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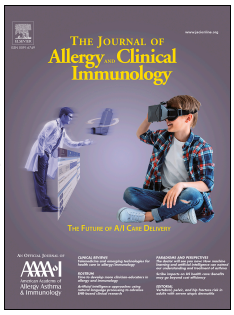

Phenotype and risk factors of venom-induced anaphylaxis: a case-control study of the European Anaphylaxis Registry

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Phenotype and risk factors of venom-induced anaphylaxis: a case-control study of the European Anaphylaxis Registry

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Conflict of interest:

A. Bauer reports personal fees from ALK, Allergopharma, Allergy Therapeutics, Diater, LETI, Thermofisher, and Stallergens outside the submitted work N. Wagner reports personal fees from ALK outside the submitted work. R. Treudler reports grants and personal fees from Sanofi-Genzyme, ALK-Abello, Takeda, Novartis, grants from Hautnetz Leipzig and Fraunhofer-IZI Leipzig, outside the submitted work. V. Cardona reports personal fees from ALK, Allergopharma, Allergy Therapeutics, Diater, LET, Thermofisher and Stallergens outside the submitted work. M. B. Bilò reports personal fees from ALK outside the submitted work. K. Scherer reports personal fees from Allergopharma, Sanofi-Aventis, and Shire outside submitted work. Franziska Ruëff reports personal fees outside the submitted work from ALK-Abelló, Allergopharma, Bencard, Boehringer Ingelheim, Bristol Myers Squibb, Circassia, Dermira, DST, LEO Pharma, Lilly, Dr. Gerhard Mann chem.-pharm. Fabrik GmbH, Mylan, Novartis, Pfizer, Thermo Fisher Scientific and UCB Claudia

Pföhler performed clinical studies for Allergy Therapeutics and received speaker honoraria and travel support from Bencard, Novartis and ALK. The rest of the authors declare that they have no relevant conflicts of interest.

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List of abbreviations:

- VIA - venom-induced anaphylaxis
- BST - baseline Serum Tryptase
- EAI - epinephrine autoinjector
- MCAS - mast cell activation syndrome
- ER - emergency room

Abstract

Background: Venom-induced anaphylaxis is a common, potentially life-threatening hypersensitivity reaction associated with specific: 1) symptom profile, 2) cofactors, and 3) management. Identifying the differences in phenotypes of anaphylaxis is crucial for future management guidelines and the development of a personalized medicine approach.

Objective: This study aimed to evaluate the phenotype and risk factors of venom-induced anaphylaxis.

Methods: Using data from the European Anaphylaxis Registry (12874 cases) we identified 3612 patients with venom-induced anaphylaxis and analyzed these in comparison to sex- and age- matched anaphylaxis cases triggered by other elicitors (non-VIA n = 3605).

Results: Venom-induced anaphylaxis more frequently involved more than three organ systems and was associated with cardiovascular symptoms. The absence of skin symptoms during anaphylaxis correlated with baseline serum tryptase and was associated with an increased risk of a severe reaction. Intramuscular or intravenous epinephrine was administered significantly less often in venom-induced anaphylaxis, in particular in patients without prior history of anaphylaxis. Baseline serum tryptase within the upper normal range (8-11.5 ng-ml) was more frequently associated with severe anaphylaxis.

Conclusion: Using a large cohort of VIA cases, we have validated that patients with intermediate baseline serum tryptase levels (8 - 11 ng/ml) and without skin involvement have higher risk of severe VIA. Patients receiving beta-blockers or ACE-I had a higher risk of developing severe cardiovascular symptoms (including cardiac arrest) in VIA and non-VIA cases. Patients undergoing VIA received epinephrine less frequently than non-VIA cases.

Clinical Implications

Allergologists should educate patients about risk of future reactions, consider prescribing 2 epinephrine autoinjectors, and performing SIT in patients with baseline serum tryptase of above eight ng/ml and a history of insect venom anaphylaxis without skin involvement.

Capsule Summary

Venom-induced anaphylaxis significantly more often presented with cardiovascular symptoms. Severe cases more often showed lack of skin involvement and were associated with higher levels of baseline serum tryptase (in range from 8 - 11 ng/ml).

Keywords: anaphylaxis, epinephrine (adrenaline), beta-blockers, insect venom allergy, Hymenoptera

Introduction

Hypersensitivity to insect venom presents as a systemic reaction (anaphylaxis) in up to 0.3–7.5% of the adult population[1]. Venom-induced anaphylaxis (VIA) can be fatal, and patients sometimes require lifelong specific immunotherapy[2]. There is a need for more precise identification of biomarkers, and better definition of phenotypes of anaphylaxis[3]. Also, in order to facilitate a precision-medicine approach[4] for the diagnosis of anaphylaxis, a better understanding of its clinical phenotypes is required.

Anaphylaxis is a clinical diagnosis with a variety of triggering factors and clinical presentations. Symptom profiles and specific cofactors for venom-induced anaphylaxis (VIA) had previously been analyzed in an uncontrolled manner, albeit in relatively small cohorts[5–7].

Controlled clinical trials in anaphylaxis are difficult to conduct due to the acuteness of this life-threatening condition and its infrequent and random occurrence. Therefore registries, gathering clinical data from patients with a well-documented (recent) history of anaphylaxis are crucial in investigating this entity.

This study aimed to identify clinical patterns of VIA regarding symptoms, cofactors, and management by a case-control comparison with other types of anaphylaxis (non-VIA) based on the data from the European Anaphylaxis Registry.

Methods

We searched the European Anaphylaxis Registry[8] (status until March 2019) for anaphylaxis cases elicited by insect venom. The flowchart in Fig. 1A represents the detailed case-selection process.

The diagnosis of anaphylaxis was based on the definition by NIAID/FAAN[9] and the severity according to the Ring and Messmer Scale[10]. Reactions of grade II were considered mild and grades III and IV (presenting with significant hypoxia, hypotension, confusion, and loss of consciousness, or incontinence or cardiac arrest) were considered severe. Mastocytosis patients were defined as having a documented diagnosis of mastocytosis in medical history prior to the reaction. The Registry is designed for reporting cases of moderate to severe anaphylaxis (Ring and Messmer grades II-IV).

Due to a large number of documented reactions in the European Anaphylaxis Registry, we were able to match the VIA with non-VIA cases according to sex and age. When we analyzed a density plot of VIA cases according to age, we determined a bimodal distribution forming two subsets of patients with a cutoff age of 22 (Fig. 1B). Subsequently, we compared the management in both groups and matched the control group according to the severity of a reaction.

Cases were matched according to sex, age, and reaction severity in order to reduce the comparison bias by propensity score matching. Propensity score is a statistical approach to quantify the similarity between two unrelated cases. Propensity scores were calculated using the "MatchIt" package for R[11]. MatchIt uses logistic regression to reduce the bias due to multiple confounding variables (i.e. sex and age) by weighing them and choosing cases with minimal differences in both groups. The results of the propensity score matching are illustrated in Fig. 1B-D and eFig. 2.

The final database included 3612 cases of venom-induced anaphylaxis reported from allergy centers in 11 countries and sex- and age-matched control group. We compared the frequency of various symptoms, cofactors — known to increase the risk of severe anaphylaxis,[12], and management in both groups.

Based on the severity and symptom profile and the previous reports[6], we defined sub-elevated baseline serum tryptase (BST) values as 8 - 11.5 ng/ml (Fig. 3C-D).

We used the R Statistical Package[13] for statistical analysis. A simple comparison of categorical variables was performed using either the Chi^2 test or Fisher's exact test (where the number of observations in a bin was less than 10). Continuous variables were analyzed using the Mann-Whitney U test. In case of comparisons with two or more independent variables, we used Factorial ANOVA or Generalized Linear Models. We defined statistical significance as $\alpha = 0.05$. Data, along with the analysis script, can be accessed online at https://github.com/wolass/venomanaphylaxiscompendium.

We developed a Random Forest classifier (using the "randomForest" package for R[14]) in order to find therapeutic approaches that varied the most between VIA / non-VIA group and presented the results as Gini importance[15]. Moreover, association analysis of therapeutic interventions and symptoms was performed. The resulting phi values were scaled and presented in a heatmap with automatic clustering using Ward's Agglomerative Hierarchical Clustering with Euclidean distances[16].

Results

VIA is more frequently associated with cardiovascular symptoms

VIA displayed a specific symptom pattern. Patients, who underwent VIA, more often experienced cardiovascular symptoms (dizziness, hypotension, unconsciousness, reduced alertness) than patients with anaphylaxis due to other elicitors and less often presented with respiratory distress, rhinitis or diarrhea (Fig. 2A).

Although the pattern of organ involvement during anaphylaxis in both groups showed similarities in gastrointestinal, skin, and respiratory systems, VIA more frequently involved more than three organ systems (2356 (65.4%) vs. 2023 (56.1%), $p < 0.001$), and predominantly involved cardiovascular system (2984 (82.8%) vs. 2244 (62.2%) $p < 0.001$ Fig. 2B).

