

Influencing factors on the safety and effectiveness of venom immunotherapy

Short title: Safety and effectiveness of VIT

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Abstract

Objectives: The safety profile of venom immunotherapy (VIT) is a relevant issue and considerable differences in safety and efficacy of VIT have been reported. The primary aim of this study was to evaluate the safety of ACE inhibitors and beta-blockers during VIT, which has already been published. For a second analysis, data concerning premedication and venom preparations in relation to systemic adverse events (AE) during the up-dosing phase and the first year of the maintenance phase were evaluated as well as the outcome of field stings and sting challenges.

Methods: The study was conducted as an open, prospective, observational, multicenter study. In total, 1,425 patients were enrolled and VIT was performed in 1,342 patients.

Results: Premedication with oral antihistamines was taken by 52.1% of patients during the up-dosing and 19.7% of patients during the maintenance phase. Taking antihistamines had no effect on the frequency of systemic AE ($p=0.11$) but large local reactions (LLR) were less frequently seen (OR: 0.74; 95% CI: 0.58-0.96; $p=0.02$). Aqueous preparations were preferentially used for up-dosing (73.0%) and depot preparations for the maintenance phase (64.5%). The type of venom preparation neither had an influence on the frequency of systemic AE nor on the effectiveness of VIT ($p=0.26$ and $p=0.80$, respectively), while LLR were less frequently seen when depot preparations were used ($p<0.001$).

Conclusions: Pretreatment with oral antihistamines during VIT significantly reduces the frequency of LLR but not systemic AE. All venom preparations used were equally effective and did not differ in the frequency of systemic AE.

Key words: Anaphylaxis. Bee venom. Effectiveness. Premedication. Systemic adverse events. Venom immunotherapy. Venom preparation. Vespid venom.

Resumen

Objetivos: El perfil de seguridad de la inmunoterapia con veneno (VIT) es un tema relevante y se han descrito diferencias considerables en su seguridad y eficacia. El objetivo principal de este estudio fue evaluar la seguridad de los inhibidores de la ECA y los betabloqueantes durante la VIT, que ya han sido descritos. En un segundo análisis, se han evaluado los datos sobre premedicación y los distintos extractos de veneno en relación con los eventos adversos (EA) sistémicos durante la fase de aumento de dosis y el primer año de la fase de mantenimiento. También se evaluaron los efectos sobre resultado de las picaduras espontáneas y las provocaciones mediante picadura.

Métodos: El diseño del estudio fue abierto, prospectivo, observacional y multicéntrico. En total, se inscribieron 1.425 pacientes y se realizó VIT en 1.342 pacientes.

Resultados: La premedicación con antihistamínicos orales fue tomada por el 52,1% de los pacientes durante la fase de subida de dosis y el 19,7% de los pacientes durante la fase de mantenimiento. La toma de antihistamínicos no tuvo efecto sobre la frecuencia de EA sistémicos ($p=0,11$), pero las reacciones locales exageradas (LLR) se observaron con menor frecuencia (OR: 0,74; IC 95%: 0,58-0,96; $p=0,02$). Se utilizaron preferentemente preparaciones de extractos acuosos para la fase de subida de dosis (73,0%) y preparaciones depot para la fase de mantenimiento (64,5%). El tipo de preparación del veneno no tuvo influencia en la frecuencia de EA sistémicos ni en la efectividad de la VIT ($p=0,26$ y $p=0,80$, respectivamente), mientras que las LLR se observaron con menor frecuencia cuando se utilizaron preparaciones depot ($p<0,001$).

Conclusiones: El tratamiento previo con antihistamínicos orales durante la VIT reduce significativamente la frecuencia de LLR, pero no los EA sistémicos. Todas las preparaciones de veneno utilizadas fueron igualmente efectivas y no difirieron en la frecuencia de EA sistémicos.

Palabras clave: Anafilaxia. Veneno de abeja. Efectividad. Premedicación. Reacciones adversas sistémicas. Inmunoterapia frente a veneno de himenópteros. Preparación del veneno. Veneno de avispa.

Summary box

What do we know about this topic?

Venom immunotherapy (VIT) is the only treatment that can potentially prevent further systemic sting reactions and the safety profile of VIT is a relevant issue; however, differences in safety and efficacy of VIT have been reported in the past.

How does this study impact our current understanding and/or clinical management of this topic?

Since the frequency of systemic adverse events is similar, quicker up-dosing protocols are preferred since patients are protected faster from future systemic sting reactions. Importantly, all venom preparations used in the present study are equally effective.

Introduction

Hymenoptera venom allergy is the most common cause of anaphylaxis in adults in Europe and a potentially life-threatening disease[1]. The rate of self-reported systemic sting reactions (SSR) ranges from 2.3% to 5.4% in European and US epidemiological studies[2-4]. Venom immunotherapy (VIT) is the only treatment that can potentially prevent further SSR[5] and is effective in 77–84% of patients treated with honeybee venom[6, 7], and in 91–96% of patients receiving vespid venom[6, 7].

The safety profile of VIT is a relevant issue and differences in safety and efficacy of VIT have been previously reported[6-10]. The most important risk factor for systemic adverse events (AE) during VIT is treatment with bee venom[9, 11]. A rapid dose increase during the up-dosing phase is a weaker, but established risk factor for systemic AE as well[8, 9]. In Europe, both purified (obtained by a filtration process which mostly removes vasoactive substances) and non-purified venom

extracts are used for VIT[12]. Purified aluminum hydroxide adsorbed preparations and tyrosine adsorbed preparations (so-called depot preparations) seem to cause large local reactions (LLR) less frequently compared to aqueous preparations; however, these results may have been biased by the up-dosing protocol used, since depot preparations are usually used for slower up-dosing protocols[13]. In addition, a lower VIT effectiveness due to a lack of venom components in some venom preparations has been postulated[14].

