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

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Vespa velutina nigrithorax is an alien species originally from Southeast Asia, although it is widely found in other regions of the world [1,2] because of its capacity to develop populous colonies with an effective predator defence and thus produce numerous founders [2]. Cases of beekeepers, farmers, and people living in rural environments who have been wounded or died owing to *Vespa velutina nigrithorax* stings are reported every year in Asia and Europe [3-5]. Most of these persons die because of the toxic effects of the venom, although *Vespa velutina nigrithorax* can also cause allergic reactions similar to those provoked by other hymenoptera species [6-8]. We previously reported extensive IgE cross-reactivity between *Vespa velutina nigrithorax* and other hymenoptera venoms, particularly *Vespula* species and *Polistes dominula* venoms [7,8]. Inhibition studies performed with *Vespula* species and *Vespa velutina* venoms suggested that *Vespula* species 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 recognized allergens [7,8]. The present study measured 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 the basophil activation test (BAT) as an additional diagnostic method. If consistent results are obtained, this approach could be used for follow-up of venom immunotherapy.

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 patients were men who lived in a rural environment. The anaphylactic event had developed less than 30 minutes after a median of 2 stings (range, 1-13).

We measured serum-specific IgE (sIgE) (ImmunoCAP-250) 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 biotinylation and binding to streptavadin-coated, high-capacity plates (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), which detects 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 a positive control, and the wash solution serves as a 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 cytometry was performed within 3 hours using a FACScan device (Becton-Dickinson Immunocytometry System) and CellQuest software. Five healthy individuals (4 atopic and 1 nonatopic) 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 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 to participate in the study, which was approved by the institutional ethics committee (code 2018/622).

All patients presented a positive sIgE result (≥0.35 kU_A/L) to *Vespa velutina nigrithorax* and Vespula species venom, and 9/10 presented sIgE to *Vespa crabro* and *Polistes dominula* venom. Levels of sIgE to *Vespula* species were higher than levels of sIgE to *Vespa velutina nigrithorax* (see Supplementary Table 1 for more detail). As can be seen in the Figure and Supplementary Table 2, all but 1 patient (#3) presented a positive result in the BAT experiments with at least 1 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 nigrithorax* whole venom, nVesp v 1, and nVesp v 5 were similar. The Spearman rank test revealed no correlations between the maximum percentage of activated basophils and the sIgE concentrations against any tested allergen (r=0.103, p=0.777 for *Vespa velutina nigrithorax* whole venom; r=0.055, p=0.881 for nVesp v 1; and r=0.067, p=0.885 for nVesp v 5) or the severity of the anaphylactic reaction (data not shown). The 5 healthy controls presented negative BAT results (Supplementary Table 3, and image from 1 control 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.

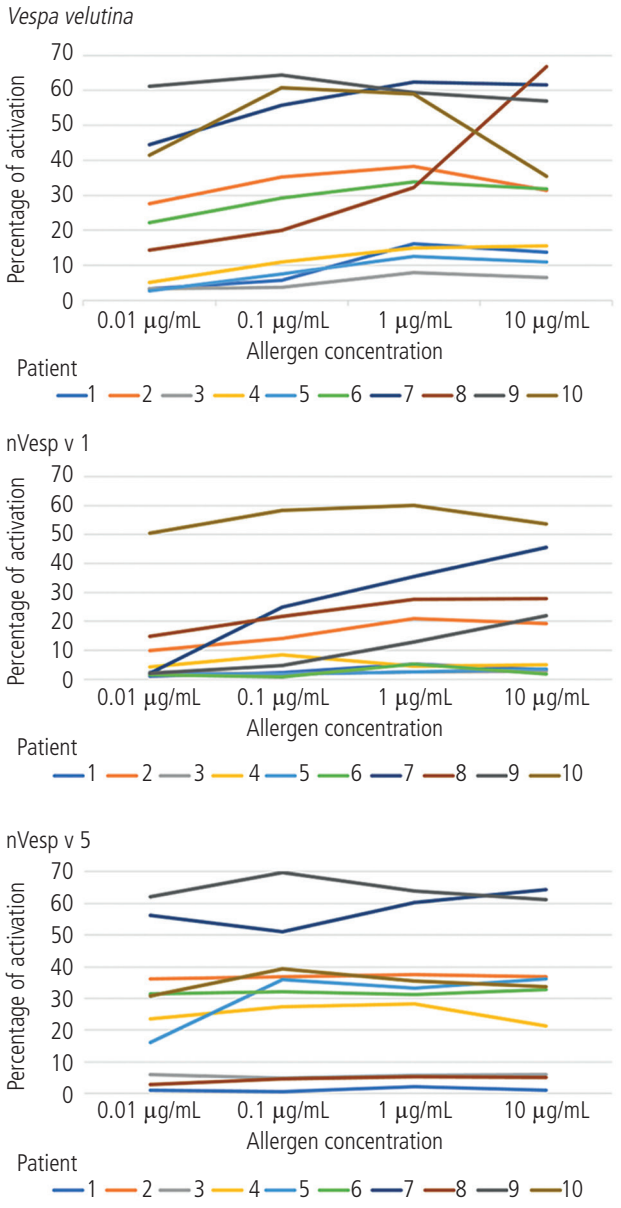

Figure. Representation of the percentage of CD63 expression on basophils after activation with different concentrations of *Vespa velutina nigrithorax* venom, nVesp v 1 (phospholipase A1), and n Vesp v 5 (antigen 5) molecules in each patient. Negative control, wash solution. Positive control, N-formyl-Met-Leu-Phe peptide. The result was considered positive when the percentage of activation was ≥15%.

The negative results from the controls ruled out nonspecific basophil activation. The results of this CD63-based BAT were consistent with the sIgE findings, since all but 1 patient (#3) with positive sIgE to *Vespa velutina nigrithorax* whole venom presented a significant percentage of basophil activation when exposed to *Vespa velutina nigrithorax* 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 nigrithorax*, as demonstrated elsewhere in wasp or bee venom allergens [10].

Regarding the level of concordance between sIgE and dichotomous BAT results (positive vs negative), it seems that *Vespa velutina nigrithorax* whole venom and nVesp v 5 behave more favorably than nVesp v 1. Therefore, considering the commercial availability of *Vespa velutina nigrithorax* whole venom for determination of sIgE and its superior performance to that of the allergenic components tested, this whole venom seems to be a good marker for studying the biological activity of the venom in clinical settings. Moreover, given that BAT has been proposed as an ex vivo method for follow-up venom immunotherapy [8,10-15] and positive BAT responses were obtained in patients clinically diagnosed with anaphylaxis due to *Vespa velutina nigrithorax* venom allergy in our study, BAT could be performed after venom immunotherapy to investigate its potential efficacy in affected patients.

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Conflicts of Interest

Rafael Monsalve is an employee of ALK-Abelló. The remaining authors declare that they have no conflicts of interest.

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