Younger patients (under 22) presented even more prominent differences in hypotension symptoms and significantly less frequently reported gastrointestinal symptoms (e.g., vomiting) when the reaction was triggered by insect venom (Fig. 2C-E).

Absence of skin symptoms during anaphylaxis is associated with more severe episodes of VIA

We found that 74 (54.4%) of patients with concomitant mastocytosis had anaphylaxis without skin symptoms (i.e., urticaria and flushing), which was significantly more frequent compared to patients without diagnosed mastocytosis (2031; 30.7%, $p < 0.001$). This finding was most prominently seen in VIA (Fig. 3A).

Similarly, in non-mastocytosis patients undergoing VIA, skin symptoms (i.e., urticaria or flushing) were less often present than if anaphylaxis was triggered by other elicitors (2356; 68% vs. 2495; 70.4% respectively, $p = 0.031$). Moreover, in this specific subgroup of patients (i.e., non-mastocytosis patients lacking skin symptoms) VIA was significantly more frequently severe (587; 52.9% in VIA vs. 498; 47.4%, $p < 0.001$, Fig. 3B).

By applying factorial logistic regression modeling (Table S1), we confirmed a significant interaction effect between the presence of skin symptoms and insect venom on the severity of anaphylaxis ($p < 0.001$). In other words, non-mastocytosis patients presenting without urticaria or flushing tended to have more severe anaphylaxis when triggered by insects. (Fig. 3B, and Tab. S1).

Absence of skin symptoms correlates with BST levels and increases the risk of severe anaphylaxis specifically in VIA

BST levels were significantly higher in patients with a prior diagnosis of mastocytosis (eFig. 7). We investigated the association of skin symptoms with the tryptase levels in non-mastocytosis patients. For this model, we excluded the cases with known mastocytosis and with BST above 11.5 ng/ml, potentially indicating non-diagnosed mast cell activation disorders. Similarly, 1) tryptase levels were higher in VIA patients, 2) correlated with the severity of anaphylaxis, and 3) this effect was significant in VIA ($p = 0.006$) but not in the non-VIA group (Fig. 3C-D).

BST over 8 ng/ml and concomitant cardiovascular conditions increase the risk of severe VIA

The cofactor most prominently associated with an increased risk of severe anaphylaxis was mastocytosis (Fig. 4). Concomitant mastocytosis increased the risk for 1) cardiac arrest and 2) loss of consciousness in patients undergoing VIA significantly more than in patients undergoing anaphylaxis due to other elicitors (Fig. 4C and eFig. 3A).

In line with the findings above, BST levels also correlated with the severity of anaphylaxis (on the Ring and Messmer scale) and, most importantly, sub-elevated BST was more

prominently associated with increasing the risk of severe anaphylaxis in VIA than in non-VIA (Fig. 2D and Fig. 4B).

Concomitant cardiovascular diseases were more prevalent in VIA than in non-VIA cases (892 (24.8%) vs. 657 (18.2%)) and were associated with higher risk of severe anaphylaxis when elicited by insects but were not relevant in non-VIA cases (Fig. 4). Interestingly, BST values were increased in patients with concomitant cardiovascular diseases, irrespectively of the reaction severity (eFig. 4).

Other cofactors of severe reactions

Severe reactions of VIA were more prevalent in patients above 22 years of age, and in VIA cases vs. non-VIA cases (eFig. 5). There were no differences in severity of reactions elicited by yellow-jackets and other insect species ($p = 0.4128$).

The effect of using ACE-I (as well as beta-blockers) on the risk of severe anaphylaxis correlated with coexisting cardiovascular diseases. ACE-I use was, however, more often associated with cardiac arrests in all anaphylaxis cases (30 (5.8%) vs. 118 (1.9%), $p < 0.001$) regardless of the elicitor (Fig. 4C). Beta-blocker use was associated with a higher severity of anaphylaxis and with the onset of cardiovascular symptoms (cardiac arrest, chest pain), but was comparable between both VIA and non-VIA, $p = 0.144$). Surprisingly, arrhythmia was more frequently reported in patients with VIA and concomitant beta-blockers (Fig. 4C).

IVA was more often severe if the reaction occurred in the first 10 minutes after exposure to venom (46.58% were severe cases) then when the reaction occurred after 10 minutes post exposure (39.75% were severe cases, $p = 0.001$).

One-third of VIA patients experience repeated reactions

940 (28.5%) of patients with insect allergy had experienced venom anaphylaxis in the past. If the reaction was elicited by other elicitors (i.e., non-VIA) — previous reactions were more frequently seen (1929; 35.7%, $p < 0.001$). We observed 227 patients with at least two fully-documented reactions. Out of these 59 (26%) had insect elicited anaphylaxis and in 6 of them (10.2%), the following reaction was more severe than before. In 43 (72.9%) cases, the reaction was similar in severity.

VIA patients receive epinephrine less often than non-VIA

We evaluated epinephrine use (administered by any route from patients themselves and medical professionals) in both ambulatory and emergency room settings.
Patients who underwent VIA significantly less often received epinephrine treatment than in other anaphylaxis cases (597; 26.9% vs. 738; 34.6%, $p < 0.001$). After adjusting both groups for similar severity - the difference in epinephrine use was still significant irrespective of the administration route ($p < 0.001$, Fig 5B).

A positive history of anaphylaxis influenced the therapy of a current episode as well. Epinephrine as a first-line treatment was given less often in VIA cases when compared to

other cases **if patients did not report a previous history of anaphylaxis** ($p < 0.001$), but in patients reporting previous reactions, there was no difference in epinephrine therapy ($p = 0.438$, Fig. 5B). Similarly, there were no differences in the epinephrine use between VIA and non-VIA when only severe reactions were taken into consideration ($p = 0.242$). However, when we restricted the analysis to moderate anaphylaxis cases — non-VIA patients received epinephrine more frequently than VIA ($p < 0.001$). The presence of skin symptoms during these mild reactions also was associated with a lower fraction of epinephrine treated patients (eFig. 6).

Patients with VIA received corticosteroids and antihistamines significantly more frequently than patients with anaphylaxis to other elicitors. On the other hand, epinephrine, beta-2 mimetics, and oxygen were given more often to patients suffering from non-VIA (Fig. 5A).

Next, we asked whether specific symptom clusters and treatment profiles could be identified within our cohort (association measured using phi coefficient). We found that patients displaying cardiovascular symptoms (cardiac arrest, hypotension, loss of consciousness) and urticaria were treated differently than patients with respiratory or gastrointestinal symptoms (Fig. 5C). The treatment of the former symptoms consisted of epinephrine autoinjector (EAI) use, i.v. epinephrine in multiple doses, 100% oxygen inhalation, an initial dose of antihistamines, and inhaled β-2 agonists. Corticosteroids, i.v. volume replacement, and i.v. β-2 agonists formed another therapy mode.

Discussion

In this study, we identified distinct symptom-profile and treatment patterns of venom-induced anaphylaxis. The data unraveled phenotypes of VIA, which may support the development of tools incorporating clinical data for predicting the severity of future episodes of anaphylaxis.

VIA was more often associated with cardiovascular symptoms than non-VIA. Previous studies suggest an essential link between the cardiovascular system and insect sting hypersensitivity[7,12,17]. VIA has been associated with Kounis syndrome (coronary arterial spasm induced by the release of mast cell mediators[18,19]) and cardiac arrhythmias usually occurring in patients with preexisting heart disease[20].

The rate of concomitant cardiovascular diseases was higher in VIA than non-VIA. They are an essential cofactor increasing the risk of a severe reaction if Hymenoptera elicited the anaphylaxis. This association was not significant in anaphylaxis elicited by other elicitors. Notably, cardiac arrest occurred more frequently in patients with elevated BST (> 8 ng/ml), especially in VIA. Nevertheless, the pathomechanism promoting cardiovascular symptoms in VIA requires further investigation.

As cardiovascular symptoms like hypotension, collapse, or cardiac arrest lead to a higher grade on the Ring and Messmer scale than skin or gastrointestinal symptoms, VIA (being associated with cardiovascular symptoms) is likely to be associated with more severe anaphylaxis.

Importantly, the absence of skin symptoms was associated with more severe VIA, which was still present after excluding patients with a known diagnosis of mastocytosis (although in non-mastocytosis cases the difference between groups was small and the clinical relevance of this needs cautious evaluation). Previous studies also observed this phenomenon[21,22]. Subsequently, the correlation of BST levels with the severity of anaphylaxis lead us to identify an interaction between the absence of skin symptoms and VIA using generalized linear regression.

