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Diagnosis and treatment of *Hymenoptera* venom allergy in adults: A single-center experience in Lithuania

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ABSTRACT

Background: Venom-specific immunotherapy (VIT) is a major treatment for patients allergic to *Hymenoptera* venom. Thus, correct diagnosis of sensitization, identification of the risk factors, and choice of venom for the treatment are the key issues.

Objective: We aimed to describe diagnostic and treatment experience data of VIT performed in a single center in Lithuania.

Methods: In this retrospective study, we analyzed 9 years of clinical data (severity of the allergic reaction, recognition of the culprit insects, diagnostics, VIT protocol safety and efficacy, sting challenge outcomes) of patients treated with cluster VIT. Sting challenge helped to reveal the influence of venom preparation quality and to adjust the dosage of venom.

Results: Data from 83 patients were analyzed. Double sensitization confirmed by component diagnosis was found in 39.4% (13/33), and double immunotherapy was initiated in 9.1% (n = 3/33). The cluster immunotherapy protocol was used in 81 patients. Systemic reactions occurred in 7.4% (n = 6/81) patients during the build-up phase. VIT failure was related to bee venom immunotherapy and systemic reactions during a build-up phase. The efficacy in the short term of our approach to cluster VIT was confirmed by the sting challenge in 97% (42/43). Nine patients (10.8%, n = 9/83) voluntarily stopped the treatment due to a lack of motivation.

Conclusion: Our protocol regarding the investigation and treatment of patients allergic to *Hymenoptera* venom has been safe and effective. Patient's motivation to continue VIT is one of the concerns, but the biggest challenge is the patients with bee venom allergy and repeated systemic reactions during VIT.

Keywords: Venom hypersensitivity, Lithuania, Cluster immunotherapy, Retrospective study

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INTRODUCTION

Hymenoptera venom allergy (HVA) is one of the most fascinating areas of allergology, curious for doctors but fearsome for patients. Although mortality rates are low, HVA greatly impairs quality of life, significantly raises anxiety levels, and restricts activities of daily living.[1] Venom-specific immunotherapy (VIT) is an effective therapy that prevents the recurrence of HVA reactions in the future, and a negative sting challenge (SC) test can greatly improve health-related quality of life.[2,3] Although the basic principles of HVA diagnostics and VIT are well known, there are differences in clinical practice in different centers regarding the treatment of these patients.

In this study, we aim to describe 9 years of experience in our center diagnosing and treating HVA with a special focus on the efficacy and safety of cluster VIT protocols and the sting challenge (SC).

MATERIALS AND METHODS

We analyzed retrospective data of adult patients, treated with *Hymenoptera* (honeybee or wasp) VIT at The name of the center where the study was conducted is Pulmonology and Allergology center of Vilnius University Hospital Santaros klinikos from January 2013 to December 2021. Our center belongs to one of the biggest tertiary hospitals in Lithuania with the highest number of HVA patients referred. Ethics permission was obtained (Vilnius Regional Bioethics Committee, No. 158200-16-847-355) before the review of clinical records. Information about the insect sting event (allergic reaction history, the number of stings induced systemic reactions, culprit insect, and symptoms), sensitization profile, tryptasemia, diagnosis of mastocytosis, venom preparation used for VIT, side effects and efficacy of the cluster VIT assessed by the SC outcomes and patients' compliance were analyzed. The evaluation of the severity of allergic reactions to bee/wasp stings was based on the patient's history and medical records when available. Allergic reactions were assigned to 1 of the 4 grades according to Ring and Messmer classification.[4]

Patients' selection for VIT and diagnostic workup

All patients included in this retrospective study had a history of immediate systemic reaction to honey bee/wasp stings and proven sensitization. In all cases, an allergological work-up was started by *in vitro* testing for specific IgE (sIgE) against bee and wasp venom with or without simultaneous skin testing. In doubtful cases, when patients' history was inconsistent or in the case of double sensitization, specific IgE testing for components (rApi m 1, rVes v 1, and rVes v 5) as a tool for differentiation of cross-reactivity and detection of the true double sensitization was done. Detection of sIgE against bee and wasp venom and tryptase was performed with ImmunoCAP FEIA (Thermo Fisher Scientific, Uppsala, Sweden). Patients with a high degree of suspicion for mastocytosis were referred for a haematologist's consultation.

