

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

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

Objective: This study aimed to evaluate the phenotype and risk factors of VIA.

Methods: Using data from the European Anaphylaxis Registry (12,874 cases), we identified 3,612 patients with VIA and analyzed their cases in comparison with sex- and age-matched anaphylaxis cases triggered by other elicitors (non-VIA cases [n = 3,605]).

Results: VIA more frequently involved more than 3 organ systems and was associated with cardiovascular symptoms. The absence of skin symptoms during anaphylaxis was correlated with baseline serum tryptase level and was associated with an increased risk of a severe reaction. Intramuscular or intravenous epinephrine was administered significantly less often in VIA, in particular, in patients without a history of anaphylaxis. A baseline serum tryptase level 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 a higher risk of severe VIA. Patients receiving β -blockers or angiotensin-converting enzyme inhibitors had a higher risk of developing severe cardiovascular symptoms (including cardiac arrest) in VIA and non-VIA cases. Patients experiencing VIA received epinephrine less frequently than did cases with non-VIA. (J Allergy Clin Immunol 2020;■■■:■■■–■■■.)

Key words: Anaphylaxis, epinephrine (adrenaline), β -blockers, insect venom allergy, Hymenoptera

Hypersensitivity to insect venom presents as a systemic reaction (anaphylaxis) in up to 0.3% to 7.5% of the adult population.¹ Venom-induced anaphylaxis (VIA) can be fatal, and patients sometimes require lifelong specific immunotherapy

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Abbreviations used

ACEI: Angiotensin-converting enzyme inhibitor
 BST: Baseline serum tryptase
 ER: Emergency room
 SIT: Specific immunotherapy
 VIA: Venom-induced anaphylaxis

(SIT).² There is a need for more precise identification of biomarkers and better definition of phenotypes of anaphylaxis.³ Also, to facilitate a precision medicine approach⁴ 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 VIA have previously been analyzed in an uncontrolled manner and in relatively small cohorts.⁵⁻⁷

Controlled clinical trials in anaphylaxis are difficult to conduct owing 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) on the basis of data from the European Anaphylaxis Registry.

METHODS

We searched the European Anaphylaxis Registry⁸ (status until March 2019) for anaphylaxis cases elicited by insect venom. The flowchart in Fig 1, A represents the detailed case selection process.

The diagnosis of anaphylaxis was based on the definition by the National Institute of Allergy and Infectious Diseases/Fellows of the American Academy of Nursing⁹ and the severity according to the Ring and Messmer scale.¹⁰ Grade II reactions were considered mild, and grade III and IV reactions (presenting with significant hypoxia, hypotension, confusion, and loss of consciousness, or incontinence or cardiac arrest) were considered severe. Patients with mastocytosis were defined as having a documented diagnosis of mastocytosis in their medical history before the reaction. The registry is designed for reporting cases of moderate-to-severe anaphylaxis (Ring and Messmer grades II-IV).

Thanks to the large number of documented reactions in the European Anaphylaxis Registry, we were able to match the cases of VIA with non-VIA cases according to sex and age. When we analyzed a density plot of VIA cases according to patient age, we determined a bimodal distribution forming 2 subsets of patients with a cutoff age of 22 years (Fig 1, B). 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 to reduce the comparison bias by propensity score matching. Propensity scoring is a statistical approach to quantify the similarity between 2 unrelated cases. Propensity scores were calculated by using the MatchIt package for R software.¹¹ MatchIt uses logistic regression to reduce the bias due to multiple confounding variables (ie, 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 1, B-D and Fig E1 (in this article's Online Repository at www.jacionline.org).

The final database included 3612 cases of VIA reported from allergy centers in 11 countries and a sex- and age-matched control group. We compared the frequency of various symptoms—cofactors known to increase the risk of severe anaphylaxis¹²—and management in both groups.

On the basis of the severity and symptom profile and the previous reports,⁶ we defined subelevated baseline serum tryptase (BST) values as those between 8 and 11.5 ng/mL.

We used the R statistical package¹³ for statistical analysis. A simple comparison of categorical variables was performed by using either the chi-square test or Fisher exact test (if there were <10 observations in a bin). Continuous variables were analyzed by using the Mann-Whitney *U* test. In the case of comparisons with 2 or more independent variables, we used factorial ANOVA or generalized linear models. We defined statistical significance as $\alpha = 0.05$. The 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 R¹⁴) to find those therapeutic approaches that varied the most between the VIA and non-VIA groups and presented the results as Gini importance.¹⁵ Moreover, association analysis of therapeutic interventions and symptoms was performed. The resulting ϕ values were scaled and presented in a heatmap with automatic clustering by using Ward 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, and reduced alertness) than did patients with anaphylaxis due to other elicitors, and they presented less often with respiratory distress, rhinitis, or diarrhea (Fig 2, A).

Although the pattern of organ involvement during anaphylaxis in both groups showed similarities in the gastrointestinal, skin, and respiratory systems, VIA more frequently involved more than 3 organ systems (2356 cases [65.4%] vs 2023 cases [56.1%]) ($P < .001$), and it predominantly involved the cardiovascular system (2984 cases [82.8%] vs 2244 cases [62.2%]) ($P < .001$) (Fig 2, B).

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

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

We found that 74 patients with concomitant mastocytosis (54.4%) had anaphylaxis without skin symptoms (ie, urticaria and flushing), which was significantly more frequent than in patients without diagnosed mastocytosis (2031 cases [30.7%]) ($P < .001$). This finding was seen most prominently in VIA (Fig 3, A).

Similarly, in patients without mastocytosis who were experiencing VIA, skin symptoms (ie, urticaria or flushing) were present less often than if anaphylaxis was triggered by other elicitors (2356 cases [68%] vs 2495 cases [70.4%], respectively) ($P = .031$). Moreover, in this specific subgroup of patients (ie, patients without mastocytosis who lack skin symptoms) VIA was significantly more frequently severe (587 cases [52.9% in VIA] vs 498 cases [47.4%]) ($P < .001$) (Fig 3, B).

