Title: Analysis of the safety and tolerance of three buildup protocols of insect venom immunotherapy frequently used in Spain

Short Title: Safety of venom immunotherapy protocols

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ABSTRACT

Introduction: Hymenoptera venom immunotherapy (VIT) is an effective treatment but not one devoid of risk as both local and systemic adverse reactions may occur especially in the initial stages of treatment. We compared the tolerance to three buildup protocols of VIT and analyzed risk factors associated with adverse reactions occurring in this phase. Methods: We enrolled 165 patients divided into three groups based on the buildup protocol used (3, 4, 9 weeks). Severity of systemic reactions was evaluated according to World Allergy Organization model. Results were analyzed using exploratory descriptive statistics and variables were compared using analysis of variance. Results: Fifty-three patients (32%) experienced some form of adverse reaction, 43 were local and 10 systemic. Local reactions were immediate in 27 patients (63%) and delayed in 16 (37%). Severity of local reaction was slight/moderate in 15 patients and severe in 13. Systemic reactions were grade 1-2. No significant association was found between the treatment modality and the appearance of local or systemic adverse reactions or the type of local reaction. We only found a statistically significant association of severity of the local reaction with female gender. As for the risk factors associated with systemic reactions at buildup phase, we found no significant differences in these values depending on protocol used or the insect responsible. Conclusions: The buildup protocols compared proved to be safe and did not differ significantly from one another. In the population studied the 9-week schedule presented no systemic reactions, so it can be considered the safest protocol.

Key words: allergy; immunotherapy; insect venom immunotherapy; buildup protocols; systemic reaction; local reaction; hymenoptera; *Apis mellifera, Vespula ssp; Polistes ssp*

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RESUMEN

Introducción: La inmunoterapia con veneno de himenópteros (ITV) es un tratamiento eficaz, pero no está desprovisto de riesgo, ya que pueden ocurrir reacciones adversas locales o sistémicas, especialmente en las etapas iniciales del tratamiento. Comparamos la tolerancia de tres protocolos de inicio de ITV, y analizamos los factores de riesgo asociados con las reacciones adversas que se produjeron en esta fase. Métodos: Se incluyeron 165 pacientes divididos en tres grupos según el protocolo de iniciación utilizado (3, 4 o 9 semanas). Evaluamos la gravedad de las reacciones sistémicas de acuerdo con el modelo de la Organización Mundial de Alergia. Analizamos los resultados mediante estadística descriptiva exploratoria, y comparamos variables mediante el análisis de la varianza. Resultados: Cincuenta y tres pacientes (32%) experimentaron algún tipo de reacción adversa; 43 eran locales y 10 sistémicas. Las reacciones locales fueron inmediatas en 27 pacientes (63%) y tardías en 16 (37%). La gravedad de la reacción local fue leve o moderada en 15 pacientes y grave en 13. Las reacciones sistémicas fueron de grado 1 ó 2. No encontramos asociación significativa entre la modalidad de tratamiento y la aparición de reacciones adversas locales o sistémicas o el tipo de reacción local. Sólo encontramos una asociación estadísticamente significativa de la gravedad de la reacción local con el sexo femenino. En cuanto a los factores de riesgo asociados con las reacciones sistémicas en la fase de inicio, no se encontraron diferencias significativas en estos valores en función de protocolo utilizado o el insecto responsable. Conclusiones: Los protocolos de inicio comparados demostraron ser seguros y no difirieron significativamente entre sí. En la población estudiada, el protocolo de 9-semanas no produjo reacciones sistémicas, por lo que se puede considerar el protocolo más seguro.

