EAACI Interest Group on Insect Venom Hypersensitivity

STUDY PROTOCOL

EADOAS-Study

The effect of antihypertensive drugs on severity of anaphylaxis and side –effects during venom immunotherapy

EAACI multicenter study

NCT04269629

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1. Study synopsis

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TITLE OF STUDY	The effect of antihypertensive drugs on severity of anaphylaxis and side-effects during venom immunotherapy
SHORT TITLE	EADOAS-Study
POPULATION	Patients with a history of anaphylactic sting reaction
OBJECTIVE(S)	The primary objective of this study is to evaluate whether subjects under antihypertensive treatment with beta-blockers and/or ACE-inhibitors show more side effects during VIT compared to subjects with no antihypertensive treatment.
INTERVENTION(S)	Venom immunotherapy (VIT)
KEY INCLUSION AND EXCLUSION CRITERIA	*Inclusion criteria:* Subjects aged from 35 to 85 years with a history of anaphylactic sting reaction *Exclusion criteria (only for phase 2):* Absolute contraindications for VIT, Pretreatment with Omalizumab
OUTCOME(S)	Proportion of side-effects during venom immunotherapy
STUDY TYPE	Prospective multicenter observational study
STATISTICAL ANALYSIS	The primary analysis will compare the proportion of objective side effects between the groups (subjects under antihypertensive treatment vs. subjects without antihypertensive treatment) using a Mantel-Haensel estimate of the odds ratio stratified by clinic. A logistic regression model will be used to control for other possible confounders
ETHICAL APPROVALS	Participating study centers have to obtain ethical approvals from their institutional review boards.
SPONSORING	Unrestricted grants from pharmaceutical companies
SAMPLE SIZE	1319
TRIAL DURATION	July 1, 2014 – December 31, 2019 (End of inclusion phase: January 31, 2018)

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SPONSORING	Unrestricted grants from pharmaceutical companies		

2. Indication

There is an ongoing debate whether antihypertensive treatment with beta-blockers and/or ACE-inhibitors comprises a risk factor for more severe anaphylactic reactions due to insect stings. Side effects under venom immunotherapy (VIT) could be more severe and more frequent. In the literature, data are controversial and originate from case reports or statistically underpowered studies.

3. Introduction

Since the introdution of beta-blockers in the early 1960s and the availability of ACE-inhibitors in the early 1980s, there is an ongoing discussion about negative effects of those substances on allergic reactions and side-effects during allergen immunotherapy. Although there is a theoretical and anectotal clinical evidence for possible interactions, data of studies are controversial and their role as risk factors is a debated issue. All available studies have drawbacks and are underpowered. Therefore, current recommendations are practice-oriented but not sufficiently evidence-based. To stop the endless debate, a well planned multicenter study ist needed.

3.1 Beta-blockers

Beta-blockers are mostly used to treat arterial hypertension and are valuable drugs in the treatment of cardiovascular disease. However, they are also prescribed for non-cardiac conditions like migraine prophylaxis or symptomatic treatment of skeletal muscle tremor.

3.1.1 Background

Four main types of adrenergic receptors (α_1, α_2, β_1, β_2) can be roughly distinguished according to their affinity to norepinephrine and epinephrine. In brief, stimulation of β_1 receptors induces a positive chronotropic and inotropic effect on the heart as well as a renin release of the kidney. β_2 stimulation results in dilatation of blood vessels and bronchioles, and α_2 stimulation induces contraction of blood vessels and bronchioles [1]. Basically, β blockade inhibits the effect of both endogenously released and therapeutically administered epinephrine on β receptors in case of anaphylaxis. Therefore epinephrine treatment can be ineffective or due to the block of β_1 and β_2 actions may facilitate unopposed α-adrenergic effects like bronchoconstriction and bradycardia [2].

3.1.2 Potential effect on immunotherapy

- *Side effects do not appear to be more frequent, but maybe more serious*
- *Side effects could be refractory to treatment*
- *Emergency treatment may cause paradoxical treatment effects*

Both beta blockers and allergen immunotherapy are frequently prescribed, therefore allergists commonly encounter patients who are candidates for immunotherapy and are treated with beta blockers. Fatality studies have shown that particularly elderly patients with pre-existing cardiovascular disease die from hymenoptera venom allergy[3]. Therefore unlike for respiratory allergies, immunotherapy with hymenoptera venoms is commonly performed in elderly patients who are more likely to be on beta blocker treatment.