Venom immunotherapy protocols

The decision to start VIT was based on the patient's medical history and allergy testing results. In every case informed consent for VIT was obtained.

Most of the patients were treated with cluster immunotherapy (Table 1) described by Tarhini et al.[5] Two patients were treated using the rush protocol (Table 2), but longer hospitalization and observation of large local reactions made our choice in favour of cluster protocol.

Our cluster VIT protocol starts with 2 one-day hospitalizations with 1-week interval (1 day–5 injections, 8 days–3 injections, all injections are subcutaneous, administered to the middle third of the outer part of the upper arm). Thus, we reach the maintenance dose of 100 μg in 2 weeks (day 15). After reaching the maintenance dose, VIT injections are done every 4 weeks. After 1 year of immunotherapy for patients without mast cell disorders and longer duration of the treatment intervals are increased by 1 week every year, reaching a maximum of 8 weeks between injections. In patients with mastocytosis, the maximum interval between injections was 6 weeks with treatment to be continued as long as possible (indefinitely). In the case of a severe reaction and confirmed mastocytosis or positive SC test we increase the dose to 200 μg of venom.

VIT build-up with both venoms in case of double sensitization and risk factors was performed on separate days of hospitalization. During the maintenance treatment, full doses of different venom

	Dosage (μg)	Cumulative dose (μg)	Duration of observation
Build – up phase (in-patient)			
Day 1	0.1/1/5/10/20 every 30 min	36.1	Until next morning
Day 8	30/30/30 every 30 min	90	Until next morning
Maintenance phase (out-patient)			
Day 15	100	100	At least 2 h
Day 44	100	100[a]	At least 1 h
Continues every 4–8 weeks			

Table 1. Cluster protocol of subcutaneous VIT. [a]*in the case of severe reaction and confirmed mastocytosis we increase dose up to 200 μg of venom with maximum interval between injections – 6 weeks*

are injected into different hands with a 60-min break.

VIT-induced systemic reaction severity was evaluated according to the WAO (*World Allergy Organization*) subcutaneous immunotherapy systemic reactions (SR) grading system.[7]

We continued VIT for 3–5 years. The stopping time of VIT was discussed with the patients. We recommend a minimum of 3 years of VIT for those with mild reactions (grade I according to Ring and Messmer) and advise to keep on VIT for up to 5 years for those with severe reactions.

Venom preparations

From 2013 to 2021 we used venoms from several different manufacturers. Alyostal (Stallergenes, France) was used at the beginning, but after prolonged supply interruption, we had to change to Venomenhal (HAL Allergy, Netherlands), Diater (Diater Laboratorios, Spain) and Venomil (Bencard Allergy, Germany). Nowadays most of our patients are treated with Venomil. Until now manufacturers are reluctant to include information about standardization of venom – they do not declare major allergens quantities in their preparations.

Sting challenge test

Sting challenge (SC) tests were performed according to the protocol in the EAACI position paper[6] during the summer – early autumn period. SC tests were carried out at the hospital with continuous monitoring of the patient's vital signs: arterial blood pressure, pulse, ECG; intravenous

	Dosage (μg)	Cumulative dose (μg)	Duration of observation
Build -up phase (in-patient)			
Day 1	0.01/0,1/1/2 every 30 min	36.1	Until next morning
Day 2	4/8/10/20 every 60 min	42	Until next morning
Day 3	40/60/80 every 60 min	180	Until next morning
Day 4	100		2 h
Maintenance phase (out-patient)			
Day 8	100		At least 1 h
Day 15	100		At least 1 h
Continue every 4–8 weeks			

Table 2. Rush protocol of subcutaneous VIT

saline infusion, oxygen supply, and anaphylactic shock treatment kit ready to use.

