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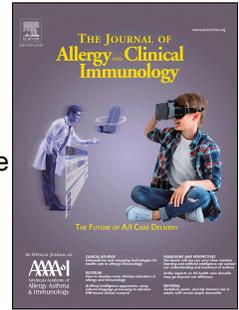
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Phenotype and risk factors of venom-induced anaphylaxis: a case-control study of the European Anaphylaxis Registry

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1 Phenotype and risk factors of venom-induced anaphylaxis: a 2 case-control study of the European Anaphylaxis Registry

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58 **Conflict of interest:**

59 A. Bauer reports personal fees from ALK, Allergopharma, Allergy Therapeutics, Diater,
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71 Pföhler performed clinical studies for Allergy Therapeutics and received speaker honoraria
72 and travel support from Bencard, Novartis and ALK. The rest of the authors declare that
73 they have no relevant conflicts of interest.

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75 **List of abbreviations:**

- 76 • VIA - venom-induced anaphylaxis
- 77 • BST - baseline Serum Tryptase
- 78 • EAI - epinephrine autoinjector
- 79 • MCAS - mast cell activation syndrome
- 80 • ER - emergency room

81

82 **Abstract**

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

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

89 **Methods:** Using data from the European Anaphylaxis Registry (12874 cases) we identified
90 3612 patients with venom-induced anaphylaxis and analyzed these in comparison to sex-
91 and age- matched anaphylaxis cases triggered by other elicitors (non-VIA n = 3605).

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

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

105 **Clinical Implications**

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

109 **Capsule Summary**

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

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

115

116 **Introduction**

117 Hypersensitivity to insect venom presents as a systemic reaction (anaphylaxis) in up to
118 0.3–7.5% of the adult population¹. Venom-induced anaphylaxis (VIA) can be fatal, and
119 patients sometimes require lifelong specific immunotherapy². There is a need for more
120 precise identification of biomarkers, and better definition of phenotypes of anaphylaxis³.
121 Also, in order to facilitate a precision-medicine approach⁴ for the diagnosis of anaphylaxis,
122 a better understanding of its clinical phenotypes is required.

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

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

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

134 **Methods**

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

138 The diagnosis of anaphylaxis was based on the definition by NIAID/FAAN⁹ and the severity
139 according to the Ring and Messmer Scale¹⁰. Reactions of grade II were considered mild and
140 grades III and IV (presenting with significant hypoxia, hypotension, confusion, and loss of
141 consciousness, or incontinence or cardiac arrest) were considered severe. Mastocytosis
142 patients were defined as having a documented diagnosis of mastocytosis in medical history
143 prior to the reaction. The Registry is designed for reporting cases of moderate to severe
144 anaphylaxis (Ring and Messmer grades II-IV).

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

151 Cases were matched according to sex, age, and reaction severity in order to reduce the
152 comparison bias by propensity score matching. Propensity score is a statistical approach to
153 quantify the similarity between two unrelated cases. Propensity scores were calculated
154 using the “MatchIt” package for R¹¹. MatchIt uses logistic regression to reduce the bias due
155 to multiple confounding variables (i.e. sex and age) by weighing them and choosing cases
156 with minimal differences in both groups. The results of the propensity score matching are
157 illustrated in Fig. 1B-D and eFig. 2.

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

162 Based on the severity and symptom profile and the previous reports⁶, we defined sub-
163 elevated baseline serum tryptase (BST) values as 8 - 11.5 ng/ml (Fig. 3C-D).

164 We used the R Statistical Package¹³ for statistical analysis. A simple comparison of
165 categorical variables was performed using either the Chi² test or Fisher’s exact test (where
166 the number of observations in a bin was less than 10). Continuous variables were analyzed
167 using the Mann-Whitney U test. In case of comparisons with two or more independent
168 variables, we used Factorial ANOVA or Generalized Linear Models. We defined statistical
169 significance as $\alpha = 0.05$. Data, along with the analysis script, can be accessed online at
170 <https://github.com/wolass/venomanaphylaxiscompendium>.

171 We developed a Random Forest classifier (using the “randomForest” package for R¹⁴) in
172 order to find therapeutic approaches that varied the most between VIA / non-VIA group
173 and presented the results as Gini importance¹⁵. Moreover, association analysis of
174 therapeutic interventions and symptoms was performed. The resulting phi values were
175 scaled and presented in a heatmap with automatic clustering using Ward’s Agglomerative
176 Hierarchical Clustering with Euclidean distances¹⁶.

