

Anaphylaxis to *Vespa velutina nigrithorax*: pattern of sensitization for an emerging problem in Western countries

Running title: Anaphylaxis to *Vespa velutina nigrithorax*

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ABSTRACT

Objective: To define the sensitization pattern of patients with anaphylaxis to *Vespa velutina nigrithorax* (VVN).

Methods: One-hundred consecutive Spanish patients with Hymenoptera anaphylaxis were studied. We systematically determined specific IgE (sIgE) to whole venoms (*Vespula spp.*, *Polistes dominula*, *Apis mellifera*, *Vespa crabro*, *Dolichovespula maculata*) and their molecular components (rApi m 1, rApi m 5, rApi m 10, rVes v 1, rVes v 5, rPol d 5, cross-reactive carbohydrates). Specific IgE to VVN venom and its antigen-5 (nVesp v 5) were measured in a subsample.

Results: Seventy-seven patients had VVN anaphylaxis. Of these, only 16 (20.8%) reported previous VVN stings but were stung by other Hymenoptera. Positive (>0.35 kU_A/L) sIgE to each of the whole venoms was detected in $>70\%$ of patients (*Vespula spp.* in 100%). Components showing $>50\%$ positivity were rApi m 5 (51.4%), rPol d 5 (80.0%), and rVes v 5 (98.7%). This pattern was similar to that of patients with *Vespula spp.* anaphylaxis (n=11) but different from that of *Apis mellifera* anaphylaxis (n=10). Specific IgE to nVesp v 5 was positive in all studied patients (n=15) with VVN anaphylaxis and was correlated with sIgE to both rVes v 5 (R=0.931) and rPol d 5 (R=0.887).

Conclusions: VVN has become the commonest cause of Hymenoptera anaphylaxis in our area. Most cases report no previous VVN stings. Their sensitization pattern is similar to that of patients with anaphylaxis to other *Vespidae*. Specific IgE to antigen-5 from VVN, *Vespula spp.*, and *Polistes dominula* are strongly correlated in patients with VVN anaphylaxis.

Key words: *Vespa velutina nigrithorax*, Anaphylaxis, Ves v 5, Pol d 5, Vesp v 5, Allergy, Hymenoptera

RESUMEN

Objetivo: Definir el patrón de sensibilización alérgica de pacientes con anafilaxia por *Vespa velutina nigrithorax* (VVN), un problema emergente en países occidentales.

Métodos: Se estudió una población de 100 pacientes adultos con anafilaxia por veneno de himenóptero en España. Se determinó IgE específica frente a los venenos completos de *Vespula spp*, *Polistes dominula*, *Apis mellifera*, *Vespa crabro*, *Dolichovespula maculata*, y algunos de sus componentes moleculares (rApi m 1, rApi m 5, rApi m 10, rVes v 1, rVes v 5, rPol d 5, carbohidratos [MUXF]). En una muestra de 15 pacientes con anafilaxia por VVN se determinó la IgE específica frente a este veneno completo y al antígeno 5 de VVN (denominado nVesp v 5).

Resultados: Setenta y siete pacientes (77.0%) padecieron anafilaxia por VVN. De ellos, tan solo 16 (20.8%) habían padecido picaduras previas por VVN si bien reconocían picaduras previas por otros himenópteros. Más del 70% de los pacientes con anafilaxia por VVN presentaron IgE específica positiva (>0.35 kU_A/L) frente a cada uno de los venenos completos estudiados (el 100% en el caso de *Vespula spp*). Los componentes moleculares reconocidos por la IgE de más del 50% de los pacientes fueron rApi m 5 (51.4%), rPol d 5 (80.0%), y rVes v 5 (98.7%). Este patrón de sensibilización fue similar al de los pacientes con anafilaxia por *Vespula spp* (n=11) y diferente al de los pacientes con anafilaxia por *Apis mellifera* (n=10). La IgE específica frente a nVesp v 5 fue positiva en el 100% de los pacientes con anafilaxia por VVN analizados (15/15). La correlación entre la IgE específica frente a nVesp v 5 y la IgE específica frente a rVes v 5 (R=0.931) y rPol d 5 (R=0.887) fue muy significativa.

Conclusiones: VVN es la principal especie de himenóptero responsable de anafilaxia en la actualidad en nuestra área. El perfil de sensibilización es similar al de los pacientes con anafilaxia por otros Véspidos. La IgE específica frente al antígeno 5 de VVN, *Vespula spp*, y *Polistes dominula* se correlaciona estrechamente en los pacientes con anafilaxia por VVN.