By applying factorial logistic regression modeling (see Table E1 in this article's Online Repository at www.jacionline.org), we confirmed a significant interaction effect of the presence of skin symptoms and insect venom on the severity of anaphylaxis ($P < .001$). In other words, patients without mastocytosis who presented without urticaria or flushing tended to have more severe

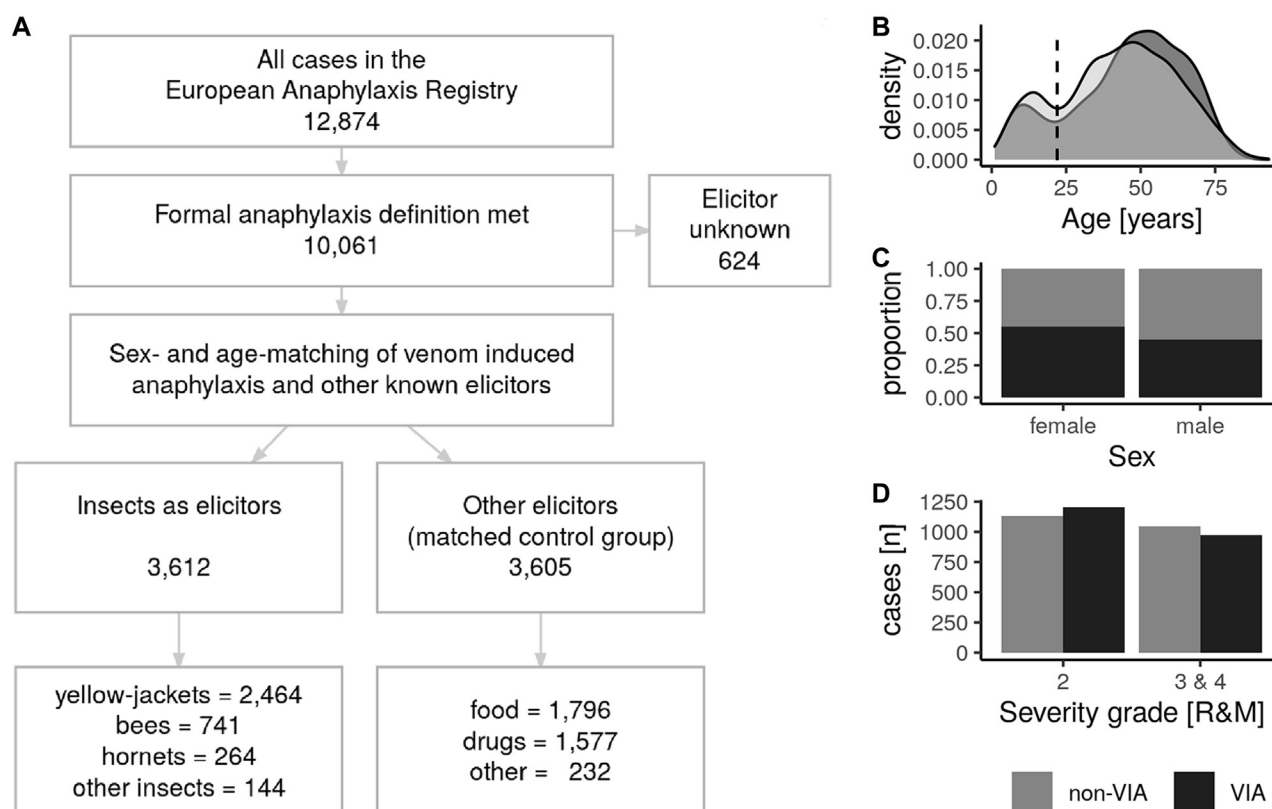


FIG 1. A, Flow diagram illustrating the rationale for case inclusion and exclusion from the final analysis. B-D, Age, sex, and severity distribution was matched in cases in both groups to allow for comparable results between VIA and non-VIA cases. Two age subsets of patients could be recognized on the basis of the density plot of age (B). R&M, Ring and Messmer.

anaphylaxis when their anaphylaxis was triggered by insects. (Fig 3, B, and see Table E1).

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 (see Fig E2 in this article's Online Repository at www.jacionline.org). We investigated the association of skin symptoms with the tryptase levels in patients without mastocytosis. For this model, we excluded the cases with known mastocytosis and with a BST level above 11.5 ng/mL, potentially indicating nondiagnosed mast cell activation disorders. Similarly, (1) tryptase levels were higher in patients with VIA, (2) the levels correlated with the severity of anaphylaxis, and (3) this effect was significant in the group with VIA ($P = .006$) but not in the group without VIA (Fig 3, C and D).

BST level greater than 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 experiencing VIA significantly more

than in patients experiencing anaphylaxis due to other elicitors (Fig 4, C and see Fig E3, A in this article's Online Repository at www.jacionline.org).

In line with the aforementioned findings, BST levels also correlated with the severity of anaphylaxis (on the Ring and Messmer scale), and most importantly, a subelevated BST level was more prominently associated with increasing the risk of severe anaphylaxis in patients with VIA than in those without VIA (Fig 2, D and see Fig 4, B in this article's Online Repository at www.jacionline.org).

Concomitant cardiovascular diseases were more prevalent in cases with VIA than in cases without VIA (892 [24.8%] vs 657 [18.2%]) and were associated with higher risk of severe anaphylaxis when elicited by insects, but they were not relevant in cases without VIA (Fig 4). Interestingly, BST values were increased in patients with concomitant cardiovascular diseases, irrespective of the reaction severity (see Fig E4 in this article's Online Repository at www.jacionline.org).

Other cofactors of severe reactions

Severe reactions of VIA were more prevalent in patients more than 22 years of age, as well as in cases with VIA versus in cases without VIA (see Fig E5 in this article's Online Repository at www.jacionline.org). There were no differences in severity of reactions elicited by yellow jackets and other insect species ($P = .4128$).

