

High long-term efficacy of venom immunotherapy after discontinuation

To the Editor

Subcutaneous venom immunotherapy (VIT) is currently the most effective treatment for hymenoptera venom allergy, with a carry-over effect lasting up to several years after its discontinuation. The current European and American guidelines/practice parameters consider 5 years of VIT protective in the majority of patients, with 3 years probably sufficient in particular groups (eg, mild reactions before VIT, children) and recommending lifelong treatment in patients with particular risk factors of severe reactions.¹⁻⁴

A number of risk factors for relapse after VIT discontinuation have been proposed so far: Older age, concomitant cardiovascular and pulmonary diseases, bee venom allergy, low threshold of skin reactivity, mast cell disorders and elevated serum tryptase levels (STL), severe reactions before VIT, systemic reactions (SRs) during VIT (to treatment injections or stings), repeated stings after VIT, and time interval after stopping VIT.^{5,6}

However, when it is safe to discontinue VIT to provide long-term protection after discontinuation and what are the reliable predictors of severe reactions after re-sting are long-standing issues. Moreover, all the current recommendations are based on outdated studies, often over short follow-up periods and on small follow-up groups of patients.⁷⁻⁹

The aim of this study was to evaluate the efficacy of VIT after a long follow-up period after discontinuation and to investigate potential predictors of severe reactions after re-stings, in clinical practice.

We analyzed 257 patients treated with vespid or honeybee venom extracts, who discontinued VIT before July 2018. Methods, baseline characteristics, and results of the follow-up group are reported in the Online Appendix S1.

Out of these 257 patients followed up for in field re-stings, 147 (57.2%) were re-stung after VIT discontinuation (re-stung group).

The median VIT duration of the re-stung group was 10 years (from 2 to 25 years), and the median follow-up time after VIT discontinuation was 4 years (from 1 to 26 years). Their characteristics are shown in Table 1.

Of note, only five patients (3.4%) experienced SRs after stopping VIT (Table S1, Figure S1), 17 had large local reactions (LLRs) (11.6%), and the remaining 125 (85%) had no reaction at all.

Our overall SR frequency of 3.4% is lower compared with that previously reported in literature (ie, from 9% to 14%),⁷⁻⁹ probably

due to the longer period of treatment (average: 10 years) and to a careful case-by-case approach for VIT discontinuation taking into account risk factors for SRs, discovered over time and so not yet known at the time of previous publications.⁷⁻⁹ For example, mast cell disorders, severe cardiovascular/respiratory comorbidities especially if associated with multidrug treatments, or occupational/recreational risks could possibly lead to avoid or delay the discontinuation. Moreover, for the first time to our knowledge, we report the outcome of re-stings on a long follow-up period up to 26 years. In particular, we included 31 patients (12% of the follow-up group) with more than 10 years of follow-up after VIT discontinuation (Table S2).

The survival analysis showed 2.6% probability of SR after 5 years off VIT (95% CI: 0.8-7.8%, SE = 0.015), 3.8% after 10 years off VIT (95% CI: 1.7-10.2%, SE = 0.019), and 10.1% after 20 years off VIT (95% CI: 2.9-31.9%, SE = 0.063; Figure 1). The survival analysis of LLRs is reported in Figure S2.

Systemic reactions were quite homogeneously distributed along the follow-up period, with no statistically significant differences in terms of VIT duration (Table S3) or off VIT period (Table S4, Figure S1).

Among the patients who experienced a reaction during VIT due to hymenoptera re-stings (n = 20) or VIT injections (n = 2), 11 were re-stung even after VIT discontinuation, reporting only LLRs in two cases.

Out of 11 patients with high STL (ie, ≥ 11.5 $\mu\text{g/L}$, from 11.6 to 32 $\mu\text{g/L}$), four were re-stung more than once after VIT, with only LLRs in two cases and no reaction in the others.

The logistic regression analysis did not show any statistically significant correlation between the occurrence of SRs after VIT discontinuation and age, gender, comorbidities, concomitant cardiovascular medications (ACE-inhibitors, sartans, beta-blockers), honeybee venom, severity of baseline reaction, occurrence of reactions on VIT, skin test reactivity before VIT and at VIT discontinuation, STL, multiple re-stings (ie, multiple stings in one single occasion), re-sting after a long period of VIT discontinuation, and ≥ 3 repeated stings after VIT (Table S5).