Palabras clave: *Vespa velutina nigrithorax*, Anafilaxia, Ves v 5, Pol d 5, Vesp v 5, Alergia, Hymenoptera

Introduction

Alien species causing biological invasions are recognised as a significant problem nowadays primarily because of the impact in biodiversity but also for provoking new health problems. *Vespa velutina nigrithorax* (VVN), vulgarly known in our country as Asian wasp, is one of the 12 colour variants of *Vespa velutina* Lepeletier 1836, which is naturally distributed in Asia from Afghanistan to eastern China, Indochina and Indonesia [1]. The first record of the presence VVN in Europe was in Lot-et-Garonne (France) in 2005 and it is thought to arrive there in 2004 [2]. This unexpected invasion seems to have happened due to an accidental displacement of, at least, one hibernating founder queen allocated in potteries or other horticultural (bonsai tree) trade products from China [3,4]. From that moment onwards, VVN has spread rapidly across France [5-7] and the Basque Country in the North of Spain [8]. In 2011, Villemant et al. published an interesting model predicting the future invasion of VVN. That model predicted that many countries of western Europe had a high probability of being invaded, the higher risk being along the Atlantic and northern Mediterranean coasts [7]. Coastal areas of the Balkan Peninsula, Turkey and Near East appeared also suitable and could potentially be colonized later [7]. Other parts of the world that show high climatic suitability for this species may also be potentially threatened by VVN since the scenario of introduction through international trade could happen again [7]. This prediction is now a reality since VVN has spread into Spain (particular in the north and northwestern areas, information available at <http://webs-gis.cesga.es/velutina/>), Portugal, Italy, and the UK [4,9,10]. In Galicia, a region in the northwest of Spain, more than 47394 nests have been identified and 24196 retrieved and destroyed in 2018 (compared to 769 in 2014)

(http://mediorural.xunta.gal/es/areas/ganaderia/apicultura/vespa_velutina/).

Vespa velutina is one of the most aggressive and fearful Hymenoptera species in China where it is known as killer-wasp because it causes a number of deaths every year [11]. Most of these subjects die after multiple stings because of multiple organ dysfunction induced toxins that are present in the venom [11,12]. Apart from these toxic effects, *Vespa velutina* can provoke allergic reactions similar to those provoked by other Hymenoptera species. In fact, up to six deaths after VVN stings due to presumably fatal allergic reactions have been reported by mass media in Spain in 2017 and 2018 (five of them in the Northwestern). For that reason, the population of the north and northwestern of Spain has been alerted against the presence of VVN and specific political campaigns have been activated to fight against the invasion. The first well-identified case of anaphylaxis due to VVN in Spain was reported in 2014 [13]. The first patient with anaphylaxis to VVN in our area was detected in June 2015. Two additional cases

were seen in 2016 and 9 in 2017. This increasing tendency of anaphylaxis to VVN led us to initiate the present study because of the lack of commercially available assays for identifying specific IgE (sIgE) against VVN or its components. The aim of this study was to identify the sensitization profile of patients suffering from anaphylaxis due to VVN in comparison to the profile of patients suffering from anaphylaxis to other Hymenoptera by using commercially available sIgE determinations against both the whole venom and molecular components of *Vespula spp*, *Polistes dominula*, *Apis mellifera*, *Vespa crabro* and *Dolichovespula maculata*. Besides, we aimed to investigate the usefulness of a customized VVN-sIgE determination by binding biotin to the whole venom extract and to its purified components, phospholipase and antigen 5 (namely Vesp v 1 and Vesp v 5).

Patients and methods

Study population and design

This is a cross-sectional study which prospectively enrolled all adult individuals with anaphylaxis due to Hymenoptera venom who attended for the first time our Allergy Department at a reference University Hospital in NW Spain from December 2017 to June 2019. Patients were submitted from either Primary Care or the Emergency Department after suffering an anaphylactic reaction due to a Hymenoptera sting. Patients with large local reactions were not included in the study. The hospital covers an area of approximately 500,000 people; nearly 90,000 live in the city of Santiago de Compostela, and the remainder lives in primarily rural areas. All eligible patients (n=100) accepted to participate. The median age was 63 years (range, 20-90 years) and 87 (87.0%) were males. The majority of the patients (97, 97%) lived in a rural environment, and 57 (57%) worked outdoors so they could be considered at high risk for exposure.

A physician-administered structured questionnaire was filled in for every patient including data on: a) identification of the Hymenoptera species involved in the reaction (bees, common wasps and VVN); b) recall of previous Hymenoptera stings (bees, common wasps and VVN); c) history of previous systemic reactions after Hymenoptera stings; d) time elapsed between exposure to the Hymenoptera and when symptoms and signs first appeared; e) signs and symptoms reported by the patient; f) signs and symptoms reported by doctors who attended patients after the reaction; and g) history of Hymenoptera stings after the reported reaction and before entering the study. The severity of the systemic reaction was classified with the information recorded and divided into 3 categories: mild (skin and subcutaneous

lesions plus nonspecific general symptoms), moderate (features suggesting respiratory, cardiovascular, or gastrointestinal involvement) or severe (hypoxia, hypotension, or neurologic compromise) [adapted from 14] as it has been accepted for Hymenoptera venom systemic reactions [15]. Information was recorded between 15 and 30 days after the reaction and all laboratory determinations were performed in serum samples obtained in baseline conditions between 1-2 months after the reaction. Finally, and following the recommendations of the European Competence Network on Mastocytosis, we measured the so-called REMA score (acronym for the Spanish Mastocytosis Network) in our patients [16] to calculate the risk of suffering from a clonal mast cell disorder that could increase the severity of the reaction .

Laboratory determinations

Commercially available specific IgE

Allergen sIgE was measured using the ImmunoCAP-250™ system (Thermo Fisher Scientific™) and included sIgE against *Vespula spp*, *Polistes dominula*, *Apis mellifera*, *Vespa crabro*, *Dolichovespula maculata*, rApi m 1, rApi m 5, rApi m 10, rVes v 1, rVes v 5, rPol d 5, and MUXF (o214) as a CCD marker. Following the manufacturer's instructions, sIgE levels ≥ 0.1 kU_A/L were deemed positive, although analyses were performed with the classic 0.35 kU_A/L threshold level for positivity.

