Managing systemic reactions and venom immunotherapy in vespid-venom allergy: observations from a retrospective study of Portuguese patients

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Abstract

Background: *Vespula* spp. and *Polistes* spp. are relevant species in South Europe, with *Vespa velutina nigrithorax* (VVN) being considered a public health problem. We aimed to characterize a cohort of Portuguese patients referred for large local reaction (LLR) and/or systemic reaction (SR) to vespids. In patients treated with venom immunotherapy (VIT), induction protocol and frequency of adverse reactions were evaluated.

Methods: Retrospective study including patients with LLR and SR to vespids referred to our Immunoallergology Department (2008-2022).

Results: A total of 129 patients were evaluated, the majority were male adults (n=77, 59.7%), from rural areas. From these, 51 patients had SR (Mueller classification: 7.8% grade I, 19.6% grade II, 37.3% grade III, 35.3% grade IV). We found no differences regarding the levels of total serum IgE, basal serum tryptase value, sIgE levels to the eliciting venom or their molecular components, regarding the severity of the SR that motivated the referral to our clinic. In the SR group, previous LLR Hymenoptera sting were reported in 15.7%. Thirty-eight patients (74.5%) initiated VIT: 22 with wasp venom, 14 with paper wasp venom and 2 with *Vespa velutina* venom. There was one mild systemic reaction, not requiring adrenaline and 4 LLR. Re-stings after VIT occurred in 16 patients, without any systemic or local reactions. Currently, eleven patients remain under VIT.

Conclusions: Most vespid-venom allergic patients are male adults from rural areas. Sensitization to wasp venom was present in 52.9%, paper wasp in 33.3% and *Vespa velutina* in 13.7%. The frequency of adverse reactions during both induction and maintenance phases appears to be low. Despite a reduced sample size, our experience with VVN VIT, suggests its safety.

Keywords: systemic reaction; ultra-rush; *Polistes*; *Vespa velutina*; *Vespula*.

Impact statement: First national study analysing the clinical and sensitization pattern of vespid venom-allergic patients. Included data from patients undergoing VIT with *Vespa velutina nigrithorax* venom, providing real-world evidence.

Introduction:

Hymenoptera venom (HV) allergy is within the principal aetiologies of anaphylactic reactions in adults, being the third most common cause in Portugal (7.4%) (1). In our country, the most prevalent Hymenoptera species is the honeybee (*Apis mellifera*), accounting for 70% of global sensitization; the wasp (*Vespula* species), implicated in 23% of cases; and the paper wasp (*Polistes dominula*), with a sensitization rate of 9% (1). In the past few years, *Vespa velutina nigrithorax* (VVN) has become a public health problem and an eminent concern in Europe. In Portugal, VVN was first identified in 2011, in the north-western province of Minho (2) and since then, the number of reported cases of anaphylaxis due to VVN has been increasing (3,4). VVN venom extract became available in Portugal in 2021, improving both diagnosis and treatment.

Epidemiological studies in Southern European countries show that sensitization to vespids is about 20.4% in rural Mediterranean areas (5). Data from Southern Spain revealed a higher sensitization to *Polistes* than to *Vespula germanica,* with 62% of subjects reacting to *Polistes dominula* in contrast to 29% reacting to *Vespula germanica*, thus, highlighting the importance of *Polistes* as an important species in this geographic area (6).

In the clinical setting, distinguishing between sensitization to *Vespula* spp. and *Polistes* spp. continues to be challenging, since sensitization to both wasp and paper wasp can be observed in up to 50.5-61.5% of anaphylactic sting reactions (7).

The most frequent clinical presentations of vespid venom allergic reactions are large local reactions (LLR) and systemic reactions (SR) (8). LLR are defined by diameters > 10cm and duration > 24 hours (8) and is the most common reported reaction (2.4% to 26.4%) (5,9). Identifying the culprit insect, in the investigation of a vespid allergic reaction, while optimal, is seldom achieved (10,11). A study revealed that approximately 50% of patients could not differentiate paper wasps and nearly one-third could not identify

wasps at all (2). Even though distinctive features can be of use, they are unreliable as a single criterion (7,10-11).