No statistically significant correlations were observed either with new potential risk predictors of SR after VIT discontinuation that we included in our study, that is, close re-stings (ie, more than one re-sting

TABLE 1 Characteristics of the patients re-stung after venom immunotherapy, grouped per type of reaction (re-stung group, n = 147)

Patients	No reactions		Large local reactions		Systemic reactions	
	125	(85%)	17	(11.6%)	5	(3.4%)
Gender (Male/female)	102	/23	13	/4	4	/1
Median age (IQR)	61	(51 - 68)	54	(49-64)	58	(53-61)
Comorbidities						
Cardiovascular	44	(35.2%)	6	(35.3%)	3	(60%)
Respiratory	8	(6.4%)	0	(0%)	1	(20%)
Diabetes mellitus	7	(5.6%)	2	(11.8%)	0	(0%)
Concomitant medications						
ACE-inhibitors	15	(12%)	1	(5.9%)	0	(0%)
Sartans	24	(19.2%)	3	(17.6%)	1	(20%)
Beta-blockers	8	(6.4%)	1	(5.9%)	2	(40%)
Proton-pump inhibitors	9	(7.2%)	0	(0%)	1	(20%)
Statins	11	(8.8%)	4	(23.5%)	1	(20%)
VIT Venom						
Vespula (100 µg)	64	(51.2%)	12	(70.6%)	2	(40%)
Vespula + Polistes (50 + 50 µg)	22	(17.6%)	3	(17.6%)	3	(60%)
Polistes (100 µg)	13	(10.4%)	0	(0%)	0	(0%)
Apis (100 µg)	24	(19.2%)	1	(5.9%)	0	(0%)
Vespa crabro (100 µg)	2	(1.6%)	1	(5.9%)	0	(0%)
Reaction before VIT ^a						
Large local reaction	1	(0.8%)	0	(0%)	0	(0%)
Mueller 1	4	(3.2%)	2	(11.8%)	0	(0%)
Mueller 2	35	(28%)	4	(23.5%)	1	(20%)
Mueller 3	40	(32%)	5	(29.4%)	3	(60%)
Mueller 4	45	(36%)	6	(35.3%)	1	(20%)
Years on VIT						
Median (IQR)	10.5	(7-13)	6.5	(4.5-10)	11	(6-11)
Total = 10 (7-13)						
<3	1	(0.8%)	0	(0%)	0	(0%)
3-<5	4	(3.2%)	4	(23.5%)	0	(0%)
5-<10	41	(32.8%)	8	(47.1%)	2	(40%)
≥10	79	(63.2%)	5	(29.4%)	3	(60%)
Years off VIT						
Median (IQR)	4	(1-7)	7	(3-10)	4	±(2-6)
Total = 4 (2-7)						
<3	50	(40%)	2	(11.8%)	2	(40%)
3-<5	20	(16%)	3	(17.7%)	1	(20%)
5-<10	44	(35.2%)	7	(41.2%)	1	(20%)
≥10	11	(8.8%)	5	(29.4%)	1	(20%)
Skin tests before VIT (µg/mL)						
0.001	28	(22.4%)	3	(17.7%)	1	(20%)

(Continues)

TABLE 1 (Continued)

Patients	No reactions		Large local reactions		Systemic reactions	
	125	(85%)	17	(11.6%)	5	(3.4%)
0.01	54	(43.2%)	5	(29.4%)	2	(40%)
0.1	31	(24.8%)	9	(52.9%)	1	(20%)
1	10	(8%)	0	(0%)	1	(20%)
Negative	0	(0%)	0	(0%)	0	(0%)
n/a	2	(1.6%)	0	(0%)	0	(0%)
Skin tests after VIT ($\mu\text{g/mL}$)						
0.001	0	(0%)	0	(0%)	0	(0%)
0.01	2	(1.6%)	2	(11.8%)	0	(0%)
0.1	25	(20%)	2	(11.8%)	2	(40%)
1	78	(62.4%)	12	(70.6%)	1	(20%)
Negative	15	(12%)	1	(5.9%)	2	(40%)
n/a	5	(4%)	0	(0%)	0	(0%)

Abbreviations: IQR, Interquartile range; VIT, venom immunotherapy.

