ORIGINAL ARTICLE

In vitro testing for the diagnosis of polysensitization to vespids: CAP inhibition

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Summary

Background. In Southern Europe countries sensitization to both *Vespula* spp. and *Polistes* spp. can be observed in 50.5 to 61.5% of anaphylactic sting reactions, hindering the distinction between vespid cross-reactivity and genuine sensitization. CAP-inhibition is regarded as the reference method in such cases. **Methods.** Retrospective study, including candidates to VIT with double sensitization (skin testing, specific IgE to whole venom extracts and to molecular components) to at least two different vespid venoms (*Vespula* spp., *Polistes* spp. and/or *Vespa velutina).* CAP-inhibition was done in all patients. **Results.** A total of 26 patients were identified (16 double sensitizations to both *Vespula* spp. and *Polistes* spp., and 10 sensitizations to all three venoms). After CAP-inhibition, VIT decision was changed in 57.7% (15/26) of cases and maintained in the remaining 11 patients (42.3%). Overall, triple VIT was changed to single VIT in 1 patient, from double VIT to single VIT in 9 cases, and from single VIT to double VIT in 3 patients. In single VIT, venom type was changed in the remaining 2 patients after CAP-inhibition. **Conclusions.** Our findings support the usefulness of CAP-inhibition in clarifying multiple positivity to vespid venoms when conventional diagnosis fails to distinguish cross-reactivity from genuine sensitization. CAP-inhibition may help to prevent costs of prolonged or unnecessary double-VIT treatment, as well as the risks of undertreatment.

Impact statement

CAP-inhibition is useful to VIT selection in patients with double sensitizations to vespid venoms, confirming genuine double sensitizations.

KEY WORDS

CAP-inhibition; cross-reactivity; *Vespula* spp.; *Polistes* spp.; *Vespa velutina*.

Introduction

In Southern European countries, sensitization to both *Vespula* spp. and *Polistes* spp. can be observed in 50.5 to 61.5% of anaphylactic sting reactions (1-4). Adding to the diagnostic complexity over the last decade is the emergence of anaphylaxis due to *Vespa velutina nigrithorax* (VVN) stings. Moreover, this problem is further enhanced by the difficulty in distinguishing between insects responsible for stings. In a previously published study, approximately 50% of patients could not distinguish paper wasps and nearly one-third could not identify wasps (5). In Portugal, *Hymenoptera* venom (HV) allergy is one of the principal aetiologies of anaphylactic reactions (7.4%), with *Vespula* spp. and *Polistes dominula* representing 23% and 9%, respectively, of global sensitizations (6).

Hymenoptera allergy is determined according to a consistent clinical history, backed up with IgE confirmed sensitization, either performed through skin testing (ST), *in vitro* measurement of whole extracts serum specific IgE (sIgE) to *Vespula* spp., *Polistes* spp. and *Vespa velutina nigrithorax* (VVN) and/or their recombinant molecular components (rVes v1, rVes v5 and rPol d5) (7). The sIgE to VVN is only available as research use only (RUO) in our country. This, all together with the lack of a specific molecular sIgE makes the confirmation of a primary sensitization more difficult. Distinguishing cross-reactivity from primary sensitization is essential to establish a correct treatment.

The introduction of component-resolved diagnostic (CRD) has improved diagnostic precision in vespid allergic patients. However, the high level of cross-reactivity documented between venoms of different vespid species is explained by the high similarity of their allergens, including phospholipase A1 (group 1), antigen 5 (group 5) and dipeptidyl peptidase IV (DPPIV) (group 3) (8-10).

Recently, the use of sIgE to whole venom extract ratios between insects have been proposed to further enhance diagnosis. Some authors have suggested a relevant cut-off of >2.68 for vespid/bees and >5.34 for bee/vespid, but conclusive results have not yet been achieved (11).

Moreover, sIgE to whole venom extract ratios between vespid species have not yet been determined.

Recent publications have proposed the use of rVes v5/rPol d5 or rPol d5/rVes v5 ratio as an alternative method to determine genuine sensitization, as values >2 can be indicative of primary sensitization (12-14). However, some advocate that this ratio has low accuracy and poor agreement with CAP-inhibition (12).