Pretreatment with different types of antihistamines was reported to reduce the frequency of LLR during the up-dosing phase[15-18], as well as generalized, cutaneous reactions such as urticaria or angioedema[16, 19, 20]. However, a potential risk of masking a beginning allergic reaction by premedication with antihistamines has also been discussed[19].

We recently published the results of an open, prospective, observational, multicenter study, recruiting 1,425 patients in 26 centers from eight European countries. We could demonstrate that β -blockers and ACE-inhibitors (ACEI) did not increase the number of systemic AE during VIT[21]. In this second analysis, we aimed to assess whether premedication with oral antihistamines as well as different venom preparations have an influence on the frequency of systemic AE and VIT effectiveness in a large study cohort. Furthermore, we compared treatment strategies for systemic sting reactions and systemic AE throughout Europe.

Materials and methods

Objectives

The study was primarily conducted to evaluate whether patients under antihypertensive treatment with β -blockers or ACEI show more systemic AE during VIT compared with patients without antihypertensive therapy. Furthermore, we evaluated whether well-known and controversially discussed risk factors were correlated with a higher frequency of systemic AE in our study cohort. These data were already published.[21] For a second analysis, we now assessed how initial sting

reactions and systemic AE were treated and evaluated the influence of premedication. Furthermore, we assessed whether some venom preparations were safer than others in respect to the occurrence of systemic AE and LLR (defined as swelling >10cm persisting for at least 24 hours) and whether there are differences in effectiveness of VIT, monitored by the outcome of sting challenges and field stings. Additionally, we evaluated different treatment strategies for initial sting reactions and systemic AE throughout Europe.

Study design and oversight

The study was conducted as an open, prospective, observational, multicenter study (Clinicaltrials.gov: NCT04269629). Patients were recruited in 26 centers in eight European countries (five centers in Austria, one in the Czech Republic, one in Germany, five in Italy, five in Poland, one in Slovenia, four in Spain and four in Türkiye). The study was approved by the ethics committee of the sponsor of the study (Medical University of Graz; approval no. 26-442 ex 13/14) as well as local ethics committees in each country, and patients gave their written, informed consent. Legally competent male and female patients aged 35 to 85 years with a history of a SSR (\geq grade I according to the classification by Ring and Messmer[22]) were eligible for the study. Absolute contraindications to VIT according to the EAACI guidelines such as active multisystem autoimmune disorders, active malignant disease, and pregnancy[5] as well as pretreatment with Omalizumab were exclusion criteria. After giving their written informed consent, patients were included after carefully reviewing all inclusion and exclusion criteria at Visit 1. All data concerning the index sting reaction as well as concomitant diseases and medications were recorded. If patients agreed to receive VIT, data concerning the up-dosing phase (premedication, venom preparation, up-dosing protocol, systemic AE (classification by Ring and Messmer[22]), and changes in concomitant diseases and medication) were recorded at Visit 2. There was no standard up-dosing protocol used for VIT. All centers used their own in-house protocols including conventional, cluster, ultrarush, and rush protocols.[5] One year after reaching the maintenance dose, Visit 3 was performed. At this visit, changes in premedication, venom preparation, concomitant diseases and

medication were recorded as well as systemic AE during the maintenance phase and, if applicable, the outcome of field stings or sting challenges. No additional study-related visits were required. All procedures (diagnosis and treatment of Hymenoptera venom allergy) had to be in concordance with current EAACI guidelines[5, 23, 24] and were conducted individually by each study center. Premedication with antihistamines (standard or double dose) was usually administered 30-60 minutes before the first injection of VIT per treatment day. All centers used a maintenance dose of 100µg for the majority of patients and 200µg for high risk patients as suggested in the EAACI guidelines[5].

Statistics

Statistical analysis

Characteristics were reported as mean and standard deviation, median with range or interquartile range, or as absolute and relative frequencies. Group comparisons, for example, between different systemic sting reaction grades, regarding parameters of interest were done using either the T-, Mann-Whitney-U, or Fisher-Exact test. Percentages and odds ratios (ORs) are given with 95% confidence interval (CI), using Clopper and Pearson procedure for percentages. A p-value <0.05 was considered to be statistically significant. All statistical analyses were performed using R Version 4.2.2[25].

Sample size calculation

Sample size calculation was done for the primary aim of this study, in other words to evaluate the safety of ACE inhibitors and beta-blockers during VIT, as reported previously[21].

Results

Patients

From August 2014 until January 2018, a total of 1,425 patients were included in the study: 330 of these patients were included in Austria, 41 in the Czech Republic, 68 in Germany, 254 in Italy, 269

in Poland, 279 in Slovenia, 44 in Spain and 140 in Türkiye. The demographic data of the patients are shown in Table 1.

Seventy-five patients refused VIT, eight patients were lost to follow-up, and therefore Visit 2 was performed with 1,342 patients. During the first year of VIT, 156 patients were additionally lost to follow-up. The majority of patients returned to the clinics for the first annual check-up and Visit 3 was performed with 1,186 patients.