Our findings indicate that patients with BST above 8 ng/ml are prone to severe anaphylaxis to insect venom. Patients with normal BST in the range of 8-11.4 ng/ml may have indolent systemic mastocytosis or concomitant undiagnosed mast cell activation syndrome (MCAS)[23]. Zanotti et al. identified mast cell disorders in 17 out of 22 patients with VIA lacking skin symptoms and concluded that patients with BST above 7.95 ng/ml and VIA should undergo extensive diagnostic procedures[24]. We recently identified that elderly patient undergoing anaphylaxis without concomitant skin symptoms tended to have more severe reactions[25]. Our finding are in concordance with a recent retrospective study from Fehr et al.[22] who identified lack of skin symptoms as a risk factor for severe VIA.

Based on these and previous findings[6,24,26] we propose to perform a peripheral blood KIT D816V mutation test in cases of BST of above 8 ng/ml and with a history of anaphylaxis presenting without urticaria or flushing. Previous studies showed 92% sensitivity of this test in patients with hymenoptera anaphylaxis, presenting without skin symptoms and with tryptase under 20 ng/ml[27].

Age is an important risk factor for severe anaphylaxis[28]. Adult patients experienced VIA more frequently. Young patients mainly suffer from food-induced anaphylaxis[8]. Emergency room (ER) admission data indicate that the frequency of insect stings hypersensitivity reactions in children is comparable to food hypersensitivity reactions (12-15% of cases of hypersensitivity reactions admitted to the ER), but pediatric anaphylaxis is triggered significantly more often by food elicitors (56% of food hypersensitivity cases vs. 5.3% of sting cases seen in the ER)[29]. Senior patients, on the other hand, suffer from drug-related hypersensitivity more often than insect sting hypersensitivity[25]. Similarly, we observed less VIA in patients with concomitant atopic diseases (eFig. 3) , as these patients more often present with food anaphylaxis[30].

The role of cardiovascular medication cannot be isolated from the effect of concomitant cardiovascular conditions; therefore, we cannot state whether ACE-I and beta-blockers increase the severity of anaphylaxis. However, we did observe that there were no significant differences between VIA and non-VIA cases regarding the symptoms and severity of an episode with concomitant use of ACE-I or beta-blockers.

Cases of VIA had been treated with epinephrine less often than the age- sex- and severity-matched cases of non-VIA. Moreover, the administration of epinephrine did not depend on the trigger if the patient experienced anaphylaxis previously, but was significantly less often used if the patients experienced their first episode of VIA (in comparison to non-VIA). The difference between groups was prominent for milder cases of anaphylaxis. The reason for this observation is unclear. One explanation could be that emergency team more often

attributed the VIA symptoms to anxiety, whereas in non-VIA, they were more often suspecting anaphylaxis. A second possibility could be that many physicians fail to diagnose anaphylaxis when no skin symptoms are present. To our knowledge, this is the only data on the comparative epinephrine usage in a case-controlled group of VIA vs. non-VIA.

Nevertheless, international guidelines of anaphylaxis state that epinephrine (i.m.) is the first-line agent in all diagnosed cases of anaphylaxis[31]. Clinicians should not underestimate the less severe VIA cases and treat them with epinephrine accordingly.

Although there are no absolute contraindications for using epinephrine in anaphylaxis, one potential scenario where clinicians tend to be reluctant to using epinephrine is a hypersensitivity reaction presenting with high blood pressure and tachycardia, which may be present at the initial phase of VIA. Nevertheless, the three exceptionally well documented cases of anaphylaxis upon sting challenge showed that the initial transient increase in blood pressure should not be interpreted as a contraindication to epinephrine and it could be safely given even if the heart rate was above 120 beats per minute[32].

IVA patients had a documented history of anaphylaxis in 28% of the cases, and systemic immunotherapy has not been initiated in these patients, what is against latest management guidelines, although this fraction may be slowly decreasing it is of utmost importance to recommend SIT to all patients who experienced VIA.

Based on our findings, insects are the most probable elicitor of anaphylaxis in Europe during summer-season, with VIA cases extending from early spring to the end of autumn (eFig. 1). Detailed information on the seasonality of insect-elicited hypersensitivity reactions is scarce[33]. The activity of *Vespula germanica* depends on the climate, and in invaded regions (e.i. Australia), it can even extend throughout the year[34]. The changing climate in Europe may influence the activity of Hymenoptera in this region in the upcoming years. However, in the period from 2007 - 2019, the perennial ratio of VIA to non-VIA cases has remained unchanged (data not shown).

Limitations

Due to the design of the European Anaphylaxis Registry, our analysis was restricted only to cases of anaphylaxis. Milder hypersensitivity reactions, as well as healthy controls, are not included in the database. Although The European Anaphylaxis Registry is ideal for investigating anaphylaxis phenotypes - it might give an incomplete perception of the populational distribution of hypersensitivity reactions and restricts us to only comparing various forms of anaphylaxis.

Nevertheless, because the European Anaphylaxis Registry has until now gathered over 12,000 cases of anaphylaxis - it was possible to perform a case-controlled analysis on a relatively large number of cases and investigate many aspects of VIA. It is worth underlining the important function of international registries, especially in diseases where targeted studies are not possible.

Conclusion

Based on our results, VIA is a distinctive phenotype of anaphylaxis, with a specific symptom profile and risk factors. Using a large cohort of VIA cases compared to sex and age matched non-VIA cases, we have validated that patients with intermediate baseline serum tryptase levels (8 - 11 ng/ml) and without skin involvement have higher risk of severe VIA. Similarly, patients receiving beta-blockers or ACE-I had higher risk of developing severe cardiovascular symptoms (including cardiac arrest) in VIA and non-VIA cases. Patients undergoing VIA received epinephrine less frequently than non-VIA cases.

VIA cases should undergo therapy according to the international management guidelines, and epinephrine should be given more often in VIA. All cases should undergo appropriate allergological testing and indication for SIT should be evaluated along with patient education regarding the risk of future anaphylaxis. Patients with BST above 8 ng/ml should undergo extensive diagnostic tests to exclude indolent systemic mastocytosis or MCAS and should be provided with two EAIs for acute self-management.

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444

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555

Figure legends

557

558 *Figure 1: A) Flow-diagram illustrating the rationale for case inclusion and exclusion from the*
559 *final analysis. B, C, D: Age, sex, and severity distribution was matched in cases in both groups*
560 *to allow for comparable results between VIA and non-VIA cases. Two age-subsets of patients*
561 *could be recognized based on the density plot of age (B).*

562

563 *Figure 2: Symptoms of venom-induced anaphylaxis (VIA) compared to other elicitors. A:*
564 *Proportional presentation of specific reaction symptoms in VIA and non-VIA according to*
565 *cardiovascular (cardio.), gastroenterologic (gastro.), and respiratory (resp.) organ systems. B:*
566 *High-level overview of involved organ systems and selected cofactors in the form of a radar*
567 *plot. C: difference in symptoms of VIA among patients under 22 and over 22 years of age. **
568 *denotes significant differences between groups.*

569

570 *Figure 3: Lack of skin symptoms (i.e., urticaria and flushing) during anaphylaxis is associated*
571 *with more severe VIA. A: lack of skin symptoms and mastocytosis in VIA and non-VIA cases. B:*
572 *Lack of skin symptoms, according to the severity in both anaphylaxis groups. C: Relation of*
573 *reaction severity according to the elicitor and the absence of skin symptoms concerning*
574 *categorized BST values. D: Continous values of BST according to the severity in both non-VIA*
575 *and VIA with subgrouping to skin symptoms.*

