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ORIGINAL ARTICLE

Drug Allergy, Insect Sting Allergy and Anaphylaxis

Large local reactions to Hymenoptera stings: Outcome of re-stings in real life

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Abstract

Background: Large local reaction to Hymenoptera stings is usually defined as a swelling >10 cm which lasts longer than 24 hours, sometimes associated with erythema, pruritus and blisters. Currently, the risk of subsequent systemic reactions after re-stings is considered low (2%-15%). Therefore, a diagnostic workup in case of large local reaction is often judged unnecessary, as well as adrenaline auto-injector and venom immunotherapy prescription. The aim of this study was to prospectively evaluate the outcome of re-stings in a real-world setting, in patients with a history of one previous large local reaction.

Abbreviations: A, *Apis mellifera*; AAI, adrenaline auto-injector; ACAAI, American College of Allergy, Asthma and Immunology; BTL, Baseline tryptase levels; CI, Confidence interval; EAACI, European Academy of Allergy and Clinical Immunology; HVA, Hymenoptera venom allergy; IQR, Interquartile range; LLR, Large local reaction; PD, *Polistes dominula*; SE, Standard error; sIgE, Specific IgE; SR, Systemic reaction; tIgE, Total IgE; VC, *Vespa crabro*; VIT, Venom immunotherapy; VS, *Vespula species*.

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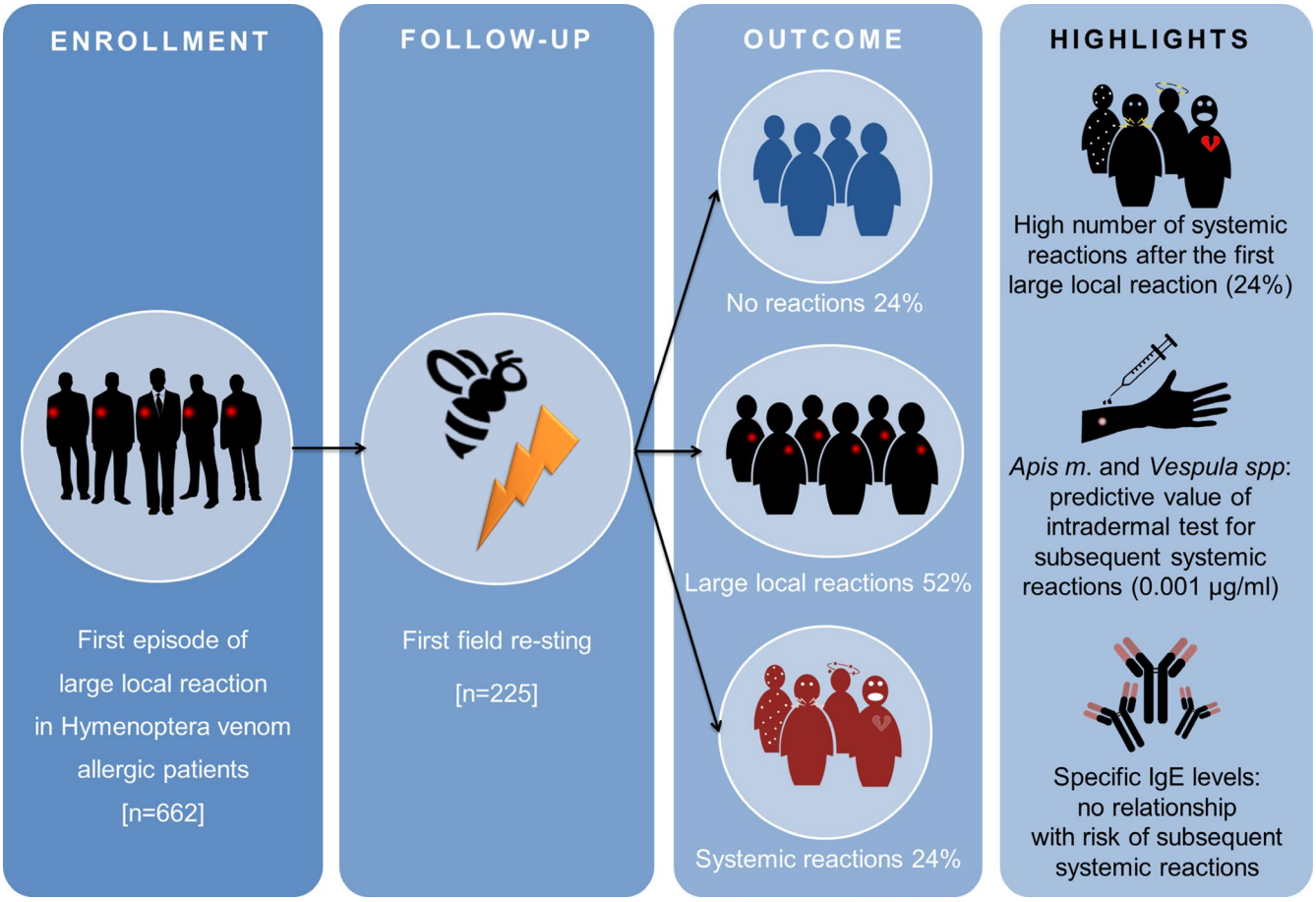
Methods: We consecutively enrolled patients who experienced their first large local reaction (as per EAACI definition), treated with antihistamine and steroids. They were followed for field re-stings and assessed for risk of subsequent systemic reactions.
Results: We enrolled 662 patients. Out of the 225 re-stung subjects, 24% did not experience reactions, 52% reported a second large local reaction and 24% had systemic reactions. The risk of subsequent systemic reactions was higher in case of skin test reactivity to *Apis mellifera* or *Vespula species* (OR 2.1 and 3.8, respectively), in particular if positive at 0.001 µg/mL concentration (OR 13.4 and 16.5, respectively).
Conclusions: Systemic reactions, after a previous large local reaction, occur more frequently than that reported by literature. After analysing the predictive role of large local reactions for systemic reactions, we demonstrated that an accurate diagnostic workup may be considered, particularly skin tests. Further studies in different countries are needed to confirm these results and large local reaction management.