Initial sting reactions

Systemic sting reactions were predominantly moderate and severe: 122 (8.6%) had grade I, 700 (49.1%) grade II, 589 grade III (41.3%) and 14 (1.0%) grade IV reactions. The median time span between the sting and the onset of symptoms was 6.5 minutes (lower and upper quartiles: 5.00 and 15.00). Severe reactions such as loss of consciousness or cardiac arrest already occurred after 5 minutes (lower and upper quartiles: 3 and 10), compared to skin symptoms such as flush, urticaria, and angioedema, with a median time to onset of 10 minutes (lower and upper quartiles: 5 and 15) ($p < 0.001$). In 1166 (81.8%) patients, initial sting reactions occurred after only one sting while 243 (17.1%) patients had multiple stings.

Stings on the head or neck did not cause more severe reactions (grade III and IV): 26.1% of patients with severe systemic sting reactions were stung on the head, compared to 73.9% stung on other parts of the body (14.0% on trunk, 32.5% on upper extremities, 13.0% on lower extremities, 14.4% unknown and several locations; Table 2).

While stings on the trunk and upper extremities did not cause severe reactions more frequently ($p = 0.120$ and $p = 0.729$, respectively), stings on lower extremities significantly caused milder reactions (grade I and II). The relative frequency of severe SSR (lower extremities vs. other sting sites) was 32.6% and 44.2%, respectively (odds ratio (OR): 0.61; 95% confidence interval (CI): 0.45-0.83; $p = 0.001$).

Antihistamines and corticosteroids were the treatment of choice for mild SSR and were used to treat 80.5% of grade I reactions and 63.8% of grade II reactions (Table 3). The usage of epinephrine

significantly increased with the severity of the reaction: 24.7% of grade I and II reactions, but 52.4% of grade III and IV reactions were treated with epinephrine ($p < 0.001$). Differences concerning the frequency of epinephrine usage were detected between the participating countries: in Austria, Germany, and Italy epinephrine was less commonly used to treat both, mild and severe SSRs (Fig 1).

Systemic AE during the up-dosing phase

In total, 93 (7.0%) patients who underwent VIT had a systemic AE, and these reactions were generally mild to moderate. Only one patient suffered from a grade III reaction with flush and bronchospasm. The majority of systemic AE occurred within the first 30 minutes after the injection (64.8%) after administering between 10 and 50 μ g of venom preparation (60.9%). Systemic AE were less frequently treated with either antihistamines or corticosteroids or epinephrine than initial systemic sting reactions (Table 4): 50.0% of grade I reactions and 31.6% of grade II reactions were not treated. The treatment of choice for grade I reactions and the only grade III reaction were antihistamines and/or corticosteroids, while the majority of the grade II reactions were treated with epinephrine.

Interestingly, the frequency of epinephrine usage for the treatment of grade I and II reactions was clearly above average in Slovenia (62.5%) (Fig 2).

Systemic AE during the maintenance phase

Twenty (1.4%) patients had a systemic AE to VIT during the first year of the maintenance phase: seven patients had a grade I, nine patients a grade II, and four patients a grade III reaction. Two patients with grade III reactions suffered from bronchospasm; the other two lost consciousness. Interestingly, all patients with grade III reactions were treated with bee venom. The median time span between the systemic AE and the end of the up-dosing phase was 10 weeks (minimum and maximum: 1 and 41). In total, 14 (70.0%) patients were treated (Table 5). Grade I and II reactions were solely or mainly treated with antihistamines and/or corticosteroids, respectively, while grade III reactions were additionally treated with epinephrine.

Premedication

Premedication with oral antihistamines was taken by more than half (52.1%) of the patients during the up-dosing phase (Table 6). Loratadine and Desloratadine were most frequently used, followed by Cetirizine and Levocetirizine. Taking antihistamines as premedication had no statistically significant effect on the frequency of systemic AE ($p=0.106$); however, the frequency of LLR was significantly lower in patients taking premedication as compared with those not taking antihistamines (23.5% vs. 29.3%; $p=0.021$) (Table 7).

During the maintenance phase, premedication was taken only by 19.7% of patients. Of the 20 patients, who had a systemic AE during the first year of the maintenance phase, 11 took a premedication. Thirty-five patients had a LLR and thereof, 15 took a premedication. Taking antihistamines had no influence on the frequency of skin symptoms (flush, urticaria, and angioedema) either, neither during the up-dosing phase ($p=0.891$) nor during the maintenance phase ($p=0.197$).

The prescription of oral antihistamines as premedication is not something that is handled individually by each country but by each center: the patients of six centers never took antihistamines during the up-dosing phase, while all patients of four other centers (respectively in Austria, Poland, Spain and Türkiye) took antihistamines during the up-dosing as well as the maintenance phase. In Slovenia, all patients took antihistamines during the up-dosing phase but none during the maintenance phase. In all the other centers, premedication was less commonly administered during the maintenance phase compared to the up-dosing phase.

Venom preparation

Generally, bee venom is obtained by electrostimulation, and vespid venom by venom sac extraction. Venom preparations from Anallergo (Anallergo SpA, Scarperia e San Piero, Italy) are obtained by capillary extraction. All purified venom preparations were from ALK-Abelló (ALK-Abelló AS, Hørsholm, Denmark), while the majority of non-purified preparations were obtained from HAL Allergy (HAL Allergy Holding B.V., Leiden, The Netherlands), followed by ALK-Abelló,

Stallergenes (Stallergenes Greer International AG, Baar, Switzerland) and Allergy Therapeutics (Allergy Therapeutics Ltd., Worthing, UK).