177 **Results**

178 **VIA is more frequently associated with cardiovascular symptoms**

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

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

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

191 **Absence of skin symptoms during anaphylaxis is associated with more** 192 **severe episodes of VIA**

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

197 Similarly, in non-mastocytosis patients undergoing VIA, skin symptoms (i.e., urticaria or
198 flushing) were less often present than if anaphylaxis was triggered by other elicitors (2356;
199 68% vs. 2495; 70.4% respectively, $p = 0.031$). Moreover, in this specific subgroup of
200 patients (i.e., non-mastocytosis patients lacking skin symptoms) VIA was significantly more
201 frequently severe (587; 52.9% in VIA vs. 498; 47.4%, $p < 0.001$, Fig. 3B).

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

207 **Absence of skin symptoms correlates with BST levels and increases** 208 **the risk of severe anaphylaxis specifically in VIA**

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

216 **BST over 8 ng/ml and concomitant cardiovascular conditions increase** 217 **the risk of severe VIA**

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

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

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

226 Concomitant cardiovascular diseases were more prevalent in VIA than in non-VIA cases
227 (892 (24.8%) vs. 657 (18.2%)) and were associated with higher risk of severe anaphylaxis
228 when elicited by insects but were not relevant in non-VIA cases (Fig. 4). Interestingly, BST
229 values were increased in patients with concomitant cardiovascular diseases, irrespectively
230 of the reaction severity (eFig. 4).

231 **Other cofactors of severe reactions**

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

235 The effect of using ACE-I (as well as beta-blockers) on the risk of severe anaphylaxis
236 correlated with coexisting cardiovascular diseases. ACE-I use was, however, more often
237 associated with cardiac arrests in all anaphylaxis cases (30 (5.8%) vs. 118 (1.9%), $p <$
238 0.001) regardless of the elicitor (Fig. 4C). Beta-blocker use was associated with a higher
239 severity of anaphylaxis and with the onset of cardiovascular symptoms (cardiac arrest,
240 chest pain), but was comparable between both VIA and non-VIA, $p = 0.144$). Surprisingly,
241 arrhythmia was more frequently reported in patients with VIA and concomitant beta-
242 blockers (Fig. 4C).

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

246 **One-third of VIA patients experience repeated reactions**

247 940 (28.5%) of patients with insect allergy had experienced venom anaphylaxis in the past.
248 If the reaction was elicited by other elicitors (i.e., non-VIA) — previous reactions were
249 more frequently seen (1929; 35.7%, $p < 0.001$). We observed 227 patients with at least two
250 fully-documented reactions. Out of these 59 (26%) had insect elicited anaphylaxis and in 6
251 of them (10.2%), the following reaction was more severe than before. In 43 (72.9%) cases,
252 the reaction was similar in severity.

253 **VIA patients receive epinephrine less often than non-VIA**

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

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

262 other cases **if patients did not report a previous history of anaphylaxis** ($p < 0.001$), but
263 in patients reporting previous reactions, there was no difference in epinephrine therapy (p
264 = 0.438, Fig. 5B). Similarly, there were no differences in the epinephrine use between VIA
265 and non-VIA when only severe reactions were taken into consideration ($p = 0.242$).
266 However, when we restricted the analysis to moderate anaphylaxis cases — non-VIA
267 patients received epinephrine more frequently than VIA ($p < 0.001$). The presence of skin
268 symptoms during these mild reactions also was associated with a lower fraction of
269 epinephrine treated patients (eFig. 6).

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

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

281 Discussion

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

286 VIA was more often associated with cardiovascular symptoms than non-VIA. Previous
287 studies suggest an essential link between the cardiovascular system and insect sting
288 hypersensitivity^{7,12,17}. VIA has been associated with Kounis syndrome (coronary arterial
289 spasm induced by the release of mast cell mediators^{18,19}) and cardiac arrhythmias usually
290 occurring in patients with preexisting heart disease²⁰.

