Consistency of sIgE Determination and Basophil Activation Test in *Vespa velutina nigrithorax* Allergy

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0716

Key words: Vespa velutina nigrithorax. nVesp v 1. nVesp v 5. Basophil activation test. sIgE.

Palabras clave: Vespa velutina nigrithorax. nVesp v 1. nVesp v 5. Test de activación de basófilos. IgE sérica.

Vespa velutina nigrithorax is an alien species originally from Southeast Asia, but widely found in other regions of the world [1,2] because of its capacity to develop populous colonies with an effective predator defence, producing numerous founders.[2]Cases of beekeepers, farmers or people living in rural environments who have been wounded or died due to *Vespa velutina nigrithorax* stings are reported every year in Asia and Europe [3-5].Most of these subjects die because of its toxic effects, but *Vespa velutina* can also provoke allergic reactions similar to those provoked by other Hymenoptera species [6-8].We have previously published extensive IgE cross-reactivity among *Vespa velutina nigrithorax* and other Hymenoptera venoms, particularly *Vespula spp* and *Polistes dominula* venoms [7,8]. Inhibition studies performed with *Vespula spp* and *Vespa velutina* venoms suggested that *Vespula spp* is likely the genuine or primary sensitizer [8]. Antigen 5 from *Vespa velutina nigrithorax* (Vesp v 5), and phospholipase A1 (Vesp v 1) are relevant, and officially recognised allergens [7,8]. The present study was aimed at measuring the basophil response to *Vespa velutina nigrithorax* venom and its allergens (Vesp v 1 and Vesp v 5) in patients with confirmed IgE-induced anaphylaxis, by using basophil activation test (BAT), as an additional diagnostic method which could be used for venom immunotherapy follow-up, if consistent results were obtained.

We prospectively studied 10 consecutive patients with *Vespa velutina nigrithorax* venom anaphylaxis from December 2020 to February 2021. The median age was 57 years (range, 26–81 years) and all were men who lived in a rural environment. The anaphylactic event had developed in less than 30 min after experiencing a median of two (range, 1–13) stings. We measured serum-specific IgE (sIgE) (ImmunoCAP-250 platform) against a panel of commercially available Hymenoptera venom allergens (including *Vespa velutina* venom [U1223]) (Thermo Fisher Scientific, Inc.) and sIgE to nVesp v 1 and nVesp v 5 after being biotinylated and bound to the high-capacity immunosorbent coupled to Streptavidin (o212, Thermo Fisher Scientific, Inc.), as previously described [7,8].

BATs were performed 3-4 months after the anaphylactic reaction with the commercially available BASOTEST® kit (Celonic™ Deutschland) that detects the translocation of CD63 from the secretory lysosomal granule to the basophil surface in heparinized whole blood samples. The test kit contains the chemotactic peptide N-formyl-Met-Leu-Phe (fMLP) as positive control, and the wash solution serves as negative background control. The specific allergens tested in patients included *Vespa velutina nigrithorax* whole venom (10, 1, 0.1, and 0.01 µg/mL), nVesp v 1, and nVesp v 5 (10, 1, 0.1, and 0.01 ng/mL) purified as previously described [9]. Flow cytometric analysis was performed within 3 hours using a FACScan™ (Becton-Dickinson Immunocytometry System, Heidelberg, Germany) and CellQuest™ software. Five healthy individuals (4 atopic and 1 non-atopic) with a negative history of venom allergy and absence of sIgE were recruited as controls. BAT experiments in control patients were performed with *Vespa velutina nigrithorax* whole venom. We defined basophil reactivity as the percentage of basophils that responded to the stimulus. The

doi: 10.18176/jiaci.0716

responses were considered positive when the percentage of activated basophils was greater than 15%, as recommended by the manufacturer and suggested by other authors [10,11].All participants gave their written informed consent for the study, which was approved by the institutional ethics committee (code 2018/622).

All patients presented a positive sIgE (≥0.35 kUa/L) to *Vespa velutina* venom and *Vespula spp*, and 9/10 presented sIgE to *Vespa crabro* and *Polistes dominula.* Levels of sIgE to *Vespula spp* were higher than levels of sIgE to *Vespa velutina* (detailed information is depicted in Supplementary Table 1).As can be seen in Figure 1 and Supplementary Table 2, all but one patient (#3) presented a positive result in the BAT experiments with at least one of the allergens tested. The allergen components nVesp v 1 and nVesp v 5 activated the basophils in 5 and 7 patients, respectively (images from 3 representative patients can be seen in Supplementary Figure 1). When positive, the percentages of activated basophils with*Vespa velutina* whole venom, nVesp v 1, and nVesp v 5 were similar. No correlation was found between the maximum percentage of activated basophils and the sIgE concentrations against any tested allergen (r=0.103, p=0.777 for *Vespa velutina* whole venom; r=0.055, p=0.881 for nVesp v 1; and r=0.067, p=0.885 for nVesp v 5) nor the severity of the anaphylactic reaction (data not shown) by using the Spearman's rank test. The five healthy control subjects presented negative BAT results (Supplementary Table 3, and image from one control subject in Supplementary Figure 1).