Before SC the insect was cooled to about 6–8 °C to slow down his mobility then taken from the jar with tweezers, transferred to a tray, and covered with the net. The insect was transferred under this net to the patient's upper arm and mechanically irritated to sting. The fact of the sting was confirmed by the patient after feeling burning pain. The insect was kept on the skin for about 60 s to allow the maximum amount of venom to enter the skin. The appearance of the wheal was considered an objective test quality sign. After SC, the insect was killed by immersing in 70 o ethanol. The bees were provided by a beekeeper and the wasps were caught by personnel instructed in wasp recognition. In case of doubt, wasp species were identified by a professional entomologist. With careful handling none of the personnel or other patients were stung. After successful SC VIT was continued for at least 6 months.

RESULTS

From 2013 to 2021 we evaluated 83 patients (41% (n = 34) males; median age 43 years, age range 18–73 years) who were treated with VIT. The data obtained from the patient's medical history (demographic, severity of anaphylaxis, culprit insect recognition, results of allergological diagnostic work-up and treatment selection, adverse events of cluster VIT, sting challenge outcomes, rate of the patients with elevated basal tryptase levels (>11.4 ng/ml) and mastocytosis, etc.) are presented in Table 3.

The majority of the patients experienced a grade II (30.1%, n = 25) and III (57.8%, n = 48) anaphylactic reaction according to Ring and Messmer classification. As noteworthy, 2 patients required resuscitation and it was consistent with a grade IV anaphylactic reaction (2.4%).

No statistically significant differences were found between gender, culprit insect, and the grade of reaction.

Some patients could not specify which insect stung them (37.4% (n = 31/83). Nearly half (48.4% (n = 15/31) of these patients were found to have double sensitization.

Thirty percent (30.1% (n = 25/83) of the patients recognized the insect as a honeybee and 32.5% (n = 27/83) of patients recognized the culprit as a wasp. In all cases when patients recognized the insect, this was confirmed by allergy work-up, and the treatment was always initiated with the same insect they indicated.

Sensitization testing

Double sensitization to bees and wasps' venom was detected in 42.2% (n = 35/83). In 33 cases CRD was used and positive result to bee (rApi m 1) and wasp (rVes v 1 and/or rVes v 5) major venom components confirmed true double sensitization in 40.6% (n = 13/33) cases. Of those with true double sensitization detected 3 patients were treated with double venom immunotherapy (2 of them were diagnosed with systemic mastocytosis (SM), and 1 patient had several severe reactions and 2 minor signs of SM in bonne marrow showing a high possibility of mast cell activation disorder). Seven double sensitized patients were treated with bee and 3 patients - with wasp VIT. CRD helped to prove monosensitization in 4 cases of bee-allergic, and 15 wasp-allergic patients. In the case of monosensitization, CRD did not give any additional value.

One patient with indolent SM and allergic to wasp venom developed anaphylactic shock after the bite of a horsefly (*Tabanidae* family). Sensitization was confirmed *in vitro* (sIgE >0.35 kUA/L, ImmunoCAP, Thermofisher Scientific, Sweden); thus, omalizumab 300 mg every 4 weeks s/c was prescribed for the period of horsefly activity, which helped the patient to tolerate bites of these bloodsuckers for the summer seasons.

Venom immunotherapy (VIT)

Fifty-five (66.3%) of the patients were treated with wasp venom (Table 1), 25 (30.1%) – with bee venom and 3 patients (3.6%) with both. Cluster VIT protocol was applied in 97.6% (n = 81/83); 22% (n = 18) of patients completed VIT. The average duration of VIT was 53.2 ± 10.1 months. 22.2% (4/18) completed >3 years of VIT, 44.4% and 27.8% more then 4 and 5 years, respectively.