Palabras clave: alergia; inmunoterapia; inmunoterapia con veneno de insectos;

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protocolos de inicio; reacción sistémica; reacción local; himenópteros; *Apis mellifera, Vespula ssp; Polistes ssp*

INTRODUCTION

In patients allergic to insect venom, immunotherapy with the venom triggering the reaction is a very effective treatment which confers protection against future stings in over 95% of cases. However, this treatment is not without risk as local and systemic adverse reactions may occur especially in the buildup phase which explains why the treatment is administered periodically and in a hospital setting. This represents a high cost both for the patient and the health care system and also a possible cause of poor compliance with treatment. In a conventional venom immunotherapy build-up schedule, injections are administered once weekly for several weeks to reach the maintenance dose capable of providing protection against a new sting. Since this immunotherapy is recommended to be administered in hospitals with the appropriate resources and experience, costs are high because of frequent visits, travel time, and waiting time. For this reason and to reduce the number of hospital visits, clustered initial treatment modalities have been proposed which have proved to be as effective and safe as conventional protocols [1-6].

The Committee on Allergy to Hymenoptera (CAH) of the SEAIC recently collected data on the three protocols most frequently used in different Spanish hospitals (Tables 1 and 2). The protocols were used in each medical center depending on the facilities available and the experience of the prescribing allergologist. The three protocols were: conventional treatment lasting 9 weeks and clustered treatments lasting 4 and 3 weeks, respectively.

The aim of the study was to assess and compare the tolerance and safety of the three protocols in the context of a prospective multicenter study involving 13 Spanish

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hospitals where immunotherapy with insect venom was administered following one of the 3 protocols of interest.

MATERIALS AND METHODS

This observational, non-randomized, prospective and multicenter study examining normal clinical practice was conducted between June 2011 and June 2013. Purposive sampling was used to ensure a 99% confidence level (Z = 2.58) and the patients enrolled were allergic to insect venom scheduled for immunotherapy in the period specified. The patients were assigned to one of the three recommended protocols (3, 4 and 9 weeks) based on the clinical practice of each participating center. Patients were included consecutively until a minimum of 55 patients for each protocol were achieved. Finally a total of 165 patients were included (121 men and 44 women), 55 patients in each one of the recommended protocols of 3, 4 or 9 weeks.

We decided to compare different protocols with different venom extracts because adverse events during venom immunotherapy may occur especially in the buildup phase with all insects. The data were collected from real life clinical practice. Each participating group used the protocol regularly they employed.

The following information was collected from each patient: background data, the reactions experienced after the sting, the insect responsible, total serum IgE levels, levels of specific IgE against the different venoms, and serum tryptase levels. Patients were also asked if they were concurrently taking angiotensin-converting-enzyme inhibitors (ACE inhibitor) or angiotensin receptor blockers (ARBs) (Table 3).

With regard to the administration of the immunotherapy, the presence of local and/or systemic reactions was recorded as was the use of premedication. Local reactions were classified according to the criteria set out in Table 3. Severity of the systemic reactions was established using the WAO classification (Table 4) [6]. The presence of local and systemic reactions and the severity of these reactions with each protocol

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were compared. This was expressed as the percentage of patients who suffered a reaction and also as the percentage of injections that caused a reaction. Other risks factors for systemic reactions like time elapsed since the last sting, responsible venom, or tryptase levels were also analyzed.

We used commercially available lyophilized and aqueous extracts obtained from different pharmaceutical companies in Spain. The study received approval from the Ethics Committees in the participating hospitals and all patients provided written informed consent.

SPSS-21.0 software (SPSS, Chicago, Illinois, USA) was used for data management and statistical analysis and exploratory descriptive techniques were applied to the different variables collected. The patients were divided into three groups depending on the protocol administered. Means, standard deviations, minimum and maximum values and 95% confidence intervals were calculated. Association between variables was analyzed using contingency tables and Chi-Squared tests were used to compare nominal variables and the U Mann-Whitney test to compare scores from different patient groups. Analysis of variance (ANOVA) was used for multiple comparisons.

RESULTS

Participants' ages ranged from 7 to 79 years for the full sample. The mean age of the patients enrolled in the study was 45.96 years (SD = 17.56). Men also outnumbered women in the sample (73.3%) and patients most frequently came from rural areas (64.2%). With regard to the insect responsible, in the case of *Apis* venom 3-week (38%) and 9-week (45%) protocols were the most used while the 4-week protocol was used most frequently for *Polistes* venom (56%). Premedication was taken prior to vaccine administration in 27% and 13% of the 3- and 9-week protocols and in 45% of cases in the 4-week protocol (Table 5). The decision of the use of premedication depended on the clinical practice of each participating center. Fifty three percent of

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patients who had taken premedication, showed local adverse reactions, while only 15.3% of patients without premedication suffered local adverse reactions ($p < 0.01$); furthermore, 19.1% of patients who had taken premedication showed systemic adverse reactions, while only 0.9% of patients without premedication suffered systemic adverse reactions ($p < 0.01$).