In the presence of unclear polysensitization, complementary *in vitro* diagnostic techniques, such as CAP-inhibition or basophil activation test (BAT), could enhance the diagnostic accuracy (12,13, 15-17). CAP-inhibition technique is considered the reference method in polysensitized patients to Hymenoptera stings (12,13,15,16). This method has demonstrated high accuracy in identifying genuine sensitizations (12). In indeterminate results, basophil activation test (BAT) can be used as an alternative method, but it is more expensive and time-consuming, as suggested in the literature (17).

The objective of this study was to identify, in a cohort of polysensitized patients to vespid venoms, the role of CAP-inhibition test in distinguishing between polysensitization and cross-reactivity, in relation to sIgE measurements.

Materials and Methods

Study Population

We conducted a retrospective study including 26 patients (candidates for venom immunotherapy (VIT) with a clinical history of systemic reaction (SR) and double sensitization to at least two different vespid venoms, including *Vespula* spp., *Polistes* spp. and/or *Vespa velutina.* Double sensitization was defined by ST positivity at same concentrations for at least two of the three tested vespid venoms.

Data regarding age, gender, concomitant allergic diseases, hobbies and occupation was collected from the clinical records. The severity of SR was classified based on the Mueller Classification (Grade I-IV) (18).

The study followed the recommendations of the Ethics Committee (approval nº 277/24) and of the World Medical Association (Declaration of Helsinki revised in 2013) and informed consent was obtained from patients.

Allergy study

ST was performed in all patients according to the European Academy of Allergy and Clinical Immunology/European Network recommendations (19). Skin prick tests (SPT) were

conducted with standardized, purified, and lyophilized venom preparations (Bial-Aristegui/Roxall®) at a concentration of 100 µg/mL, and intradermal tests (IDT) with diluted venom preparations at 0.001 µg/mL, 0.01 µg/mL, 0.1 µg/mL and 1 µg/mL. A positive (histamine 0.1% solution) and negative control (normal saline solution) were used as controls for SPT. For IDT a negative control with normal saline solution was used. If a clinical history of *Vespa velutina* stings was identified, this venom was added to the evaluation.

In all patients sIgE for *Vespula* spp. and *Polistes* spp. were determined. Additionally, sIgE to *Vespa velutina* was determined whenever patients had history of velutina stings. In 24 patients we also performed measurement of sIgE values to rVes v1, rVes v5 and rPol d5. Total serum IgE and baseline serum tryptase were also determined. CAP-inhibition was carried out for all patients after testing for cross-reactive carbohydrate determinant (CCD) (20). Even though we determined sIgE to Ves v1, we did not have the possibility to measure sIgE to rPol d1. At the time of the study, we were unable to measure sIgE to rApi m5 as a marker of sensitization to vespid group 3 allergens (DPPIV).

Quantification of specific IgE levels from serum samples to whole extract of *Vespa velutina* (sIgE-U1223, ThermoFisher Scientific®), *Vespula* spp. (sIgE-i3, ThermoFisher Scientific®) and *Polistes* spp. (sIgE-i4, ThermoFisher Scientific®), and recombinant molecular components for wasp and paper wasp venom, rVes v1 (phospholipase A1), rVes v5 (antigen 5) and rPol d5 (antigen 5) were determined by fluorescent enzyme immunoassay in a ImmunoCAP Phadia 200 analyser (ThermoFisher Scientific®, Uppsala, Sweden). All results > 0.35 kUA/L were considered positive. All measurements were performed on serum samples frozen prior to the initiation of VIT. Basal serum tryptase was also collected and values < 11.4 ng/mL were considered normal.

CAP-inhibition

CAP-inhibition was conducted according to the methodology developed by Straumann et al. (15). The main goal of this protocol was to assess the presence or absence of cross-reactivity between vespid venoms based on the determination of homologous and heterologous inhibition percentage. For this purpose, venom preparations from *Vespula* spp., *Polistes* spp. and *Vespa velutina* provided by Bial-Aristegui/Roxall® were used. We agree that using *Polistes dominula* venom would have been more appropriate for our geographical area than *Polistes spp.* venom, given that cross-reactivity between *Polistes spp.* (i4) and *Polistes dominula* (i77) is incomplete. However, at the time of the study, there was a complete global shortage of *Polistes dominula* venom, leaving *Polistes spp.* as the only available option.