KEYWORDS
Hymenoptera, large local reaction, skin test, specific IgE, venom allergy



GRAPHICAL ABSTRACT
The frequency of systemic reactions, after one first large local reaction, is considerably high (24%). Skin reactivity to *Apis mellifera* or *Vespula species* seems a good risk predictor, particularly at low concentration (0.001 µg/ml). An accurate diagnostic workup should be considered for large local reactions, particularly skin testing, to assess the risk of subsequent systemic reactions and the consequent management.

1 | INTRODUCTION

Large local reaction (LLR) to Hymenoptera stings is usually defined as a swelling exceeding a diameter of 10 cm which lasts longer than 24 hours, sometimes associated with erythema, pruritus and blisters; the underlying mechanism can be IgE, cell-mediated or even toxic (ie not allergic).[1,2] The prevalence of LLRs ranges from 2.4% to 26.4% in the general population,[3] being lower in children, and higher in beekeepers (38%) and in the common cases of asymptomatic sensitization to bee or wasp venom (44%).[4] The large variability in the reported prevalence is probably the result of a nonuniform definition of LLR across studies, different methodology of surveys, and considered populations.[3] The prevalence of systemic reactions (SRs) after a previous LLR is about 2%-15%,[2,5-9] and it seems to be even lower after two consecutive episodes of LLR,[10] but the literature reports limited data based on small patient sample sizes. Currently, the available diagnostic tests are not able to distinguish or foresee LLRs and SRs on a case-by-case basis.[4] However, assessing the risk of SRs in patients with a history of LLRs would be crucial for their clinical management, for diagnosis as well as for treatment. In some countries, it is considered that there is no need to refer patients with LLRs to a diagnostic workup, while in others it is an option.[11] Based on the assumed negligible risk of SRs following a history of LLRs, the ACAAI practice parameter (2016 update)[5] states that most patients who have had a LLR do not need to be tested, and adrenaline auto-injector (AAI) prescription is usually not necessary, but might be considered only in special circumstances (ie comorbidities, high risk of re-sting). Similarly, a European Consensus Statement, according to the vast majority of Hymenoptera venom allergy (HVA) experts who consider the risk in LLR negligible, does not propose the indication of AAI.[12]

On the other hand, concerning venom immunotherapy (VIT), both American and European Guidelines advise that it could be an acceptable option or recommended, in recurrent and troublesome LLR, to reduce the duration and size of future LLR, but only in special circumstances (ie frequent exposure, lifestyle factors) and after evaluating the cost/benefit profile.[5,13-15]

The aim of this study was to prospectively evaluate the outcome of re-stings in a real-world setting, in patients with a history of one previous LLR.

2 | METHODS

2.1 | Design

Fourteen allergy medical centres, in Italy (12 centres), Poland (1 centre) and United Kingdom (1 centre), consecutively enrolled, over a period of five years (from 2012 to 2017), all the adults and children who presented at the centres and that experienced their first LLR (as per EAACI Interest Group on Insect Venom Hypersensitivity definition[1]) after Hymenoptera sting. All the subjects included in the study were referred to the allergist after evaluation of the General Practitioners or Emergency Departments that prescribed antihistaminic and steroid treatments, but only those complying with the definition of LLR, after the allergists' evaluation, were enrolled. Normal/mild reactions, as well as SRs, were excluded from the study.