Diagnostic testing should be performed when clinical history is consistent with VIT indication (12). The presence of venom-specific IgE is usually confirmed through skin testing (ST), including both skin prick test (SPT) and intradermal tests (ITD), using a maximum venom concentration of 1.0 μg/mL. *In vitro* measurement of serum specific IgE (sIgE) to Hymenoptera venom extracts (*Vespula spp.*, *Polistes dominula* and *Vespa velutina nigrithorax*) and their molecular allergens such as Ves v1 (phospholipase A1), Ves v5 (antigen V) and Pol d5 (antigen V) should be used as a complementary test in VIT candidates, or as alternative test, particularly if ST yields a negative result. Despite the availability of sIgE to VVN in our country, the lack of a specific molecular testing makes the confirmation of a primary sensitization more difficult.

VIT is the only preventive treatment with reported outcomes in both reducing the risk for future severe sting reactions and improving patients´ quality of life (13). The protective success rate ranges from 91 to 96% for wasp venom allergy (12). In Portugal, the available venoms for VIT are *Vespula* spp., *Polistes dominula* and *Vespa velutina* venom. Multiple induction protocols, differing in the duration required to reach the maintenance dose of 100 μg, have been proposed over the years (14-17). Treatment should be considered for 3 to 5 years, although extended administration can be carried out in selected candidates (12). Sting challenges in patients on maintenance VIT are not routinely implemented in our country due to safety concerns.

The objective of the present study is to characterize a cohort of Portuguese patients referred to our Immunoallergology Department for LLR and/or SR to vespids. Additionally, in SR patients sensitized to *Vespa velutina*, *Vespula* spp. and/or *Polistes* spp., *in vivo* and *in vitro* sensitization profiles were evaluated. In VIT candidates, induction protocol and frequency of adverse reactions was assessed.

Materials and Methods:

Study Population

We reviewed the clinical files of all patients with a suggestive clinical history of LLR and/or SR to vespids that were referred and followed in our Immunoallergology Department between 2008 and 2022. Data regarding age, gender, concomitant allergic diseases, seasonality of sting episodes, hobbies, occupation, and previous history of sting reactions were collected from all patients' records.

For the patients referred for SR, information regarding ST results, sIgE for *Vespula* spp., *Polistes* spp., *Vespa velutina*, total serum IgE, baseline serum tryptase and sIgE values to Ves v1, Ves v5 and Pol d5 was also collected. Reaction severity was assessed using the Mueller Classification (Grade I-IV) (18). For patients proposed to VIT, data regarding its duration, and the occurrence of adverse reactions (frequency and type of adverse events) during induction or maintenance phase were also evaluated.

This study protocol was approved by our hospital´s Ethics Committee, and the study was conducted in accordance with the ethical standards established in the Declaration of Helsinki of 1946, revised in 2013, of the World Medical Association. Informed consent was obtained from all patients before enrolment in the study.

Allergy study

Skin tests were performed in an accelerated protocol, consisting of SPT followed by simultaneous ITD, due to the reported low rate of adverse events associated with this approach. (19-21) SPT were performed with standardized, purified, and lyophilized venom preparations (Bial-Aristegui/Roxall®) at a concentration of 100 µg/mL. IDT were conducted simultaneously using diluted venom preparations at concentrations of 0.01 µg/mL, 0.1 µg/mL and 1 µg/mL. (19-21) Positive (histamine 0.1% solution) and negative controls (normal saline solution) were used as controls for SPT. For IDT a negative control with normal saline solution was used.

The determinations for sIgE for *Vespa Velutina*, *Vespula* spp. and *Polistes* spp., and molecular components (Ves v1, Ves v5 and Pol d5) were performed using *ImmunoCAP, ThermoFisher Scientific* (Uppsala, Sweden). All results > 0.35 kUL were considered positive. Basal serum tryptase was also collected, with values < 11.4 ng/mL were considered normal.