Eleven patients discontinued the VIT earlier voluntarily (average after 18.6 ± 14.1 months) due to the fear of possible SR or lost on follow-up. In 10 of the cases treatment duration was less then 3

Total number of patients	83
Mean age, years	43 (18–73)
Males	34/83 (40.96%)
No of patients, suffering from more than 1 systemic sting reaction	36/83 (43.37%)
Grade of sting reaction (according to Ring/Messmer) I II III IV	 8/83 (9,64%) 25/83 (30,12%) 48/83 (57,83%) 2/83 (2,41%)
Number of sIgE/skin-tested patients	83/53
Sensitization	
- Bee venom	12/83 (14.46%)
- Wasp venom	36/83 (43.37%)
- Double sensitization	35/83 (42.17%)
- Double-sensitized patients after CRD	13/33 (39,39%)
Detected sIgE level for venoms	
- at >0.35 kU_A/L	80/83 (96.39%)
- at $>0.1 < 0.35$ kU_A/L	3/83 (3.61%)
Mastocytosis (systemic and cutaneous)	7/83 (8,43%)
Tryptase in patients with mastocytosis, ng/ml	22.22 ± 17.89
No of patients with ISM and tryptase <11.4 ng/ml	3/7 (42.9%)
Elevated tryptase $>11,4$ ng/ml	10/82 (12.2%)
- Tryptase in patients with a single sting reaction	$6,84 \pm 9.1$ ng/ml
- Tryptase in multiple sting reaction	$8,63 \pm 5.97$ ng/ml
VIT	
- Bee VIT	25/83 (30.12%)
- Wasp VIT	55/83(66.27%)
- Double VIT	3/83 (3.61%)
Number of patients with SR induced by VIT	8/83 (9.64%)
- Wasp VIT-related adverse events	1/58 (1.72%)
- Bee VIT-related adverse events	7/28 (25.00%)
Treatment duration of patients who completed VIT	53.2 ± 10.1 months
Maintenance treatment of <12 months >12 months and <24 months >24 months and <36 months >36 months and <48 months	 20/83 (21.9%) 13/83 (15.7%) 13/83 (15.7%) 17/83 (20.5%)

(continued)

>48 months and <60 months >60 months	13/83 (15.7%) 7/83 (8.4%)
Number of SC patients	43/83 (51.81%)
- Bee	13/43
- Wasp	30/43
Number of field stings (without allergic reactions)	7/83 (8.43%)

Table 3. (Continued) The demographic data of the patients, anaphylaxis grade, sensitization, tryptase levels, treatment and mastocytosis, specific IgE levels to insect venoms for study cohort from January 2013 to December 2021. *CRD – component resolved diagnosis, ISM – indolent systemic mastocytosis, VIT – venom immunotherapy, SC – sting challenge, SR – systemic reactions*

years. Nine patients decided to stop VIT earlier voluntarily despite recommendations.

Two patients became pregnant, but the treatment was not stopped. No adverse reaction occurred.

Adverse events during VIT

Local erythema, swelling, and pruritus/pain of various degrees were observed in all of the patients during the first days of treatment introduction. In a few cases, local reactions persisted for a few days. Eight patients of all treated groups experienced SR (9.6%). In 6 (7.2%) patients SR occurred during a build-up phase: 5 during bee cluster VIT, and 1 – wasp cluster VIT (Table 4). Four patients experienced SR while receiving maintenance doses of bee venom. Two of them stopped VIT (both also had SR during build-up).

One patient with cutaneous mastocytosis whose SC was positive while she was treated with the usual 100 μg dose, developed hypotension after a doubled bee venom dose (200 μg). This patient continues VIT with omalizumab pretreatment.

In total 7/28 (25%) patients treated with bee cluster VIT and just 1 patient treated with wasp cluster VIT (1.7%, n = 1/58) developed systemic reactions. The majority of participants at the end of this study were still undergoing VIT. However, throughout the ongoing treatment, none of them encountered systemic reactions for 2 consecutive years.

Sting challenge (SC), field stings, and treatment modification

Sting challenge (SC) was performed in 51.8% (n = 43/83) of treated patients (13 of them were treated with bee venom and 30 with wasp venom). On average SC was performed in 15.5 ± 12.0 months after starting VIT. In 6 cases SC was positive (in 2 patients treated with bee VIT and in 4 treated with wasp VIT).

After positive SC, an adjustment of the VIT dosage was done (Table 5).