The study centers in the Czech Republic, Germany, Spain, and Türkiye solely used preparations of ALK-Abelló, while the other centers used venom preparations of at least two different companies. Aqueous preparations, both purified and non-purified, were preferentially used for up-dosing while depot preparations were the first choice for the maintenance phase (Table 8 and Online Supplement 1).

The type of venom preparation had no influence on the frequency of systemic AE during up-dosing: 18 (5.6%) patients treated with purified depot preparations, 23 (7.7%) patients treated with purified aqueous preparations, and 51 (7.6%) of patients treated with non-purified aqueous preparations had a systemic AE ($p=0.258$).

However, the frequency of LLR was significantly increased when aqueous preparations were used: 77 (24.1%) patients treated with purified depot preparations, 138 (46.5%) patients treated with purified aqueous preparations, 129 (19.3%) patients treated with non-purified aqueous preparations and one patient (2.9%) treated with a non-purified depot preparation had a LLR. The odds of getting a LLR were 2.7 times higher for patients treated with purified aqueous preparations compared to patients treated with purified depot preparations (OR: 2.73; 95% CI: 1.94-3.86; $p<0.001$).

Effectiveness of VIT

The effectiveness of VIT can solely be monitored by the outcome of sting challenges or field stings. In total, 210 (17.7%) patients were stung: sting challenges were performed in 18 patients and 192 patients experienced field stings within the first year of the maintenance phase. The majority (91.0%) of patients tolerated the sting without systemic symptoms.

Eighteen SSRs occurred after field stings: 12 patients experienced a grade I reaction, five patients had a grade II reaction and two had a grade III reaction. These reactions were primarily treated with antihistamines and corticosteroids; seven patients (38.9%) used their prescribed adrenaline

autoinjector. Only one systemic reaction occurred after a sting challenge: this patient had a grade I reaction with general fatigue and a feeling of warmth 10 minutes after the sting.

Taking antihistamines as premedication had no influence on the effectiveness of VIT: seven (8.3%) patients, who were taking a premedication had a systemic reaction after a field sting or sting challenge compared to 12 (9.5%) patients, not taking antihistamines as premedication (OR: 0.86; 95% CI: 0.28-2.51; $p=0.812$).

The type of venom preparation (used for the maintenance phase) had no influence on the effectiveness of VIT either: 11 (10.2%) patients who were treated with purified venom preparations, did not tolerate a sting compared to seven (8.3%) patients treated with non-purified venom preparations (OR: 1.25; 95% CI: 0.42-3.98; $p=0.804$).

Discussion

All venom preparations used in the present study were equally effective and all caused similar frequencies of systemic AE; however, the risk of developing LLR was 2.7 times higher for patients treated with purified aqueous preparations compared to patients treated with purified depot preparations. That aqueous preparations evoke LLR more frequently has also been reported previously[12, 13, 26].

Unexpectedly, the frequency of LLR was higher in patients treated with purified aqueous preparations than in patients treated with non-purified aqueous preparations in our study. Contrary results have been reported by Biló et al for bee venom immunotherapy: purified aqueous preparations resulted in fewer systemic AE and smaller local reactions compared to non-purified preparations using the same rush protocol[27]. The superiority of purified aqueous and/or purified depot preparations compared to non-purified aqueous extracts in terms of safety (fewer LLR) has also been reported in other studies[28-31]. Therefore, we do not have an explanation for our conflicting results.

The major reason for fewer LLR when using purified venom preparations is the absence of peptides and active amine components; purified venom extracts do not contain low molecular components such as vasoactive amines and comprise only a reduced concentration of small peptides, which are present in native venom extracts[27]. Another reason for fewer LLR after using depot preparations is the fact that allergens, adsorbed to substances like aluminum hydroxide or tyrosine, are released slowly from the injection site[29, 30, 32].

In 2001, it was reported that the switch from aqueous to depot extracts for VIT has occurred almost exclusively in German-speaking European countries[30, 33]. This assumption has changed over the years since in our European multicenter study, depot preparations were used not only in German-speaking countries but also in Italy, Poland, and the Czech Republic. In Türkiye, the only venom extract available for VIT was a purified depot preparation. However, since the majority of patients were treated using rush, cluster, or ultrarush up-dosing protocols, more than 70% of patients were treated with aqueous preparations, which are commonly used for these dosing regimens. In the present study, quicker up-dosing protocols (conventional vs. rush, cluster, and ultrarush) did not cause more frequent systemic AE during VIT but LLR were seen more frequently when quicker up-dosing protocols were used[21]. It has been reported previously that systemic AE appear to occur more frequently in patients on rush VIT[13] and rapid dose increase has been established as a risk factor for systemic reactions[8, 9]. Rueff et al also concluded that the aluminum hydroxide adsorbed bee venom preparation caused fewer LLR than the aqueous preparation; however, different up-dosing protocols were used for the different venom preparations[13].

Usually, depot preparations were used in up to 16 week long up-dosing phases, which is time-consuming and unacceptable for Hymenoptera venom allergic patients, who need immediate protection; however, two safe and efficient up-dosing protocols using aluminum hydroxide adsorbed venoms for up-dosing in 7-weeks have been reported since 2019[34, 35].

All venom preparations used in the present study were equally effective as detected by the outcome of field stings and sting challenges. This has also been observed by several previous studies[13, 28,

29, 36, 37]. Furthermore, pretreatment with antihistamines did not negatively influence VIT effectiveness[15, 38]. These findings were also confirmed by the results of the present study.