The patients were then followed for field re-stings and assessed for subsequent reactions, if any. All the participants were instructed to report any stings to the centres, regardless of the type of reaction. In addition, they underwent at least annual follow-up visits in case of re-stings causing SR, or a telephone contact in October/November, every year, if not re-stung or re-stung without SR. None of them started VIT at baseline. Patients gave an informed consent to in vivo and in vitro investigations and to the use of their clinical data for research purposes in an anonymous form. The protocol was approved by the members of the EAACI Interest Group on Venom Hypersensitivity. Since all the interventions were part of routine clinical practice, a formal approval by the Ethical Committee was not needed and was not requested.

2.2 | Diagnosis

Hymenoptera venom allergy was diagnosed, at least four weeks after the index LLR, by clinical history, skin tests and/or specific serum IgE antibodies detection (sIgE).[1]

All the patients were questioned for clinical symptoms and, particularly, for the type of insect responsible of the sting.

Skin tests were performed using purified (ALK-Abellò®) and nonpurified aqueous (Stallergenes®) extracts for *Apis mellifera* (A) and *Vespula species* (VS),[1] and capillary extracted aqueous extracts (Anallergo®) for *Polistes dominula* (PD) and *Vespa crabro* (VC). PD and VC were investigated only in the Mediterranean area, taking into account the different geographical distributions of these Hymenoptera species. Patients were tested at 0.001, 0.01, 0.1 and 1 µg/mL extract concentrations, intradermally injected. Positive results were defined as a wheal ≥3 mm diameter with flare after 15 - 20 minutes after the injection.[1] Since all the centres involved in the study were skilled and specialized in HVA management, fresh extracts for intradermal testing were always available, and prick testing not routinely performed, taking into account its lower diagnostic sensitivity.

ImmunoCAP (Thermo Fisher Scientific, Uppsala) was used for sIgE measure of A, VS and, in the Mediterranean countries, PD and VC as well. Limit of quantitation for ImmunoCAP has been reported to be 0.10 kUA/L; therefore, all results >0.1 kUA/L were interpreted as positive, according to the company's instructions. However, a secondary analysis was performed using the old cut-off value of ≥0.35 kUA/L.

Total IgE (tIgE) and baseline serum tryptase levels (BTL, normal values: 0 - 11.5 µg/L) (ImmunoCAP—Thermo Fisher Scientific, Uppsala) were also measured in the vast majority of patients.

2.3 | Statistical analysis

Logistic regression analysis was used for comparing the effect of all the considered exposure factors on the outcome (SR), showed as odds ratios (OR). A stratification was performed based on the exposure

factors, and the pattern of ORs was examined in the different strata to rule out any possible effect modification, testing for homogeneity. Control for confounding was performed by Mantel-Haenszel test and logistic regression for the final analysis, adopting a stepwise selection procedure for the choice of exposure variables to be included.

The main analysis was conducted comparing SR I-IV events (according to Mueller classification[16]) *versus* LLR and negative cases, after re-sting. An additional analysis, performed as confirmatory check, grouped severe SR (SR III-IV) *versus* mild/moderate SR (SR I-II), LLR and negative cases (Table S1, online repository).

The agreement assessment between tests was checked by Cohen's Kappa test (k), reporting the standard error (SE), values <0 indicate no agreement, 0 - 0.20 poor, 0.21 - 0.40 fair, 0.41 - 0.60 moderate, 0.61 - 0.80 good and 0.81 - 1 very good agreement.

Wilcoxon rank-sum and χ^2 tests were used to compare quantitative and categorical variables, respectively (significance level 0.05). All data were analysed using STATA software version 15.

3 | RESULTS

3.1 | Patients

Out of 662 enrolled patients, 360 (54%) were male and 302 (46%) female. Median age was 46 years, (interquartile range, IQR: 31 - 58 years; min-max: 8 - 86 years).

Overall, the Italian centres enrolled 580 (88%) patients, 75 (11%) were from Poland and 7 (1%) from United Kingdom.

All of them reported the first LLR after Hymenoptera sting. Vespids (VS, PD or VC) were recognized by the patients in 350 (53%) cases, and A in 84 (13%) cases. The remaining 228 (34%) patients were unable to identify the insect.

The patients were assessed for risk of frequent re-stings on the basis of frequent exposure, that is occupational/recreational risk, beekeepers or their relatives. The risk of frequent re-stings was considered high in 171 (26%) cases.

In 174 patients (26.3%), only intradermal tests were done; on the other hand, 62 subjects (9.4%) underwent in vitro sIgE measure without skin test. All the other subjects (n = 426, 64.3%) were studied with both methods.