In positive SC patient's average serum baseline tryptase was 8.9 ng/ml, and in negative SC patients – 8.1 ng/ml, but after exclusion of patients with mastocytosis average serum baseline tryptase is 6.1 ng/ml In 2 cases, mastocytosis was confirmed after negative SC despite being treated with a standard 100 μg dose. In 10 patients we had to change Alyostal Stallergenes (France) to Venomenhal (The Netherlands) because of supply interruption. On changing preparation, the loading dose of 30/30/40 mcg every 30 min was performed and the maintenance dose was continued every 3–4 weeks. This was done in 9 patients without any complications. One patient developed an immediate skin itch after the first dose of switching.

DISCUSSION

The relevant season for HVA patients in Lithuania lasts from March to October. As in most other north-eastern European countries, the main insects in the *Hymenoptera* order that cause allergic reactions are honeybees (*Apis mellifera)* and social wasps, including yellow jackets (*Vespula* and *Dolichovespula*) and hornets (*Vespa*). Due to climate change, paper wasps became more common in our region. In Lithuania *Polistes dominulus* first time was noticed about 25 years ago and is spreading,[8] but they do not pose additional concerns from a diagnostical perspective.

	Venom/ Commercial name	Dose of VIT build-up SR	Reaction on build-up (WAO)	Treatment modification	SC	Risk factors	Reaction on maintenance (WAO)	Further decision/ maintenance dose
1	Honeybee (Diater apis mellifera)	At 60 µg cumulative dose	I	No	Nd	None	No reaction	100 µg
2	Honeybee (Venomil biene)	At 90 µg cumulative dose	IV (shock)	Rush IT	Neg.	SM	No reaction	100 µg
3	**Honeybee (Venomil biene)**	**At 20 µg**	**II**	**Rush IT**	**Nd**	**None**	**IV (shock)**	**STOP**
4	Honeybee (Diater apis mellifera)	At 100 µg	I	No	Neg.	None	No reaction	100 µg
5	**Honeybee (Venomenhal bee)**	**Multiple (10,30,50 µg)**	**II**	**No**	**Nd**	**None**	**IV (shock)**	**STOP**
6	Wasp (Venomil wespe)	20 µg	I	No	Neg.	None	No reaction	100 µg
7	Honeybee (Diater apis mellifera)	No reaction	–	–	Nd	Hypertension, ACEI	II	100 µg
8	Honeybee (Venomil biene)	No reaction	–	–	Pos.	cutaneous mastocytosis	IV	Omalizumab

Table 4. Characteristics of patients with immunotherapy-induced systemic reactions. *ACEI – angiotensin-converting enzyme inhibitor; Nd – not done; Pos. – positive; WAO - the WAO subcutaneous immunotherapy systemic reactions grading system; Venomenhal - HAL Allergy, Netherlands; Venomil - Bencard Allergy, Germany; Diater - Diater Laboratorios, Spain*

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Patient No	Venom	VIT duration	SC	Comorbidities	The adjustment of the VIT	Further history
1	Wasp (Diater vespula)	6 months	Generalized urticaria	None	Changed to Venomil wespe	SC after 12 months, negative
2	Honeybee (Venomil biene)	6 months	Hypotension, tachycardia	Skin mastocytosis	Dose: 100 → 200 mcg/ml	SR after maintenance dose → (omalizumab) The second SC is not done
3	Wasp (Venomil wespe)	12 months	Generalized flushing, tachycardia	Systemic mastocytosis	Dose: 100 → 200 mcg/ml	SC after 18 months, negative
4	Wasp (Diater vespula)	19 months	Generalized urticaria	None	Changed to Venomil wespe	SC after 12 months, negative
5	Honeybee (Diater apis mellifera)	6 months	Hypotension	2 minor criteria of SM in BMB	Dose: 100 → 200 mcg/ml	Field stings few time - negative
6	Wasp (Diater vespula)	12 months	Generalized urticaria	None	Changed to Venomil wespe	SC after 12 months, negative
TOTAL number of SC patients: 43/83 (51.81%)			**Positive SC after first attempt:** 6/43 (14%)		**Reactions (SC or field sing) after treatment modification:** 0/6	