No significant differences were found in baseline serum tryptase levels depending on the protocol ($p = 0.89$), in mean initial specific IgE values ($p = 0.53$) or initial total IgE values ($p = 0.54$). The level of REMA score of the Spanish Network on Mastocytosis (Red Española de Mastocitosis [REMA]) did not show any statistical association with adverse reactions.

In the 165 patients, *Polistes* was the species responsible in 65 patients (40%), *Apis* in 61 patients (37%), *Vespula* in 37 patients (22%) and *Bombus* in 2 patients (1%). In total 1265 injections were administered, of which 385 corresponded to the 3- and 4-week protocols (30.4% each one) and 495 (39.2%) to the 9-week protocol.

Ten systemic reactions were recorded representing 6% of all the patients (0.8% of all injections). Five occurred in the 3-week protocol (9% of the patients in this group) and five in the 4-week protocol (9% of the patients). As for local reactions, 43 were recorded (26% of all the patients and 3.4% of the total number of injections), most of them (81.4%) corresponding to the 3 and 4 weeks protocols (Table 5).

Based on the classification system used [6], the severity of the systemic reactions was grade 1 in 7 patients and grade 2 in 3 patients. There were no cases of grade 3 or 4 reactions.

With regard to the local reactions, these were immediate in 27 patients (63%) and delayed in 16 patients (37%); and the severity of the reaction was slight in 13 patients (8%), moderate in another 15 (9%) and severe in 14 (8%).

In half of the patients experiencing a systemic reaction – 5 patients – there was no concurrent local reaction. The local reactions in the other 5 patients who experienced a

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systemic reaction were immediate in 4 cases and delayed in the rest. The severity of these local reactions was slight in one patient, moderate in two and severe in the other two.

As for the buildup protocol employed, we found no significant association with the occurrence of local adverse (p = 0.47) or systemic (p = 0.70) reactions or relative to the severity of the systemic reaction (p = 0.54) in the 3- and 4-week protocols. In contrast, there were significant differences in the severity of the local reaction depending on the initial treatment with a higher percentage of slight reactions for the 9-week protocol as is reported in Table 6; however this result is influenced by the lower number of local adverse reactions in the 9-week protocol, while the 3-week and 4-week protocols had at least a double number of local adverse reactions.

Bearing in mind the association between initial treatment and reactions, an analysis stratified by gender was performed and this revealed that the severity of local reactions was significant depending on the initial treatment used in the case of women (χ^2 (6) = 20.687, p = 0.002) but not for men (χ^2 (6) = 7.234, p = 0.30). Women receiving the 3-week protocol experienced moderate reactions in 31% of cases.

Of the total number of patients enrolled in the study, 9 (7.5%) were found to be concurrently taking ARBs medication and 8 (8.3%) ACE inhibitors although no significant association was found with the occurrence of systemic reactions in patients either taking ARBs (p = 0.34) or ACE inhibitors (p = 0.07).

As for the time elapsed since the last sting, 73.9% of patients began immunotherapy treatment in the following 10 months. Regarding the levels of serum tryptase recorded, no significant association was found with the occurrence of systemic reactions (p = 0.68).

With regard to the patients' jobs, it is worth outlining that 8 of the patients who received immunotherapy with *Apis* extract were bee-keepers. There were 41 patients who

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declare to be beekeeper relatives: 36 received *Apis* extract, two received *Polistes* extract and three received *Vespula* extract.