^aAccording to Mueller classification.

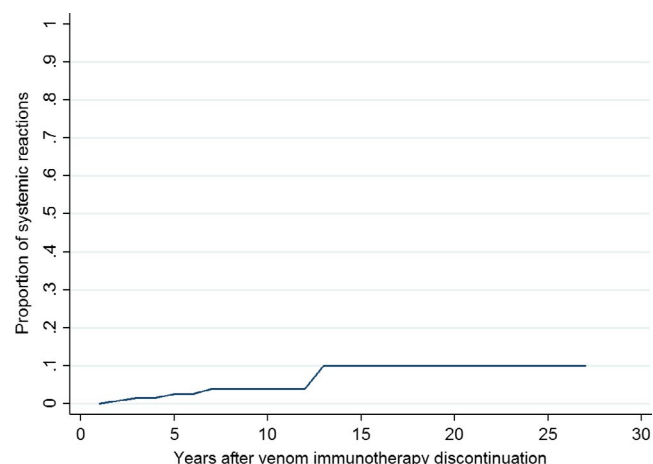


FIGURE 1 Survival analysis—probability of systemic reactions 5, 10, and 20 y after venom immunotherapy discontinuation

within few days or weeks), concomitant treatment with statins and proton-pump inhibitors, and previous LLRs (Table S5).

Treatment with low VIT maintenance dosage (50 μg), compared with the standard 100 μg , does not reach the statistical significance as a risk factor either (OR = 5.3, 95% CI: 0.8–33.7, $P = .075$), but it is noteworthy that three (10.7%) out of 28 patients who received the Vespula + Polistes mix (50 + 50 μg) experienced SRs, compared with two SRs (2.2%) among the 91 patients who received 100 μg of Vespida VIT. The use of 50 μg instead of 100 μg is not recommended by the guidelines, but it was necessary in these exceptional circumstances for cost and reimbursement issues.

The low rate of SRs, even if remarkable from a clinical perspective, hinders a robust statistical analysis. Therefore, even if the

above-considered risk factors seem to be not statistically significant in our study, they can still have a role that should be investigated in larger sample sizes.