Table 5. Characteristics of the patients who had a positive sting challenge test. *BMB – bone marrow biopsy; SC – sting challenge; SM – systemic mastocytosis*

Since the treatment must be administered with the venom of the culprit insect, patient testimony may be important. According to the data of various authors, they are not very reliable.[9] According to the data we have, in all cases when patients could recognize the insect (52 patients, 62.7%) their suspicion was confirmed by allergy work up to 100%, which could testify to a fairly good entomologically well-educated part of the population. One could argue, that double sensitization was found in 21.2% (11/52) cases. Three of these patients were treated with double VIT. Double sensitization was detected in 15 of 31 cases when the patient could not identify the correct insect. In these cases, the decision to choose venom for VIT was based on the circumstances of the sting event: day/night time, hives/nests found nearby, type of sting, and whether the stinger remained or not.

When evaluating the choice of diagnostic method, the international consensuses give priority to skin testing (ST) as a first step,[10] but we usually start with *in vitro* testing as more reliable. Current diagnostic systems are sufficiently sensitive in detecting sIgE starting from 0.1 kU/ml. Also, in the case of double sensitization, ST is not useful and only CRD can help. We found 1 benefit of skin testing. In several patients with clear sensitization to wasp venom, ST was negative with Diater vespula (Diater laboratorios, Spain) venom (intradermal test at maximal 1 µg/ml concentration) and positive with Venomil wespe (Bencard Allergy, Germany). This assumed insufficient composition of the Diater wasp venom preparations. Insufficient quality of venom extracts affecting diagnostic accuracy and treatment efficacy has been described in the literature.[11,12] Three positive SC in the patients treated with Diater vespula who did not have any additional risk factors for ineffective VIT strengthened this suspicion. We could not prove what was missing but decided to change to a different venom brand and this decision was correct (the second SC turned negative). Thus, it can be concluded that even in the presence of clear sensitization, detected by *in vitro* method, it could be worth performing ST before prescribing treatment with a specific preparation to make sure that specific preparation contains at least some components that are important for the patient.

In double-sensitized (confirmed by CRD) mastocytosis patients we prescribe double VIT, as these patients' sensitization is rarely silent.[13]

Most of the patients were treated with the same cluster VIT scheme, which allowed us to carry out a safety analysis of the build-up and maintenance phases of the VIT. The overall rate of cluster VIT-induced anaphylaxis during build-up was 7.2%. This rate was comparable with other rush protocols described in the literature.[14–16] Due to a small number of patients, we could not identify the dose of venom that predisposes to the development of SR and find which step is the most dangerous.

Binbaum et al showed that cumulative venom dose correlates with the risk of VIT-induced anaphylaxis (>100 mcg), and Brehler et al. found that the incidence of anaphylaxis is related to the number of injections.[16,17] In the cluster VIT protocol that we use the total venom dose of the day does not exceed 100 µg at the build-up and the maximal number of injections is 5 on Day 1.

Manufacturers of venom preparation in their drug information leaflets recommend to increase gradually the interval between the injections by 1 or 2 weeks after reaching 100 mcg dose.[18] Our data show that there is no need for this approach. We did not observe any risk related to monthly injections right after build-up completion. This approach is more appropriate for the patient and saves time and venom.

After evaluating SR during the maintenance period, we could conclude that the major risk factor for unsuccessful immunotherapy is treatment with bee VIT and SR developed at the build-up phase. These observations suggest to use of a different approach for patients with SR on bee VIT during build-up: a modification of the VIT protocol or premedication with omalizumab could be an option.[2] Kranert et al propose to reduce temporarily the maintenance dose for 12 weeks before a second attempt of build-up.[19]

But when talking about safety bees and wasp VIT are incomparable. In our experience wasp venom immunotherapy with cluster protocol could be initiated in an outpatient setting safely.

Local reactions were observed in almost all patients during the first days of injections. If the

patient does not react locally, suspicion of venom preparation quality should arise.