Serum samples were obtained from all patients and the values of sIgE for i3, i4 and U1223 (whole extracts) were determined. For patients presenting with sIgE between 1 and 50 kUA/L a

1/3 dilution (1-part serum, 2-parts venom) was performed. For those with sIgE above 50, a 1/10 dilution (1-part serum, 9-parts venom) is conducted to avoid the saturation of venom components in the measurements. A mixture of serum sample and the corresponding venom preparation for both the homologous and heterologous inhibition was incubated at 4°C overnight. Venom preparations (Bial-Aristegui/Roxall®) at concentrations of 100 µg/mL were used to both inhibitions. After the incubation period, sample preparations for sIgE-i3, sIgE-i4 and sIgE-U1223 levels of whole extracts were measured in the ImmunoCAP Phadia 200 analyser (ThermoFisher Scientific®, Uppsala, Sweden), and the extent of homologous inhibition (blockage of venom-sIgE by the same venom) and heterologous inhibition (blockage of venom-sIgE by the other venom) were obtained.

A homologous inhibition percentage of ≥70% was required to perform heterologous inhibition. Heterologous inhibitions of ≥ 70 % are considered positive in the CAP-inhibition test and suggestive of cross-reactivity if similar or superior to homologous inhibition. A result of less than 70% is considered indeterminate or inconclusive (15). The venom preparations tested were the same as those used for ST.

VIT selection

In this study, we aimed to compare the selection of VIT composition before and after incorporating the results of the CAP inhibition study. The first VIT selection before CAP-inhibition results was determined by two independent investigators (SCF and JC) based on serum sIgE levels to whole venom extracts and molecular allergens, according to the predefined criteria shown in table I. In cases of disagreement, the final decision was made in consultation with a third investigator (MBF). VIT selection was reassessed by the same investigators after CAP-inhibition results were known (table III). Despite using *Polistes spp.* venom for the CAP inhibition assays, we considered it to be indicative of genuine sensitization to *Polistes dominula*. Consequently, *Polistes dominula* venom immunotherapy was initiated as soon as it became commercially available again.

Statistical analysis

Descriptive analysis of demographic and clinical data was performed. Categorical variables were presented as absolute (n) and relative frequencies (%), and continuous variables as mean ± standard deviation (SD) (minimum; maximum) or median value and the first and third quartiles [Q1, Q3]. Mann-Whitney U test was used to calculate differences between variables and p-values < 0.05 were considered statistically significant. All descriptive statistical analyses were performed using Software IBM SPSS (version 25.0, SPSS Inc., Chicago, lll).

Results

Demographic and clinical characterization of the studied population

In the study period, 26 patients were included. Table II summarizes the baseline clinical characteristics of the study population. Most patients lived in rural areas (n=23, 88.5%) and/or had hobbies in such settings (n=14, 53.8%). All patients included in the series were referred to our department due to SR to *Hymenoptera* sting. Most individuals reported a grade III Mueller classification reaction (n=9, 34.6%).

Allergy workup

In the twenty-six allergic patients, 9 showed sensitization to all three venoms and the remainder seventeen patients had sensitization to *Vespula vulgaris & germanica* venom and *Polistes dominula* venom (all from Bial-Aristegui/Roxall®).

The results of *in vitro* diagnostic tests are summarized in table III**.** Specific IgE to rVes v1 was measured in 24 patients, being positive in 19 patients, with a median value of 4.9±0.7 [0.37-3.9] kUA/L. The median value of total serum IgE was 182 [85.5-401] IU/mL, and of basal tryptase was 5 [3.8-7.6] mcg/L. One patient included in this series was diagnosed with systemic mastocytosis. All patients were negative for CCD.

CAP-inhibition results

A homologous inhibition higher than 70%, for at least one venom, was detected in all sera. No patients were excluded from this analysis after the CAP-inhibition assay.

CAP-inhibition was able to reduce the number of genuine double sensitizations (*Vespula* spp. and *Polistes* spp.) to 7 patients (26.9%), whereas single sensitization to *Vespula* spp. was found in 11 patients (42.3%) and to *Polistes* spp. in 8 patients (30.8%). No genuine sensitizations to *Vespa velutina* were established after CAP-inhibition in this cohort of patients. Given the exponential increase in the prevalence of *Vespa velutina* venom allergy in our region, it is likely that genuine sensitization to this insect's venom will be increasingly detected in the future.