In conclusion, we reported the outcome of re-stings on a long follow-up period after VIT discontinuation (up to 26 years), in a considerable high number of re-stung patients (147, out of 257) with an average VIT duration of about 10 years, showing a low frequency of SRs (3.4%), compared with that reported by previous studies (9%–14%) (Figure 1). Even if the current guidelines are quite in agreement that 5 years of VIT might be protective in the majority of patients,^{1,2} our results may imply that a low rate of SRs might be achieved if VIT is discontinued after a case-by-case assessment, based on individual risk factors of relapse.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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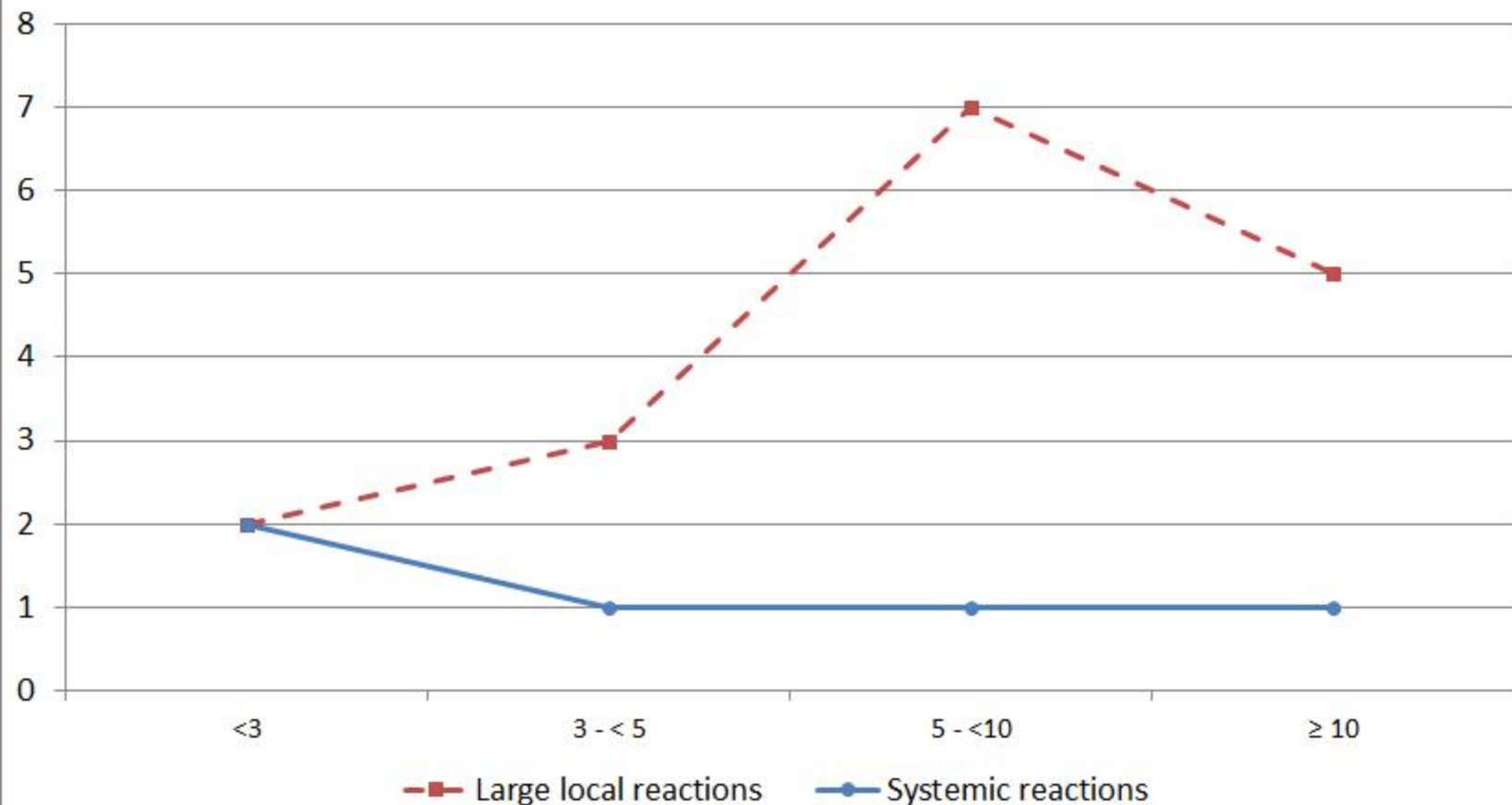
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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Number of reactions over the years after venom immunotherapy discontinuation (re-stung group, n=147)