One of the important questions is what are the criteria of effective VIT. In the short term, there could be no systemic reactions during VIT and negative SC or field sting. In a long-term period, the effectiveness of VIT is shown when field sting is tolerated after stopping VIT. In our cohort, we had 2 patients who decided to stop bee VIT because of SR induced by VIT. In this case, bee VIT effectiveness would be 92.9%. None of the patients decided to stop VIT with wasp venom because of adverse events.

The first SC attempt revealed the efficacy of VIT at 86.1%, but after treatment correction and the second SC, the efficacy is 97.7% (theoretically it could be 100%, but 1 patient continues the treatment in another hospital and the second SC was not performed). SC test proved to be an important tool for VIT efficacy evaluation, but it should be regarded as an important tool to detect unprotected patients. In 3 cases venom quality was the major reason for VIT failure. This presumption was based on our empiric experience of negative skin testing with Diater wasp venom in patients with clear sensitization proven by *in vitro* testing. After changing venom preparation repeated SC was negative. In 2 cases the dose increase to 200 μg (1 bee and 1 wasp) was effective. The last patient with cutaneous mastocytosis was more complicated as developed SR on a bee venom maintenance dose of 200 μg. Omalizumab was started and she could keep the treatment and gradually increase the dose.

Wasp VIT efficacy is 100% in our group of patients in the short term.

Arbitrary and early termination of treatment should also be considered as some failure of treatment, as it is related to the lack of motivation. Such failure occurred in 9 cases (10.8%, average after 23.5 ± 13.7 months).

International guidelines recommend increasing the maintenance dose to 150 or 200 μg in the case of relapsing sting-induced anaphylaxis, severe reactions, mastocytosis, and cases where patients have accumulated risk factors for treatment failure.[2] Half of our positive SC was related to mast cell proliferation disorders. Therefore, we suggest increasing the dose of the venom early during VIT in such patients, even if a proliferative mast cell disorder is suspected (e.g., in patients with 2 minor SM criteria and severe reactions). Tryptase concentration is not the main criterion – the majority of the patients with increased tryptase receiving the usual 100 μg venom dose do not develop a reaction during SC and 3 of our patients with indolent systemic mastocytosis had tryptase levels below 11.4 ng/ml, so additional risk factors should guide the decision to increase the dose without waiting for SC results.

CONCLUSION

The effectiveness of VIT is related to several factors: identification of culprit insects, correct diagnosis of sensitization, safety of the treatment protocol, and appropriate dosage for the patient.

A closer look at data collected at 1 center can help to optimize allergy work-up and treatment plans. Our experience shows that the 3-day cluster VIT protocol is the best, it has a good safety and efficacy profile but the venom quality control is necessary. Monthly injections can be given right after reaching the maintenance dose. Despite that, some patients with SM could be protected with the usual 100 μg doses, the doubling of the dose gives more safety in these cases. The biggest challenge is the patients with bee venom allergy and repeated adverse reactions during VIT. The guidelines for the management of difficult cases are needed.

Abbreviations

VIT, Venom-specific immunotherapy; HVA, *Hymenoptera* venom allergy; CRD, Component-resolved diagnosis; SC, Sting challenge; SM, Systemic mastocytosis; SR, Systemic reaction.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
Kestutis Cerniauskas (KC), Laura Malinauskiene (LM) were involved in the conceptualization and methodology of the study. KC, Kotryna Linauskiene (KL), Linas Giguola (LG), Anzelika Chomiciene (AC), LM provided clinical data for analysis. KC and JR curated the data and performed the formal analysis. KC and JR were involved in writing of original draft. The writing and reviewing process involved KC, JR, KL, AC, LG, LM. KC and LM were responsible for project supervision.

Ethics approval and consent to participate
The study was approved Vilnius Regional Bioethics Committee (No. 158200-16-847-355) before the review of clinical records.

Authors' consent for publication
All authors approve this manuscript to be submitted to the World Allergy Organization Journal.

Declaration of competing interest
All authors have no conflict of interest within the scope of the submitted work.

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