Customized specific IgE

In a subsample of 15 patients with anaphylaxis to VVN, sIgE to VVN-whole venom extract and to VVN phospholipase and antigen 5 (nVesp v 1 and nVesp v 5, respectively) were measured. The nVesp v 1 and 5 components, were purified from an extract of VVN venom (ALK-Source Materials) by successive chromatographic steps [17], in a similar procedure than that used for purifying the allergenic components of *Vespula spp* and *Polistes dominula* [18]. VVN whole venom extract, nVesp v 1 and nVesp v 5 proteins were biotinylated [19] and bound to the high-capacity immunosorbent coupled to Streptavidin (o212, Thermo Fisher Scientific, Inc.), and subsequently used as specific reagents in the ImmunoCAP-250 platform.

Serum total IgE

Total IgE was measured in serum samples by using a chemiluminescence immunoassay (CLIA) in a Centaur XP System analyzer (Siemens™).

Serum tryptase

Serum tryptase was determined in serum samples with the ImmunoCAP 250 tryptase assay™ (Thermo Fisher Scientific™).

Statistical analyses

The Chi-squared test (with continuity correction and analysis of trend, when needed) was used to compare proportions. The Mann-Whitney test was used to compare numerical variables between groups. The Jonckheere-Terpstra test was used to analyze the trend of numerical variables in relation to ordinal categories. The Pearson test was used to assess correlation. Linear regression was used to predict the value of a dependent variable (VVN-sIgE) based upon the values independent variables (commercially available sIgE to related allergens).

Ethics

The study was approved by the Institutional Ethics Committee and complied with the recommendations of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Results

Clinical data

Seventy-seven patients (77.0%) identified VVN as the culprit insect for the reaction. Only 11 (11.0%) and 10 (10.0%) patients identified the common wasp (*Vespula spp*) and honeybee (*Apis mellifera*) as the culprit insect, respectively. Two patients (2%) did not recognize the insect involved in the reaction.

All patients with anaphylaxis to *Vespula spp* and *Apis mellifera* reported previous stings by the culprit insect. In contrast, only 20.8% (16/77) of patients with anaphylaxis to VVN reported a previous sting by this species. However, all patients with anaphylaxis to VVN reported previous stings by either *Vespula spp* (75/77, 97.4%) or *Apis mellifera* (41/77, 53.2%). Twenty-three patients reported past episodes of anaphylaxis before the present attack even though they had not been sent for evaluation (14 episodes related to *Vespula spp*, 5 to VVN and 4 to *Apis mellifera*). No additional stings after the last systemic reaction and before the inclusion in the study were recorded. No differences were found regarding the culprit insect with respect to age, sex, environment (rural or urban), or occupation (outdoors or indoors) (Table 1).

The majority of the reactions happened in the first 15 minutes after the sting with no differences regarding the culprit insect (Table 1). The anaphylactic reaction was mild in 17 cases (17%), moderate in 45 (45%) and severe in 38 (38%) cases. Severity of anaphylaxis to VVN was similar to that of anaphylaxis to *Vespula spp* or *Apis mellifera* (Table 1). Adrenaline was used in 48 patients (48%). The more severity of the reaction, the more frequent the use of adrenaline (17.6% in mild reactions; 44.4% in moderate reactions, and 65.8% in severe reactions; $p < 0.001$, trend test). Baseline serum tryptase levels (available in all patients) tended to increase in relation with the severity of the anaphylactic reaction. Median and (IQR) tryptase was 3.9 ng/mL (3.6-5.4 ng/mL), 4.5 ng/mL (3.5-5.9 ng/mL) and 5.4 ng/mL (4.3-6.7 ng/mL), in patients with mild, moderate and severe reaction, respectively ($p = 0.015$, trend test). However, serum tryptase levels were similar in patients with anaphylaxis to VVN, *Vespula spp* and *Apis mellifera* (Table 2). REMA score was lower than 2 in 98 patients. Only two patients, who belonged to the VVN group, presented a calculated REMA higher than 2 but the haematological study performed let us rule out the presence of a clonal mast cell disorder.

Sensitization profile

Commercially available specific IgE determinations

Specific IgE against *Vespula spp* was available in 76/77 patients with anaphylaxis to VVN and all of them showed a positive result (> 0.35 kU_A/L). The majority ($> 70\%$) of patients with anaphylaxis to VVN also showed positive sIgE to the whole venom extract of *Dolichovespula maculata*, *Polistes dominula*, *Vespa crabro*, and *Apis mellifera*, in that order (Figure 1). Regarding molecular components, patients with anaphylaxis to VVN showed sIgE positivity to *Vespula spp* rVes v 5 more frequently than to rVes v 1. Specific IgE to *Polistes dominula* rPol d 5 was present in the majority of patients with anaphylaxis to VVN. The only molecular component from *Apis mellifera* showing a sIgE positivity rate $> 50\%$ in patients with anaphylaxis to VVN was rApi m 5 (Figure 1).