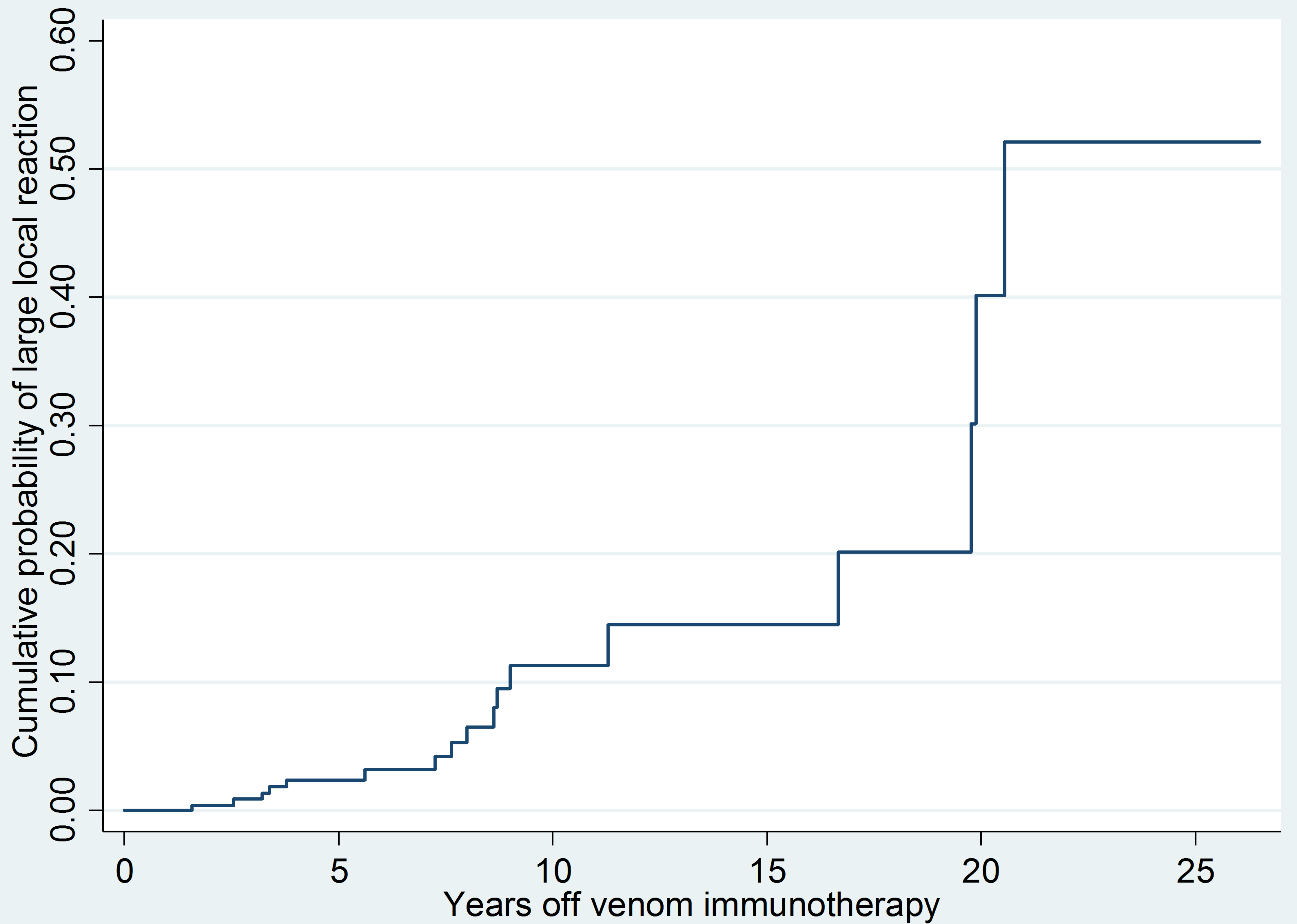


Table S1. Characteristics of the patients with re-sting systemic reactions after venom immunotherapy discontinuation (n=5)

Patient	A	B	C	D	E
Gender	Female	Male	Male	Male	Male
Age	61	32	53	69	58
VIT Venom	V / P	V / P	V	V	V / P
Reaction	M 3	M 3 (twice)	M 2	M 3	M 4 (twice)
[culprit insect]	[one multiple sting by 3 Ve]	[3 Ve stings within 2 weeks]	[1 Ve sting]	[1 Ve sting]	[1 P and 1 C sting within 2 months]
Reaction before starting VIT	M 3	M 2	M 3	M 3	M 4
Re-stings on VIT	2	≥3	2	≥3	≥3
Re-sting reactions on VIT*	No	No	No	No	No
Years on VIT	11	5	12	11	6
Years off VIT	1	2	12	4	6
Comorbidities	No	Hypertension	Hypertension	Ischemic cardiopathy	No
Therapies	No	beta-blocker	beta-blocker	Sartan, statin, IPP	No
Baseline Tryptase (μg/l)	2.6	2.3	4.7	4	7.1
Skin Tests before VIT (μg/ml)	0.01 / 0.01	0.01 / 0.01	0.1	1	0.001 / 0.001
Skin Tests at VIT discontinuation (μg/ml)	Neg / 1	0.1 / 0.1	1	Neg	0.1 / 1
Skin Tests after the reaction (μg/ml)	Neg / Neg	Neg / 0.1	1	n/a	0.1 / 0.1
Previous re-stings	4 (no reaction)	1 (no reaction)	0	2 (no reaction)	>5 (no reaction)
Subsequent re-stings	2 (M 3)	≥3 (no reaction)	0	0	0

C, *Vespa crabro*; M, Mueller score; V, *Vespula* sp; Ve, *Vespid*; P, *Polistes dominula*; VIT, venom immunotherapy.

*Due to extract injections or to re-stings.

Table S2 – Re-stings in patients with ≥ 10 years of follow-up after VIT discontinuation, grouped per follow-up time intervals (n=31).