Venom immunotherapy ultra-rush protocol

Data regarding the induction protocol used and any adverse reactions (local or systemic) reported during either the induction phase or the maintenance phases were evaluated. All patients were submitted to a 210-minute ultra-rush (UR) protocol proposed by Birnbaum (consisting of a cumulative dose of 101.1 µg, divided in 6 injections) (12,17). This procedure was carried out in an Immunoallergology Day Hospital, featuring continuous cardiovascular monitoring, and equipped with medical personnel and necessary equipment for managing possible systemic reactions. All injections were administered subcutaneously in

the mid-lateral posterior region of the upper arm. Erythema and swelling ≥10 cm at the injection site were considered as LLR during VIT. Systemic reactions were evaluated according to the World Allergy Organization (WAO) systemic allergic grading system (22).

Any administered premedication was also recorded from patient´ files, including premedication administrated during maintenance phase and all reported adverse reactions throughout this phase.

Statistical analysis

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

Results:

Demographic and clinical characterization of the studied population

In the last 15 years, 129 patients were referred to our Immunoallergology Department for LLR and SR to vespids. The study population included mostly male patients (n=77, 59.7%) with a mean age of 54.5 years (minimum 9 years; maximum 86 years). Eleven patients (8.5%) were under 18 years of age (81.8% boys; mean age 12 years, minimum age 9, maximum age 16). Thirty-nine patients (30.2%) had concomitant atopic disease, 36 of whom had allergic rhinitis and 10 had asthma. Demographic and clinical characteristics of the referred patients with a suggestive clinical history of vespid allergic reactions are displayed in **table I**.

When considering the season of stinging episodes, most patients were unable to determine it (n=72, 55.8%). Considering potential risk factors for new stings, 74.4% (n=96) of patients either lived or frequently travelled to rural areas in line with their occupation/hobbies. Regarding clinical characteristics of the last sting reaction, 60.5% (n=78) had LLR and 39.5% (n=51) had SR.

Characterization of patients referenced by SR

Fifty-one patients had a previous history of SR following insect sting (Mueller classification: 7.8% grade I, 19.6% grade II, 37.3% grade III and 35.3% grade IV). Among them, twenty-seven patients exhibited sensitization to wasp venom, 17 to paper wasp venom and 7 to *Vespa velutina* venom. Most patients were male (n=35, 68.6%), with a mean age of 55.5 years (minimum 19 years; maximum 86 years). Demographic and clinical data of this population are shown in **table II**.

Within the SR group, 23.5% (n=12) of patients reported a previous history of Hymenoptera sting reaction. Of these, 8 patients (15.7%) reported previous LLR and 4 (7.8%) a SR (Mueller grade I-II), despite not having undergone allergy evaluation previously. For the remaining population (n=39, 76.5%) no prior sting history was mentioned.

ST was performed in 51 patients, with positive results in 88.2% of cases (21.6% in SPT, 43.1% in 0.01 μg/mL ITD, 66.7% in 0.1 μg/mL ITD and 87.5% in 1 μg/mL ITD). For those with negative ST (n=6, 11.8%), sIgE was positive in all of them, thus confirming sensitization. In three patients (2 with sensitization to wasp and 1 with sensitization do paper wasp) simultaneous ITD was only performed up to the concentration of 0.1 μg/mL, due to severe SR grade 5, with neurological symptoms. The median value of total serum IgE was 161.5 [62.3-322] (median, first and third quartiles [Q1, Q3]) IU/mL, and the mean value of basal serum tryptase was 5.1±2.9 [1.0; 11.4] (mean, standard deviation [minimum; maximum]) mcg/L. None of the included patients had the diagnosis of mastocytosis or mast cell disorder. Detailed results of *in vivo* and *in vitro* diagnostic tests are summarized in **table II.**

We found no differences regarding the levels of total serum IgE, basal serum tryptase value, sIgE levels to the eliciting venom or their molecular components, concerning the severity of the SR that motivated the referral to our clinic.