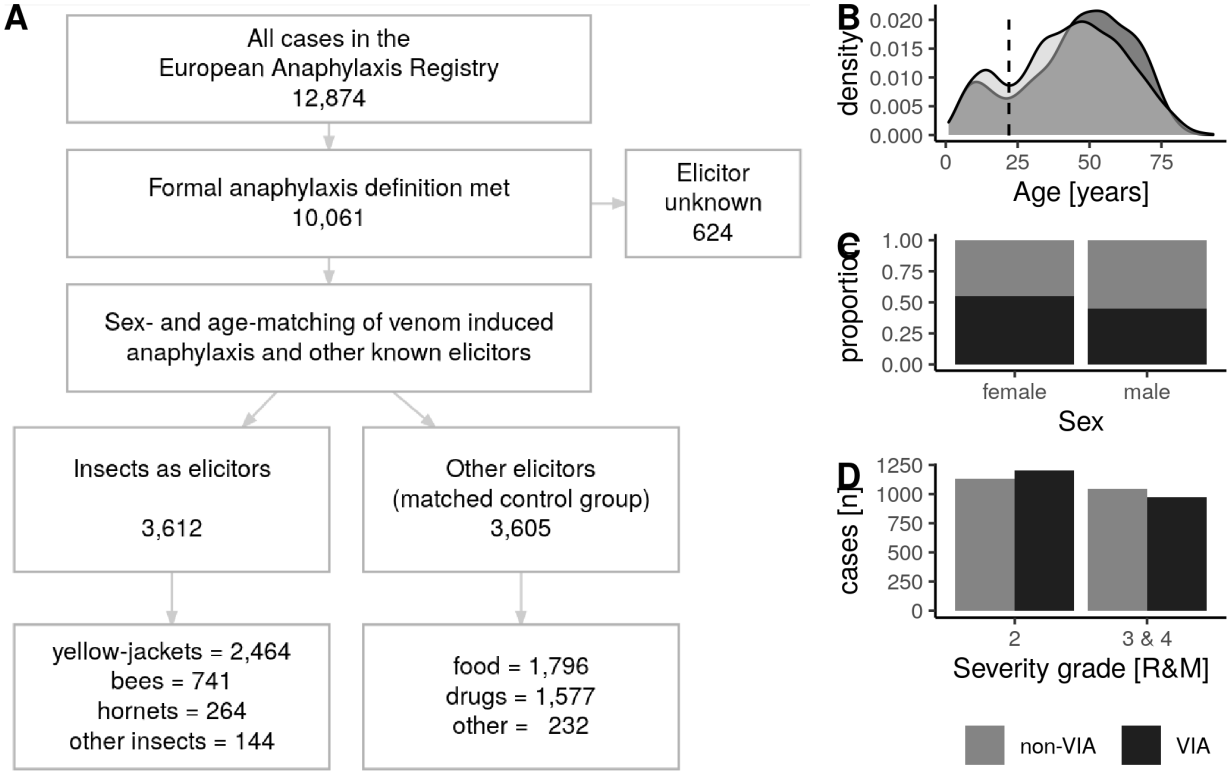
576

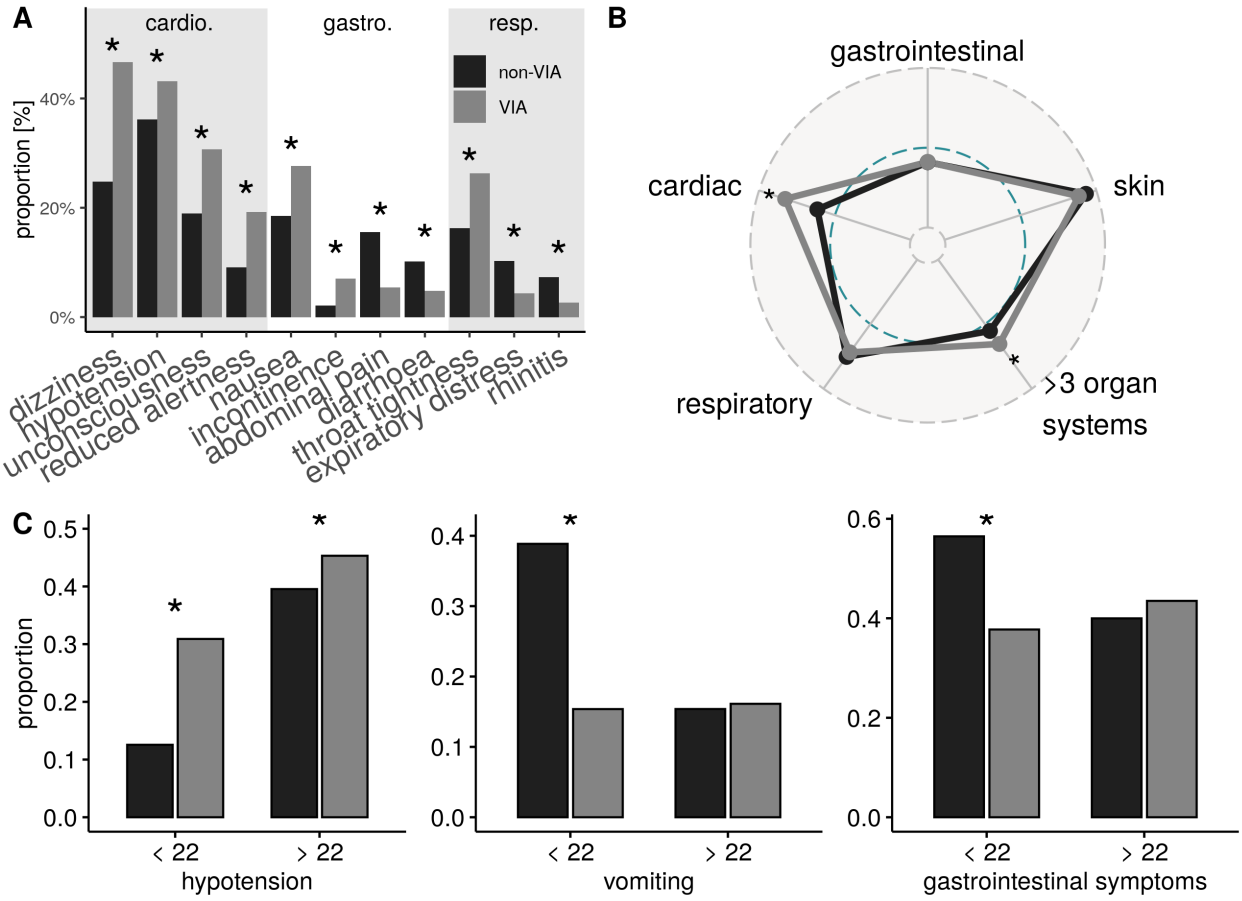
577 *Figure 4: Cofactors of insect venom anaphylaxis. A: Odds ratios of eliciting severe anaphylaxis.*
578 *B: Proportion of cases elicited by insects or other elicitors (upper panels) according to*
579 *tryptase levels and cardiovascular symptoms.*