These results support the biological activity of *Vespa velutina nigrithorax* venom through an IgE-mediated mechanism. The allergen components nVesp v 1 and nVesp v 5 also induced strong positive responses, even at very low concentrations. The

negative results from the control subjects ruled out non-specific basophil activation. The results of this CD63-based BAT were consistent with sIgE since all but one patient (#3) with positive sIgE to *Vespa velutina* whole venom presented a significant percentage of basophil activation when exposed to *Vespa velutina* whole venom or its allergens (nVesp v 1 or nVesp v 5). Nevertheless, we could not find a good correlation between the degree of basophil activation and sIgE reactivity to *Vespa velutina* as it has been demonstrated by wasp or bee venom allergens in former studies [10].Regarding the level of concordance between sIgE and BAT dichotomous results (positive vs negative), it seems that *Vespa velutina* whole venom and nVesp v 5 have a better behaviour than nVesp v 1. Thus, considering the commercial availability of the *Vespa velutina* whole venom for sIgE determinations and its better performance in comparison with the allergenic components tested, this whole venom seems to be a good marker to study the biological activity of this venom, in clinical settings. Moreover, taken into account that BAT has been proposed as an *ex vivo* method to follow-up venom immunotherapy [8,10-15], and positive BAT responses were obtained in our patients clinically diagnosed with anaphylaxis due to *Vespa velutina* venom allergy, BAT could be performed after venom immunotherapy to investigate its potential efficacy in these particular patients.

Funding

The study was supported by the Instituto de Salud Carlos III (Fondo de Investigaciones Sanitarias, Spanish Ministry of Health, PI16/1401 and PI19/01023) and Fundación de la Sociedad Española de Alergología e Immunología Clínica (SEAIC).

doi: 10.18176/jiaci.0716

Conflicts of interest

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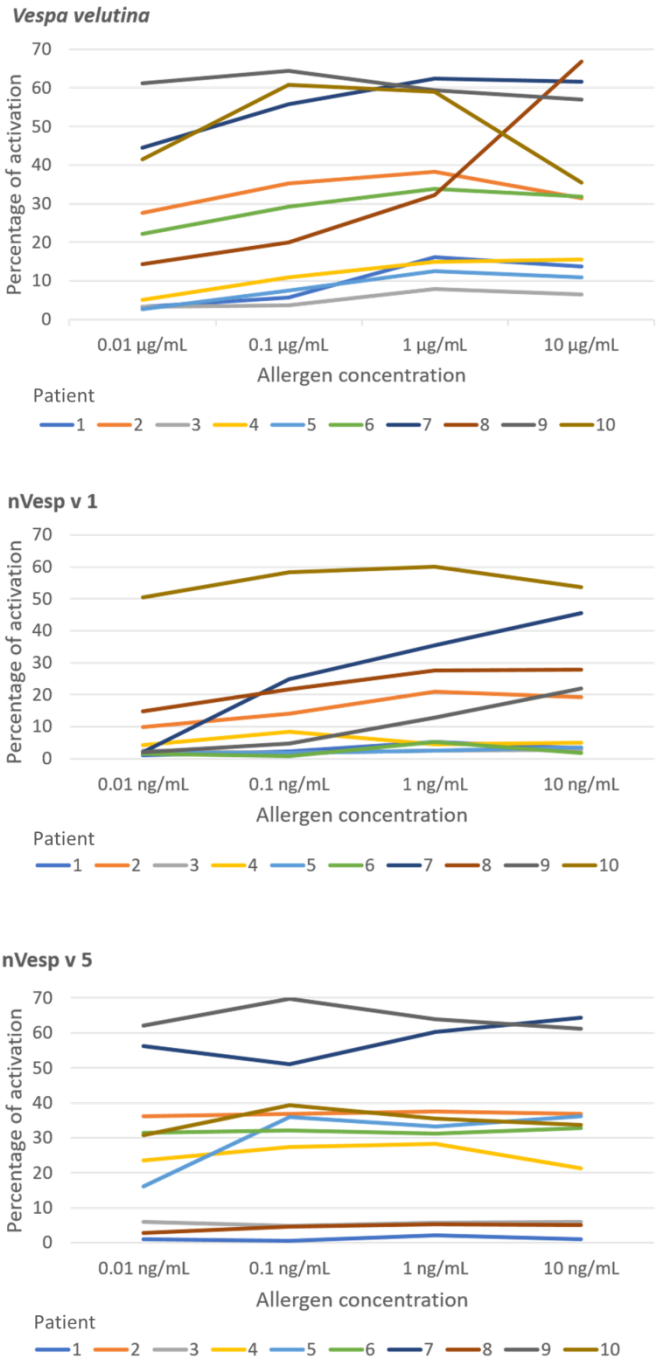
LEGEND FIGURE 1

Representation of the percentage of CD63 expression on basophils after activation with different concentrations of *Vespa velutina nigrithorax* venom, and nVesp v 1 (phospholipase A1), and n Vesp v 5 (antigen 5) molecules in every patient.



Negative control, wash solution. Positive control, N-formyl-Met-Leu-Phe peptide. Positive result when percentage of activation was ≥15%.