It has been shown in several studies that pretreatment with H1 antihistamines reduces the number of local as well as systemic reactions[15, 16, 19, 20]. While Levocetirizine significantly reduced the number of systemic AE, especially cutaneous reactions, during the up-dosing phase of bee VIT[15], local reactions, as well as cutaneous, systemic AE occurred less frequently with Fexofenadine pretreatment[16]. In the present study, more than half of the patients took antihistamines as pretreatment during up-dosing. Loratadine and Desloratadine were most frequently used, followed by Cetirizine and Levocetirizine. The frequency of systemic AE on the whole was not reduced and we did not even detect a positive effect on the frequency of systemic skin symptoms; however, the number of LLR was significantly reduced in patients taking premedication as compared with those not taking antihistamines.

Antihistamines, together with corticosteroids, have also been the treatment of choice for mild SSR. Even though SSR were predominantly moderate and severe, only 8% of all reactions have not been treated with either antihistamines, corticosteroids, or epinephrine. The median time span between the sting and the appearance of mild systemic reactions was 10 minutes, while severe SSR already occurred after 5 minutes. As expected, stings in the head and neck region did not cause more severe reactions, as reported previously[39, 40]; however, stings in the lower extremities significantly caused milder reactions in our study cohort. Systemic AE usually occurred within 30 minutes post-injection and after administering 10 to 50 μ g of venom preparation. In previous studies, most systemic AE occurred after administering 40 to 60 μ g of venom[17, 34], which is in good agreement with the present study.

The present analysis has two main limitations: first, the study was primarily designed to assess whether taking β -blockers and ACEI has an influence on the frequency of systemic AE during VIT; therefore, the results of the secondary endpoints must be interpreted with caution as the patient number was not calculated to show these effects. Second, it has been reported that both, quicker up-

dosing protocols and aqueous venom preparations cause more frequent AE, especially LLR; however, since aqueous preparations are preferentially used for rush, cluster and ultrarush protocols, the present study cannot generate sufficient evidence since different preparations have been used for different up-dosing protocols and not only one venom preparation for different dosing regimens or vice versa.

The safety profile of VIT is a relevant issue and considerable differences in safety and efficacy have been reported in the past, due to several reasons. The strength of recommendations concerning risk factors and the management of side effects in the current EAACI guidelines are often weak since only case series studies or case reports are available.[5] Taken together, this prospective multicenter study with 1,425 patients clearly shows that taking β -blockers and ACEI does not increase the frequency of systemic AE during VIT[21] and that all venom preparations used were equally effective and none was superior to others concerning the frequency of systemic AE. Pretreatment with oral antihistamines during VIT significantly reduced the frequency of LLR. The potential higher frequency of LLR, when using aqueous preparations for rapid up-dosing can be reduced by using antihistamines as pretreatment. Depot preparations are commonly used and well tolerated during the maintenance phase. Due to the similar frequency of systemic AE, quicker up-dosing protocols are preferred since patients are protected much faster from future systemic sting reactions.

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References

1. Worm M, Moneret-Vautrin A, Scherer K, Lang R, Fernandez-Rivas M, Cardona V, et al. First European data from the network of severe allergic reactions (NORA). *Allergy*. 2014;69(10):1397-404. doi: 10.1111/all.12475.
2. Golden DB, Marsh DG, Kagey-Sobotka A, Freidhoff L, Szklo M, Valentine MD, et al. Epidemiology of insect venom sensitivity. *Jama*. 1989;262(2):240-4.
3. Bilo BM, Bonifazi F. Epidemiology of insect-venom anaphylaxis. *Curr Opin Allergy Clin Immunol*. 2008;8(4):330-7. doi: 10.1097/ACI.0b013e32830638c5.
4. Bokanovic D, Aberer W, Griesbacher A, Sturm GJ. Prevalence of hymenoptera venom allergy and poor adherence to immunotherapy in Austria. *Allergy*. 2011;66(10):1395-6. doi: 10.1111/j.1398-9995.2011.02659.x.
5. Sturm GJ, Varga EM, Roberts G, Mosbech H, Bilo MB, Akdis CA, et al. EAACI guidelines on allergen immunotherapy: Hymenoptera venom allergy. *Allergy*. 2018;73(4):744-64. doi: 10.1111/all.13262.
6. Muller U, Helbling A, Berchtold E. Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. *J Allergy Clin Immunol*. 1992;89(2):529-35.
7. Rueff F, Vos B, Elberink JO, Bender A, Chatelain R, Dugas-Breit S, et al. Predictors of clinical effectiveness of Hymenoptera venom immunotherapy. *Clin Exp Allergy*. 2014;44(5):736-46. doi: 10.1111/cea.12275.
8. Mosbech H, Muller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. *European Academy of Allergology and Clinical Immunology. Allergy*. 2000;55(11):1005-10.
9. Rueff F, Przybilla B, Bilo MB, Muller U, Scheipl F, Aberer W, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase. *J Allergy Clin Immunol*. 2010;126(1):105-11 e5. doi: 10.1016/j.jaci.2010.04.025.
10. Lockey RF, Turkeltaub PC, Olive ES, Hubbard JM, Baird-Warren IA, Bukantz SC. The Hymenoptera venom study. III: Safety of venom immunotherapy. *J Allergy Clin Immunol*. 1990;86(5):775-80.
11. Sturm G, Kranke B, Rudolph C, Aberer W. Rush Hymenoptera venom immunotherapy: a safe and practical protocol for high-risk patients. *J Allergy Clin Immunol*. 2002;110(6):928-33. doi: 10.1067/mai.2002.129124
12. Bilo MB, Antonicelli L, Bonifazi F. Purified vs. nonpurified venom immunotherapy. *Curr Opin Allergy Clin Immunol*. 2010;10(4):330-6. doi: 10.1097/ACI.0b013e328339f2d1.
13. Rueff F, Wolf H, Schnitker J, Ring J, Przybilla B. Specific immunotherapy in honeybee venom allergy: a comparative study using aqueous and aluminium hydroxide adsorbed preparations. *Allergy*. 2004;59(6):589-95.
14. Frick M, Fischer J, Helbling A, Rueff F, Wiczorek D, Ollert M, et al. Predominant Api m 10 sensitization as risk factor for treatment failure in honey bee venom immunotherapy. *J Allergy Clin Immunol*. 2016;138(6):1663-71 e9. doi: 10.1016/j.jaci.2016.04.024.
15. Muller UR, Jutel M, Reimers A, Zumkehr J, Huber C, Kriegel C, et al. Clinical and immunologic effects of H1 antihistamine preventive medication during honeybee venom immunotherapy. *J Allergy Clin Immunol*. 2008;122(5):1001-7 e4. doi: 10.1016/j.jaci.2008.08.007.
16. Reimers A, Hari Y, Muller U. Reduction of side-effects from ultrarush immunotherapy with honeybee venom by pretreatment with fexofenadine: a double-blind, placebo-controlled trial. *Allergy*. 2000;55(5):484-8. doi: 10.1034/j.1398-9995.2000.00520.x.