Patients with anaphylaxis due to VVN had similar levels of sIgE against *Vespula spp*, *Polistes dominula*, rVes v1, rVes v 5, rPol d 5, *Dolichovespula maculata* and *Vespa crabro* than patients with anaphylaxis due to *Vespula spp*. In contrast, patients with anaphylaxis to VVN have lower levels of sIgE against *Apis mellifera* and its main allergen rApi m 1 than patients with anaphylaxis to *Apis mellifera*. Of note, sIgE levels to rApi m 5, rApi m 10, rVes v 1 and to the CCD marker (MUXF, o214) were similar in all patients irrespectively the culprit insect (Table 2).

Patients with anaphylaxis to *Vespula spp* presented higher levels of total IgE than patients with anaphylaxis to *VVN* or *Apis mellifera* (Table 2). When concentrations of venom-specific IgE were considered as the ratio to total IgE, a similar profile was observed (i.e., patients with anaphylaxis to *VVN* displayed a pattern of sIgE sensitization similar to that of patients with anaphylaxis to *Vespula spp* and different from that of patients with anaphylaxis to *Apis mellifera* (Table 3).

Customized specific IgE determinations

In a subsample of 15 patients with anaphylaxis to *VVN*, 13 (86.6%) patients showed positive (>0.35 kU_A/L) sIgE against a whole venom extract of *VVN*. Levels of sIgE to *VVN* were low (median 1.1 kU_A/L, [IQR] 0.12-20.5 kU_A/L). Using the same methodology, 100% of these patients presented positive sIgE to nVesp v 5 while only 28.8% presented positive sIgE levels to nVesp v 1. Levels of sIgE to nVesp v 5 (median 2.71, [IQR] 0.52-37.2 kU_A/L, -n=15-) tended to be higher than those of sIgE to nVesp v 1 (median 0.17 kU_A/L, [IQR] 0.99-27.1 kU_A/L, -n=14-) and also tended to be higher than those of sIgE to whole venom extract of *VVN* (median 2.71, [IQR] 0.52-37.2 kU_A/L, n=15). A significant correlation was found between sIgE to *VVN* and sIgE to *Vespula spp* (R=0.669, p=0.009), *Polistes dominula* (R=0.620, p=0.014), *maculata* (R=0.973, p<0.001) and *Vespa crabro* (R=0.968, p<0.001). There was also strong correlation between sIgE to nVesp v 5 and sIgE to rVes v 5 (R=0.931, p<0.001) and between sIgE to nVesp v 5 and rPol d 5 (R=0.887, p<0.001). Figure 2 shows a scatterplot of nVesp v5 in relation to rVes v5 and rPol d 5 and linear regression parameters to predict the level of sIgE to nVesp v5 given the values of rVes v 5 and rPol d 5. Finally, no significant correlation was found between sIgE to nVesp v 1 and sIgE to rVes v 5 and rVes v 1 (R=0.429, p=0.143, R= -0.002, p=0.993).

Discussion

The present study shows that: (a) Anaphylaxis to *VVN* has quickly increased in recent years in our area, where it represents the most common form of Hymenoptera anaphylaxis nowadays; (b) The majority of patients with anaphylaxis to *VVN* do not report previous stings by *VVN*, but they report previous stings to other Hymenoptera, specially common wasps (*Vespula spp*); (c) The profile of IgE sensitization of patients with anaphylaxis to *VVN* is similar to that of patients with anaphylaxis to other *Vespidae*; and (d) Most patients with *VVN* anaphylaxis show sIgE to antigen 5 from *Vespula spp* (rVes v 5) and antigen 5 from *Polistes dominula* (rPol d 5), which is

strongly correlated with sIgE to antigen 5 from VVN (n Vesp v 5) in the same patients. Taken together, these findings suggest a degree of cross reactivity between VVN and other *Vespidae*, which may be important for sensitization and relevant for diagnostic and therapeutic purposes.

Our data confirm VVN anaphylaxis as a significant emerging problem. The number of patients reporting anaphylaxis due to VVN has exponentially increased from the first case in 2015 in our area. The number of incident cases of anaphylaxis to other Hymenoptera remained stable in those years, but nowadays more than three-quarters of incoming patients reporting Hymenoptera anaphylaxis identify VVN as the culprit insect. As an example and considering the number of patients receiving venom immunotherapy, while 60.3% of 126 patients were being treated with *Apis mellifera* venom in 2015, 68.2% of 245 were being treated with *Vespula spp* venom in June 2019 (the majority of them after suffering anaphylaxis due to VVN). To the best of our knowledge, this is the largest series of VVN anaphylaxis that has been reported in the English literature. This paucity of previous reports is noteworthy. From originary countries in Asia, we found scientific reports of neither anaphylaxis to VVN nor anaphylaxis to *Vespa velutina* variants. From European countries where VVN has expanded, we found only one case of VVN anaphylaxis in the literature, corresponding to the first case described in 2014 in Spain [13]. Cases of VVN anaphylaxis are commonly seen in mass media in our country, but underreporting of epidemiological, clinical, and immunological characteristics of VVN anaphylaxis prompted us to describe this series in an attempt to preliminary fill that gap for an emerging, relevant clinical problem.