VIT – Prescription and reported adverse reactions

A total of 38 patients (74.5%) were approved and agreed to VIT. In all of them an induction 210-minute UR protocol was used (17). Among them, 22 (57.9%) received VIT with wasp venom, 14 (36.8%) with paper wasp venom and 2 (5.3%) with *Vespa velutina* venom. The UR protocol was performed with aqueous extracts purified from Hymenoptera venom (Bial-Aristegui/Roxall®), with specific venoms including *Vespula vulgaris & germanica, Polistes dominula* and *Vespa velutina,* respectively. Thirteen patients did not receive immunotherapy, ten of whom were recommended but refused, while the remaining three are awaiting cardiovascular evaluation before starting treatment.

Among the 38 patients who underwent UR protocol, only one (2.6%) experienced a SR during the induction phase, receiving paper wasp venom. The reaction was mild, grade II, accordingly to Cox L. classification (22), and did not require adrenaline. The patient achieved a cumulative dose of 71.1 µg on the UR day, and the cumulative protective dose of 100 µg was achieved in the second session, 15 days after the UR, with a reinforcement in premedication (administered 30 minutes prior to the venom injection: clemastine 2 mg i.v. and hydrocortisone 100 mg i.v.).

Four patients (10.5%) reported a LLR during the induction phase. Among them, two occurred with *Vespula* spp. VIT, one with *Polistes dominula* VIT and one with *Vespa velutina* VIT. Despite these LLR, all patients completed the 210-minutes UR on the first attempt, and these reactions were treated with topical corticosteroids.

For the remaining patients (n=33, 86.8%), there were no reports of any systemic or local reactions (immediate or delayed). Additionally, no systemic reactions were reported during the maintenance phase, and there were no fatalities reported.

Currently, eleven patients are still undergoing VIT (28.9%). From these, two patients receiving VIT with wasp venom have already been re-stung, without any systemic reactions. None of the patients receiving VIT with paper wasp venom have been re-stung.

Among those who have completed VIT (n=27), 16 (59.3%) have been re-stung. No systemic or local reactions were reported after concluding the VIT procedure thus far, as indicated in **table II.** The average duration of VIT was 4.1±1.3 [min 0.2; max 6.4] years.

We found no differences regarding the levels of total serum IgE, basal serum tryptase value, sIgE levels to the eliciting venom or their molecular components, concerning the severity of adverse events during VIT protocol. Clinical and laboratory characteristics of SR patients is described in **table II.**

Discussion:

This study is a retrospective analysis spanning the last 15 years, encompassing all patients referred to our outpatient Immunoallergology Clinic with a suggestive clinical history of LLR and/or SR after a vespid field-sting. Among our cohort of vespid allergic patients, the majority were male adults (n=77, 59.7%) from rural areas (n=96, 74.4%). In the SR group, 27 patients (52.9%) showed sensitization to wasp venom, 17 (33.3%) to paper wasp venom and 7 (13.7%) to *Vespa velutina* venom.

In our cohort, vespid sensitization was confirmed in all included patients, 88.2% (n=45) of cases through skin testing. The methodology applied, concerning simultaneous IDT, seems to be a safe procedure, as no adverse events were recorded, mirroring the literature (19-21). One can also discuss that this accelerated protocol can provide a quick overview of the patient sensitization pattern, while reducing testing duration, costs and patient anxiety and discomfort.

Vespula spp. and *Polistes* spp. are relevant species in the Southern European countries where patients are frequently exposed to these species (23). Our finding regarding the frequency of sensitization to *Polistes* is similar to the one reported by Vega et al (24), who documented a sensitization to *Polistes* spp. in Spain ranging from 23.4% to 36.8%. Notably, our cohort diverges from the Spanish data, exhibiting a higher number of patients with *Vespula* spp. sensitization, compared to the reported 27.3% (24). We believe that this variance may be attributed to differences in environmental exposure in our country, as highlighted in previous studies (5,6,23,25).