3.2 | In vivo tests

Intradermal tests were performed in 600 patients (91%) and resulted positive for A, VS, VC and PD venom in 241 (36%), 418 (63%), 216 (33%) and 319 (48%) patients, respectively; multi-sensitized patients according to skin tests were 373 (56%), and mono-sensitization was observed in 209 cases (32%), namely to A, VS, C and P in 66 (10%), 87 (13%), 3 (<1%) and 53 (8%) subjects, respectively; skin testing was not performed in 62 (9%) patients and resulted negative in 18 (3%) patients.

The diagnostic agreement between clinical history (ie type of insect described by the patients, at baseline) and skin tests was 98% (k = 0.95, SE 0.08), when distinguishing A from Vespids (VS, PD, and VC), in the mono-sensitized patients, showing a skin test sensitivity and specificity >97% (AUC 99%, SE 0.007). On the other hand, after adding the multi-sensitized subjects to the analysis, the agreement decreased between clinical history and skin test, for both A (79%, k = 0.50, SE 0.05) and Vespids (89%, k = 0.60, SE 0.05).

3.3 | In vitro tests

Specific IgEs were measured in 488 patients (74%) and resulted positive to A, VS, C and P venom in 191 (29%), 301 (45%), 317 (48%) and 354 (53%) patients, respectively. Specific IgE multiple sensitization was observed in 261 patients (39%) and mono-sensitization in 120 (18%) to A (35, 5%), VS (53, 8%), C (3, <1%) and P (29, 4%); specific IgE assessment was not performed in 174 (27%) patients and resulted negative in 107 (16%) patients.

The diagnostic agreement between clinical history and sIgE was 93% (k = 0.85, SE 0.10) in the mono-sensitized patients, with a sIgE sensitivity and specificity >96% (AUC 94%, SE 0.02). In the total population, including multi-sensitized subjects, the agreement was 74% (k = 0.41, SE 0.05) for A, and 88% (k = 0.55, SE 0.06) for Vespids.

Median baseline tIgE and BTL were 97.3 kU/L (IQR 45.4 - 205 kU/L) and 4.5 μg/L (IQR 3.4 - 6 μg/L), respectively. Fifteen (2%) patients had high BTL (≥11.5 μg/L).

3.4 | Re-stung patients

Out of 662 total enrolled patients, 225 (34%) were re-stung after their first LLR, and 78 (35%) of them had been considered at high re-sting risk at baseline. Fifty-five (24% of the re-stung patients) did not experience any reaction, 116 (52%) reported a second LLR and 54 (24%) had a SR. In particular, a mild/moderate SR (grade I or II, according to Mueller classification[16]) occurred in 29 (13%) patients, and severe SR (grade III or IV) in 25 (11%). All the subjects with SR, according to the severity reaction, were treated by the Emergency Departments and, when necessary, followed by General Practitioners.

The culprit insects of the re-sting, according to the patients, were Vespids in 102 (45%) cases, A in 29 (13%) and not identified in 94 (42%) cases. Among the 35 patients clearly re-stung by the same insect—according to their history, 9 (26%) did not report any reaction, 22 (63%) had a LLR, and 4 (11%) reported a SR (grade I - II). Seven patients with high BTL were re-stung reporting no reaction in two cases, LLR in four and SR I in one case. The time interval between the index LLR and the subsequent re-sting was known in 73 (32%) patients, and it was ≥2 months.

The characteristics of patients, grouped by type of reaction after re-sting (ie no reaction, LLR, and SR), are shown in Table 1 and Table 2. No statistically significant differences were observed between these groups in terms of age, gender, type of insect and high risk of frequent re-sting.

Similarly, no relationship between SR and positive sIgE for a particular Hymenoptera species was found, and the sIgE value (high *versus* low levels) was not related to the type or severity of reaction after re-sting.

TABLE 1 Characteristics of re-stung patients, after a previous large local reaction, grouped by type of reaction (n = 225)