Importantly, the majority of our patients with anaphylaxis to VVN did not recall previous stings from this insect. This fact, together with the results of sIgE determinations, support the idea that allergic reactions to VVN may develop after having been stung by other Hymenoptera, mainly *Vespula spp*, in our patients. It could also explain why patients reported here more frequently reacted against *Vespula spp* than against *Vespa crabro* a nearer specie to VVN than *Vespula spp*. Likewise, cases of anaphylaxis to *Vespa orientalis* (a different member of the *Vespidae* family) without previous stings by this insect were reported in USA soldiers deployed in Afghanistan [20]. The existence of cross-reactivity among allergens from different *Vespidae* could explain these reactions [18,21-23]. Specific reasons for a potentially increased susceptibility to VVN anaphylaxis after sensitization via different Hymenoptera species are not known.

Most of the anaphylactic reactions to *VVN* were moderate to severe. The proportion of cases with moderate-to-severe reactions was similar to that of reactions to *Vespula spp* and *Apis mellifera* in this series of patients with Hymenoptera anaphylaxis. Our data cannot answer the question of a potentially more frequent anaphylaxis after *VVN* sting than after sting by other Hymenoptera species. Taken into account the size of *VVN*, a more severe reaction could be expected, as it happens with *Vespa crabro* sting, which is known to induce three times more life-threatening reactions than *Vespula spp* or *Apis mellifera* stings [22]. Of note, 5 deaths after a *VVN* sting were reported during years 2017 and 2018 in Galicia region in Spain with an approximate population of 2.7 million. Thus, the number of deaths attributable to *VVN* was higher than expected for the general population according to UK national databases that sets the risk of death after Hymenoptera stings at 0.09 cases per million inhabitants per year (95% confidence interval 0.07-0.10) [24]. This further supports the clinical importance of emerging *VVN* anaphylaxis.

The lack of specific tools to diagnose and treat allergic patients to *VVN* is a challenge for the physician who has to choose the best option to treat these patients. In the studied population, all patients with anaphylaxis to *VVN* presented sIgE to *Vespula spp* and almost all of them presented sIgE to rVes v 5 and also to rPol d 5. Besides, we could confirm the presence of sIgE to the antigen 5 from *VVN* by using the novel allergen nVesp v 5 in all cases from a subsample of patients with anaphylaxis to *VVN*. Of note, sIgEs to antigen 5 from *Vespula spp*, *Polistes dominula*, and *VVN* were closely correlated in patients with *VVN* anaphylaxis. Hence, antigen 5 from *VVN* appears to be a good candidate as major allergen. On the contrary a poorer diagnostic value was depicted for nVesp v 1. In addition, sIgE to rApi m 5 (dipeptidylpeptidase IV homologous to Ves v 3 from *Vespula spp*) [25] was detected in more than 50% of patients with anaphylaxis to *VVN* so it could be also considered a major allergen, as well. The fact that more than 70% of the studied patients with anaphylaxis to *VVN* reacted against all the venoms used in the study suggest a high level of cross-reactivity that cannot be explained though CCD sensitization. So, further studies are needed to identify other relevant allergens in *VVN* venom that likely would react to other venoms as well.

Analogies in the profile of sensitization in patients with anaphylaxis to *VVN* and *Vespula spp* led us to systematically use a commercially available *Vespula spp*. immunotherapy in patients with *VVN* anaphylaxis (data not shown). Up to date, only five patients with anaphylaxis due to *VVN* and treated with *Vespula spp*. venom were stung by *VVN* after initiating immunotherapy and none presented systemic reactions (data not shown). Likewise, immunotherapy with *Vespula spp*. in patients with anaphylaxis to *Vespa orientalis* has proven

to be efficacious [23]. Similarly, immunotherapy with *Vespula* spp. was efficacious in patients with anaphylaxis to *Vespa crabro* [26]. Further studies are needed to develop a specific immunotherapy for VVN and a longer follow-up is needed to support the efficacy of immunotherapy initiated in our patients. Meanwhile, commercially available *Vespula* spp immunotherapy seems to be a wise option in patients with VVN anaphylaxis.

The study has limitations that should be acknowledged. First, the cause of anaphylaxis was classified according to the identification of the culprit insect provided by patients because samples of the insects were not available. This problem of classification is always present when dealing with anaphylaxis due to Hymenoptera species. However, the fact that almost all patients live or work in a rural environment where they are used to recognizing Hymenoptera decreases the risk of misidentification. Besides, VVN has become very popular in our region after its proliferation, therefore, photographs of them and their nests are frequently displayed in local newspapers and on television. Only two patients did not identify the culprit insect because they could not see them. Second, determinations of sIgE to VVN venom and nVesp v 5 were available for only a small subsample of patients; therefore, findings should be confirmed in larger studies. Finally, as already mentioned, further molecular and follow-up studies are needed to add information to the profile and outcome of patients that cannot be provided from this cross-sectional, preliminary study.

In conclusion, VVN anaphylaxis is a relevant, emerging problem which has quickly become the most common type of Hymenoptera anaphylaxis in our area. Similarities in the profile of sensitization between VVN and other *Vespidae* (particularly, *Vespula spp*) could serve to help in both diagnosis and therapy of VVN anaphylaxis while specific determinations and immunotherapy are not readily available.

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Conflicts of interest:

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The remaining authors have nothing to disclose.