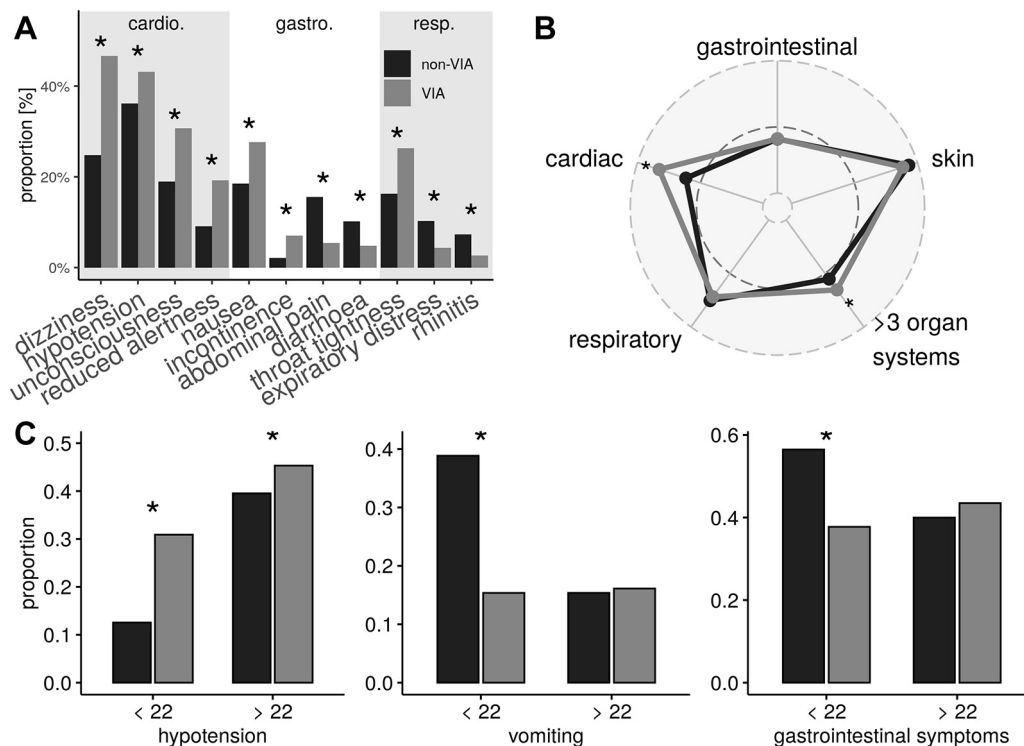


FIG 2. Symptoms of VIA compared with other elicitors. **A**, Proportional presentation of specific reaction symptoms in VIA and non-VIA according to cardiovascular (cardio.), gastroenterologic (gastro.), and respiratory (resp.) organ systems. **B**, High-level overview of involved organ systems and selected cofactors in the form of a radar plot. **C**, Difference in symptoms of VIA among patients younger versus older than 22 years. *Denotes significant differences between groups.

The effect of using angiotensin-converting enzyme inhibitors (ACEIs) (as well as β -blockers) on the risk of severe anaphylaxis correlated with coexisting cardiovascular diseases. ACEI use was, however, more often associated with cardiac arrest in all anaphylaxis cases (30 cases [5.8%] vs 118 cases [1.9%]) ($P < .001$) regardless of the elicitor (Fig 4, C). β -Blocker use was associated with a higher severity of anaphylaxis and with the onset of cardiovascular symptoms (cardiac arrest and chest pain), but was comparable between those with and without VIA ($P = .144$). Surprisingly, arrhythmia was more frequently reported in patients with VIA and concomitant β -blockers (Fig 4, C).

VIA was more often severe if the reaction occurred within the first 10 minutes after exposure to venom (46.58% of cases were severe) than when the reaction occurred more than 10 minutes after exposure (39.75% of cases were severe [$P = .001$]).

One-third of patients with VIA experience repeated reactions

In all, 940 patients with insect allergy (28.5%) had experienced venom anaphylaxis in the past. If the reaction was elicited by other elicitors (ie, case patients without VIA), previous reactions were more frequently seen (1929 cases [35.7%]) ($P < .001$). We observed 227 patients with at least 2 fully documented reactions. Of these patients, 59 (26%) had insect-elicited anaphylaxis, and in 6 of them (10.2%), the subsequent reaction was more severe than the previous reaction. In 43 cases (72.9%), the reaction was similar in severity.

Patients with VIA receive epinephrine less often than do patients without VIA

We evaluated epinephrine use (administered by any route by the patients themselves and by medical professionals) in both ambulatory and emergency room (ER) settings. Patients who experienced VIA often received epinephrine treatment significantly less than did other cases with anaphylaxis (597 cases [26.9%] vs 738 cases [34.6%]) ($P < .001$). After adjustment of both groups for similar severity, the difference in epinephrine use was still significant irrespective of the administration route ($P < .001$ [Fig 5, B]).

A positive history of anaphylaxis influenced the therapy provided for a current episode as well. Epinephrine as a first-line treatment was given less often in VIA cases when compared with other cases *if the patients in those cases did not report a history of anaphylaxis* ($P < .001$), but in patients reporting previous reactions, there was no difference in epinephrine therapy ($P = .438$ [Fig 5, B]). Similarly, there were no differences in the epinephrine use between the group with VIA and that without VIA when only severe reactions were taken into consideration ($P = .242$). However, when we restricted the analysis to cases of moderate anaphylaxis cases, patients without VIA received epinephrine more frequently than did patients with VIA ($P < .001$). The presence of skin symptoms during these mild reactions was also associated with a lower fraction of epinephrine-treated patients (see Fig E6 in this article's Online Repository at www.jacionline.org).

Patients with VIA received corticosteroids and antihistamines significantly more frequently than did patients with anaphylaxis