	No reaction	LLR	SR	Combined	*P*-value*
Patients	55 (24%)	116 (52%)	54 (27%)	225	
Median age [min-max]	42 [9-81]	50 [12-82]	48 [13-78]	48 [9-82]	0.71
Gender					0.07
M	36 (26%)	73 (54%)	27 (20%)	136	
F	19 (21%)	43 (48%)	27 (30%)	89	
Median tIgE (kU/L) [IQR]	85 [59-117]	95 [51-215]	74.5 [29-167]	84 [48-194]	0.40
Median BTL (µg/L) [IQR]	4.2 [3.2-5.4]	4.5 [3.6-6.3]	4.2 [3.4-6.1]	4.4 [3.4-6]	0.80
Presumed insect					0.55
A	7 (24%)	11 (38%)	11 (38%)	29	
Ve	26 (25%)	52 (51%)	24 (24%)	102	
Risk of re-sting					0.30
Y	19 (24%)	43 (55%)	16 (21%)	78	
N	36 (25%)	68 (48%)	38 (27%)	142	
Median sIgE (kU/L) [IQR]					
A	0.35 [0.1-4.2]	0.16 [0.09-1.2]	0.36 [0.1-2.42]	0.34 [0.1-2.2]	0.25
VS	1.00 [0.11-3.5]	2.10 [0.31-7.41]	1.90 [0.34-5.26]	1.59 [0.28-5.35]	0.70
C	0.35 [0.1-1.01]	0.27 [0.1-1.5]	0.52 [0.14-3.95]	0.32 [0.1-1.58]	0.20
P	1.02 [0.14-3.95]	2.10 [0.28-11]	2.15 [0.6-6.4]	1.90 [0.24-6.4]	0.41
Skin test					
A+	26 (28%)	38 (40%)	30 (32%)	94	0.02 OR 2.1 (1.05-4.21)
A−	25 (22%)	69 (60%)	21 (18%)	115	
VS+	29 (19%)	77 (51%)	45 (30%)	151	<0.01 OR 3.8 (1.49-11.59)
VS−	22 (37%)	32 (53%)	6 (10%)	60	
C+	14 (17%)	51 (63%)	16 (20%)	81	0.31
C−	36 (36%)	49 (49%)	14 (14%)	99	
P+	27 (22%)	75 (61%)	20 (16%)	122	0.67
P−	21 (36%)	26 (54%)	11 (19%)	58	

Abbreviations: LLR, large local reaction; SR, systemic reaction; IQR, interquartile range; tIgE, total IgE; BTL, baseline serum tryptase level; A, Apis mellifera; Ve, Vespids; VS, Vespula species; C, Vespa crabro; P, Polistes dominula; OR, odds ratio (95% CI).
*No reaction and LLR *versus* SR I - IV analysis. Wilcoxon rank-sum and χ^2 tests for quantitative and categorial variables, respectively.

Total IgEs were studied comparing, among the groups of re-stung patients (ie no reaction, LLR, and SR), both the numerical levels and the quartiles (ie ≤ 45.5, >45.5 - 97.5, >97.5 - 205, >205 kU/L). Furthermore, the risk of subsequent SR, for patients having tIgE >250 kU/L, was particularly examined, since this value has been previously set as a cut-off of lower risk of severe SR, according to Sturm et al.[17] The above analyses were also performed within SRs, comparing tIgE among SR I, II, III and IV. All the results did not show any significant difference, among re-stung patients, in terms of tIgE.

Overall, BTL were similar among SR, LLR and no reaction group; subgroup analyses were also performed to investigate potential tryptase differences, within gender (Male *versus* Female) and type of insect (Vespids *versus* A), with no significant results.

On the contrary, a significant increased OR for SR was found in case of positive skin test to A (OR = 2.1, *P* = 0.022, 95% CI 1.05 - 4.21) or VS (OR = 3.8, *P* = 0.002, 95% CI 1.49 - 11.59), compared to the others (Table 1). Furthermore, logistic regression analysis revealed a significant relationship between SR and high intradermal reactivity (ie 0.001 µg/mL) for A (OR = 13.4, *P* = 0.002, 95% CI 3.35 - 53.89) and VS (OR = 16.5, *P* < 0.001, 95% CI 4.48 - 60.8) (Figure 1). On the other hand, PD and VC data did not give statistically significant results.

Re-sting reactions, after a previous LLR, were analysed comparing mono-sensitized *versus* multi-sensitized patients, and no significant differences were observed.

4 | DISCUSSION

The results of the study showed that in our patients with HVA who experienced a first LLR, re-stings led to a second LLR in about half of them (52%), SR in one-quarter (24%) and no reaction in the others

TABLE 2 Characteristics of patients with systemic reactions, after a previous large local reaction, grouped by severity of reaction (n = 54)

	SR I	SR II	SR III	SR IV
Patients (%)	18 (11%)	11 (5%)	14 (6%)	11 (5%)
Median age [min-max]	41 [14-78]	52 [13-76]	44 [16-62]	54 [36-76]
Gender (M/F)	10/8	7/4	4/10	6/5
Median tIgE (kU/L) [IQR]	75.3 [56-140]	119 [74-237]	68.2 [28-120]	21.7 [17.1-194]
Median BTL (μg/L) [IQR]	3.9 [3.2-4.6]	4.4 [3.56-5.8]	4.2 [3.2-5.3]	7.58 [4.3-9.2]
Presumed insect (A/Ve)	5/8	2/4	1/7	3/5
Risk of re-sting (Y/N)	11/3	2/6	0/13	3/6
Median sIgE (kU/L) [IQR]				
A	0.99 [0.09-4]	0.34 [0.09-0.93]	0.34 [0.34-1.7]	2.28 [0.34-3.75]
VS	2.33 [0.44-7.38]	1.58 [0.18-10.3]	3.32 [0.78-5.26]	1.11 [0.28-1.9]
C	0.25 [0.14-4.31]	1.20 [0-3.9]	0.35 [0.14-4]	0.70 [0.7-0.7]
P	1.58 [0.31-11]	6.02 [3.92-11.65]	1.14 [0.43-3.84]	1.9 [1.9-1.9]
Skin test				
A±	10/7	5/6	10/3	5/5
VS±	13/4	10/1	13/0	9/1
C±	6/10	5/3	2/1	3/0
P±	9/8	5/3	3/0	3/0