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

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

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

308 Our findings indicate that patients with BST above 8 ng/ml are prone to severe anaphylaxis
309 to insect venom. Patients with normal BST in the range of 8-11.4 ng/ml may have indolent
310 systemic mastocytosis or concomitant undiagnosed mast cell activation syndrome
311 (MCAS)²³. Zanotti et al. identified mast cell disorders in 17 out of 22 patients with VIA
312 lacking skin symptoms and concluded that patients with BST above 7.95 ng/ml and VIA
313 should undergo extensive diagnostic procedures²⁴. We recently identified that elderly
314 patient undergoing anaphylaxis without concomitant skin symptoms tended to have more
315 severe reactions²⁵. Our finding are in concordance with a recent retrospective study from
316 Fehr et al.²² who identified lack of skin symptoms as a risk factor for severe VIA.

317 Based on these and previous findings^{6,24,26} we propose to perform a peripheral blood KIT
318 D816V mutation test in cases of BST of above 8 ng/ml and with a history of anaphylaxis
319 presenting without urticaria or flushing. Previous studies showed 92% sensitivity of this
320 test in patients with hymenoptera anaphylaxis, presenting without skin symptoms and
321 with tryptase under 20 ng/ml²⁷.

322 Age is an important risk factor for severe anaphylaxis²⁸. Adult patients experienced VIA
323 more frequently. Young patients mainly suffer from food-induced anaphylaxis⁸. Emergency
324 room (ER) admission data indicate that the frequency of insect stings hypersensitivity
325 reactions in children is comparable to food hypersensitivity reactions (12-15% of cases of
326 hypersensitivity reactions admitted to the ER), but pediatric anaphylaxis is triggered
327 significantly more often by food elicitors (56% of food hypersensitivity cases vs. 5.3% of
328 sting cases seen in the ER)²⁹. Senior patients, on the other hand, suffer from drug-related
329 hypersensitivity more often than insect sting hypersensitivity²⁵. Similarly, we observed
330 less VIA in patients with concomitant atopic diseases (eFig. 3) , as these patients more often
331 present with food anaphylaxis³⁰.

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

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

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

347 Nevertheless, international guidelines of anaphylaxis state that epinephrine (i.m.) is the
348 first-line agent in all diagnosed cases of anaphylaxis³¹. Clinicians should not underestimate
349 the less severe VIA cases and treat them with epinephrine accordingly.

350 Although there are no absolute contraindications for using epinephrine in anaphylaxis, one
351 potential scenario where clinicians tend to be reluctant to using epinephrine is a
352 hypersensitivity reaction presenting with high blood pressure and tachycardia, which may
353 be present at the initial phase of VIA. Nevertheless, the three exceptionally well
354 documented cases of anaphylaxis upon sting challenge showed that the initial transient
355 increase in blood pressure should not be interpreted as a contraindication to epinephrine
356 and it could be safely given even if the heart rate was above 120 beats per minute³².

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

361 Based on our findings, insects are the most probable elicitor of anaphylaxis in Europe
362 during summer-season, with VIA cases extending from early spring to the end of autumn
363 (eFig. 1). Detailed information on the seasonality of insect-elicited hypersensitivity
364 reactions is scarce³³. The activity of *Vespula germanica* depends on the climate, and in
365 invaded regions (e.i. Australia), it can even extend throughout the year³⁴. The changing
366 climate in Europe may influence the activity of Hymenoptera in this region in the upcoming
367 years. However, in the period from 2007 - 2019, the perennial ratio of VIA to non-VIA cases
368 has remained unchanged (data not shown).

369 **Limitations**

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

376 Nevertheless, because the European Anaphylaxis Registry has until now gathered over
377 12,000 cases of anaphylaxis - it was possible to perform a case-controlled analysis on a
378 relatively large number of cases and investigate many aspects of VIA. It is worth
379 underlining the important function of international registries, especially in diseases where
380 targeted studies are not possible.

381 Conclusion

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

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

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444

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555

556 **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: Continuous values of BST according to the severity in both non-VIA*
575 *and VIA with subgrouping to skin symptoms.*