In the total number of systemic reactions, the venom of the insect involved was principally from the *Apis* (6 patients; 60%) and *Polistes* (3 patients; 30%) species with the *Vespula* species being much less frequently involved (1 patient; 10%). The most common range of systemic reactions was found in the 10 and 50 μg concentrations in the buildup protocol of immunotherapy. When this issue was analyzed with reference to the protocol used, in the group of patients with systemic reactions receiving the 3-week protocol, the insect most frequently responsible was *Apis* with 4 patients followed by *Polistes* with one. For the initial 4-week treatment, both *Apis* and *Polistes* were involved in 2 cases and *Vespula* in only one. Interestingly, there were no cases in the 9-week protocol (Table 7). The occurrence of systemic reactions showed no significant association with the insect responsible ($p = 0.453$) as was the case with gender for men ($p = 0.126$) or women ($p = 0.891$). Neither were there any significant differences in mean initial specific IgE values ($p = 0.135$) depending on the insect responsible.

DISCUSSION

The three buildup protocols with insect venom examined proved to be safe and well tolerated in the group of patients studied with only 6% of patients experiencing a systemic reaction, which represents 0.8% of all injections given. There are few prospective studies which have compared buildup protocols of venom immunotherapy [7-10] and to date there are no studies comparing a conventional 9-week protocol with two clustered 3- and 4-week protocols. Buildup protocols are difficult to compare given the many differences that exist between them (patients selected, protocols used, extracts, recording of adverse reactions, among other factors) which no doubt explains why the prevalence of systemic reactions reported in the literature is so variable, ranging from 2%[11] to 21%[12]. The true figure most probably lies between 5 and 15%

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[13]. In a recent prospective multicenter study by the EAACI, the frequency of systemic reactions was 8.4% across the different protocols used [14]. The type of buildup protocol that should be used to reach the maintenance dose remains a matter of debate. Standard protocols (lasting 8-15 weeks), rush protocols (lasting 4-7 days) and ultra-rush protocols (lasting 1-2 days) can all be used. Although in different studies these latter protocols have been shown to be safe [9,12,15,16,17], other prospective studies have shown a rapid increase in dose to be an independent risk factor for adverse systemic reactions [13,18]. In the case of ultra-rush protocols, this risk is increased 1.8-fold, although they are also cost-effective [19,20]. In our study we chose three protocols that had already been successfully tested by different groups in our country [8,21], and furthermore are those used by the majority of patients treated in Allergy departments in Spain.

As for the descriptive data from the study, we found that the majority of patients were male (a ratio of 2.75:1) which is in line with practically all published studies [11,13,14], and they lived in a rural setting. With regard to the frequency of atopic patients (31% in our case) this figure is also in line with previously published studies and is no higher than in the general population [13].

The primary objective of this study was to compare tolerance to three buildup protocols of immunotherapy with insect venom and we thus included in the data collection form not only adverse reactions but all the risk factors that have been associated with systemic reactions during the initial phases of treatment. One aspect that requires highlighting is that of baseline tryptase values [14], which in our patients was determined in 62% of patients. The mean value was 4.93 µgr/L and there were no significant differences in this parameter depending on the protocol used (p = 0.89) or the insect responsible (p = 0.29). The mean value of tryptase in the 3-weeks protocol was 4.95 µgr/L (n = 55 patients); in the 4-weeks protocol was 5.02 µgr/L (n = 38 patients), and in the 9-weeks protocol was 4.48 µgr/L (n = 10 patients). These data

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contrast with those from other prospective studies which obtained tryptase levels of >11.4 µgr/L in 10% of patients and >20 µgr/L in 2.6% of participants [14] while in the patients in our series the maximum tryptase value observed was 10 µgr/L. However, tryptase was only measured in 62% of the patients included and we don´t know the results if it would be measured in all the patients.

As for other risk factors associated with systemic reactions in the initial phases of treatment (anti-hypertensive medication, patient age or time since the sting occurred and the beginning of immunotherapy), we found no significant associations with any local or systemic reaction.