17. Gorska L, Chelminska M, Kuziemski K, Skrzypski M, Niedozytko M, Damps-Konstanska I, et al. Analysis of safety, risk factors and pretreatment methods during rush hymenoptera venom immunotherapy. *Int Arch Allergy Immunol*. 2008;147(3):241-5. doi: 10.1159/000142048.
18. Wohrl S, Gamper S, Hemmer W, Heinze G, Stingl G, Kinaciyan T. Premedication with montelukast reduces local reactions of allergen immunotherapy. *Int Arch Allergy Immunol*. 2007;144(2):137-42. doi: 10.1159/000103225.
19. Brockow K, Kiehn M, Riethmuller C, Vieluf D, Berger J, Ring J. Efficacy of antihistamine pretreatment in the prevention of adverse reactions to Hymenoptera immunotherapy: a prospective, randomized, placebo-controlled trial. *J Allergy Clin Immunol*. 1997;100(4):458-63. doi: 10.1016/s0091-6749(97)70135-0.
20. Berchtold E, Maibach R, Muller U. Reduction of side effects from rush-immunotherapy with honey bee venom by pretreatment with terfenadine. *Clin Exp Allergy*. 1992;22(1):59-65.
21. Sturm GJ, Herzog SA, Aberer W, Alfaya Arias T, Antolin-Amerigo D, Bonadonna P, et al. beta-blockers and ACE inhibitors are not a risk factor for severe systemic sting reactions and adverse events during venom immunotherapy. *Allergy*. 2021;76(7):2166-76. doi: 10.1111/all.14785.
22. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet*. 1977;1(8009):466-9. doi: 10.1016/s0140-6736(77)91953-5
23. Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. *Allergy*. 2005;60(11):1339-49. doi: 10.1111/j.1398-9995.2005.00963.x.
24. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U. EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. *Allergy*. 2005;60(12):1459-70.
25. R Core Team. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2022.
26. Valerio P, Marina M, Federica R, Dario C, Camilla C, Alessandra CG, et al. Venom immunotherapy: safety and tolerability of the build-up phase with depot versus aqueous preparations. *Clin Exp Allergy*. 2022;52(10):1230-3. doi: 10.1111/cea.14209.
27. Biló MB, Cinti B, Brianzoni MF, Braschi MC, Bonifazi M, Antonicelli L. Honeybee venom immunotherapy: a comparative study using purified and nonpurified aqueous extracts in patients with normal Basal serum tryptase concentrations. *Journal of allergy*. 2012;2012:869243. doi: 10.1155/2012/869243.
28. Mosbech H, Malling HJ, Biering I, Bowadt H, Soborg M, Weeke B, et al. Immunotherapy with yellow jacket venom. A comparative study including three different extracts, one adsorbed to aluminium hydroxide and two unmodified. *Allergy*. 1986;41(2):95-103.
29. Cadario G, Marengo F, Ranghino E, Rossi R, Gatti B, Cantone R, et al. Higher frequency of early local side effects with aqueous versus depot immunotherapy for hymenoptera venom allergy. *J Investig Allergol Clin Immunol*. 2004;14(2):127-33.
30. Quercia O, Rafanelli S, Puccinelli P, Stefanini GF. The safety of cluster immunotherapy with aluminium hydroxide-adsorbed honey bee venom extract. *J Investig Allergol Clin Immunol*. 2001;11(1):27-33.
31. Biló MB, Severino M, Cilia M, Pio A, Casino G, Ferrarini E, et al. The VISYT trial: Venom Immunotherapy Safety and Tolerability with purified vs nonpurified extracts. *Ann Allergy Asthma Immunol*. 2009;103(1):57-61.
32. Zubeldia JM, Ferrer M, Davila I, Justicia JL. Adjuvants in Allergen-Specific Immunotherapy: Modulating and Enhancing the Immune Response. *J Investig Allergol Clin Immunol*. 2019;29(2):103-11. doi: 10.18176/jiacci.0349.