Ratios of rVes v5/rPol d5

In our population, only 8/26 cases showed a ratio >2 (4 with rPol d5/ rVes v5 and 4 with rVes v5/rPol d5). Among these 8 patients, agreement between this ratio and CAP-inhibition was seen in 5 cases (62.5%). In the remaining 3 patients, one showed a discordant single sensitization, while the other two were in fact genuine double sensitizations to both venoms.

VIT selection

Among the 26 patients evaluated, CAP-inhibition led to a change in VIT selection in 15 cases (57.7%). Of these, 9 patients who had initially been considered for double VIT with *Vespula vulgaris* & *germanica* and *Polistes dominula* were reclassified as requiring single VIT (5 for *Polistes dominula* and 4 for *Vespula vulgaris* & *germanica*). CAP-inhibition further revealed that 2 patients previously selected for *Vespula vulgaris* & *germanica* VIT and 1 patient previously selected for *Polistes dominula* VIT were in fact genuinely double-sensitized to both venoms, while 2 patients selected for VIT with *Polistes dominula* were actually only sensitized to *Vespula vulgaris* & *germanica.* Additionally, 1 patient originally assigned to triple-venom VIT had confirmed sensitization only to *Polistes dominula.*

The initial prescription was maintained in 11 patients (42.3%): 4 patients kept the indication for double VIT with *Vespula vulgaris* & *germanica* and *Polistes dominula*, 5 for single VIT with *Vespula vulgaris* & *germanica* and 2 for single VIT with *Polistes dominula.*

Overall, no indication for triple VIT was maintained, nor for single VIT with *Vespa velutina*. Double VIT with *Vespula vulgaris* & *germanica* and *Polistes dominula* was maintained in 4 patients and changed in 3, while single VIT was maintained in 7 patients and altered in 12. The results of homologous and heterologous inhibition are summarized in table III.

Discussion

This study confirms the ability of CAP-inhibition to distinguish between cross-reactivity and double sensitization in patients with double ST and IgE positivity to vespid venoms. CAP inhibition changed previous VIT prescriptions based on sIgE values in 15 patients (57.7%).

In our cohort, CAP-inhibition confirmed the identification of 7 genuine double sensitizations to *Vespula* spp. and *Polistes* spp., with an actual need for double-VIT (26.9%). This finding aligns with previous reports that showed a double genuine sensitization profile to *Vespula* spp. and *Polistes* spp. after CAP-inhibition in only eleven out of 41 patients (26.8%) previous admitted for double-VIT (16). Another study reported that only 25% of patients maintained a double-VIT indication after CAP-inhibition (12). Double-VIT prescription after ST alone was proposed in 65% of cases, while after sIgE to whole venom extract was 73% and CRD 72%. In this study, CAP-inhibition agreement with CRD was poor (29%), as was the agreement with ST (42%) (12).

In our analysis, according to the criteria shown in table I, sIgE values were not helpful to determine VIT selection in eleven patients. In the remaining 15 patients (60%), sIgE values suggested a VIT selection that was only confirmed by CAP inhibition results. Several studies

have been published on this subject, comparing CAP-inhibition and standard diagnostics, but results have been controversial, probably due to small sample sizes (12-15).

In southern Europe, *Vespula* spp. and *Polistes dominula*, and in the last decade, also *Vespa velutina,* are among the most frequent insects leading to systemic reactions, and cross-reactions between them are well documented (21-23). However, current routine *in vitro* and *in vivo* diagnostics tools are not able, by themselves, to effectively distinguish between irrelevant cross-reactivity and VIT relevant double sensitization to vespid venoms. This limitation often leads to the prescription of inappropriate VIT, either in excess or in default. Apart from its costs, double-VIT also implies double risk for adverse reactions (17).

In general terms, it is considered that CRD adds important accuracy to allergy diagnoses through the identification of major allergens (21). Nevertheless, CRD fails to demonstrate superiority in distinguishing between sensitization to vespid species, as no specific molecular components are available for all vespid allergies, especially regarding *Polistes* spp. sensitization, whose antigen 1 is not currently available for routinely diagnostic use (22). Without suitable antigens to distinguish genuine sensitizations from cross-reactivity patterns, CAP-inhibition seem to be a highly accurate method for addressing this unmet diagnostic need (24), as we have also shown in our study.