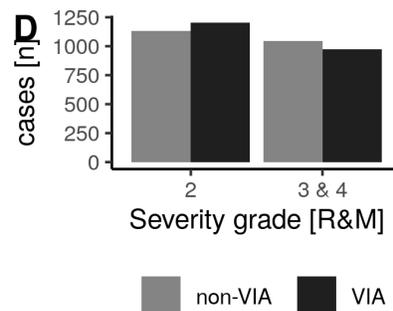
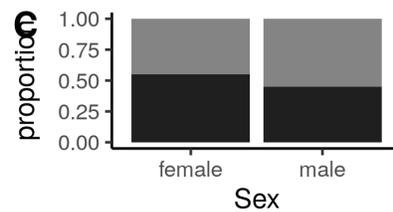
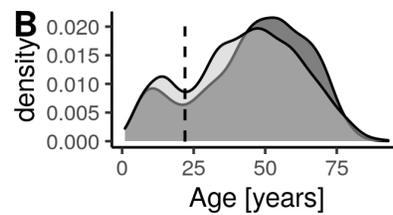
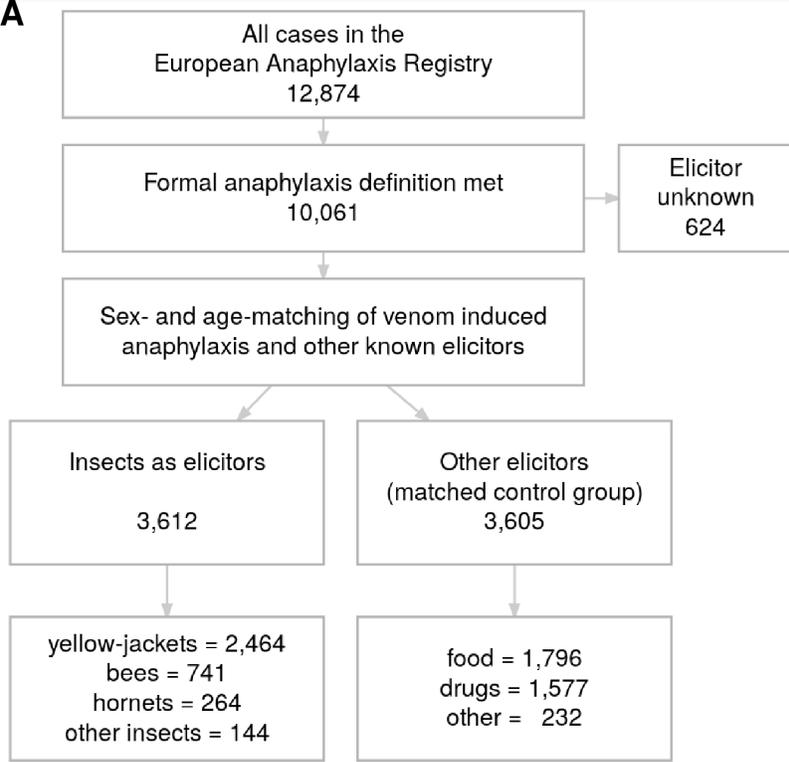
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577 *Figure 4: Cofactors of insect venom anaphylaxis. A: Odds ratios of eliciting severe anaphylaxis.*
578 *B: Proportion of cases elicited by insects or other elicitors (upper panels) according to*
579 *tryptase levels and cardiovascular symptoms.*