33. Poli F, Longo G, Parmiani S. The safety and efficacy of immunotherapy with aluminum hydroxide-adsorbed venom extract of *Vespula* spp. An open, retrospective study. *Allergol Immunopathol (Madr)*. 2001;29(5):191-6. doi: 10.1016/s0301-0546(01)79054-4.
34. Schrautzer C, Arzt-Gradwohl L, Bokanovic D, Schwarz I, Cerpès U, Koch L, et al. A safe and efficient 7-week immunotherapy protocol with aluminum hydroxide adsorbed vespid venom. *Allergy*. 2019. doi: 10.1111/all.14012.
35. Sozener ZC, Kendirlinan R, Cerci P, Ayd NO, Mungan D, Bavbek S, et al. Field sting reactions in patients receiving Hymenoptera venom immunotherapy: real-life experience. *Asian Pac J Allergy Immunol*. 2022. doi: 10.12932/AP-011221-1282.
36. Quercia O, Emiliani F, Pecora S, Burastero SE, Stefanini GF. Efficacy, safety, and modulation of immunologic markers by immunotherapy with honeybee venom: comparison of standardized quality depot versus aqueous extract. *Allergy Asthma Proc*. 2006;27(2):151-8.
37. Alessandrini AE, Berra D, Rizzini FL, Mauro M, Melchiorre A, Rossi F, et al. Flexible approaches in the design of subcutaneous immunotherapy protocols for Hymenoptera venom allergy. *Ann Allergy Asthma Immunol*. 2006;97(1):92-7. doi: 10.1016/S1081-1206(10)61376-2.
38. Muller U, Hari Y, Berchtold E. Premedication with antihistamines may enhance efficacy of specific-allergen immunotherapy. *J Allergy Clin Immunol*. 2001;107(1):81-6.
39. Arzt L, Bokanovic D, Schwarz I, Schrautzer C, Massone C, Horn M, et al. Hymenoptera stings in the head region induce impressive, but not severe sting reactions. *Allergy*. 2016;71(11):1632-4. doi: 10.1111/all.12967.
40. Solley GO. Stinging and biting insect allergy: an Australian experience. *Ann Allergy Asthma Immunol*. 2004;93(6):532-7. doi: 10.1016/S1081-1206(10)61259-8.

TABLES

Table 1. Demographic data. The percentages refer to the total number of observations. Age at Visit 1 was the age at index sting, age at Visit 2 was the age when venom immunotherapy was started.

	Visit 1 (n=1,425) index sting	Visit 2 (n=1,342) immunotherapy induction	Visit 3 (n=1,186) maintenance phase
<i>Age range (mean age) [years]</i>	35–80 (52)	35–84 (54)	36–85 (55)
<i>Sex, n (%)</i>			
male	810 (56.8)	774 (57.7)	679 (57.3)
female	615 (43.2)	568 (42.3)	507 (42.7)
<i>Grade of SSR (index sting), n (%)</i>			
Grade I	122 (8.6)
Grade II	700 (49.1)
Grade III	589 (41.3)
Grade IV	14 (1.0)
<i>Causal venom, n (%)</i>			
bee	320 (22.5)	351 (26.2)	297 (25.0)
vespid/Vespa/Polistes	838 (58.8)	924 (68.9)	832 (70.2)
bee & vespид/Vespa/Polistes	206 (14.5)	67 (5.0)	57 (4.8)
unknown	61 (4.3)	0 (0.0)	0 (0.0)

Table 2. Location of sting of initial sting reactions. Missing data are not explicitly stated in the table.

location of sting	Grade 1 and II	Grade III and IV	Overall
head, n (%)	199 (24.5)	155 (26.1)	354 (25.2)
trunk, n (%)	91 (11.2)	83 (14.0)	174 (12.4)
upper extremities, n (%)	257 (31.6)	193 (32.5)	450 (32.0)
lower extremities, n (%)	159 (19.6)	77 (13.0)	236 (16.8)
several locations, n (%)	26 (3.2)	27 (4.5)	53 (3.8)
unknown, n (%)	81 (10.0)	59 (9.9)	140 (10.0)

Table 3. Treatment of systemic sting reactions. Missing data are not explicitly stated in the table.

	Grade I	Grade II	Grade III	Grade IV	Total
no treatment, n (%)	12 (10.6)	52 (8.6)	37 (6.9)	0 (0.0)	101 (7.9)
antihistamines and/or corticosteroids, n (%)	91 (80.5)	388 (63.8)	225 (41.8)	0 (0.0)	704 (55.4)
epinephrine, n (%)	10 (8.9)	168 (27.6)	276 (51.3)	12 (100.0)	466 (36.7)

Table 4. Treatment of systemic AE during up-dosing. Most of the patients were treated with antihistamines, corticosteroids or epinephrine. Two patients were treated with ipratropium bromide/fenoterol hydrobromide and benzodiazepine and ipratropium bromide, respectively and for additional two patients the drug was not documented. Missing data are not explicitly stated in the table.

	Grade I	Grade II	Grade III	Total
no treatment, n (%)	26 (50.0)	12 (31.6)	0 (0.0)	38 (41.8)
antihistamines and/or corticosteroids, n (%)	20 (38.5)	11 (28.9)	1 (100.0)	32 (35.2)
epinephrine, n (%)	6 (11.5)	13 (34.2)	0 (0.0)	19 (20.9)
other treatment, n (%)	0 (0.0)	2 (5.3)	0 (0.0)	2 (2.2)

Table 5. Treatment of systemic AE during the maintenance phase.