Years off VIT	Patients with follow-up	Re-stung after VIT (in the overall period)	Re-stung after VIT (in the specific time frame)
$\geq 10 - 14$ years off VIT	15	8	7 ($\geq 10 - 14$ years off VIT)
$\geq 15 - 19$ years off VIT	10	8	7 ($\geq 15 - 19$ years off VIT)
$\geq 20 - 26$ years off VIT	6	6	3 ($\geq 20 - 26$ years off VIT)
Total	31	22	17

Table S3. Numbers and rates of reactions occurred after venom immunotherapy discontinuation, grouped per duration of venom immunotherapy (re-stung group, n=147)

Years ON VIT	Systemic reactions	Large local reactions	No reactions	Total
0 - <7*	2 (5.4%)	9 (24.3%)	26 (70.3%)	37
7 - <10	0 (0.0%)	5 (13.5%)	32 (86.5%)	37
10 - <13	3 (8.1%)	1 (2.7%)	33 (89.2%)	37
13 - 25	0 (0.0%)	2 (5.6%)	34 (94.4%)	36
	n.s.	<i>p=0.020</i>[†]	-	
Total (2 – 25)	5 (3.4%)	17 (11.6%)	125 (85.0%)	147

VIT, venom immunotherapy.

*Nine subjects underwent VIT for <5 years, four of them had LLRs and the others no reactions after discontinuation.

[†] Fisher's exact test.

Table S4. Rate of reactions, over follow-up time intervals after discontinuation of venom immunotherapy (re-stung group, n=147)

Years off VIT	Systemic reactions	Large local reactions	No reactions	Total
0 - <2	1 (2.7%)	1 (2.7%)	35 (94.6%)	37
2 - <4	2 (5.4%)	4 (10.8%)	31 (83.8%)	37
4 - <7	1 (2.7%)	4 (10.8%)	32 (86.5%)	37
7 - 21	1 (2.8%)	8 (22.2%)	27 (75.0%)	36
	n.s.	<i>p=0.077</i>[*]	-	
Total (1 – 26)	5 (3.4%)	17 (11.6%)	125 (85.0%)	147

VIT, venom immunotherapy.

*Fisher's exact test.

Table S5. Risk factors of re-sting reactions after venom immunotherapy discontinuation (re-stung group, n=147).

Risk factors	Overall reactions	Systemic reactions
Gender	n.s.	n.s.
Age	n.s.	n.s.
Baseline tryptase levels	n.s.	n.s.
Comorbidities		
Cardiovascular	n.s.	n.s.
Respiratory	n.s.	n.s.
Diabetes	n.s.	n.s.
Medications		
ACE-inhibitors	n.s.	n.s.
Beta-blockers	n.s.	n.s.
Sartans	n.s.	n.s.
Statins	n.s.	n.s.
Pump-inhibitors	n.s.	n.s.
Severity of reaction		
Before VIT	n.s.	n.s.
During VIT*	n.s.	n.s.
Skin tests		
High reactivity before VIT	n.s.	n.s.
Negative at VIT discontinuation	OR=0.17 (0.02-1.36), p=0.092	n.s.
Low VIT dosage (50 µg)	n.s.	OR=5.34 (0.84-33.74), p=0.075
Short VIT duration	OR=0.84 (0.74-0.96), p=0.008	n.s.
Long period after VIT discontinuation	OR=1.1 (1.01-1.2), p=0.029	n.s.
Repeated stings after VIT	OR=3.8, (1.01-14.24), p=0.047	n.s.
Close re-stings[†]	n.s.	n.s.
Multiple re-stings	OR=4.15 (1.49-11.55), p=0.006	n.s.
Large local reactions after VIT discontinuation	n.a.	n.s.

n.a., not applicable; n.s. not statistically significant; OR, Odds Ratio (95% Confidence intervals); VIT, venom immunotherapy. *due to extract injections or re-stings. [†] more than one re-sting within few days/weeks.