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LEGENDS OF FIGURES

Figure 1. Percentage of patients with anaphylaxis due to *Vespa velutina nigrithorax* with positive (>0.35 kU_A/L) sIgE to whole Hymenoptera venoms (*Vespula spp* [n=76], *Polistes dominula* [n=77], *Apis mellifera* [n=76], *Vespa crabro* [n=75], and *Dolichovespula maculata*. [n=76], left side), and their molecular components (rApi m 1 [n=76], rApi m 5 [n=72], rApi m 10 [n=75], rVes v 1 [n=77], rVes v 5 [n=76], rPol d 5 [n=75], and o214 [MUXF, as a CCD marker, n=77], right side). Every venom and its molecular components are represented with the same color to facilitate identification.

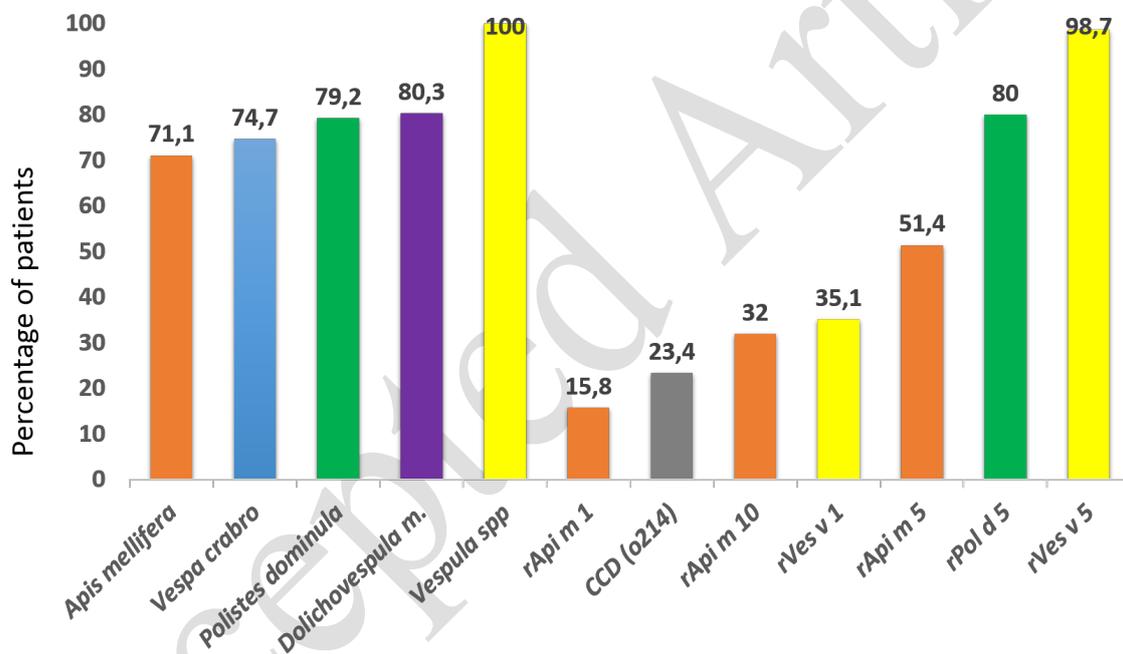


Figure 2. Scatterplots representing the relationship of serum specific IgE to rVes v 5 and rPol d 5 with specific IgE to *Vespa velutina nigrithorax* antigen 5 (nVesp v 5). Linear regression models were developed to predict nVesp v 5 concentrations as a function of rVes v 5 and rPol d 5 concentrations, which explain 86% and 78% of the variability of nVesp v 5, respectively.