When considering the frequency of sensitization to *Vespa velutina*, the number of reported cases of SR due to this Hymenoptera is increasing worldwide, with Portugal mirroring this trend (3,4,26,27). Recently, sensitization profiles of Portuguese patients with VVN reactions have been published helping to further understand VVN hypersensitivity (26,27).

In our study, considering the SR patients, 23.5% presented a history of previous reactions that occurred before their referral (7.8% had a previous SR (grade I-II) and 15.7% a previous LLR). Although SR following previous LLR have been described in literature as relatively rare, ranging from 2% to 15% (28,29), a recent study by Bilò *et al* reported that 24% of patients that had a second sting episode with SR had previously experienced a LLR (30).

Consistent with findings from other studies, most patients were unable to identify the culprit insect or recall the season of the sting (27,29,30). However, for the ones who did, the summer season was the most commonly mentioned (n=54, 41.9%), aligning with the observations in a Spanish study (5). This association with summer and fall season corresponds to the typical activity periods of vespids (10).

No statistically significant differences were found regarding the severity of the SR and levels of total serum IgE, basal serum tryptase or sIgE for the eliciting venom or for their molecular components. These results are in line with previous studies (12,14,31).

In our study, VIT UR protocol, as proposed by Birnbaum (12,17), was initiated in 38 patients (74.5%), with an overall completion rate of 97.4% (n=37). This protocol, carried out in the Day Hospital

setting, allows for a quicker achievement of the protective dose. However, it is not a risk-free procedure (12,17).

We identified only one patient (2.6%) who experienced an adverse systemic reaction during the UR phase. Nevertheless, the SR was mild, and adrenaline administration was not required. This patient successfully achieved a cumulative dose of 71.1 μg during the UR and tolerated the 100 μg dose administration 15 days post-UR, with proper premedication adjustment.

The global frequency of SR during VIT UR protocols documented ranges from 0% to 30%, including both bee and vespid immunotherapy extracts (17,32-34). The risk of SR during VIT seems to be higher with bee venom. This may be, beside other explanations, because vespid venom is obtained from the venom sac, which includes proteases that can degrade vespid venom allergens, thereby making it potentially less allergenic (35). Additionally, in accordance with this data, adverse systemic events in patients treated with wasp venom varies from 6.1% to 7.5% (17,30), which is slightly more consistent with our findings.

We also documented 10.5% of LLR during the induction phase. For the remaining population (86.8%), VIT did not induce any immediate or delayed reactions.

Recurrence of sting reactions after VIT conclusion have been reported in about 0% to 10% of cases within one to five years following completion (12,36-38). In our study, we found that 16 patients (59.3%) had already been re-stung, with no systemic or LLR reported after concluding VIT.

VIT with *Vespa velutina* venom extracts has become recently available in Portugal. Recently published studies have demonstrated the efficacy and safety of treating patients with SR to *Vespa velutina* with *Vespula* spp. venom (3,4,39). This could be a logical choice since most patients also had sensitization, to some extent, to *Vespula* spp. or *Polistes* spp., or even to their recombinants, Ves v1, Ves v5 and Pol d5 (3,4,39). Given that the majority of these patients had no prior exposure to VVN stings, sensitization likely occurred through cross-reactivity with other Hymenoptera, especially *Vespula* ssp., due to high homology of major allergens, as previously noted (3,4,39). The two patients sensitized to VVN in our cohort are receiving VVN venom VIT. While our sample size is limited, and patients have had a relatively short follow up period, our findings do not allow us to conclude about its efficacy, however, suggest that VVN VIT appears to be safe, as no adverse systemic reactions were described.

In Southern Europe, there is a high percentage of patients that are sensitized to both *Vespula* spp. and *Polistes* spp. (7). This fact may be explained by the presence of cross-reactivity, as it is well documented in several previous studies. For VIT prescription, in patients sensitized to both *Vespula* spp. and *Polistes*

spp., attending physicians used a bottom-up diagnostic strategy, starting with the patient and environmental information (geographical area, seasonal distribution, and insect characteristics) and concluding with complementary tests (sIgE to Ves v1, Ves v5, Pol d5). No additional laboratory evaluation (e.g. basophilic activation test) was performed regarding cross-reactivity.