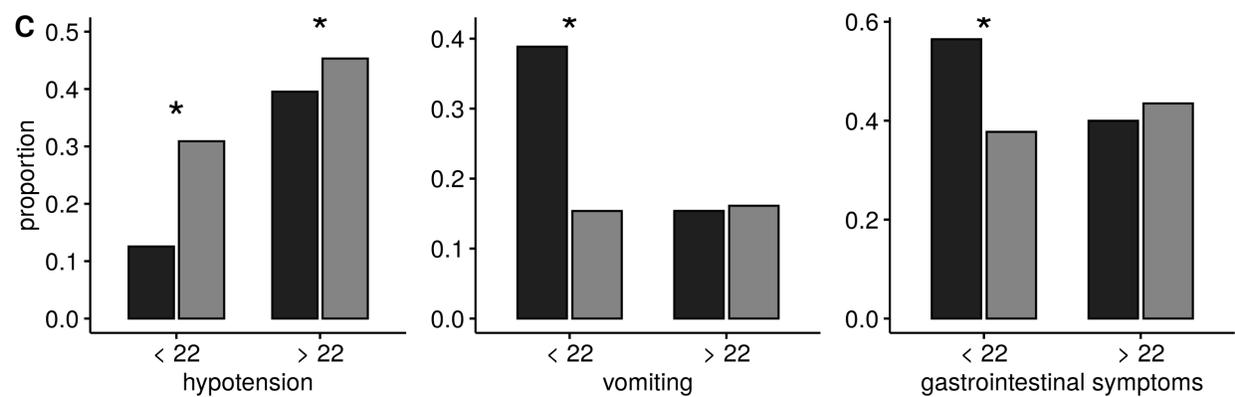
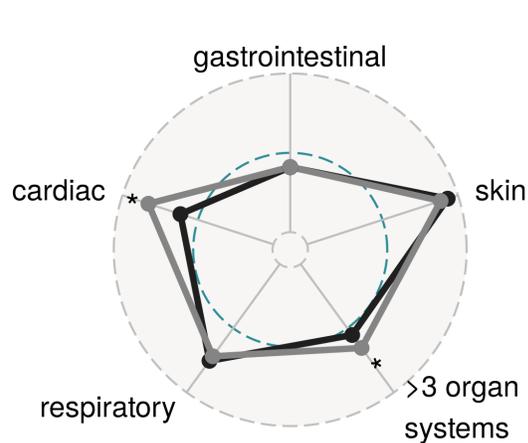
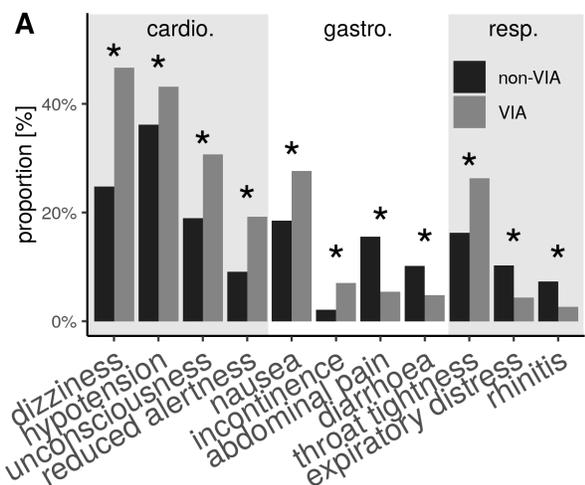
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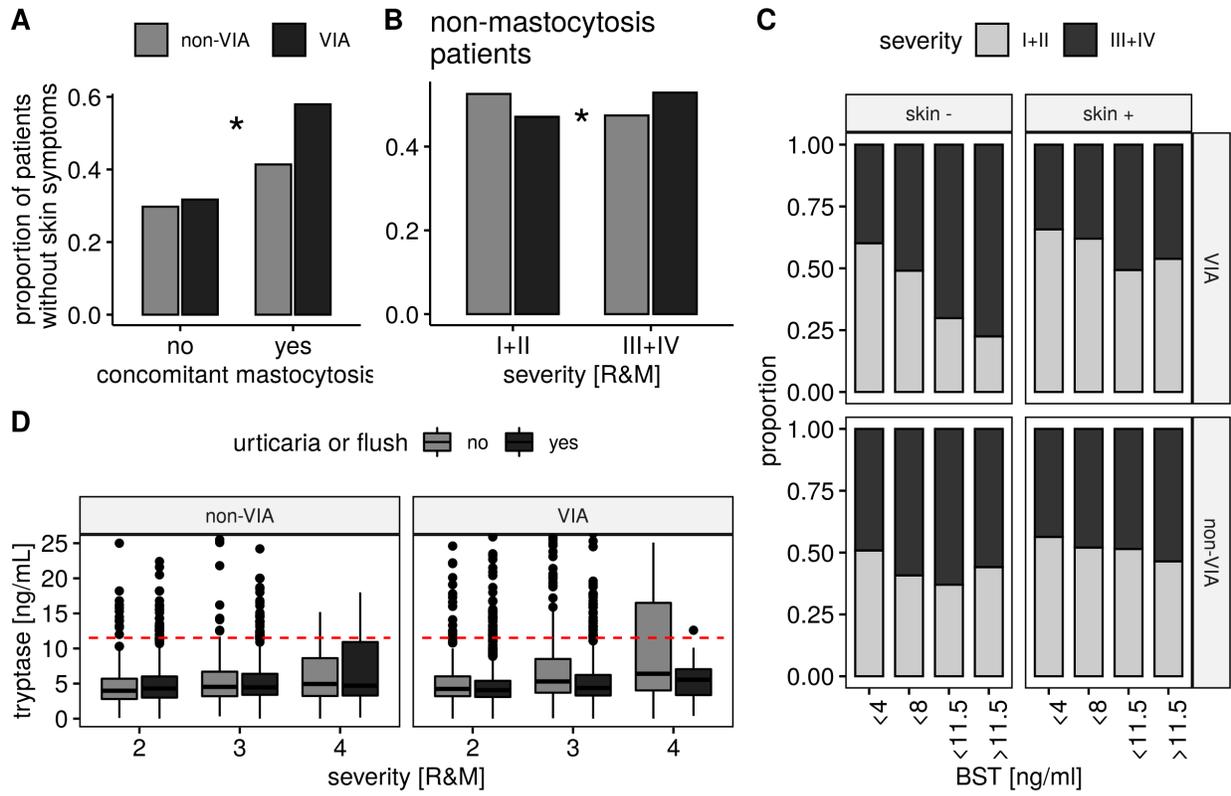
581 *Figure 5: Therapy in patients with VIA compared to other elicitors, cases matched according*
582 *to sex, age, and severity of a reaction. A: Proportional use of therapy measures in both*
583 *anaphylaxis groups. B: C: Heatmap visualizing the association of symptoms and*
584 *corresponding treatment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