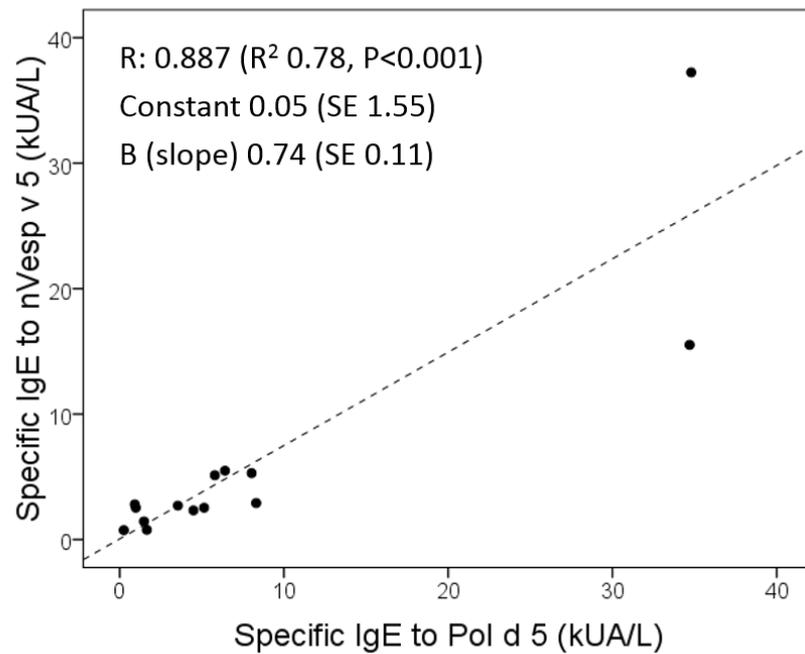
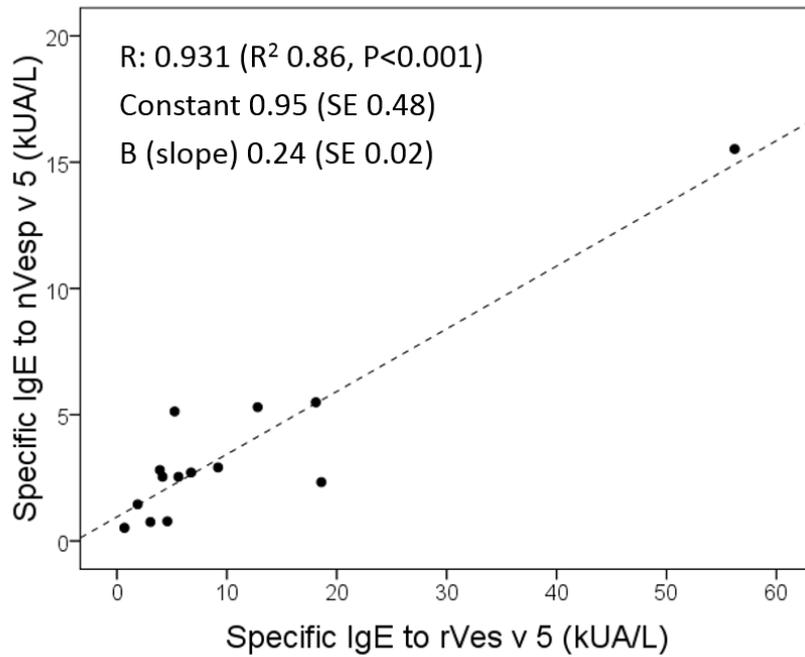


Table 1. Clinical data in patients of the study, stratified by the culprit insect.

| | Culprit insect | | | P-value | |
|---|------------------------------|---|---------------------------------|-----------|-----------|
| | <i>Vespula spp</i> (n=11) | <i>Vespa velutina nigrithorax</i> (n=77) | <i>Apis mellifera</i> (n=10) | VVN vs VV | VVN vs AM |
| Age (years) | 58 (56-65) | 63 (54-69) | 54 (41-68) | 0.618 | 0.348 |
| Sex (male) | 11 (100) | 66 (85.7) | 9 (90.0) | 0.394 | 0.712 |
| History of previous stings | | | | | |
| <i>Vespula spp</i> | 11 (100) | 75 (97.4) | 8 (80.0) | 0.999 | 0.095 |
| <i>Vespa velutina nigrithorax</i> | 2 (18.2) | 16 (20.8) | 0 (0.0) | 0.999 | 0.245 |
| <i>Apis mellifera</i> | 6 (54.5) | 41 (53.2) | 10 (100) | 0.936 | 0.013 |
| Previous systemic reactions | | | | | |
| <i>Vespula spp</i> | 1 (9.1) | 13 (16.9) | 0 (0.0) | 0.826 | 0.349 |
| <i>Vespa velutina nigrithorax</i> | 0 (0.0) | 5 (6.5) | 0 (0.0) | 0.862 | 0.914 |
| <i>Apis mellifera</i> | 0 (0.0) | 0 (0.0) | 3 (30.0) | 0.999 | <0.001 |
| Habitat (rural) | 11 (100) | 75 (97.4) | 9 (90.0) | 0.999 | 0.775 |
| Occupational exposure (outdoors) | 8 (72.7) | 43 (55.8) | 5 (50.0) | 0.463 | 0.991 |
| Grade of anaphylaxis | | | | | |
| Grade I | 3 (27.3) | 12 (15.6) | 2 (20.0) | 0.628 | 0.419 |
| Grade II | 4 (36.4) | 33 (42.9) | 6 (60.0) | | |
| Grade III | 4 (36.4) | 32 (41.6) | 2 (20.0) | | |
| Elapsed time to reaction | | | | | |
| Less than 15 minutes | 7 (63.6) | 61 (79.2) | 7 (70.0) | 0.514 | 0.431 |
| 15-30 minutes | 3 (27.3) | 12 (15.6) | 3 (30.0) | | |
| More than 30 minutes | 1 (9.1) | 4 (5.2) | 0 (0.0) | | |
| Use of adrenaline | 5 (45.5) | 37 (48.1) | 5 (50.0) | 0.872 | 0.908 |

Age data are median and interquartile range (within parenthesis). The remainder are absolute numbers and percentages (within parenthesis).

VVN vs VV: comparison of *Vespa velutina nigrithorax* with *Vespula spp*. VVN vs AM: comparison of *Vespa velutina nigrithorax* with *Apis mellifera*.