In conclusion, our results confirm that CAP-inhibition can be useful to confirm genuine double sensitizations to vespid venoms, and to differentiate from cross-reactivity patterns. Although technically demanding, CAP-inhibition may help to prevent both overtreatment and undertreatment.

Contributions: SCF: conceptualization, methodology, data collection, investigation, formal analysis and interpretation, writing - original draft, writing - review and editing. DFS: conceptualization, methodology, investigation, formal analysis and interpretation, writing - review and editing. FV: methodology, data collection, formal analysis and interpretation, writing - original draft, writing - review and editing. EP: writing - review & editing and supervision. MBF: formal analysis, writing - review & editing and supervision. SLS: conceptualization, writing - review & editing, supervision. MLH: methodology, writing - review & editing. JC: conceptualization, data collection, formal analysis and interpretation, writing - review & editing, supervision.

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Table I. Criteria for VIT selection before and after CAP-inhibition.

Criteria for VIT selection based on sIgE levels to whole venom extracts and to molecular venom extracts	VIT composition
sIgE Vespula/sIgE Polistes and sIgE Vespula/sIgE Velutina > 2	Only Vespula
sIgE Polistes/sIgE Vespula and sIgE Polistes/sIgE Velutina > 2	Only Polistes
sIgE Velutina/sIgE Vespula and sIgE Velutina/sIgE Polistes >2	Only Velutina
If the patient doesn't fit any of the previous criteria but has sIgE Velutina levels higher than 0.5 of Vespula and/or Polistes sIgE levels	Velutina + other venom
If the patient doesn't fit in any of the previous criteria, molecular sIgE levels would be analysed for VIT selection between Vespula and Polistes in the following manner	
rVes v1, rVes v5 and rPol d5 are unknown or not determined	Vespula and Polistes
rVes v5/rPol d5 >2	Only Vespula
rPol d5/rVes v5 >2	Only Polistes
rVes v5/rPol d5 <2 AND rPol d5/rVes v5 <2	Vespula and Polistes
Criteria for VIT selection based on CAP-inhibition	**VIT composition**
Negative homologous inhibition with Vespula venom	VIT without Vespula
Negative homologous inhibition with Polistes venom	VIT without Polistes
Negative homologous inhibition with Velutina venom	VIT without Velutina
Positive homologous inhibition with two venoms and negative heterologous inhibition with the same venoms	Double VIT with the two venoms
Positive homologous inhibition with three venoms and negative heterologous inhibition with the same venoms	Triple VIT
Single Positive heterologous inhibition Vespula/Polistes (Vespula venom inhibits sIgE Polistes AND Polistes venom DOES NOT inhibit sIgE Vespula)	Single VIT with Vespula
Single Positive heterologous inhibition Polistes/Vespula (Polistes venom inhibits sIgE Vespula AND Vespula venom DOES NOT inhibit sIgE Polistes)	Single VIT with Polistes
Single Positive heterologous inhibition Vespula/Velutina (Vespula venom inhibits sIgE Velutina AND Velutina venom DOES NOT inhibit sIgE Vespula)	Single VIT with Vespula
Single Positive heterologous inhibition Velutina/Vespula (Velutina venom inhibits sIgE Vespula AND Vespula venom DOES NOT inhibit sIgE Velutina)	Single VIT with Velutina
Single Positive heterologous inhibition Polistes/Velutina (Polistes venom inhibits sIgE Velutina AND Velutina venom DOES NOT inhibit sIgE Polistes)	Single VIT with Polistes
Single Positive heterologous inhibition Velutina/Polistes (Velutina venom inhibits sIgE Polistes AND Vespula venom DOES NOT inhibit sIgE Polistes)	Single VIT with Velutina
Double Positive heterologous inhibition between Vespula and Polistes (Vespula venom inhibits sIgE Polistes AND Polistes venom inhibits sIgE Vespula) OR Double Positive heterologous inhibition between Vespula and Velutina (Vespula venom inhibits sIgE Velutina AND Velutina venom inhibits sIgE Vespula) OR Double Positive heterologous inhibition between Polistes and Velutina (Polistes venom inhibits sIgE Velutina AND Velutina venom inhibits sIgE Polistes)	Single VIT with the venom that had the higher sIgE value to whole venom
Triple Positive heterologous inhibition (Each one of the three venoms inhibits sIgE to the other two venoms)	Single VIT with the venom that had the higher sIgE value to whole venom.
Triple negative homologous inhibition	Inconclusive result. Consider additional studies.