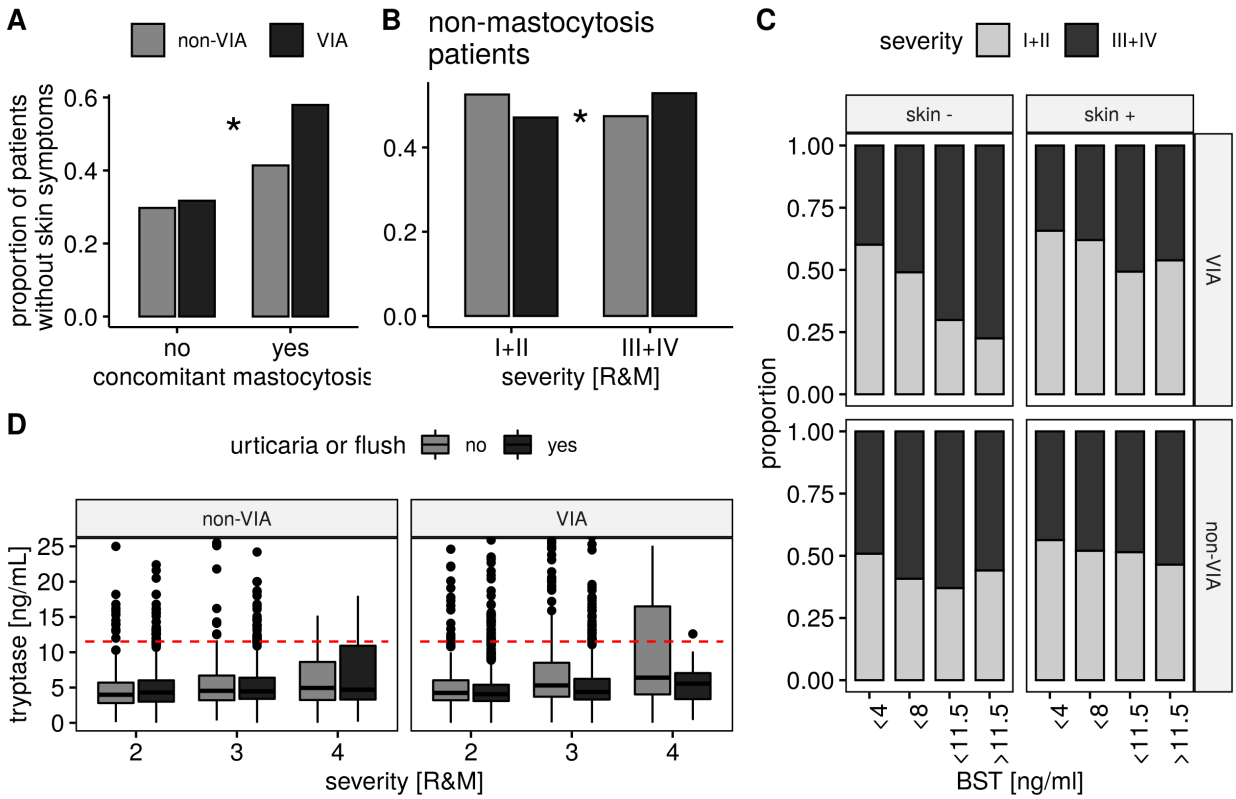
580

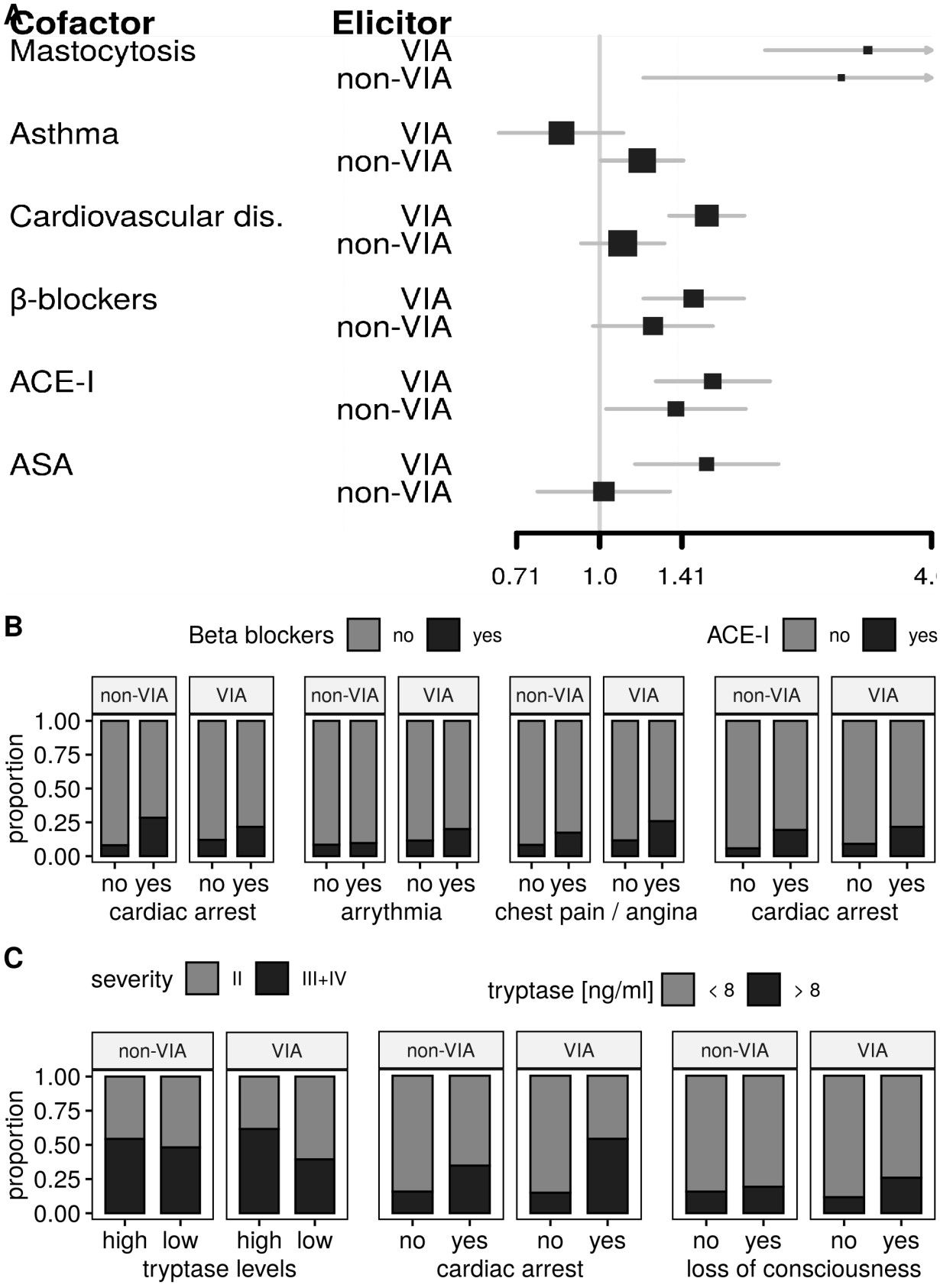
581 *Figure 5: Therapy in patients with VIA compared to other elicitors, cases matched according*
582 *to sex, age, and severity of a reaction. A: Proportional use of therapy measures in both*
583 *anaphylaxis groups. B: C: Heatmap visualizing the association of symptoms and*
584 *corresponding treatment - presented as a scaled correlation coefficient (phi). * - p-value <*
585 *0.05 after false discovery rate correction.*