In the present study, 43 patients (26%) experienced local reactions but only 13 (8%) were considered to be severe (> 10 cm). No significant association was found between the appearance of the local reactions and the protocol used (p = 0.47) although the severity of the reaction was significant for buildup protocols (p < 0.05) with the reactions being significantly less severe in the 9-week protocol. Furthermore, when analyses were performed on the data stratified by gender, the association was highly significant in women (p = 0.002) but not in men (p = 0.30). Although this finding has not been reported in other analyses, some studies have shown female sex to be a risk factor, albeit a less important one, for experiencing adverse reactions in the initial stages of immunotherapy with insect venom [13]. However, systemic reactions are generally more severe in men [21]. We also found no significant association between local reactions and the insect responsible (p = 0.082).

Ten patients in our study experienced systemic reactions, which represents 6% of the total number of patients and 0.8% of the total number of injections. All the systemic reactions were slight and adrenaline was not used in any case. This prevalence of systemic reactions is on the lower limit of the published mean [13] and is similar to that for immunotherapy with inhalant allergens. In five of these 10 patients the 3-week protocol had been used and in the other five, patients received the 4-week protocol.

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None of the patients included in the 9-week protocol experienced systemic reactions. Analysis of the 10 patients with systemic reactions failed to identify any associated risk factors (age, gender, time between last sting and beginning of immunotherapy or atopy). However, with regard to time between last sting and beginning of immunotherapy, this was over two months in all patients – the cut-off point established by some authors to consider this a risk factor [14].

No significant differences were found in the levels of total or specific IgE or tryptase levels in the group of patients with systemic reactions. As a result, we have not identified any of the significant risk factors proposed by different authors [14,16,22].

No significant differences were found regarding the insect responsible (p = 0.45) although six of the ten patients with systemic reactions were receiving immunotherapy with bee venom. Many studies have shown that bee venom is an independent risk factor for adverse reactions during immunotherapy [12,14,18,23-25], other showed no significant difference in the number of systemic reactions comparing patients receiving wasp or honeybee venom extract [26]; but our results may well be due to the sample size.

There is a preferential association between hymenoptera venom allergy (HVA) and mastocitosis and a high prevalence of insect venom allergy in patients with any form of mastocitosis [27,28]. The Spanish Network on Mastocytosis (Red Española de Mastocitosis [REMA]) score ≥2 can predict the presence of mastocitosis [24], however it was not related to the presence of adverse reactions during hymenoptera venom immunotherapy in our patients.

Premedication seemed to be unable to prevent adverse reactions; paradoxically, premedication was associated to more adverse reactions; probably because it was more frequently employed in shorter protocols.

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In conclusion, the three buildup protocols compared in this study were shown to be safe with no significant differences between them. Of interest, however, is the lack of systemic reactions in the 9-week protocol.

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TABLE 1. Conventional protocol

Initial 9-week protocol			
Week	Concentration (mg/ml)	Dose (ml)	Dose (mg)
1	1 mg/ml	0.1	0,1
2	10 mg/ml	0.1	1
3	10 mg/ml	0.5	5
4	100 mg/ml	0.1	10
5	100 mg/ml	0.2	20
6	100 mg/ml	0.4	40
7	100 mg/ml	0.6	60
8	100 mg/ml	0.8	80
9	100 mg/ml	1	100

Maintenance treatment: once the maintenance dose is reached in any type of protocol (1 ml of the 100 µg/ml concentration), the interval between administrations will be 4 weeks. This may be increased to 6/8 weeks at the allergist´s discretion

TABLE 2. Cluster schedules

Initial 4-week protocol			
Day	Concentration (mg/ml)	Dose (ml)	Dose (mg)
1	10 mg/ml	0.5	5
	100 mg/ml	0.1	10
8	100 mg/ml	0.2	20
	100 mg/ml	0.3	30
15	100 mg/ml	0.5	50
	100 mg/ml	0.5	50
29	100 mg/ml	1	100

Initial 3-week protocol			
Day	Concentration (mg/ml)	Dose (ml)	Dose (mg)
1	10 mg/ml	0.5	5
	100 mg/ml	0.1	10
	100 mg/ml	0.2	20
	100 mg/ml	0.2	20
8	100 mg/ml	0.5	50
	100 mg/ml	0.5	50
22	100 mg/ml	1	100