Abbreviations: SR, systemic reaction (according to Mueller classification[16]); IQR, interquartile range; tIgE, total IgE; BTL, baseline serum tryptase level; A, Apis mellifera; Ve, Vespids; VS, Vespula species; C, Vespa crabro; P, Polistes dominula.

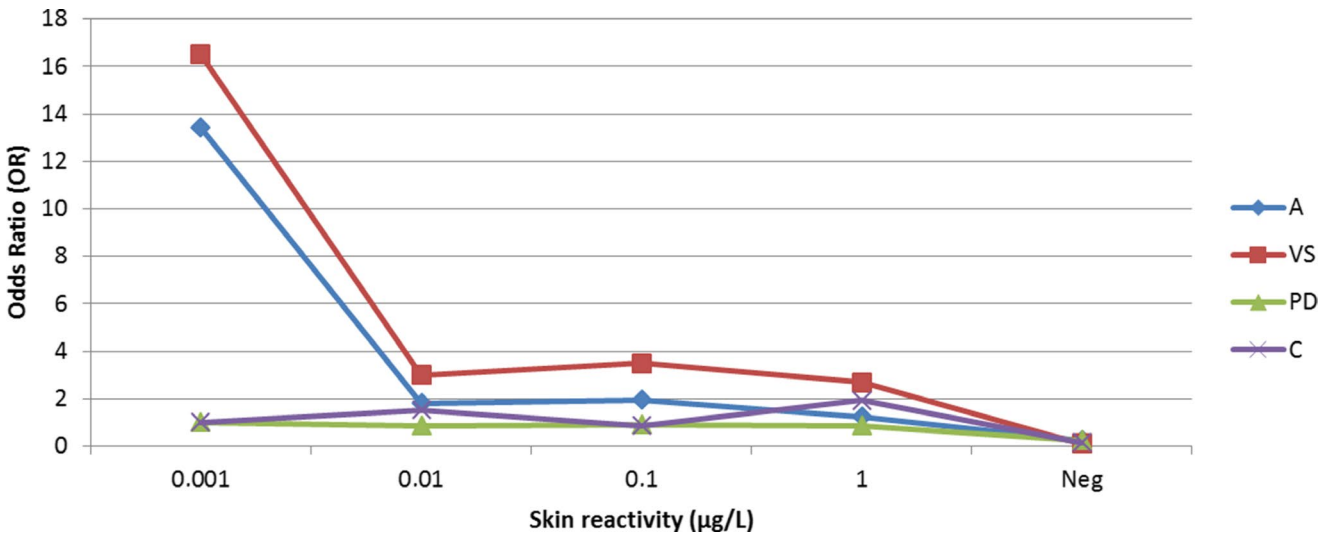


FIGURE 1 Relationship between grade of intradermal test reactivity and systemic reactions in re-stung patients, after the first large local reaction

(24%). Nearly half of the SRs were reported as severe (SR III - IV, 11%).

Therefore, the risk of SR appears to be remarkable and by far higher than the rates of other studies, which enrolled a considerable lower number of patients compared to our sample. Sturm et al[4] have reported 7.4% of SRs using a sting challenge out of 25 subjects who have previously experienced LLRs, but the sample size was quite small and the number of previous LLRs has not been specified. Pucci et al[10] have observed a low risk of SR (4.2%) in case of a single previous LLR, and no risk after at least two previous LLRs; however, the first result has been collected retrospectively, and the latter analysing a too small sample size of LLRs, for a statistical analysis (n = 81).

The discrepancies between our results and these studies may be caused by the larger sample size of our population (n = 662), the prospective design of the study and the homogeneity of our data in terms of LLR criteria. We cannot exclude that some LLR cases, occurred in the general population, did not seek medical attention, and we think that this is a limitation affecting all the "traditional" epidemiologic studies, including SRs. In addition, differences in terms of time interval between first LLR and subsequent re-sting

may be another confounder when comparing results from different studies, as SRs could be more frequent if re-stings occur within a short time span. However, this information is rarely available (32% of cases, in our study). Some patients were studied only by in vivo or in vitro testing due to the workload and economic considerations that limited the examinations, if not necessary to the routine clinical practice.