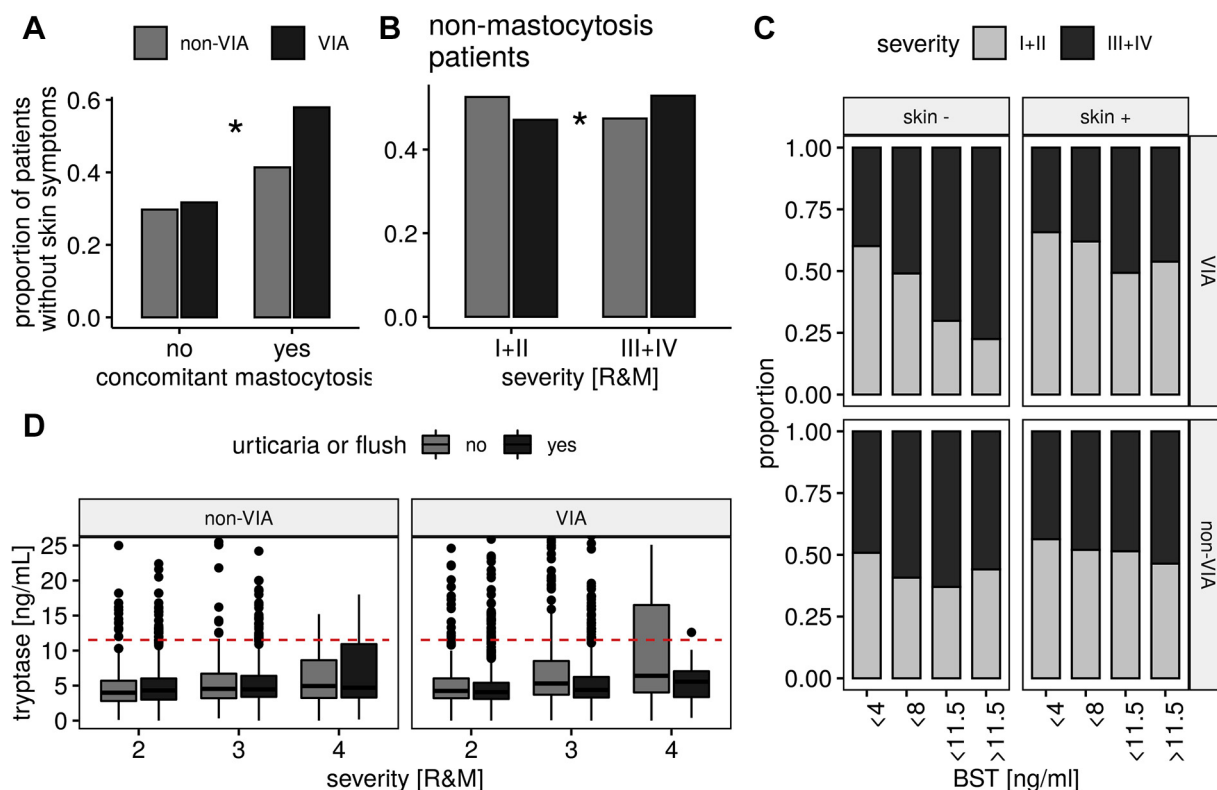


FIG 3. Lack of skin symptoms (ie, urticaria and flushing) during anaphylaxis is associated with more severe VIA. **A**, Lack of skin symptoms and mastocytosis in VIA and non-VIA cases. **B**, Lack of skin symptoms, according to the severity in both anaphylaxis groups. **C**, Relation of reaction severity according to the elicitor and the absence of skin symptoms concerning categorized BST values. **D**, Continuous values of BST according to the severity in both non-VIA and VIA with subgrouping to skin symptoms.

in response to other elicitors. On the other hand, epinephrine, β_2 mimetics, and oxygen were given more often to patients experiencing non-VIA (Fig 5, A).

Next, we asked whether specific symptom clusters and treatment profiles could be identified within our cohort (association measured by using ϕ coefficient). We found that patients displaying cardiovascular symptoms (cardiac arrest, hypotension, and loss of consciousness) and urticaria were treated differently from patients with respiratory or gastrointestinal symptoms (Fig 5, C). The treatment of the former symptoms consisted of epinephrine autoinjector use, intravenous epinephrine in multiple doses, 100% oxygen inhalation, an initial dose of antihistamines, and inhaled β_2 agonists. Corticosteroids, intravenous volume replacement, and intravenous β_2 agonists formed another therapy mode.

DISCUSSION

In this study, we identified distinct symptom profile and treatment patterns of VIA. 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 was. Previous studies have suggested 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 in 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 an elevated BST level (>8 ng/mL), especially in VIA. Nevertheless, the pathomechanism promoting cardiovascular symptoms in VIA requires further investigation.

As cardiovascular symptoms such as hypotension, collapse, or cardiac arrest lead to a higher grade on the Ring and Messmer scale than skin or gastrointestinal symptoms do, 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 exclusion of patients with a known diagnosis of mastocytosis (although in cases without mastocytosis the difference between groups was small and the clinical relevance of this finding needs cautious evaluation). Previous studies also observed this phenomenon.^{21,22} Subsequently, the correlation of BST levels with the severity of anaphylaxis led us to identify an interaction between the absence of skin symptoms and VIA by using generalized linear regression.

Our findings indicate that patients with a BST level higher than 8 ng/mL are prone to severe anaphylaxis in response to insect venom. Patients with a normal BST level in the range of 8 to 11.4 ng/mL may have indolent systemic mastocytosis or