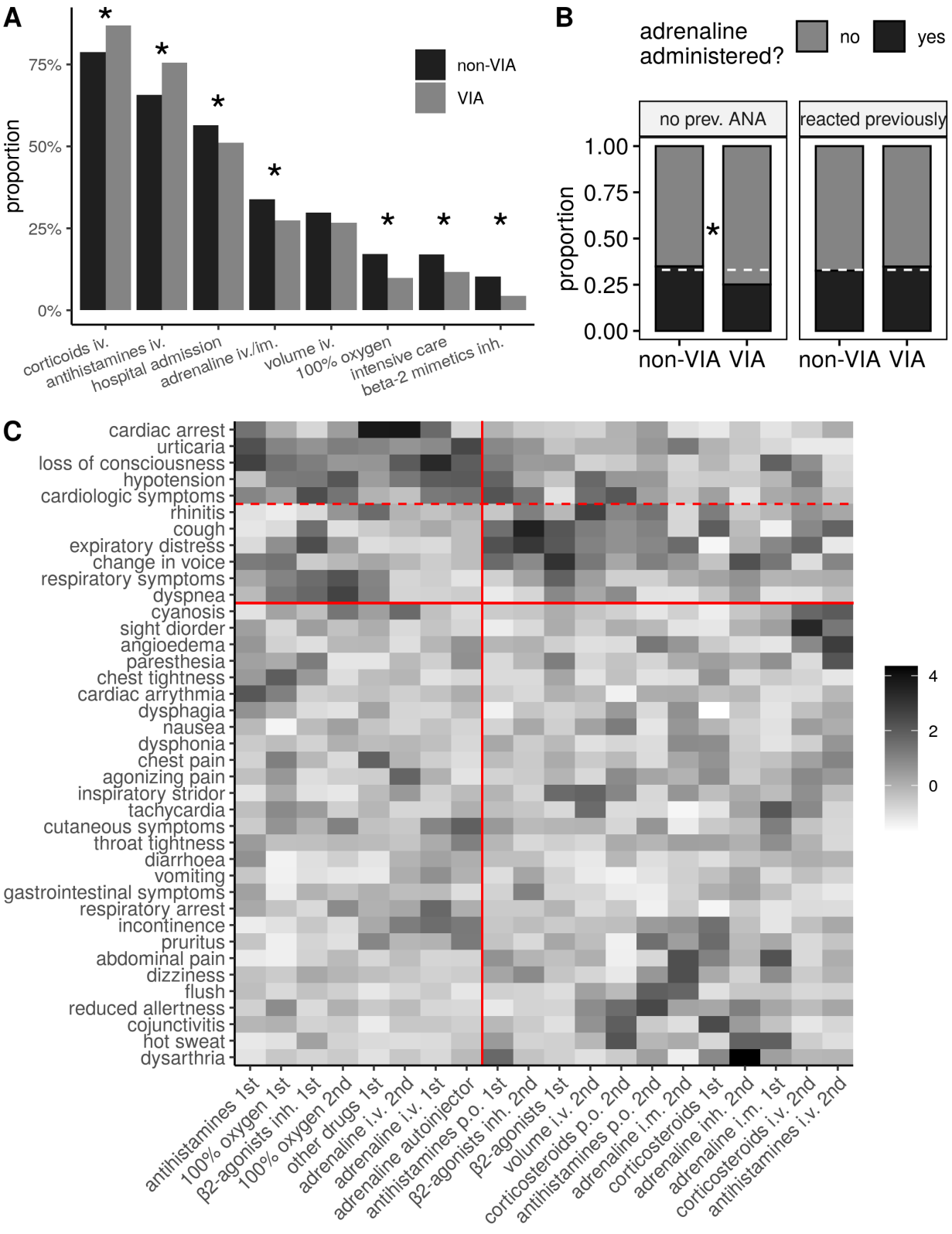
586

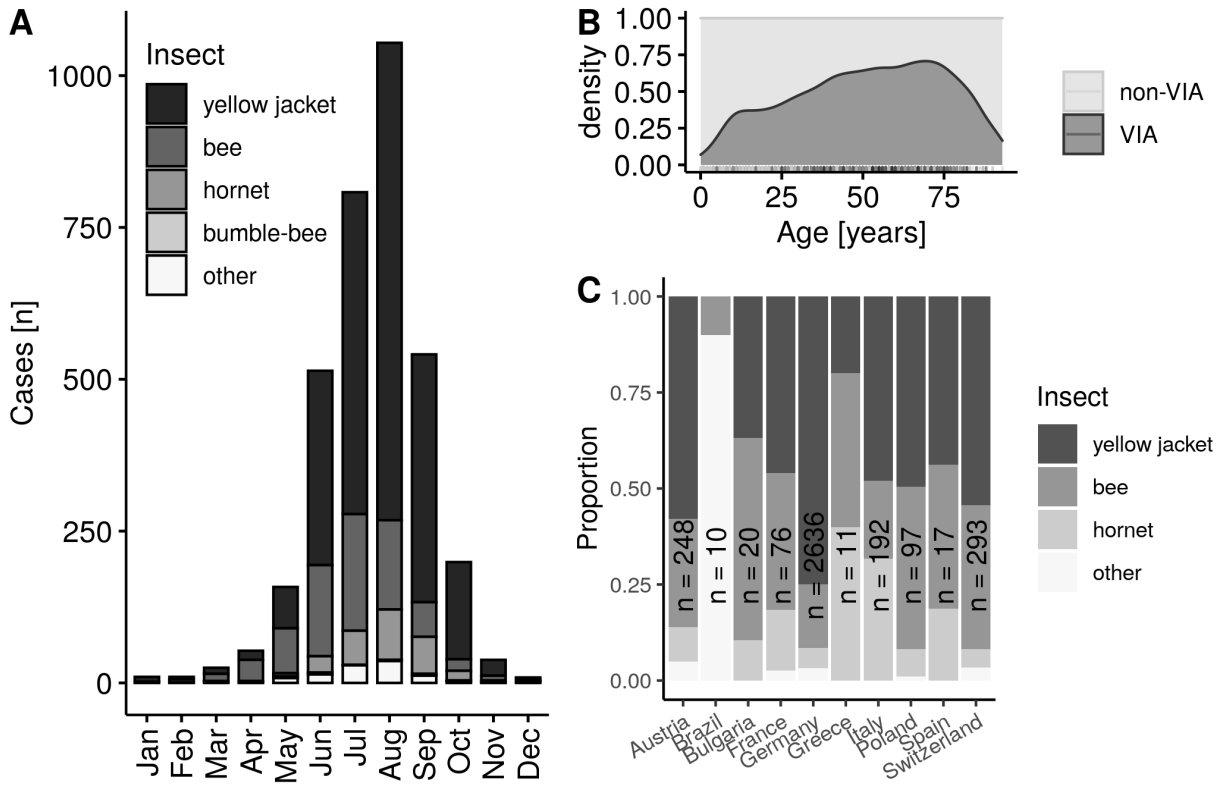


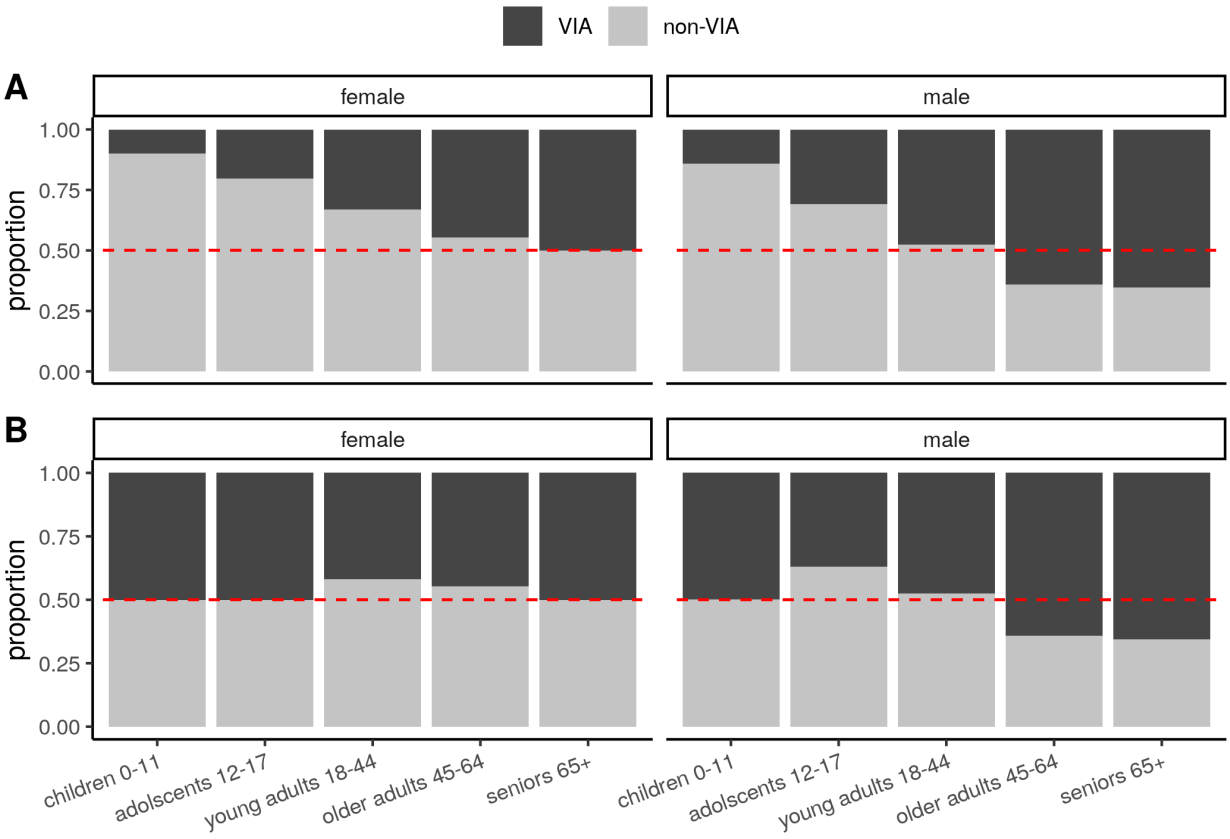

A
cardio.
gastro.
resp.
non-VIA
VIA
proportion [%]
40%
20%
0%
dizziness
hypotension
unconsciousness
reduced alertness
nausea
incontinence
abdominal pain
diarrhoea
throat tightness
expiratory distress
rhinitis
B
gastrointestinal
cardiac
skin
respiratory
>3 organ systems
C
proportion
0.5
0.4
0.3
0.2
0.1
0.0
< 22
> 22
hypotension
vomiting
0.6
gastrointestinal symptoms