The risk of beta blocker treatment is still questionable. There is plenty of experimental human[4, 5] and animal studies[6-8] mainly performed in the 1970s and 80s as well as anecdotal clinical evidence[9-15] that anaphylaxis may be influenced by pharmacodynamics of β-blockade. In theory, β-blockade perturbs the control of endogenously produced mediators of anaphylaxis resulting in an enhanced generation, release, and reduced effects of these mediators on the end organs[2]. If anaphylaxis occurs in a patient on beta-blocker, it may therefore be more severe and protracted. There is also evidence that even beta-blocker eye drops have systemic effects[16-19]. However, the plurality of uncontrolled variables makes the susceptibility of individual patients to this effect of topically and orally applied beta-blockers largely unpredictable[2]. The second point is that emergency treatment with epinephrine may be ineffective or promote undesired α-adrenergic and vagotonic effects[20]. β-blockade dramatically alters pharmacotherapeutic actions of epinephrine and isoproterenol, as up to 80-fold higher doses may be necessary[5, 21]. Additionally, the block of β_1 and β_2 actions of epinephrine may facilitate unopposed α-adrenergic and reflex vagonotic effects, which can result in increased mediator release, bronchoconstriction and bradycardia[5]. The pronounced α-receptor activation in the presence of epinephrine may also constrict coronary arteries or exaggerate systemic pressor effects of epinephrine leading to severe hypertensive episodes.

There are three clinical studies dealing with the question whether patients taking beta-blockers are at increased risk of having side effects during immunotherapy. Hepner et al[22] prospectively studied 3178 patients; 68 patients were taking beta-blockers. Although statistically 3 patients were expected to have systemic reactions, only one patient taking a beta-blocker had a systemic reaction. In a retrospective study of Mueller et al[23], 1389 patients were included, and 25 patients were on beta-blockers during immunotherapy. They also did not find an increased incidence of systemic reactions, and reactions were not more severe than in the control group. Most recently, an EAACI observational prospective multicenter study on 680 patients (15 on beta-blockers) could not identify beta-blocker therapy as independent predictor for an emergency intervention[24] during immunotherapy. Interestingly, in a former EAACI observational study on 962 patients[25] (52 on beta-blockers) beta blocker were not identified as independent risk factor for severe anaphylaxis, which

was consistent with a large Australian study[26]. In this study, case records of 1149 patients were retrospectively analyzed. Univariate analyses suggested that reaction severity was influenced by beta-blockers. However, in the multivariate analysis beta-blockers were not identified as independent risk factor. Recently, 657 patients suitable for VIT were included in a large single-center observational study. 59 were on beta-blocker treatment; 27 on both, ACE-inhibitors and beta blockers. Again, beta blockers were not identified to be correlated with severe anaphylaxis[27]. More recently, again controversial data have been published: Beta-blockers were not associated with severe anaphylactic symptoms such as syncope or hypotension, but with 3 or more organ involvement and hospitalization[28].

3.1.3 Current recommendations

There is good evidence in the literature that anaphylaxis is not more frequent in patients receiving beta-blockers. On the other side patients may be at increased risk from more severe systemic reactions and from ineffective emergency treatment. Therefore there is still a relative contraindication for beta blockers in subjects allergic to hymenoptera venom.

3.2 ACE-inhibitors

ACE-inhibitors are primarily used to treat arterial hypertension and congestive heart failure.

3.2.1 Background

The renin angiotensin aldosterone system (RAS) regulates blood pressure. The system in general increases blood pressure by reduced salt and water excretion and vasoconstriction if blood pressure acutely drops[1]. ACE inhibitors block conversion of angiotensin I to angiotensin II, which results in vasodilatation and reduced blood volume. Therefore, hypotension during anaphylaxis may not sufficiently counteracted by the inhibited RAS.

3.2.2 Potential effect on immunotherapy

- *Diminished ability to counteract allergy-induced hypotension*

There is some anecdotal clinical evidence that a subset of patients receiving venom immunotherapy tend to have more serious hypotension in case of side effects[29, 30]. In theory, the RAS is part of the compensatory physiologic response to anaphylaxis and thus ACE-inhibitors may hamper an effective response in some instances of anaphylaxis. However, also a dysfunctional RAS in which overall RAS activity is diminished might aggravate symptoms due to an insufficient response to anaphylaxis[31-33].