Abbreviations: VIT – venom immunotherapy; sIgE – specific serum IgE.

Table II – Demographical, social and clinical characterization of vespid polysensitized patients performing CAP inhibition.

	Total
Patients – n	26
Gender	
Male – n (%)	19 (73)
Female – n (%)	7 (27)
Age ($M_e \pm SD$ [min; max] years old)	53±3 [18; 79]
Season of last sting – n (%)	
Summer	17 (65.4)
Spring	1 (3.8)
Fall	1 (3.8)
Unknown	7 (27)
Presence of allergic diseases – n (%)	
Rhinitis	13 (50)
Asthma	2 (7.7)
Chronic Spontaneous Urticaria	1 (3.8)
Rural area of residence – n (%)	23 (88.5)
Rural occupation/hobbies – n (%)	14 (53.8)
Severity of SR* – n (%)	
Grade I	7 (27)
Grade II	5 (19.2)
Grade III	9 (34.6)
Grade IV	5 (19.2)

Abbreviations: n – total number; % - percentage; M_e- mean value; SD – standard deviation; min – minimum; max – maximum; SR – systemic reactions; VIT – venom immunotherapy. *Mueller classification (18).

Table III. Results obtained for CAP-inhibition and VIT selection before and after CAP-inhibition results.

	Whole venom extracts sIgE (kUA/L)			**Recombinant allergens sIgE (kUA/L)**			**Venom VIT before CAP-inhibition**			**CAP-inhibition (% of inhibition)**									**Venom ~~for~~ VIT after CAP-inhibition**		
N	*Vespula spp.*	*Polistes sp.*	*Vespa velutina*	Ves v 1	Ves v 5	Pol d 5	*Vespula* spp.	*Polistes* spp.	*Vespa velutina*	*Vespula* spp. sIgE – *Vespula* spp. venom	*Polistes* spp. sIgE – *Vespula* spp. venom	*Vespa velutina* sIgE – *Vespula* spp. venom	*Polistes* spp. sIgE – *Polistes* spp. venom	*Vespula* spp. sIgE – *Polistes* spp. venom	*Vespa velutina* sIgE – *Polistes* spp. venom	*Vespa velutina* sIgE – *Vespa velutina* venom	*Vespula* spp. sIgE – *Vespa velutina* venom	*Polistes* spp. sIgE – *Vespa velutina* venom	*Vespula* spp.	*Polistes* spp	*Vespa velutina*
1	8.07	3.27		2.48	5.18	3.24	X			**(+) (96.0)**	**(+) (90.5)**		**(+) (74.0)**	NS (26.0)					X		
2	9.27	23.2		8.24	0.28	1.18		X		NS (56.6)			**(+) (82.8)**	NS (41.2)						X	
3	2.64	1.53		1.72	0.29	0.75		X		**(+) (86.1)**	NS (28.6)		NS (1.0)						X		
4	7.91	10.7		ND	ND	ND	X	X		**(+) (84.8)**	NS (42.5)		**(+) (89.6)**	**(+) (77.0)**						X	
5	38.4	3.69	13.2	0.02	40.9	4.96	X			**(+) (92.7)**	**(+) (95.8)**	**(+) (70.7)**	NS (45.0)			**(+) (72.3)**	NS (50.4)	NS (27.5)	X		
6	52.1	97.2	20.4	4.01	92.4	>1000	X	X		**(+) (96.4)**	**(+) (81.4)**	**(+) (96.7)**	**(+) (96.9)**	**(+) (92.8)**	**(+) (97.2)**	**(+) (97.7)**	**(+) (89.2)**	**(+) (85.7)**		X	
7	7.38	10.2	1.14	1.73	7.84	10.6	X	X		**(+) (85.8)**	NS (66.0)	**(+) (82.9)**	**(+) (97.4)**	**(+) (77.7)**	**(+) (71.4)**	**(+) (85.7)**	NS (62.7)	NS (69.7)		X	
8	50.2	27.6		1.59	46.3	40.3	X	X		**(+) (80.0)**	**(+) (78.0)**		NS (59.0)						X		