Table 2. Laboratory data in patients of the study, stratified by the culprit insect.

| | Culprit insect | | | | | | P-value | |
|--|--------------------|------------------------|-----------------------------------|------------------------|-----------------------|------------------------|-----------|-----------|
| | <i>Vespula spp</i> | | <i>Vespa velutina nigrithorax</i> | | <i>Apis mellifera</i> | | | |
| | n | Median and (IQR range) | n | Median and (IQR range) | n | Median and (IQR range) | VVN vs VV | VVN vs AM |
| Serum specific IgE (kU_A/L) | | | | | | | | |
| MUXF | 11 | 0.20 (0.00-0.92) | 77 | 0.05 (0.00-0.34) | 10 | 0.09 (0.00-1.55) | 0.362 | 0.562 |
| <i>Apis mellifera</i> | 11 | 1.12 (0.31-3.79) | 76 | 0.91 (0.22-3.67) | 10 | 7.48 (2.88-27.4) | 0.655 | <0.001 |
| rApi m 1 | 10 | 0.07 (0.00-0.35) | 76 | 0.01 (0.00-0.12) | 10 | 2.45 (1.50-7.79) | 0.332 | <0.001 |
| rApi m 5 | 10 | 1.17 (0.11-10.2) | 72 | 0.50 (0.00-3.93) | 9 | 1.15 (0.02-15.4) | 0.432 | 0.710 |
| rApi m 10 | 10 | 0.04 (0.01-0.07) | 75 | 0.03 (0.00-0.86) | 10 | 0.31 (0.04-2.25) | 0.802 | 0.053 |
| <i>Vespula spp</i> | 11 | 14.0 (8.51-57.3) | 76 | 5.38 (2.03-14.2) | 9 | 0.08 (0.00-7.23) | 0.019 | 0.007 |
| rVes v 1 | 11 | 0.07 (0.03-5.98) | 77 | 0.15 (0.03-1.44) | 9 | 0.00 (0.00-1.29) | 0.870 | 0.820 |
| rVes v 5 | 11 | 12.4 (0.52-65.0) | 76 | 4.45 (1.48-12.3) | 9 | 0.00 (0.00-0.75) | 0.322 | <0.001 |
| <i>Polistes dominula</i> | 11 | 4.22 (2.95-36.1) | 77 | 1.54 (0.41-6.72) | 9 | 0.06 (0.00-2.43) | 0.060 | 0.024 |
| rPol d 5 | 11 | 6.89 (0.85-15.7) | 75 | 1.65 (0.50-8.31) | 9 | 0.01 (0.00-0.57) | 0.151 | 0.001 |
| <i>Dolichovespula m.</i> | 7 | 4.78 (1.10-13.5) | 76 | 1.34 (0.42-4.05) | 8 | 0.15 (0.00-8.85) | 0.046 | 0.185 |
| <i>Vespa crabro</i> | 7 | 2.98 (0.98-3.79) | 75 | 0.93 (0.34-3.34) | 9 | 0.02 (0.00-8.48) | 0.137 | 0.073 |
| Serum total IgE (kU/L) | 11 | 189 (126-264) | 76 | 78.5 (33.0-198) | 10 | 45.0 (16.2-182) | 0.017 | 0.247 |
| Serum tryptase (ng/mL) | 11 | 4.3 (3.5-5.2) | 77 | 5.1 (3.9-6.4) | 10 | 4.5 (4.1-6.3) | 0.304 | 0.868 |

n, number of patients with available determinations. IQR, interquartile range.

MUXF (o214), marker of cross-reactive carbohydrate determinant. *Dolichovespula m*, *Dolichovespula maculata*.

VVN vs VV: comparison of *Vespa velutina nigrithorax* with *Vespula spp*. VVN vs AM: comparison of *Vespa velutina nigrithorax* with *Apis mellifera*.

Table 3. Ratio of serum specific IgE to serum total IgE in patients of the study, stratified by the culprit insect.

| | Culprit insect | | | | | | P-value | |
|---|--------------------|------------------------|-----------------------------------|------------------------|-----------------------|------------------------|-----------|-----------|
| | <i>Vespula spp</i> | | <i>Vespa velutina nigrithorax</i> | | <i>Apis mellifera</i> | | | |
| Ratio of serum specific IgE (kU _A /L) to total IgE (kU/L) (x100) | n | Median and (IQR range) | n | Median and (IQR range) | n | Median and (IQR range) | VVN vs VV | VVN vs AM |
| <i>Apis mellifera</i> | 11 | 0.51 (0.34-1.43) | 76 | 1.07 (0.31-2.92) | 10 | 16.7 (12.3-38.3) | 0.358 | <0.001 |
| <i>Vespula spp</i> | 11 | 8.45 (5.93-21.0) | 75 | 5.54 (3.12-15.7) | 9 | 0.22 (0.0-1.64) | 0.419 | <0.001 |
| <i>Polistes dominula</i> | 11 | 3.27 (1.01-13.8) | 76 | 2.02 (0.84-5.63) | 9 | 0.18 (0.0-4.16) | 0.319 | 0.040 |
| <i>Vespa crabro</i> | 7 | 1.12 (0.58-3.46) | 74 | 1.91 (0.59-3.35) | 9 | 0.11 (0.0-1.81) | 0.814 | 0.043 |
| <i>Dolichovespula maculata</i> | 7 | 1.83 (1.25-7.11) | 75 | 1.97 (0.85-3.35) | 8 | 0.83 (0.0-2.52) | 0.578 | 0.112 |

n, number of patients with available determination. IQR, interquartile range.

VVN vs VV: comparison of *Vespa velutina nigrithorax* with *Vespula spp*. VVN vs AM: comparison of *Vespa velutina nigrithorax* with *Apis mellifera*.