The role of ACE-inhibitors as risk factor for more severe anaphylaxis is still a debated issue. In a large Australian retrospective analysis done by Brown[26], 1149 patients who have had anaphylaxis were included. Among these, fifty-seven patients were on ACE-inhibitor treatment. Multivariate analysis did not identify ACE-inhibitors as independent risk factor. However, in an EAACI observational prospective multicenter study on 962 patients with hymenoptera allergy, the 42 patients receiving ACE inhibitors had an approximately 2.3-fold increased risk for severe anaphylaxis (odds ratio 2.26, 95% CI 1.13 - 4.56) [25].

The second EAACI observational multicenter study on 680 VIT patients (18 patients on ACE-inhibitors) did not identify ACE-inhibitors as independent risk factor for side effects.

Recently, 657 patients suitable for VIT were included in a large single-center observational study. 32 were on ACE and 27 on beta-blocker treatment. Again, ACE-inhibitors were not identified to be correlated with severe anaphylaxis[27]. Another retrospective analysis revealed that ACE-inhibitors were not associated with severe anaphylactic symptoms such as syncope or hypotension, but with 3 or more organ involvement and hospitalization[28]. Most recently, in a study on 743 patients (thereof 90 on ACE inhibitor treatment) ACE inhibitors did not increase the frequency of side-effects during the buildup phase of VIT[34].

3.2.3 Current recommendations

If ACE-inhibitors increase the risk of more serious anaphylaxis is still a debated issue. Currently, the recommendation that patients receiving ACE-inhibitors are more likely to have side effects during immunotherapy is only based on case reports. Nevertheless, until there will not be new evidence, current guidelines recommend to replace ACE-inhibitors, if feasible.

4. Justification of the study

Although a considerable number of studies on this topic were performed, all studies were underpowered to identify beta blockers and/or ACE inhibitors as risk factors. The number of included patients was usually high; however, the proportion of patients on antihypertensive treatment was low ranging from 2-11%[22, 34]. In the current study we plan to enroll 1319 patients aged 35-85. Considering retrospective data from our own institution it is assumed that 24% (317) will be on beta blockers and/or ACE inhibitors. Based on these data, a clear statement on the risk of antihypertensive treatment will be possible.

5. Study criteria

5.1 Primary objective

- The primary objective of this study is to evaluate whether subjects under antihypertensive treatment with beta-blockers and/or ACE-inhibitors show more side effects during VIT compared to subjects with no antihypertensive treatment.

5.2 Secondary objectives

- To evaluate whether subjects under antihypertensive treatment with beta-blockers and/or ACE-inhibitors have more severe sting reactions.
- To correlate the prevalence of cardiovascular diseases and/or hypertension with the risk for more severe systemic sting reactions and with more severe and more frequent side effects under VIT.
- To evaluate whether bee venom is associated with a higher frequency of side-effects.
- To evaluate whether high sIgE levels are correlated to a higher frequency of side-effects.
- To evaluate whether high tryptase levels are correlated to a higher frequency of side-effects.
- To evaluate whether quicker up-dosing protocols are correlated to a higher frequency of side-effects.
- To evaluate efficacy of VIT by sting challenges or field stings and look for differences between patients with and without antihypertensive treatment

6. Patients and methods

6.1 Subject eligibility

Patient selection takes place on the basis of the inclusion and exclusion criteria

6.2 Inclusion criteria

- Legally competent male and female individuals aged from 35 to 85 years with a history of systemic sting reaction (≥ grade I according to the classification by Ring and Messmer, *Table 1*).
- Age ≥35 and ≤85 years

6.3 Exclusion criteria for phase 2 (no exclusion criteria for phase 1)

- Absolute contraindications for VIT
- Pretreatment with Omalizumab

6.4 Methods

6.4.1 Personal history

6.4.2 Personal history is recorded at Visit 1 if patients have met inclusion and exclusion criteria. Previous sting reactions, allergies, diseases and current medication will be recorded in the CRF.

Symptoms will be divided into subjective and objective symptoms.
Subjective symptoms are: feeling of warmth, isolated pruritus, headache, general fatigue, anxiety, dysphagia, throat tightness, chest tightness, nausea, vertigo, perception of impending doom

Objective symptoms are: flush, urticarial, angioedema, dyspnea, hoarseness, drop in blood pressure, tachycardia, vomiting, abdominal cramping, bronchospasm, involuntary urination, involuntary defecation, loss of consciousness, cardiac arrest, apnoea

6.4.3 Laboratory tests

Total and specific IgE (bee and vespid venom, rApi m 1, rVes v 1, rVes v 5) as well as serum tryptase will be determined.
Measurements will be performed as part of clinical routine procedures by ImmunoCAP® (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions.