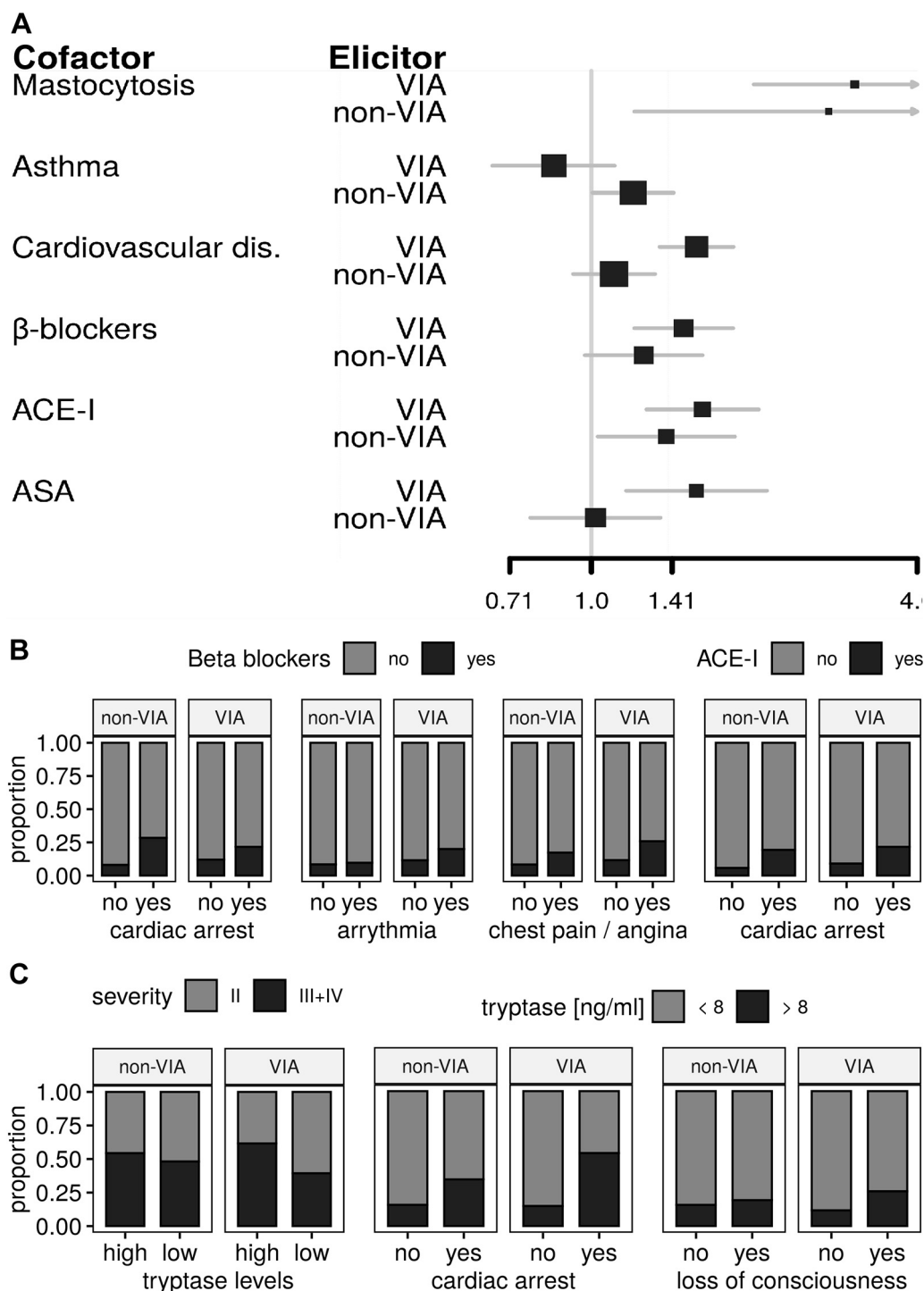


FIG 4. Cofactors of insect venom anaphylaxis. **A**, Odds ratios of eliciting severe anaphylaxis. **B**, Proportion of cases elicited by insects or other elicitors (upper panels) according to tryptase levels and cardiovascular symptoms. ACE-I, Angiotensin-converting enzyme inhibitor; ASA, acetylsalicylic acid; dis, disease.

concomitant undiagnosed mast cell activation syndrome.²³ Zanotti et al identified mast cell disorders in 17 of 22 patients with VIA lacking skin symptoms and concluded that patients with a BST level higher than 7.95 ng/mL and VIA should undergo extensive diagnostic procedures.²⁴ We recently determined that elderly patient experiencing anaphylaxis without concomitant skin symptoms tended to have more severe reactions.²⁵ Our

findings are in concordance with those of a recent retrospective study from Fehr et al,²² who identified lack of skin symptoms as a risk factor for severe VIA.

On the basis of these and previous findings,^{6,24,26} we propose that a peripheral blood *KIT* D816V mutation test be performed in cases with BST levels higher than 8 ng/mL and with a history of anaphylaxis presenting without urticaria or flushing. Previous