METHODS

Design

We analyzed all the patients treated with vespid (*Vespula species*, V; *Polistes dominula*, P; *Vespa crabro*) or honeybee (*Apis mellifera*) venom extracts, who discontinued VIT before July 2018, and enrolled only the ones with a follow-up of at least one year, after VIT discontinuation (follow-up group). The follow-up visits were performed at our center, during and after VIT. Some patients had not come to visit since 2016, and they were assessed again by telephone contacts for an update, from April to July 2019. All the interventions were part of routine clinical practice, and a formal approval by the Local Ethics Committee was not needed. Hymenoptera venom allergy was diagnosed by clinical history, skin tests and, in the majority of patients, specific serum IgE antibodies detection¹. Before starting VIT, in all patients a medical evaluation was performed to identify comorbidities and treatments of special interest, including respiratory function tests, ECG (with cardiology consultation, if needed), blood testing (including tryptase, since 2000) and REMA score for mast cell disorders (since 2013)². During VIT, follow-up visits were performed in all patients at least after three and five years of VIT, repeating skin tests, laboratory tests (in specific cases), and a medical re-assessment for comorbidities and concomitant medications (ACE-inhibitors, sartans, beta-blockers, statins, proton-pump inhibitors). The duration of VIT was decided according to the guidelines and literature available at the time of discontinuation³⁻⁶, and then tailored case-by-case taking into special account all the possible individual risk factors. In case of cardiovascular, respiratory, mast cell diseases, and/or remarkable occupational/recreational risks, the standard 5-year term for VIT was advised to be extended, when appropriate. All the patients, at the follow-up visits, were questioned for new comorbidities and treatments, re-stings and clinical outcome, management, and the type of insect responsible of the sting. A re-stung population was identified for the analysis (re-stung group). The efficacy of VIT was evaluated by in-field stings. Systemic reactions (SRs) were recorded if documented by Emergency Rooms or General Practitioners. Skin testing was repeated at each visit, while laboratory tests were

repeated in specific cases. Skin tests were performed injecting intradermally 0.001, 0.01, 0.1, and 1 µg/mL venom extract concentrations to raise a bleb of diameter 3-5 mm. Positive results were defined as an increase in wheal diameter of ≥3 mm after 15 - 20 minutes after the injection⁶.

Statistical analysis

Chi-squared or Fisher's exact test, when appropriate, were used to compare categorical variables (significance level 0.05). Logistic regression was used to investigate correlations between all the considered covariates and the outcome of re-stings after VIT discontinuation. The differences between STL were tested with t-test, after log-transformation for normality. A survival analysis, taking into account time and lost-to-follow up subjects, was performed in the re-stung population, considering the occurrence of reaction as the endpoint, over the time from VIT discontinuation to the last follow-up visit. All data were analyzed using STATA software version 13.

RESULTS

Follow-up group

The median age of the follow-up group (n=257) was 62 years old (IQR: 50 – 71), 76% males and 24% females, they started venom immunotherapy (VIT) from 1986 to 1997 and stopped it between 1992 and 2018. The most severe reaction before VIT, according to Mueller's score⁷, was Grade 4 in 93 patients (36.2%), Grade 3 in 91 (35.4%), Grade 2 in 60 (23.4%), Grade 1 in 9 (3.5%), and a large local reaction (LLR) in four (1.6%). Even if exclusively cutaneous reactions (i.e. Grade 1) have been thought suitable for observation without VIT, given their relatively low rates of more severe future reactions, these patients were treated with VIT due to their

Supporting Information

high risk of exposure to re-stings. The skin test reactivity to the venom used for immunotherapy, before starting VIT, was 0.001 µg/ml in 60 (24%) patients, 0.01 µg/ml in 91 (35.8%), 0.1 µg/ml in 81 (31.9%), and 1 µg/ml in 21 (8.37%); skin tests were not performed on three patients due to compliance issues. Ninety-eight patients (38.1%) had cardiovascular diseases (hypertension, ischemic cardiopathy), 13 (5.1%) diabetes mellitus, 17 (6.6%) asthma/COPD, in treatment with ACE-inhibitors (29, 11.3%), beta-blockers (24, 9.3%), sartans (51, 19.8%), statins (26, 10.1%), and/or proton-pump inhibitors (16, 6.2%). Eleven patients had high serum tryptase levels (STL), i.e. ≥ 11.5 µg/l (from 11.6 to 32 µg/l), measured before or during VIT (for those who started VIT when tryptase determination was not yet available).