6.4.4 Skin testing (Intradermal skin test and/or prick testing)

Skin testing is performed routinely within the standard diagnostic procedure of each center.

6.4.5 Venom immunotherapy (VIT)

There is no standard buildup phase; centers use their own standard protocols. Typically, conventional (outpatient), Cluster/Ultrarush- (partial inpatient) and Rush-protocols (inpatient) will be performed. The respective venom that is used for VIT will be recorded (bee or wasp venom, trade name, company). Injections will be administered subcutaneously in the dorsal part of the upper arm, one hand's breadth above the elbow, or in the volar part of the thigh. All interventions will be performed according to the in-house protocols of participating centers.

7. Overall study design and plan

The study is conducted as an open, prospective observational study on an outpatient basis. After giving the Informed Consent subjects will be screened at Visit 1. Subjects meeting the inclusion and exclusion criteria will be included (phase 1). If subjects agree to receive VIT, side effects during the buildup phase will be recorded (phase 2). At the first annual control visit side effects during maintenance phase and, if applicable, the outcome of field stings of sting challenge tests will be recorded (phase 3). No additional study-related visits are required.

Participation in this study will have no influence on decision-making for venom-immunotherapy. All procedures have to be in concordance with current EAACI guidelines[35, 36] and will be conducted individually by each study center.

Detailed schedule of events please see Table 2.

8. Schedule of study procedures

Phase 1 (Screening, inclusion) – Visit 1. (week 0)

The following procedures will be performed:

- Ask the subject to read and sign the Informed Consent
- Review of inclusion and exclusion criteria
- Collect medical and allergy history, recording of co-medication
- Collect demographics
- Perform skin testing and determination of IgE and serum tryptase (CAP)
- Record decision regarding venom immunotherapy (yes/no)

Phase 2 (up-dosing of VIT) – Visit 2. (0-4 weeks after reaching maintenance dose)

- Re-collect medical history, recording of co-medication
- Record of up-dosing scheme and of the respective venom used for VIT
- Record pre-treatment during VIT
- Record of side-effects during VIT up-dosing

Phase 3 (maintenance phase) – Visit 3/3a (at least 12 months [+/- 2 months] after reaching maintenance dose)

- Re-collect medical history, recording of co-medication
- Record of side-effects during VIT maintenance phase

Phase 3 (maintenance phase) – Visit 3b/3c optional

- Document field stings and sting challenges
- Record outcome of field sting or sting challenge

9. Early termination of study

The subject will be advised in the Informed Consent Form that he/she has the right to withdraw from the trial at any time without prejudice. Reasons for a premature treatment stop will be documented, if feasible.

The enrolment of new subjects will be suspended if more than 5 serious anaphylactic reactions (≥grade III according to Ring & Messmer; except bronchoconstriction) during buildup phase in patients with antihypertensive treatment occur.

10. Data recording and documentation

Data are recorded in case report forms (CRFs). Data will also be collected using a web based form. Data regarding this study is recorded, analyzed, and archived in anonymized form.

11. Statistical methods

All patients who participate in this study belong to one of the following two groups for the analyses: the group without antihypertensive medication, and the group on antihypertensive medication (beta blockers and/or ACE inhibitors). The analyses will be based on the patients who completed buildup phase of immunotherapy.

Categorical variables will be described with percentages, and continuous variables will be expressed as means ± standard deviation or median with interquartile range.

Statistical analysis will be performed using IBM SPSS Statistics 21. A 5% level of significance will be used throughout. In all analyses, an appropriate transformation may be used to obtain approximate normality.

11.1 Analysis of primary endpoint

The primary endpoint for the study is the presence of objective side effects during immunotherapy which is defined as binary outcome: yes if any grade of objective side effect occurred, no otherwise.

The primary analysis will compare the proportion of objective side effects between the groups using a Mantel-Haensel estimate of the odds ratio stratified by center. A logistic regression model will be used to control for other possible confounders: age, gender, cardiovascular disease, hypertension, chronic bronchial disease or asthma, total IgE, venom-specific IgE, tryptase levels, mastocytosis, skin test reactivity, and study centers.