Despite its insights, our study has some limitations. Firstly, it is retrospective in nature, relying on patient recall and clinical records review. Secondly, the small sample of patients under VIT with *Vespa velutina* venom could limit data extrapolation. Lastly, the unavailability of commercialized recombinant allergens for VVN remains a limitation, hindering the distinction between cross-reactivity and/or primary sensitization.

Nonetheless, the authors suggest that these results have important added value in clinical practice, contributing to further data on this subject.

Conclusion

In conclusion, over the past 15 years, in our Immunoallergology Department, 129 patients were evaluated for suspected hypersensitivity reaction to vespids. Most patients allergic to vespids are male adults from rural areas. Fifty-one patients were referred for SR reactions (7.8% grade I, 19.6% grade II, 37.3% grade III and 35.3% grade IV) with confirmed sensitization: 52.9% to wasp venom, 33.3% to paper wasp venom and 13.7% to *Vespa velutina* venom. ST was performed in all patients with VIT indication, with positive results in 88.2% of cases (positive SPT in 21.6% and positive ITD in 87.5%, the majority for 1 µg/mL). For those with negative ST, sIgE was positive in all of them, thus confirming sensitization. No differences were found regarding the levels of total serum IgE, basal serum tryptase value, sIgE levels to the eliciting venom or their molecular components, and the severity of the SR, consistent with existing literature.

VIT UR protocol was initiated in 38 patients, with an overall completion rate of 97.4% (n=37). The documented frequency of systemic reactions during VIT procedure was 2.6%. For this patient, adrenaline administration was not required, and the cumulative protective dose was achieved in the second session, 15 days after the UR.

While *Vespa velutina* VIT remains understudied, our limited data suggest that immunotherapy targeting VVN seems to be a safe procedure. However, further investigations are needed. The authors consider that this study adds important value in clinical practice, being, to our knowledge, the first national

study to analyse the clinical and sensitization patterns of vespid venom-allergic patients. Furthermore, inclusion of data from patients undergoing VIT VVN, provides valuable real-world evidence.

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Patients – n	**129**
Gender Male – n (%) Female – n (%)	 77 (59.7) 52 (40.3)
Adults – n (%) **Children** – n (%)	118 (91.5) 11 (8.5)
Age (M_e±SD [min; max] years old)	54.5 ± 17.8 [9; 86]
Age at diagnosis (M_e±SD [min; max] years old)	47.3 ± 17.8 [9; 84]
Stinger season – n (%) Summer Spring Fall Unknown	 54 (41.9) 2 (1.6) 1 (0.7) 72 (55.8)
Presence of allergic diseases – n (%) Rhinitis Asthma	 36 (27.9) 10 (7.8)
Rural occupation/hobbies – n (%)	96 (74.4)
Referred for LLR – n (%)	78 (60.5)
Referred for SR – n (%)	51 (39.5)

Table I – Demographics, sting season, previous allergic diseases, and rural occupations of vespid allergic patients. Abbreviations: n – total number; % - percentage; M_e- mean value; SD – standard deviation; min – minimum; max – maximum; SR - systemic reactions; LLR – local large reaction.