Interestingly, after analysing the re-stung patients to find a possible predictor of SR risk, according to our results, intradermal skin test reactivity to A or VS defines the most accurate high-risk profile (OR 2.1 and 3.8, respectively), compared to the other tests. On the contrary, skin positivity to PD or VC does not lead to a higher risk (Table 1), but these results might be mainly related to the different habits of the Hymenoptera species, that makes the risk of PD and VC stings lower than A and VS stings. Namely, subjects can easily stay away from nests of PD and VC, especially after experiencing a previous LLR and if well instructed by their allergists; on the other hand, the nests of VS are hidden under the ground, and the stings happening by chance are more frequent, as well as for A stings, due to the high exposure in beekeepers. The different quality of the allergen extracts used for PD and VC testing could be another possible explanation.

In addition, our results describe a novel correlation between A and VS skin reactivity and reaction severity, being the SR risk considerably higher in case of positivity at 0.001 μg/mL concentration (OR 13.4 and 16.5 for A and VS, respectively). A similar relationship was not observed in PD or VC (Figure 1).

Therefore, we demonstrate how skin tests might play an important role in the diagnostic workup in the assessment of cases of LLR with risk of SR, in addition to the other traditional clinical, occupational or recreational risk factors. Taking into account the correlation between SR risk and skin reactivity at low venom concentration (ie 0.001 μg/mL), the skin tests for LLR should always be started at the lowest concentration, not to overlook the real threshold of skin reactivity. Skin testing is a simple, quick, safe and inexpensive diagnostic technique, compared to other procedures, and, as confirmed by our study, with a good sensitivity and specificity, particularly in monosensitized patients (>95%). Even if prick tests would be easier to perform for safety and cost/time reasons, than intradermal tests, they were not used because not routinely adopted in the HVA specialized centres of many countries, due to a lower diagnostic sensitivity.[1]

Overall, no significant relationships were observed between SR risk and all the other exposure variables, among different subgroups of patients (Table 1).

The lack of correlation between in vitro sIgE and SR risk has been previously observed in other studies[4] and is confirmed by our results. On the contrary, patients with HVA and high levels of tIgE (>250 kU/L) have been found to predominantly develop a SR of grade I and grade II than grade III, according to Sturm et al.[17] Our study shows no correlation between tIgE and risk of subsequent SR after a previous LLR, nor statistically significant tIgE differences between SR I – II and SR III – IV, even if the different baseline characteristics of our subjects make difficult a comparison with the conclusions of Sturm et al.[17]

Neither subjects with high BTL seem to have a different risk profile, after a previous LLR, compared to the others. Ruëff et al have shown the importance of BTL as predictor of severe SR in HVA,[18] but the characteristics of their enrolled population were different, as tryptase concentration was collected in patients who had a SR after a field sting, and 9.9% of them had already had one or more SRs before the index sting. On the contrary, only patients reporting their first LLR, with no previous reactions, were enrolled in our study. However, only seven, out of the 15 total patients with high BTC, were re-stung in our study, and this sample size is too small for robust conclusions. Further characterizations of re-stung patients, based on the responsible insects of the re-stings, were not possible, and this is a possible weakness of the study. However, the importance of insect identification, in case of re-sting, is questionable, taking into account that in a real-world setting we cannot foresee which type of insect will re-sting a patient who had a previous LLR. Another possible limitation is the lack of sting challenges to confirm the re-sting reactions, but it would have been applicable only for mild reactions, as all the SR have been documented by the Emergency Departments' medical records, and this kind of documentation was mandatory for the diagnosis of SR. Additionally, sting challenge is not recommended for diagnostic use, due to several related issues (ie difficult choice of the right insect to test—based on uncertain clinical history and diagnostic test outcome, low accuracy of the vespid sting challenge and, especially, ethical limitations).[19]

Since the majority of the subjects, in this study, were enrolled in Italy, additional multicentre international studies would be needed to confirm our results worldwide. In fact, the Italian setting allowed the collection of a high number of LLRs that would probably be unfeasible elsewhere, as in clinical practice, in Italy, the majority of LLRs are usually referred to the allergist, due to the high incidence of deaths by Hymenoptera stings, reported by the media, and the consequent high awareness of the general population.

5 | CONCLUSIONS

Currently, a comprehensive allergological examination, in case of LLR, is not a globally accepted procedure in the HVA framework, and this may be caused by low attention and/or resources in the allergy field, in some countries, and/or by previous studies describing low rates of severe reactions after LLRs.[2,5-9]

However, this approach may underestimate the risk of subsequent re-stings after LLRs, possibly leading to very severe SRs. Our study, after analysing the predictive role of LLRs for SRs, reveals that SRs, and in particular severe SRs, occur in a remarkably higher percentage of previous LLR cases (24% and 11%, respectively), suggesting the need of an accurate diagnosis of LLRs.

