (IST) and specific IgE (sIgE) levels. IST was performed with commercial lyophilized venoms (Polistes dominula and Vespula species, Pharmalgen, ALK-Abelló SA), which were diluted with albumin-based specific solvent according to the manufacturer’s instructions (Pharmalgen, ALK-Abelló SA), reaching a concentration of 100 μg/mL. The venom was prepared in the same way for sIgE-INH/CAP inhibition (sIgE-INH) and the basophil activation test (BAT). For the remaining techniques, phosphate-buffered saline was used as a reconstituent. IST performed with Vespula and Polistes venoms was positive for both allergens.
at 0.1 μg/mL and 1 and 3 months after the sting reaction. sIgE levels (ImmunoCAP) were as follows: total IgE, 984 kU/L; *Vespula* species, 88.60 kU/L; *Polistes* species, >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 the patient’s serum revealed sIgE for *Polistes* to be 192 kU/L and for Ves v 1 to be 110 kU/L. In addition, after obtaining the patient’s informed consent, we determined sIgG4 to be 0.86 mg/L for *Polistes* species and 0.30 mg/L for *Vespula* species.

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

sIgE-INH was carried out by incubating separately two 50-μL aliquots of the patient’s serum at room temperature for 1 hour with 100 μL of 100 μg/mL *Vespula* species and *Polistes dominula* venom (ALK Pharmalgen) [adapted from 3-5]. Heterologous inhibition of 70%-75% is considered strongly suggestive of sequence identity [4,5]. Inhibition higher than 70% was detected in the sera that had been preincubated with the venom of *Polistes dominula* (98% homologous inhibition and 97% heterologous inhibition). In the case of inhibition with *Vespula* species venom, the patient presented 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 3 concentrations of *Vespula* and *Polistes* venoms. A percentage of CD63-positive basophils was obtained from 5 μg/mL of both venoms [6]. Degranulation for each concentration was almost double after stimulation with *Polistes* than after stimulation with *Vespula* (Supplementary figure 1).

As routine techniques were not sufficient for an accurate diagnosis, we performed nonconventional tests: protein slot blotting (PSB), Western blot, and peptide microarray immunoassays (PMI).

PSB was performed using 50 μL of venom at different concentrations and the patient’s serum diluted 1:10 [7]. The protein was revealed using α-hIgE-HRP (Southern Biotech) and visualized using chemiluminescence with the Clarity Western ECL Substrate (Bio-Rad). IgE-binding to *Polistes* was higher than to *Vespula* at all concentrations (Figure, A). A slight reduction in *Vespula* recognition was observed when the serum was incubated in the membrane with both venoms simultaneously (Figure, AII).

*Vespula* and *Polistes* extracts (5.6 μg protein/lane) were resolved in a 10%-15% SDS-PAGE gel under denaturing conditions. Western blot showed recognition of a ~33-kDa protein by IgE in *Polistes*, probably corresponding to Pol d 1 (Figure, B). A much less intense band recognized in *Vespula* was probably Ves v 1 [1].

We performed an IgE and IgG4 binding analysis using 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 µL of the patient’s serum diluted 1:10. Quantification analysis revealed a positive correlation between the fluorescent signal obtained and the venom 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. These were clearly visible, even at 0.125 µg/µL.

sIgE showed DS for the complete extracts of both venoms, with values being higher in Polistes. However, considering that phospholipase A1 (Ves v 1) is a major allergen in vespid and with no information available for Pol d 1, it was not possible to accurately determine the most relevant allergen.

sIgE-INH indicates that Polistes is dominant over Vespula, although this cannot be definitively established owing to the indeterminate value of the percentage of inhibition obtained for Vespula. However, it can be explained by the fact that there is only 52% sequence identity between Pol d 1 and Ves v 1, suggesting low cross-reactivity.

BAT was useful in the identification of the dominant allergen [9]. BAT with complete venom extracts resulted in considerable activation of basophils to Polistes at all concentrations while remaining positive to Vespula at 5 and 10 µg/mL.

Because of the increasingly polarized results for Polistes and the very low Pol d 5 value with respect to the total extract, we performed PSB and Western blot as alternative methods for assessing IgE. These enabled us to identify higher sIgE binding to the Polistes component Pol d 1.

PMI could not identify primary sensitization, since there was no significant difference between the signal obtained for sIgE in both extracts, although it did reveal greater binding of IgG4 to Polistes, possibly owing to greater exposure to that species. This is consistent with the sIgG4 ImmunoCAP result.

sIgE-INH and BAT suggested Polistes as the primary allergen, although PSB and Western blot pointed to Polistes and Pol d 1 as the primary sensitizers, respectively. We report a complex case of hymenoptera allergy in which conventional techniques cannot identify the clinically relevant allergen. The combined application of unusual in vitro techniques enabled us to achieve an accurate diagnosis for Polistes.

**Funding**

The authors declare that no funding was received for the present study.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**References**