Clinical and Laboratory characteristics of *Vespula* spp., *Polistes* spp. and *Vespa velutina* patients with SR				
	Wasp	**Paper Wasp**	**Vespa velutina**	**Total**
Patients – n	27	17	7	51
Gender				
Male – n (%)	18 (66.7)	14 (83.4)	3 (42.9)	35 (68.6)
Female – n (%)	9 (33.3)	3 (17.6)	4 (57.1)	16 (31.4)
Adults – n (%)	27 (100)	17 (100)	7 (100)	51 (100)
Children – n (%)	0	0	0	0
Age (M_e±SD [min; max] years old)	54.7±18.1 [20; 76]	53.6±17.4 [19; 86]	53.0±22 [20; 78]	55.5±16.8 [19; 86]
History of previous Hymenoptera sting – n (%)	4 (14.8)	3 (17.6)	1 (14.3)	8 (15.7)
LLR	1 (3.7)	3 (17.6)	0	4 (7.8)
AR				
Severity of SR[#] – n (%)				
Grade I	2 (7.4)	0	2 (28.6)	4 (7.8)
Grade II	8 (29.6)	2 (11.8)	0	10 (19.6)
Grade III	8 (29.6)	9 (52.9)	2 (28.6)	19 (37.3)
Grade IV	9 (33.3)	6 (35.3)	3 (42.9)	18 (35.3)
ST – n (%)				
SPT 100 µg/mL	6/27 (22.2)	2/17 (11.8)	3/7 (42.9)	11/51 (21.6)
ITD 0.01 µg/mL	13/27 (48.1)	6/17 (35.3)	3/7 (42.9)	22/51 (43.1)
ITD 0.1 µg/mL	19/27 (70.4)	11/17 (64.7)	4/7 (57.1)	34/51 (66.7)
ITD 1 µg/mL	23/25 (92.0)*	15/16 (93.8)**	4/7 (57.1)	42/48 (87.5)
Negative	2/27 (7.4)	1/17 (5.9)	3/7 (42.9)	6/51 (11.8)
Laboratory findings - SR group				
Serum tryptase (mcg/L) – (M_e±SD [min; max])	5.0±2.9 [1.0; 10.6]	5.0±2.6 [1.5; 9.6]	5.7±3.7 [1.8; 11.4]	5.1±2.9 [1.0; 11.4]
Total IgE (IU/mL) – (M_d [Q1-Q3])	208 [57-331]	110 [64.1-418.3]	193 [51.7-256]	161.5 [62.3-322.0]
Vespula sIgE (kUA/L) – (M_d [Q1-Q3])	2.8 [0.9-10.3]			
Ves v1 (kUA/L) – (M_d [Q1-Q3])	1.2 [0.4-3.1]			
Ves v5 (kUA/L) – (M_d [Q1-Q3])	0.2 [0.02-2.3]			
Polistes sIgE (kUA/L) – (M_d [Q1-Q3])		11 [1.3-16.5]		
Pol d5 (kUA/L) – (M_d [Q1-Q3])		1.1 [0.5-3.3]		
			3.6 [0.4-22.7]	

Vespa Velutina sIgE (kUA/L) – (M_d [Q1-Q3])				
Patients proposed to VIT – n (%)	22 (81.5)	14 (82.4)	2 (28.6)	38 (74.5)
Completed VIT – n	17	10	0	27
Still on VIT – n	5	4	2	11
Reactions during VIT protocol - n (%)	2 (9.1)	1 (7.1)	1 (50)	4 (10.5)
LLR	0	1 (7.1)	0	1 (2.6)
SR				

Table II – Demographics, reaction's severity, skin test, laboratory findings, molecular evaluation, and VIT in patients with documented SR. Abbreviations: n – total number; % - percentage; M_e- mean value; SD – standard deviation; min – minimum; max – maximum; M_d– median value; Q1-Q3 – first and third quartiles; ST - skin test; SPT – skin prick test; ITD – intradermic test; SR - systemic reactions; LLR – local large reaction; VIT - Venom immunotherapy; UR – Ultra rush. Reference values: Serum tryptase < 11.4 mcg/L, Total IgE < 100 IU/mL, sIgE Ves v1, Ves v5, Pol d5 < 0.35 kUA/L.

 # Mueller classification (18)

*** Two patients with anaphylaxis grade 5 and neurological symptoms were not tested for the concentration of 1.0** µg/mL.

**** One patient with anaphylaxis grade 5 and neurological symptoms was not tested for the concentration of 1.0** µg/mL.