11.2 Analysis of secondary endpoints

For the analysis of secondary endpoints, comparison between groups will be made by using $\chi 2$ statistics or the Fisher exact test for categorical variables and by t-test or the Mann-Whitney U test for continuous variables.

11.3 Sample size

It is assumed that 24% of the patients will be on beta blockers and/or ACE inhibitors. A $\chi 2$ test with a two-sided 5% significance level has a 80% power to detect the difference between the group without antihypertensive medication with 6% side effects during VIT and the group on beta blockers and/or ACE inhibitors with 12.3% side effects during VIT (OR = 2.2) when the sample sizes are 631 and 200 (a total sample size of 831), respectively.

The drop-out rate includes study dropouts (30%) who do not start immunotherapy and study dropouts (10%) who do not end immunotherapy. This results in a drop-out rate of 37% and a required number of 1319 patients.

12. Regulatory and ethical obligations

12.1 Institutional Review Board

Each study center will follow the respective regulations of the local ethical review board. Approval of the study seems necessary particularly with respect to the statistical evaluation of patient data, which have to be processed in an anonymized form. Only routine diagnostic procedures will be performed. No additional diagnostic test, treatment or check of efficacy with sting challenges will be done solely for the study

12.2 Informed consent

Informed consent has to be obtained from each patient before inclusion in the study. The local policies have to be followed. Translations to the national language have to be done by study centers. Informed consent has to be documented. The documentation will remain with the study documents throughout the study, and must be available for inspection by any authorized personnel.

12.3 Study initiation

The following documents must be on file at the study sites before the start of this study:

- Current curricula vitae of all investigators involved in the study
- Documentation of Institutional Review Board approval of the following:
    - Protocol with version number and date, and date of approval
    - Informed Consent Form with version number and date, and date of approval

12.4 Case report forms

The study coordinator will supply copies of the forms. A center code will be assigned to each study center. Consecutive patients receive consecutive numbers by the study center. The anonymization key is kept together with the patients' written informed consent at each center. Data of each paper-CRF will be entered into an online e-CRF form in anonymised form. The originals are kept in the center.

13. Tables

13.1 Table 1

13.1.1 Table 1A

Classification of systemic reactions modified according to J. Ring and Messmer[35, 37]

Grade I	Generalised skin symptoms (e.g. flush, generalised urticaria, angioedema)
Grade II	Mild to moderate pulmonary, cardiovascular (tachycardia, hypotension, dizziness), and/or gastrointestinal symptoms (nausea)
Grade III	Anaphylactic shock, loss of consciousness, life-threatening spasm of smooth muscles (bronchi, uterus, etc.)
Grade IV	Cardiac and/or respiratory arrest

13.1.2 Table 1B

Classification of systemic reactions according to Mueller[38]

Grade I	Generalised urticaria, itching, malaise and anxiety
Grade II	Any of the above plus two or more of following: Generalised oedema; constriction in chest; wheezing; abdominal pain, nausea and vomiting; and dizziness.
Grade III	Any of the above plus two or more of following: Dyspnea; dysphagia; hoarseness or thickened speech; confusion; and feeling of impending disaster.
Grade IV	Any of above plus two or more of following: Cyanosis; fall in blood pressure; collapse incontinence; and unconsciousness.

13.2 Table 2

Study procedures	**Visit 1**	**Visit 2**	**Visit 3/3a**	**Visit 3b/c** [a]
	Screening/Inclusion	**Up-dosing**	**Maintenance Phase**	**Field sting / sting challenge**
Informed consent	X			
Inclusion/Exclusion review	X			
Allergy history	X			
Medical history / co-medication	X	X	X	X
Demographics	X			
Collect results of IDT and CAP	X			
Recording of decision regarding venom immunotherapy (yes/no)	X			
Recording of venom used for VIT		X		
Recording of side-effects		X	X	
Recording of stinging insect				X
Recording of sting reaction				X

[a] if applicable.

14. Abbreviations

ACE	angiotensin converting enzyme
CAP	ImmunoCAP® (test for sIgE determination)
CI	confidence interval
CRF	case report form
EAACI	European Academy of Allergology and Clinical Immunology
IDT	intradermal test
OR	odds ratio
RAS	renin angiotensin (aldosterone) system
sIgE	specific IgE antibodies
tIgE	total IgE
VIT	venom immunotherapy

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