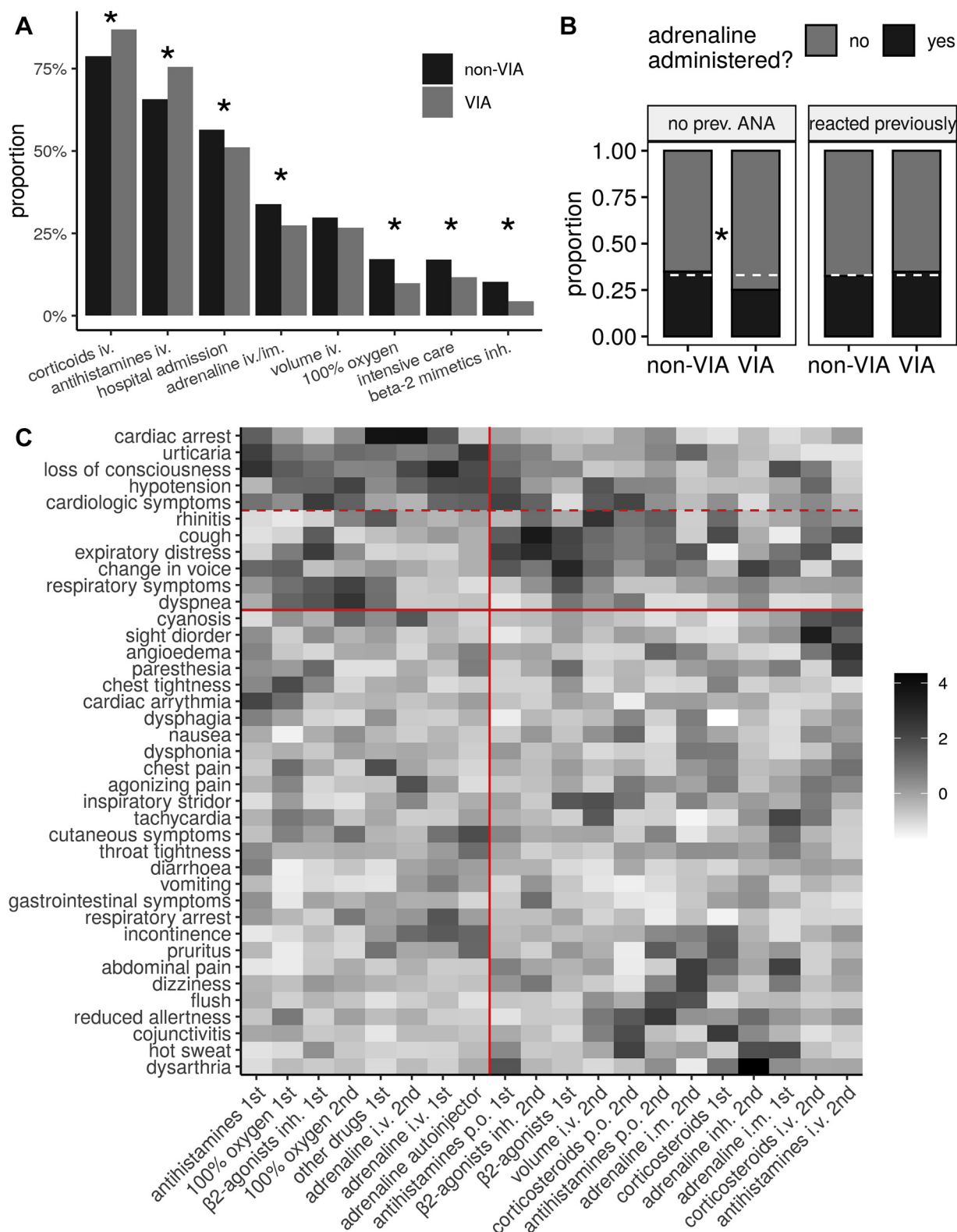


FIG 5. Therapy in patients with VIA versus in patients with anaphylaxis due to other elicitors; cases matched according to sex, age, and severity of a reaction. **A**, Proportional use of therapy measures in both anaphylaxis groups. **B** and **C**, Heatmaps visualizing the association of symptoms and corresponding treatment are presented as a scaled correlation coefficient (ϕ). * $P < .05$ after false discovery rate correction.

studies showed 92% sensitivity of this test in patients with hymenoptera anaphylaxis who present without skin symptoms and have a tryptase level less than 20 ng/mL.²⁷

Age is an important risk factor for severe anaphylaxis.²⁸ Adult patients experienced VIA more frequently. Young patients mainly experience food-induced anaphylaxis.⁸ Emergency room (ER) admission data indicate that the frequency of insect sting hypersensitivity reactions in children is comparable to that of 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 (in 56% of food hypersensitivity cases vs in 5.3% of sting cases seen in the ER).²⁹ Senior patients, on the other hand, experience drug-related hypersensitivity more often than insect sting hypersensitivity.²⁵ Similarly, we observed less VIA in patients with concomitant atopic diseases (see Fig E3), 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 ACEIs and β -blockers increase the severity of anaphylaxis. However, we did observe that there were no significant differences between cases with VIA and cases without VIA in terms of the symptoms and severity of an episode with concomitant use of ACEIs or β -blockers.

Cases of VIA had been treated with epinephrine less often than did the age-, sex-, and severity-matched cases of non-VIA. Moreover, the administration of epinephrine did not depend on the trigger if the patient had experienced anaphylaxis previously, but epinephrine was used significantly less often if the patients were experiencing their first episode of VIA (in comparison with patients experiencing their first episode of 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 cases of 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, these are the only data on the comparative epinephrine use in a case-controlled groups of patients with and without VIA.

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

Although there are no absolute contraindications for using epinephrine in anaphylaxis, 1 potential scenario in which clinicians tend to be reluctant to use epinephrine is a hypersensitivity reaction presenting with high blood pressure and tachycardia, which may be present at the initial phase of VIA. Nevertheless, the 3 exceptionally well documented cases of anaphylaxis in response to sting challenge showed that the initial transient increase in blood pressure should not be interpreted as a contraindication to epinephrine and that epinephrine could be safely given even if the heart rate was greater than 120 beats per minute.³²

The patients with VIA had a documented history of anaphylaxis in 28% of cases, and systemic immunotherapy was not initiated in these patients, which goes against the latest management guidelines, and although this percentage may be

slowly decreasing, it is of utmost importance to recommend SIT to all patients who have experienced VIA.

On the basis of our findings, insects are the most probable elicitor of anaphylaxis in Europe during the summer season, with VIA cases extending from early spring to the end of autumn (see Fig E7 in this article's Online Repository at www.jacionline.org). 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 (ie, 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 to 2019, the perennial ratio of VIA cases to non-VIA cases remained unchanged (data not shown).

Limitations

Because of the design of the European Anaphylaxis Registry, our analysis was restricted to cases of anaphylaxis only. Patients experiencing 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 population distribution of hypersensitivity reactions and restrict us to comparing only various forms of anaphylaxis.

Nevertheless, because the European Anaphylaxis Registry has thus far gathered more 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 in which targeted studies are not possible.

Conclusion

On the basis of our results, VIA is a distinctive phenotype of anaphylaxis, with a specific symptom profile and risk factors. By using a large cohort of VIA cases compared with 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 β -blockers or ACEIs had a higher risk of developing severe cardiovascular symptoms (including cardiac arrest) in VIA and non-VIA cases. Patients experiencing VIA received epinephrine less frequently than did patients in non-VIA cases.

VIA cases should undergo therapy according to the international management guidelines, and epinephrine should be given more often in those with VIA. In all cases, patients should undergo appropriate allergologic testing, the indication for SIT should be evaluated, and patient education regarding the risk of future anaphylaxis should be provided. Patients with a BST level higher than 8 ng/mL should undergo extensive diagnostic tests to exclude indolent systemic mastocytosis or mast cell activation syndrome, and they should be provided with 2 epinephrine autoinjectors for acute self-management.

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Clinical implications: Allergologists should educate patients about risk of future reactions and consider prescribing 2 epinephrine autoinjectors and performing specific immunotherapy in patients with a baseline serum tryptase level greater than 8 ng/mL and a history of insect venom anaphylaxis without skin involvement.

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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 cases to anaphylaxis cases elicited by other elicitors during the summer seasons reached 60% but was less than 1% of cases during winter.

Nevertheless, 116 cases of VIA (bee [*Apis mellifera*] in spring and yellow jacket [*Vespula spp*] in autumn) were triggered in March, April, and November. The 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.