Maintenance treatment: once the maintenance dose is reached in any type of schedule (1 ml of the 100 µg/ml concentration), the interval between administrations will be 4 weeks. This may be increased to 6/8 weeks at the allergist´s discretion

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TABLE 3. Data collection form

PATIENT/HOSPITAL CODE	
AGE	
SEX	[ ] MALE [ ] FEMALE
CLINICAL ATOPY AND SENSITIZATIONS	
RELATIVE OF BEE-KEEPER?	[ ] YES [ ] NO
PROFESIÓN	
HOME SETTING	[ ] Rural [ ] Urban (> 10.000 inhabitants)
TIME SINCE LAST STING UNTIL BEGINNING OF IMMUNOTHERAPY (MONTHS)	
INSECT RESPONSIBLE	*[ ] Bee [ ] Polistes [ ] Véspula [ ] Bombus [ ] Other [ ] Not known*
BASELINE SPECIFIC IgE (kU/L)	
EXTRACT USED AND COMPOSITION	
INITIAL SCHEDULE USED	[ ] 9 weeks [ ] 4 weeks [ ] 3 weeks
PREMEDICACION	[ ] YES [ ] NO
Total IgE	
Serum tryptase	
REMA score	
Taking ACE inhibitors	[ ] YES [ ] NO
Taking ARBs	[ ] YES [ ] NO

LOCAL ADVERSE REACTIONS [ ] NO (WITH EITHER MEDICATION) [ ] YES (COMPLETE AS NECESSARY)			
DATE	DOSE	TYPE (IMMEDIATE, DELAYED)	SEVERITY (Slight < 5cm, Moderate (5-10 cm), Severe (>10 cm)

SYSTEMIC ADVERSE REACTIONS [ ] NO (WITH EITHER MEDICATION) [ ] YES (COMPLETE AS NECESSARY)			
DATE	DOSE	SEVERITY (GRADE 1, 2, 3 or 4)	TIME TO APPEARANCE OF FIRST SYMPTOM (min)

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TABLE 4. World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System (modified from [3])

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Symptom(s)/ sign(s) of one organ system present [(1)] **Cutaneous** Generalized pruritus, urticaria, flushing or sensation of heat or warmth **or** Angioedema (not laryngeal, tongue or uvular) **or** **Upper respiratory** Rhinitis (e.g., sneezing, rhinorrhea, nasal pruritus and/or nasal congestion) **or** Throat-clearing (itchy throat) **or** Cough perceived to come from the upper airway, not the lung, larynx, or trachea **or** Conjunctival erythema, pruritus or tearing **Other** Nausea, metallic taste, or headache	***Symptom(s)/ sign(s) of more than one organ system present*** **or** **Lower respiratory** Asthma: cough, wheezing, shortness of breath (e.g., less than 40% PEF or FEV1 drop, responding to an inhaled bronchodilator) **or** **Gastrointestinal** Abdominal cramps, vomiting, or diarrhea **or** **Other** Uterine cramps	**Lower respiratory** Asthma (e.g., 40% PEF or FEV1 drop, NOT responding to an inhaled bronchodilator) **or** **Upper respiratory** Laryngeal, uvula or tongue edema with or without stridor	**Lower or Upper respiratory** Respiratory failure with or without loss of consciousness **or** **Cardiovascular** Hypotension with or without loss of consciousness	Death

Patients may also have a feeling of impending doom, especially in grades 2, 3, or 4.
Children with anaphylaxis seldom convey a sense of impending doom and their behavior changes may be a sign of anaphylaxis, e.g., becoming very quiet or irritable and cranky. Scoring includes a suffix that denotes if and when epinephrine is or is not administered in relationship to symptom(s)/sign(s) of the SR: a, ≤ 5 minutes; b, >5 minutes to ≤10 minutes; c, >10 to ≤ 20 minutes; d, >20 minutes; z, epinephrine not administered. The final grade of the reaction will not be determined until the event is over, regardless of the medication administered. The final report should include the first symptom(s)/sign(s) and the time of onset after the subcutaneous allergen immunotherapy injection and a suffix reflecting if and when epinephrine was or was not administered, e.g., Grade 2a; rhinitis:10 minutes.
(1) Each Grade is based on organ system involved and severity. Organ systems are defined as: cutaneous, conjunctival, upper respiratory, lower respiratory, gastrointestinal, cardiovascular and other. A reaction from a single organ system such as cutaneous, conjunctival, or upper respiratory, but not asthma, gastrointestinal, or cardiovascular is classified as a Grade 1. Symptom(s)/sign(s) from more than one organ system or asthma, gastrointestinal, or cardiovascular are classified as Grades 2 or 3. Respiratory