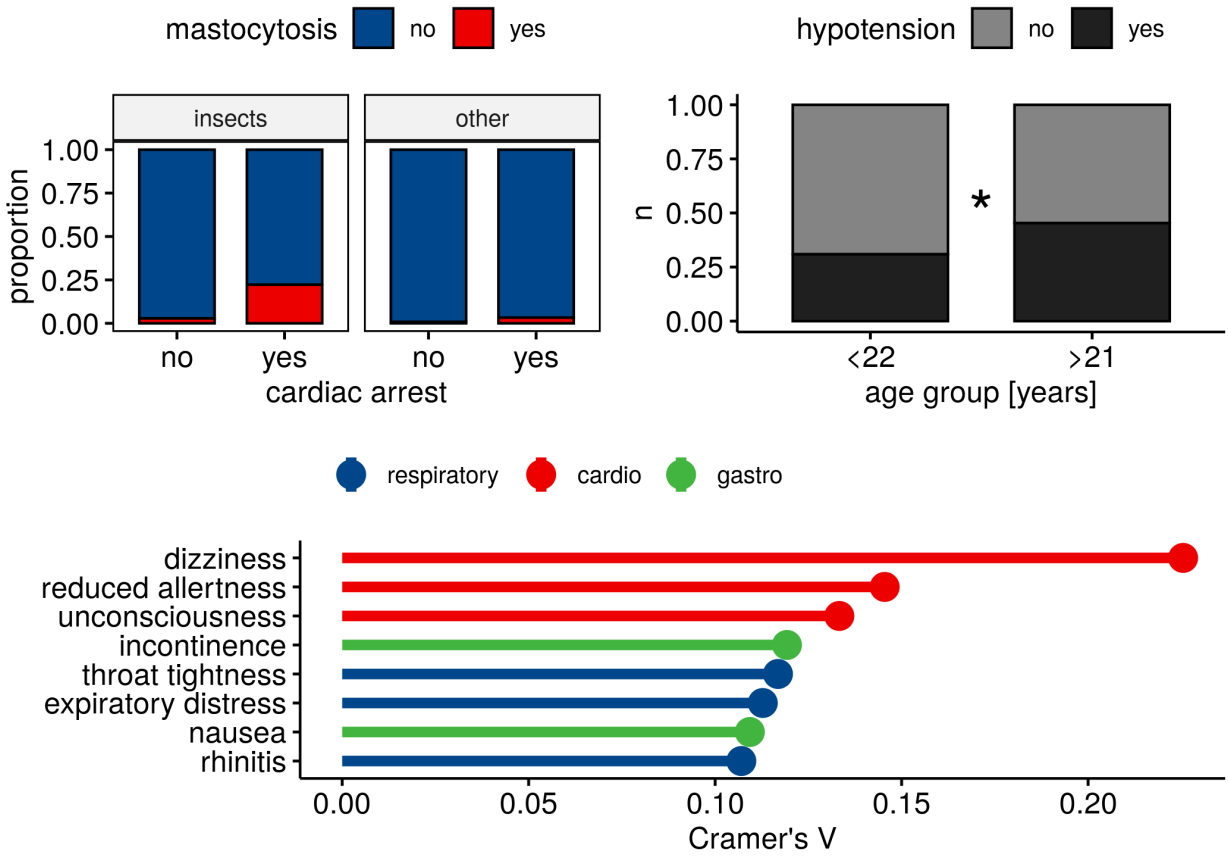



A
Cofactor
Elicitor
Mastocytosis
VIA
non-VIA
Asthma
VIA
non-VIA
Cardiovascular dis.
VIA
non-VIA
β-blockers
VIA
non-VIA
ACE-I
VIA
non-VIA
ASA
VIA
non-VIA
0.71
1.0
1.41
4.
B
Beta blockers
no
yes
ACE-I
no
yes
non-VIA
VIA
proportion
1.00
0.75
0.50
0.25
0.00
no yes
cardiac arrest
arrythmia
chest pain / angina
C
severity
II
III+IV
tryptase [ng/ml]
< 8
> 8
high low
tryptase levels
cardiac arrest
loss of consciousness

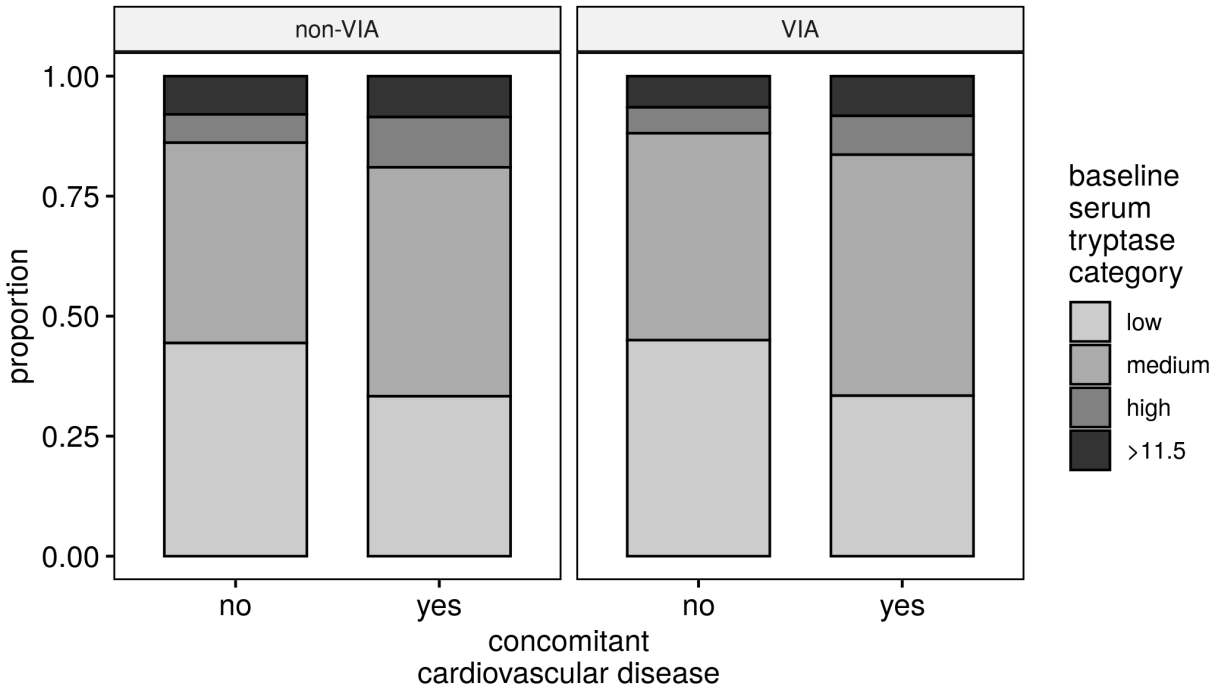

A
proportion
75%
50%
25%
0%
non-VIA
VIA
corticoids iv.
antihistamines iv.
hospital admission
adrenaline iv./im.
volume iv.
100% oxygen
intensive care
beta-2 mimetics inh.
B
adrenaline administered?
no
yes
no prev. ANA
reacted previously
proportion
1.00
0.75
0.50
0.25
0.00
non-VIA VIA
non-VIA VIA
C
cardiac arrest
urticaria
loss of consciousness
hypotension
cardiologic symptoms
rhinitis
cough
expiratory distress
change in voice
respiratory symptoms
dyspnea
cyanosis
sight diorder
angioedema
paresthesia
chest tightness
cardiac arrythmia
dysphagia
nausea
dysphonia
chest pain
agonizing pain
inspiratory stridor
tachycardia
cutaneous symptoms
throat tightness
diarrhoea
vomiting
gastrointestinal symptoms
respiratory arrest
incontinence
pruritus
abdominal pain
dizziness
flush
reduced allertness
cojunctivitis
hot sweat
dysarthria
antihistamines 1st
100% oxygen 1st
β2-agonists inh. 1st
100% oxygen 2nd
other drugs 1st
adrenaline i.v. 2nd
adrenaline i.v. 1st
adrenaline autoinjector
antihistamines p.o. 1st
β2-agonists inh. 2nd
β2-agonists 1st
volume i.v. 2nd
corticosteroids p.o. 2nd
antihistamines p.o. 2nd
adrenaline i.m. 2nd
corticosteroids 1st
adrenaline inh. 2nd
adrenaline i.m. 1st
corticosteroids i.v. 2nd
antihistamines i.v. 2nd
4
2
0