	Grade I	Grade II	Grade III	Total
no treatment, n (%)	3 (42.9)	2 (12.5)	1 (40.0)	6 (30.0)
antihistamines and/or corticosteroids, n (%)	4 (57.1)	5 (62.5)	0 (0.0)	9 (45.0)
epinephrine, n (%)	0 (0.0)	2 (25.0)	3 (60.0)	5 (25.0)

Table 6. Usage of premedication during the up-dosing and maintenance phase.

	Visit 2 (up-dosing phase)	Visit 3 (maintenance phase)
no premedication, n (%)	643 (47.9)	953 (80.4)
Cetirizine-Levocetirizine, n (%)	140 (10.4)	90 (7.6)
Loratadine-Desloratadine, n (%)	435 (32.4)	98 (8.3)
Dimetindene n (%)	45 (3.4)	0 (0.0)
Other antihistamines, n (%)	79 (5.9)	45 (3.8)

Table 7. Impact of premedication on the frequency of systemic adverse events and large local reactions during the up-dosing phase of VIT.

	no premedication	premedication	OR (95% CI)	p-value
<i>systemic AE, n (%)</i>				
no	606 (94.2)	634 (91.9)	1.45 (0.92-2.29)	0.106
yes	37 (5.8)	56 (8.1)		
<i>large local reaction, n (%)</i>				
no	454 (70.7)	520 (76.5)	0.74 (0.58-0.96)	0.021
yes	188 (29.3)	160 (23.5)		

Table 8. Venom preparations used for up-dosing and during the maintenance phase.

	Visit 2 (up-dosing phase)	Visit 3 (maintenance phase)
purified depot preparation, n (%)	326 (24.4)	675 (60.0)
purified aqueous preparation, n (%)	305 (22.8)	9 (0.8)
non-purified depot preparation, n (%)	34 (2.5)	62 (5.5)
non-purified aqueous preparation, n (%)	671 (50.2)	379 (33.7)

FIGURE LEGENDS

Figure 1. Frequency of epinephrine usage in participating countries. (A) 24.7% of grade I and II reactions were treated with epinephrine compared to (B) 52.4% of grade III and IV reactions (vertical red lines). In the Czech Republic, Germany and Slovenia only one center each was participating in the study.

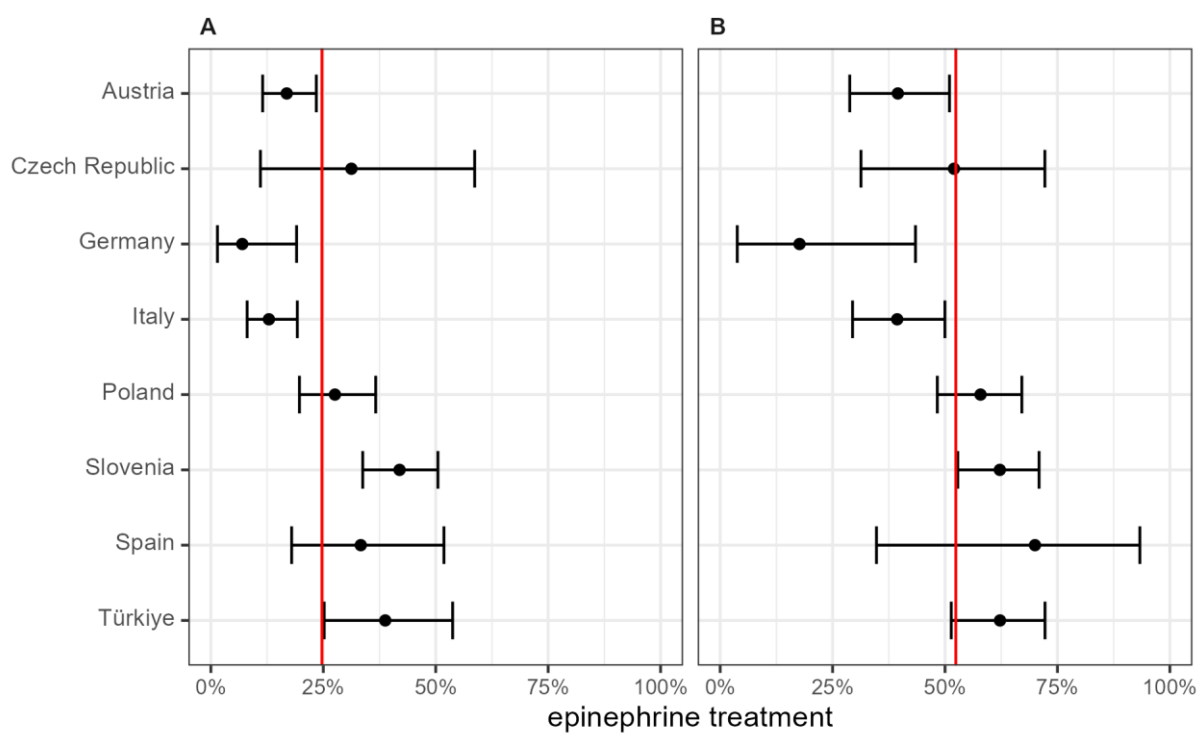


Figure 2. Frequency of epinephrine usage to treat grade I and II reactions during up-dosing.