The type of venom for immunotherapy was V in 135 patients (52.5%), P in 25 (9.7%), V+P mix in 44 (17.1%), C in 6 (2.3%), and A in 47 (18.3%). Almost all the patients underwent the standard 100 µg VIT maintenance dosage (250, 97.3%), including 51 patients undergoing V+P mix VIT (50+50 µg, respectively); five (2%) received 200 µg (as the culprit insect was a hornet), and in one subject the dosage was increased to 150 µg after a SR caused by a multiple hymenoptera sting during VIT. During VIT, within the follow-up group, 222 patients (86.4%) were re-stung with no reaction in 202 patients (91%), LLR in 13 (5.9%), Grade 2 reaction in 2 (0.9%), Grade 3 in 2 (0.9%), and Grade 4 in 3 (1.4%). The most severe reactions (Grade 4) were all caused by hornet re-stings in patients with V allergy at baseline, stung by V before VIT, and one of them had high STL. During VIT, out of the 222 re-stung patients, 49 (22.1%) received only one sting, 34 (15.3%) two stings, and 139 (62.6%) three or more stings; 35 (15.8%) reported multiple stings. Only two patients (0.8%) experienced one SR during VIT caused by the extract injection, with V+P and A venom extract, respectively, but all the subsequent injections were well tolerated; the vespid allergic patient was also re-stung during VIT, with no reaction. All the patients with high STL safely received VIT without any reaction to the injections. Ten of them were re-stung during VIT (≥ 2 re-stings in eight patients) with reactions in two cases: LLR in one subject and Grade 4 reaction in the only patient with a confirmed diagnosis of mastocytosis. These patients discontinued VIT, despite of their high STL, because of patients' personal decision or, in some cases, unavailability of diagnostic work-up/scores

for mast cell disorders at the time of their VIT. The REMA score, often calculated after VIT discontinuation, has resulted abnormal (i.e. ≥ 2) in seven of them. The mean VIT duration of the follow-up group was 10.9 ± 4.9 years (from 2 to 25 years), and the mean follow-up time after VIT discontinuation was 6.0 ± 4.4 years (from 1 to 26 years).

Analysis of overall reactions (LLR and SR)

Out of 147 re-stung patients after VIT discontinuation, 22 patients (15%) had a reaction of any grade (LLR or SR), and the remaining 125 (85%) had no reaction at all. Amongst the 22 patients who reacted after re-sting, 17 (77.3%) had LLRs, and five (22.7%) SRs, a lower SR frequency compared to that previously reported in literature⁸⁻¹³.

The risk of overall reaction (LLR or SR) was higher in case of multiple re-stings (i.e. multiple stings in one single occasion) (OR=4.2, 95% CI: 1.5-11.6, $p=0.006$). Some studies showed that the decrease in skin testing reactivity, after VIT discontinuation, is not a reliable predictor of safety in case of further re-stings^{8,14,15}. We observed a borderline significance level for the protective role of negative skin tests at VIT discontinuation (83% reduction in odds of reactions, 95% CI: from 98 to -34%, $p=0.092$), if all types of post VIT reactions are considered (i.e. SRs and LLRs). Our results suggested that the risk of SRs might be the same over the time after VIT discontinuation (Table 4_SupplInfo, Figure_2_SupplInfo), confirming the observations published by Golden et al^{8,14}. However, we showed that the risk of LLRs could increase over time after VIT (Table 4, Figure_3_SupplInfo), with a 10% odds increase (95% CI: 1 – 20%, $p=0.029$) of post VIT overall reactions (LLRs and SRs) for every year off VIT. Moreover, as previously reported for SRs^{8,10,14,16,17}, our data confirmed that repeated re-stings after VIT could be a risk factor of reactions (overall LLRs and SRs), with an OR=3.8 (95% CI: 1–14.2, $p=0.047$) for patients re-stung ≥ 3 times. As previously reported^{9,10}, a lower VIT duration seems related to higher risk of reactions after VIT, and longer VIT gives superior long-term effects. In particular, Lerch and Muller have observed 4.8% and

17.8% of SRs in patients who underwent VIT for ≥ 4 years and < 4 years, respectively⁹. Our results confirmed that short VIT duration might be a risk factor, with a 16% odds reduction of post VIT overall reactions (LLRs and SRs) for every year on VIT (95% CI: 26-4, $p=0.008$) (Table 3_SupplInfo). However, due to the limited occurrence of SRs in our study, caution is suggested in extending these conclusions to SRs alone (Table 2_SupplInfo).

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Supporting Information

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