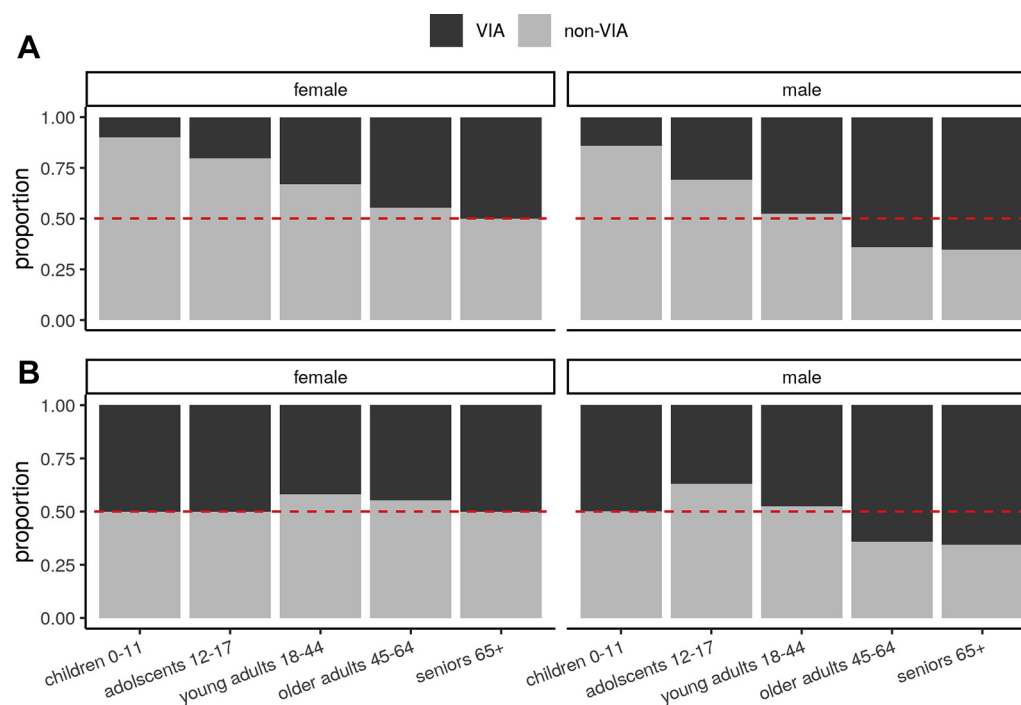


FIG E1. Results of matching the cohort according to sex and age to perform a case-controlled study. **A,** The original distribution of VIA and non-VIA cases according to age group and sex. Note the uneven distribution of VIA and non-VIA cases in age groups. **B,** The distribution of VIA and non-VIA after age and sex matching with the use of the MatchIt package for R. Note how the ratio of VIA to non-VIA cases is approaching 50%, indicating balanced matching according to sex and age variables.

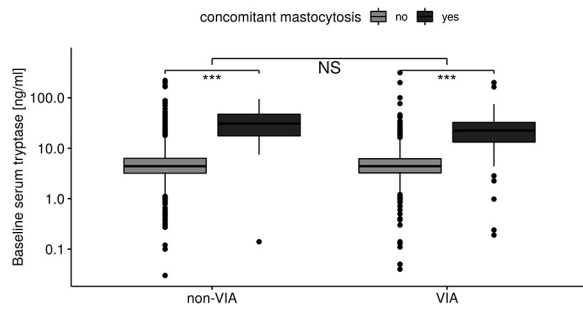


FIG E2. Levels of baseline serum tryptase in patients with VIA and non-VIA. Significant difference in BST level between patients with concomitant mastocytosis and other patients (***). There was no significant (NS) difference between anaphylaxis elicited by insects and other elicitors. Tested by 2-way ANOVA.

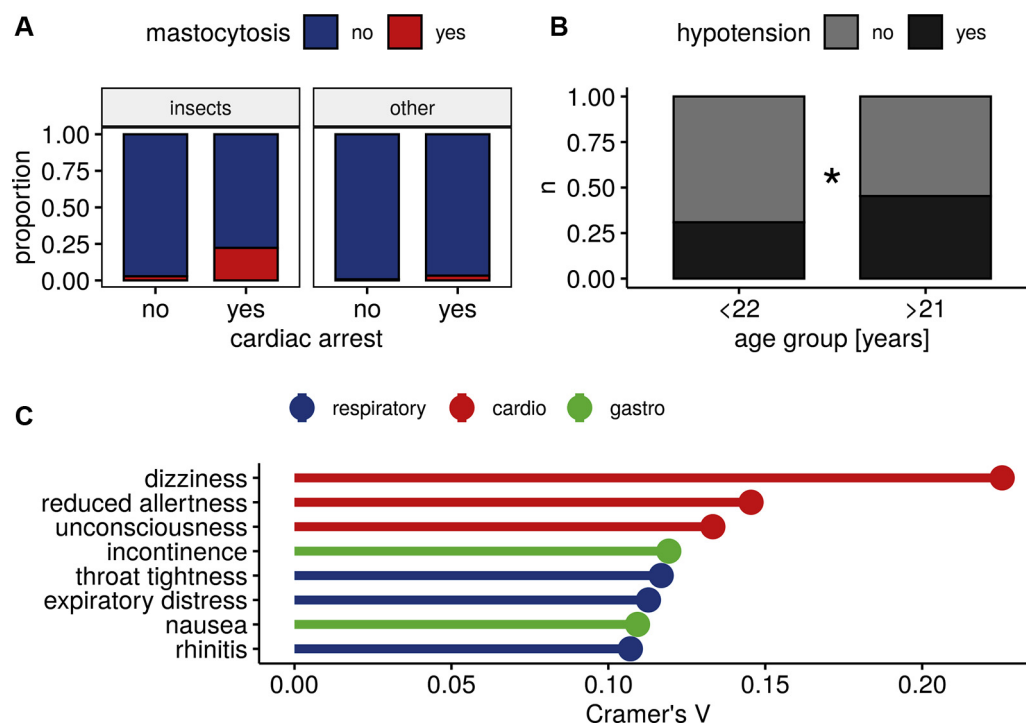


FIG E3. Symptoms of anaphylaxis. **A**, The association between cardiac arrest and concomitant mastocytosis in VIA and non-VIA. **B**, Hypotension frequency in 2 age groups of patients with anaphylaxis. **C**, The Cramer V as the measure of association between anaphylaxis groups (VIA vs non-VIA). Higher values indicate a stronger association with VIA.

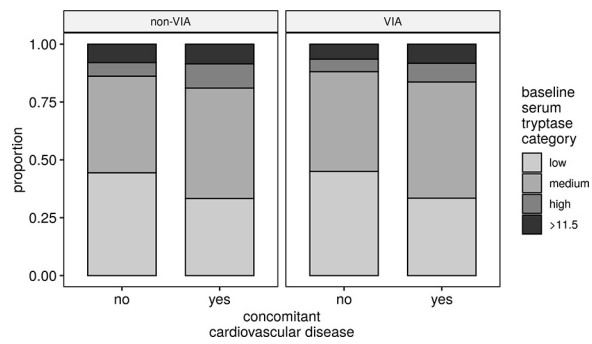


FIG E4. Tryptase levels in patients with concomitant cardiovascular diseases. Low indicates a level less than 4 ng/mL, medium indicates a level of 4 to 8 ng/mL, and high indicates a level of 8 to 11.5 ng/mL.