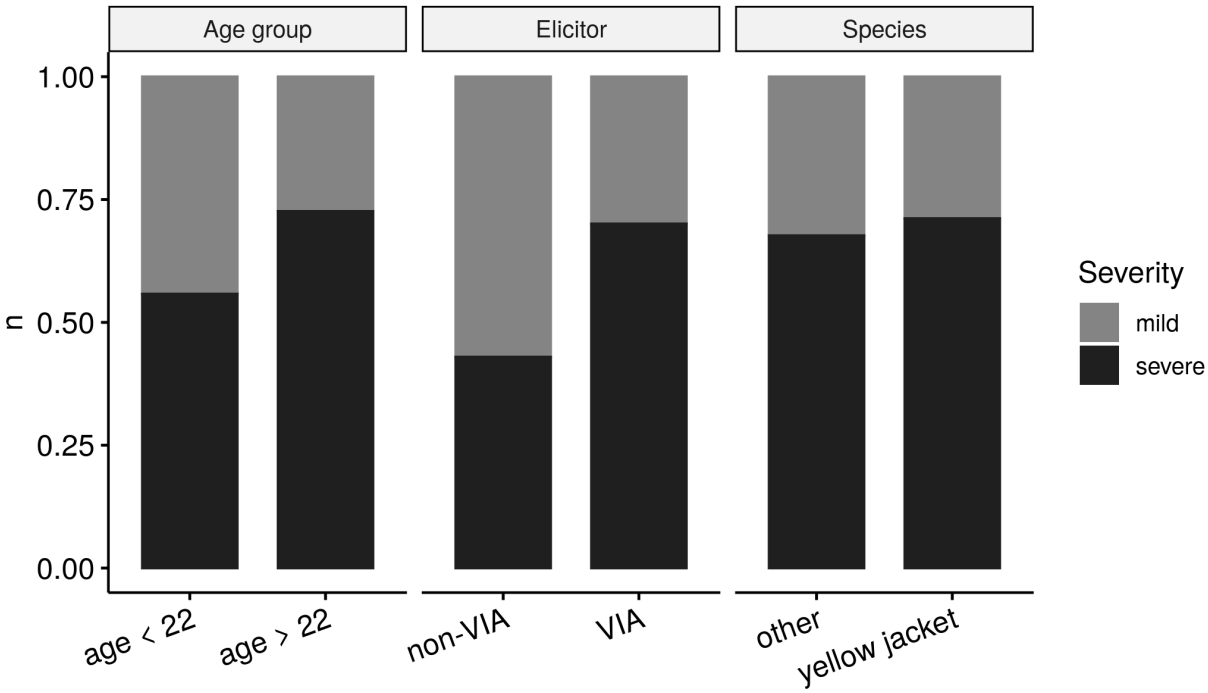


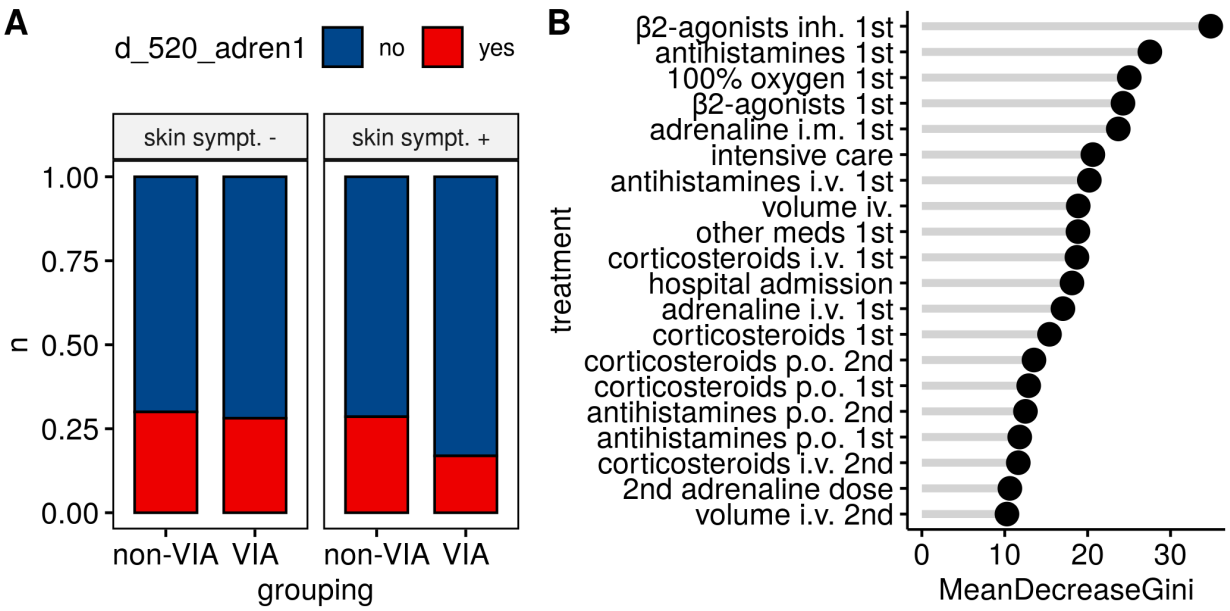

VIA
non-VIA
A
female
male
proportion
1.00
0.75
0.50
0.25
0.00
B
female
male
proportion
1.00
0.75
0.50
0.25
0.00
children 0-11
adolscents 12-17
young adults 18-44
older adults 45-64
seniors 65+
children 0-11
adolscents 12-17
young adults 18-44
older adults 45-64
seniors 65+

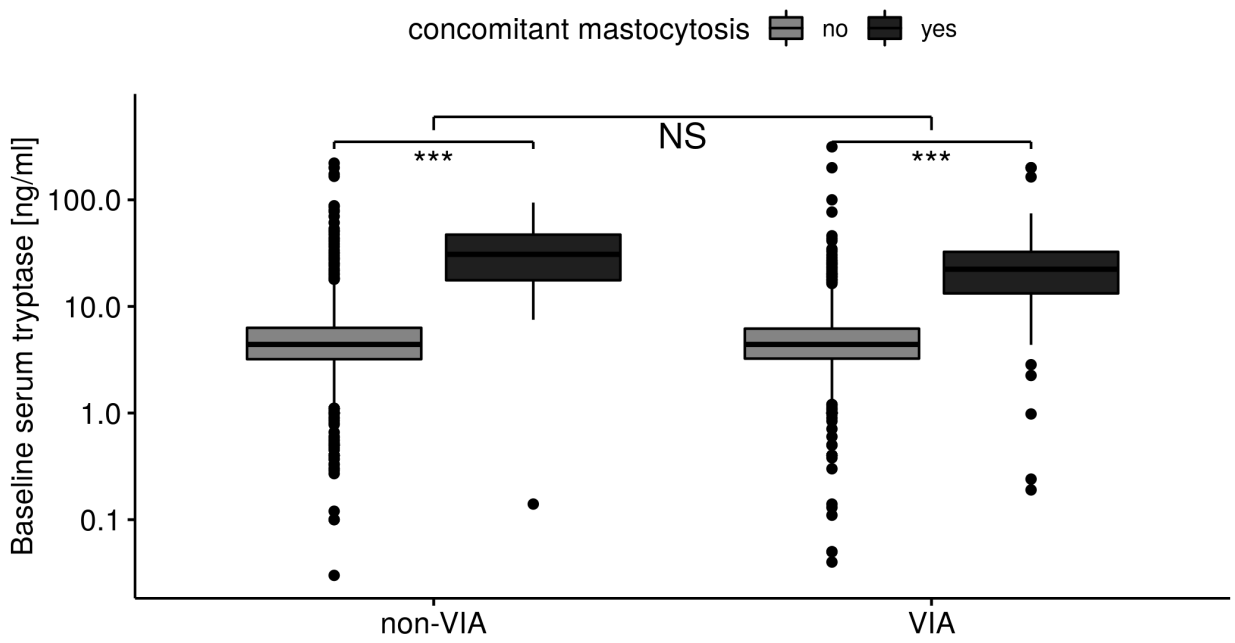

mastocytosis
no
yes
insects
other
proportion
1.00
0.75
0.50
0.25
0.00
no
yes
no
yes
cardiac arrest
hypotension
no
yes
n
1.00
0.75
0.50
0.25
0.00
*
<22
>21
age group [years]

respiratory
cardio
gastro
dizziness
reduced allertness
unconsciousness
incontinence
throat tightness
expiratory distress
nausea
rhinitis
0.00
0.05
0.10
0.15
0.20
Cramer's V








concomitant mastocytosis
no
yes
NS
***
***
Baseline serum tryptase [ng/ml]
100.0
10.0
1.0
0.1
non-VIA
VIA

Supplementary Figures (online-only material)

Insect venom anaphylaxis is a seasonal disease.

VIA in contrast to other elicitors showed a significant seasonal fluctuation and was most frequently reported from May to October. The proportion of VIA to anaphylaxis cases elicited by other elicitors during the summer seasons reached 60% and was below 1% of cases during winter. Nevertheless, 116 cases of VIA (bee – *Apis mellifera* in spring; yellow jacket – *Vespula spp.* in autumn) were triggered in March, April, and November. Yellow-jacket was the most prominent VIA-causing insect followed by bees. The VIA-causing insects differed in European countries with hornets (*Vespa crabro*) being more prominent in southern Europe.

eFig. 1: A: Proportion of anaphylaxis cases elicited by specific insects according to the month in which the reaction occurred. Less common insect species grouped as 'other'. B: The density distribution of VIA cases to cases elicited by other elicitors considering the patient's age. C: Geographical differences in the most common elicitors of VIA. Countries which reported less than 10 VIA cases were not illustrated in this figure. Fire ants and insects that could not be identified formed the 'other' group.

eFig. 2: Results of matching the cohort according to sex and age in order to perform a case-controlled study. A: The original distribution of VIA and non-VIA cases according to age group and sex. Please note the uneven distribution of VIA and non-VIA cases in age groups. B: The distribution of VIA and non-VIA after age and sex matching with the use of MatchIt package for R. Please notice how the ratio of VIA to non-VIA cases is approaching 50% indicating balanced matching according to sex and age variables.

eFig. 3: Symptoms of anaphylaxis. A: The association between cardiac arrest and concomitant mastocytosis in VIA and non-VIA. B: Hypotension frequency in two age groups of anaphylaxis. C: Crammer's V as the measure of association between groups anaphylaxis (VIA vs. non-VIA). Higher values indicate stronger association with IVA.

eFig. 4: Tryptase levels in patients with concomitant cardiovascular diseases. Low < 4 ng/ml, medium 4-8 ng/ml, high 8-11.5 ng/ml.

eFig. 5: Severity of anaphylaxis in subgroups. The severity of patients with VIA in two age groups (left), according to elicitor type (center) and according to the responsible insect species (right)

eFig. 6: Therapy of anaphylaxis. A: Patients who presented with skin symptoms and VIA less often received epinephrine than if skin symptoms were absent during the reaction. B: Variable importance in the unsupervised classification between VIA and non-VIA using Random Forest classifier.

eFig. 7: Levels of baseline serum tryptase in patinent with VIA and non-VIA. Significant difference in BST between patients with concomitant mastocytosis and other patients (***). There was no significant difference between anaphylaxis elicited by insects and other elicitors (NS). Tested by two way ANOVA.

Table S1: The results of a factorial logistic regression. Regression coefficients.

	Dependent variable:
	Severity [R & M II vs III-IV]
Non-VIA	-0.234***
	(0.087)
Skin symptoms	-0.627***
	(0.074)
Interaction of elicitor and skin symptoms	0.585***
	(0.105)
Constant	0.123**
	(0.060)
Observations	6,883
Log Likelihood	-4,688.151
Akaike Inf. Crit.	9,384.303
Note:	***p<0.01