Moreover, we suggest how skin tests may provide some indication of the risk of severe reactions to some insect species and should be used in clinical practice to achieve a more tailored management of LLR, identifying the specific circumstances at high risk of subsequent SR, after re-sting, that might need AAI. Controlled prospective studies of patients with one and more than one previous LLR would be urgently needed to confirm the weight of our new evidence and to shed light on the predictive role of repeated LLRs.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

M. Severino planned, designed the study and collected patients' data. M.B. Bilò and M. Martini conducted data analyses and wrote the manuscript. All the authors collected patients' data and performed *in vivo* and *in vitro* tests. All the authors contributed to the interpretation of data and revision of the article. All authors had access to the data and read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Table SUPP1 - Characteristics of re-stung patients, after a previous large local reaction, grouped by type of reaction (No reaction, LLR, and SR I - II *versus* SR III - IV analysis; n=225)

	No reaction	LLR	SR I	SR II	SR III	SR IV	Combined	p-value*
Patients	55 (24%)	116 (52%)	18 (11%)	11 (5%)	14 (6%)	11 (5%)	225	
Median age [min-max]	42 [9-81]	50 [12-82]	41 [14-78]	52 [13-76]	44 [16-62]	54 [36-76]	48 [9-82]	0.51
Gender M/F	36 / 19	73 / 43	10/8	7/4	4/10	6/5	136 / 89	**0.03** **OR 0.4 (0.15-0.99)**
Median tIgE (kU/L) [IQR]	85 [59-117]	95 [51-215]	75.3 [56-140]	119 [74-237]	68.2 [28-120]	21.7 [17.1-194]	84 [48-194]	0.53
Median BTL (µg/L) [IQR]	4.2 [3.2-5.4]	4.5 [3.6-6.3]	3.9 [3.2-4.6]	4.4 [3.56-5.8]	4.2 [3.2-5.3]	7.58 [4.3-9.2]	4.4 [3.4-6]	0.09 OR 1.1 (0.99-1.22)
Presumed insect (A/Ve)	7 / 26	11 / 52	5/8	2/4	1/7	3/5	29 / 102	0.98
Risk of re-sting (Y/N)	19 / 36	43 / 68	11/3	2/6	0/13	3/6	78 / 142	**0.006** **OR 0.2 (0.04-0.71)**
Median sIgE (kU/L) [IQR]								
A	0.35 [0.1-4.2]	0.16 [0.09-1.2]	0.99 [0.09-4]	0.34 [0.09-0.93]	0.34 [0.34-1.7]	2.28 [0.34-3.75]	0.34 0.1-2.2]	0.14
VS	1.00 [0.11-3.5]	2.10 [0.31-7.41]	2.33 [0.44-7.38]	1.58 [0.18-10.3]	3.32 [0.78-5.26]	1.11 [0.28-1.9]	1.59 [0.28-5.35]	0.56
C	0.35 [0.1-1.01]	0.27 [0.1-1.5]	0.25 [0.14-4.31]	1.20 [0-3.9]	0.35 [0.14-4]	0.70 [0.7-0.7]	0.32 [0.1-1.58]	0.64
P	1.02 [0.14-3.95]	2.10 [0.28-11.05]	1.58 [0.31-11]	6.02 [3.92-11.65]	1.14 [0.43-3.84]	1.9 [1.9-1.9]	1.90 [0.24-6.4]	0.70
Skin tests								
A +/-	26 / 25	38 / 69	10/7	5/6	10/3	5/5	94 / 115	**0.039** **OR 2.5 (0.95-7.24)**
VS +/-	29 / 22	77 / 32	13/4	10/1	13/0	9/1	151 / 60	**0.007** **OR 10.1 (1.54-422)**
C +/-	14 / 36	51 / 49	6/10	5/3	2/1	3/0	81 / 99	0.05 OR 6.5 (0.69-308)
P +/-	27 / 21	75 / 26	9/8	5/3	3/0	3/0	122 / 58	N/A

LLR, large local reaction; SR, systemic reaction; IQR, interquartile range; tIgE, total IgE; BTL, baseline serum tryptase level; A, Apis mellifera; Ve, Vespids; VS, Vespula species; C, Vespa crabro; P, Polistes dominula; OR, odds ratio (95% CI); N/A, not applicable.
* No reaction, LLR, and SR I - II *versus* SR III - IV analysis. Wilcoxon rank-sum and χ^2 tests for quantitative and categorial variables, respectively.