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failure or hypotension, with or without loss of consciousness, defines Grade 4 and death Grade 5. The Grade is determined by the physician's clinical judgment.

TABLE 5. Patient characteristics by protocol

Parameter	3 weeks	4 weeks	9 weeks
Age			
Mean	49.64	39.55	48.71
Standard Deviation	16.80	18.59	15.63
Range	72 (7-79)	70 (7-77)	64 (14-78)
Gender			
Female	15 (27%)	17 (31%)	12 (33%)
Male	40 (73%)	38 (69%)	43 (78%)
Home setting			
Rural	29 (53%)	35 (64%)	42 (76%)
Urban	26 (47%)	20 (36%)	13 (24%)
Atopy			
Yes	37 (67%)	31 (56%)	46 (84%)
No	18 (33%)	24 (44%)	9 (16%)
Premedication			
Yes	15 (27%)	25 (45%)	7 (13%)
No	40 (73%)	30 (55%)	48 (87%)
Insect responsible			
Polistes	20 (36%)	31 (56%)	14 (26%)
Bee	21 (38%)	15 (27%)	25 (45%)
Véspula	14 (26%)	8 (15%)	15 (27%)
Bombus	0 (0%)	1 (2%)	1 (2%)
Local reaction			
Yes	16 (29%)	19 (34%)	8 (14%)
No	39 (71%)	36 (66%)	47 (86%)
Systemic reaction			
Yes	5 (9%)	5 (9%)	0 (0%)
No	50 (91%)	50 (91%)	55 (100%)
Serum tryptase			
Mean value	4.94	5.02	4.48
(Standard deviation)	(2.73)	(4.09)	(2.35)
Patients analyzed	55/55	38/55	10/55

TABLE 6. Buildup protocols and reactions

		Initial schedule				
		3-week	4-week	9-week	*p-value*	*Sig**
Local adverse reaction	No Yes	39(32%) 16 (37%)	36 (30%) 19 (44%)	47 (38%) 8 (19%)	6.102 (2)	0.47
Severity of local reaction	Slight Moderate Severe	2 (14%) 7 (47%) 7 (50%)	7 (50%) 7 (47%) 5 (36%)	5 (36%) 1 (6%) 2 (14%)	12.380 (6)	0.05
Systemic adverse reaction	No Yes	50 (32%) 5 (50%)	50 (32%) 5 (50%)	55 (36%) 0 (0%)	5.323 (2)	0.70
Type of reaction	No Immediate Delayed	3 (60%) 2 (50%) 0 (0%)	2 (40%) 2 (50%) 1 (100%)	0 (0%) 0 (0%) 0 (0%)	1.200 (2)	0.54
Severity of systemic reaction	Grade 1 Grade 2	3 (43%) 2 (67%)	4 (57%) 1 (33%)	0 (0%) 0 (0%)	0.476 (1)	0.49

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	Grade 3 Grade 4	0 (0%) 0 (0%)	0 (0%) 0 (0%)	0 (0%) 0 (0%)		

* *Sig* denotes statistical significance.

TABLE 7. Insect responsible for systemic reactions by buildup protocol

Initial schedule	**Systemic reactions**	**Insect responsible**		
	Totals	Bee	*Polistes*	*Vespula*
3-week	5	4 (80%)	1 (20%)	0 (0%)
4-week	5	2 (40%)	2 (40%)	1 (20%)
9-week	0	0 (0%)	0 (30%)	0 (10%)
TOTAL	10	6 (60%)	3 (30%)	1 (10%)

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