9	16.4	7.69	5.74	0.37	14.6	12.9	X			**(+) (97.7)**	**(+) (91.5)**	**(+) (96.1)**	**(+) (78.6)**	NS (40.2)	NS (64.3)	**(+) (96.5)**	**(+) (84.8)**	**(+) (89.0)**	X		
10	1	1.54		0.2	1.06	0.76	X	X		**(+) (82.4)**	**(+) (94.8)**		NS (65.0)						X		
11	1.71	2.48		1.25	2.65	1.25	X			**(+) (95.1)**	**(+) (80.0)**		NS (57.5)						X		
12	14.5	19.3		14.2	0.06	0.46		X		**(+) (71.0)**	NS (53.4)		**(+) (96.4)**	**(+) (97.2)**						X	
13	2.04	1.54		2.75	2.12	0.3	X			**(+) (88.6)**	**(+) (72.2)**		**(+) (70.4)**	NS (26.7)					X		
14	6.68	6.71		1.04	5.02	6.24	X	X		**(+) (89.8)**	**(+) (78.8)**		**(+) (94.6)**	**(+) (89.4)**						X	
15	71.4	>1000		9.3	>1000	>1000	X	X		**(+) (87.4)**	NS (69.7)		**(+) (77.1)**	NS (41.2)					X	X	
16	0.44	0.63	0.26	0.09	0.27	0.43	X	X		**(+) (71.4)**	NS (48.0)	NS (28.6)	**(+) (72.0)**	NS (35.7)	NS (14.3)	NS (14.3)			X	X	
17	21.9	8.33	8.69	6.45	9.38	3.63	X			**(+) (74.9)**	NS (59.3)	NS (40.3)	**(+) (75.6)**	NS (37.8)	NS (45.7)	NS (68.0)	NS (36.0)	NS (46.2)	X	X	
18	4.111	4.21		2.77	1.64	3.58		X		**(+) (81.9)**	NS (23.4)		**(+) (81.8)**	NS (28.2)					X	X	
19	7.63	10.4	4.06	10.5	0.02	0.03	X	X		NS (69.5)	NS (38.0)	NS (61.9)	**(+) (98.5)**	**(+) (98.0)**	**(+) (97.9)**	**(+) (78.3)**	**(+) (70.1)**	NS (47.9)		X	
20	5.55	4.23		0.01	4.29	4.83	X	X		**(+) (84.3)**	**(+) (79.9)**		**(+) (78.4)**	NS (63.9)					X		
21	1.24	1.31		1.77	1.04	1.18	X	X		**(+) (72.6)**	NS (13.0)		**(+) (83.2)**	NS (42.0)					X	X	

22	4.97	2.36	0.68	0.81	4.61	2.93	X			**(+) (91.2)**	NS (15.5)	NS (52.4)	**(+) (73.0)**	NS (66.0)	NS (62.0)	NS (25.0)	NS (1.0)	NS (8.1)	X	X	
23	0.74	1.51		0.01	1.61	2.19		X		**(+) (86.2)**	NS (28.3)		NS (41.6)						X		
24	52.2	58.4	23.7	13.4	44.2	44.7	X	X		**(+) (97.9)**	**(+) (81.5)**	**(+) (93.2)**	**(+) (91.6)**	**(+) (78.5)**	**(+) (91.6)**	**(+) (91.9)**	**(+) (83.8)**	**(+) (73.8)**	X		
25	1.69	3.21		ND	ND	ND	X	X		**(+) (87.4)**	NS (53.3)		**(+) (70.0)**	NS (60.0)					X	X	
26	8.53	9.43	5.65	9.94	0	0	X	X	X	**(+) (75.0)**	**(+) (72.4)**	**(+) (94.5)**	**(+) (99.4)**	**(+) (99.4)**	**(+) (97.0)**	NS (57.5)				X	

Abbreviations: N – patient number; % - percentage; VIT – venom immunotherapy; ND – not determined; (+) – significative inhibition (≥70%); NS – not significative inhibition (<70%).