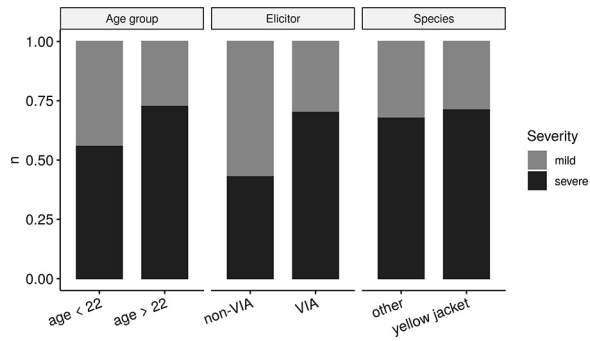


FIG E5. Severity of anaphylaxis in subgroups. The severity of patients with VIA in the 2 age groups (*left*), according to elicitor type (*center*) and according to the responsible insect species (*right*).

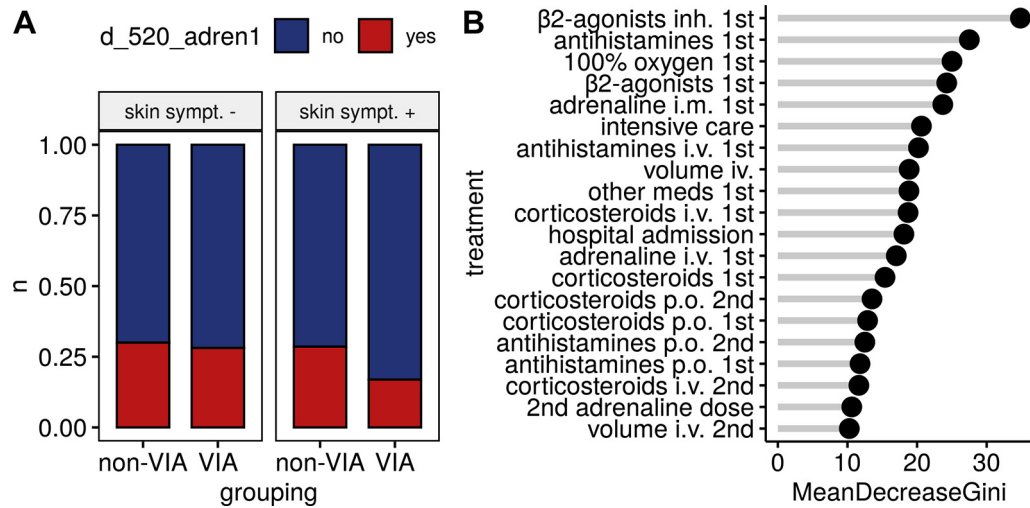


FIG E6. Therapy for anaphylaxis. **A**, Patients who presented with skin symptoms and VIA received epinephrine less often than if skin symptoms were absent during the reaction. **B**, Variable importance in the unsupervised classification between VIA and non-VIA by using a random forest classifier. *adren*, Adrenaline; *inh.*, inhaled; *i.m.*, intramuscularly; *i.v.*, intravenously; *p.o.*, orally; *sympt.*, symptoms.

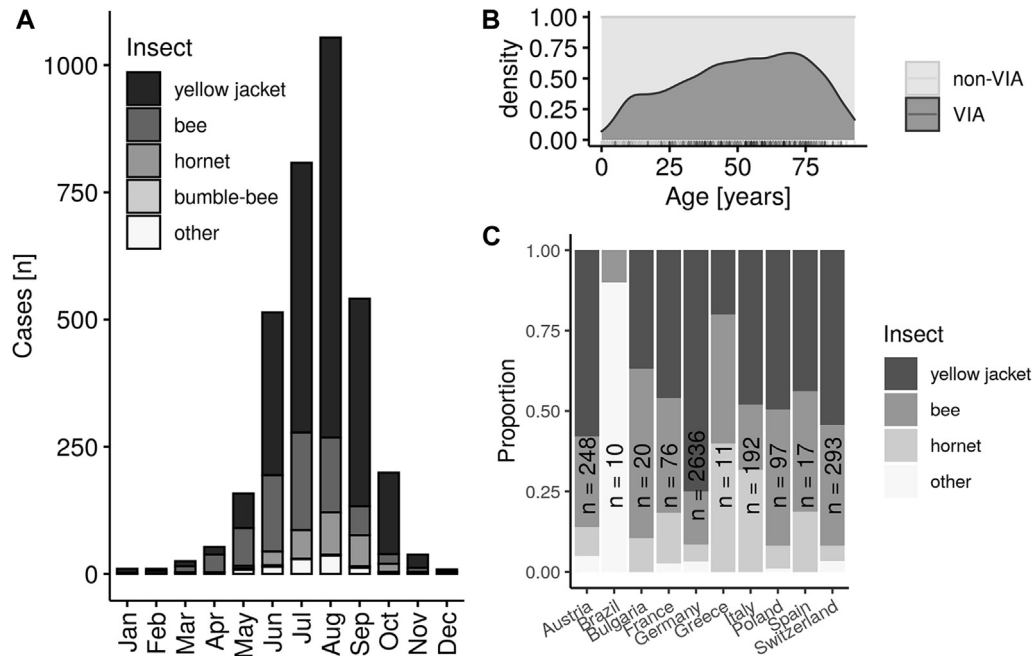


FIG E7. **A**, Proportion of anaphylaxis cases elicited by specific insects according to the month in which the reaction occurred. Less common insect species grouped as "other." **B**, The density distribution of VIA cases to cases elicited by other elicitors, with consideration of the patient's age. **C**, Geographic differences in the most common elicitors of VIA. Countries that reported fewer than 10 VIA cases were not illustrated in this figure. Fire ants and insects that could not be identified formed the other group.

TABLE E1. Results of a factorial logistic regression: Regression coefficients

Dependent variable	Severity*
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	6883
Log likelihood	−4688.151
AIC	9384.303

AIC, Akaike information criterion.

*Ring and Messmer grade II versus grade III-IV.

[†] $P < .01$.[‡] $P < .05$.