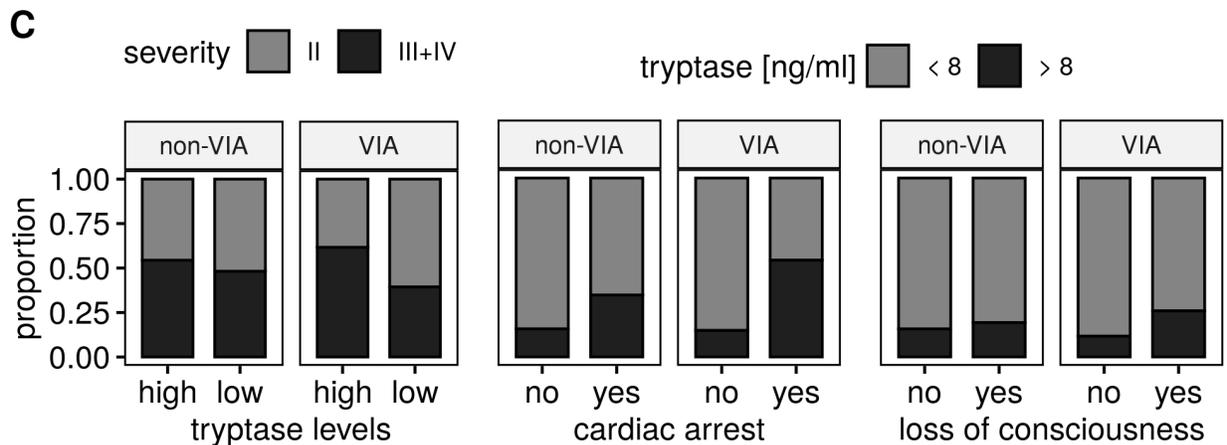
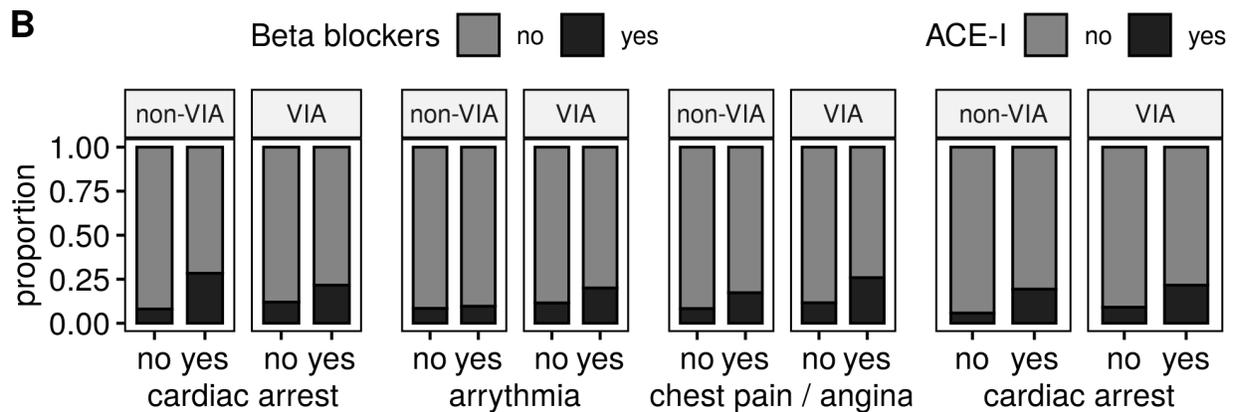
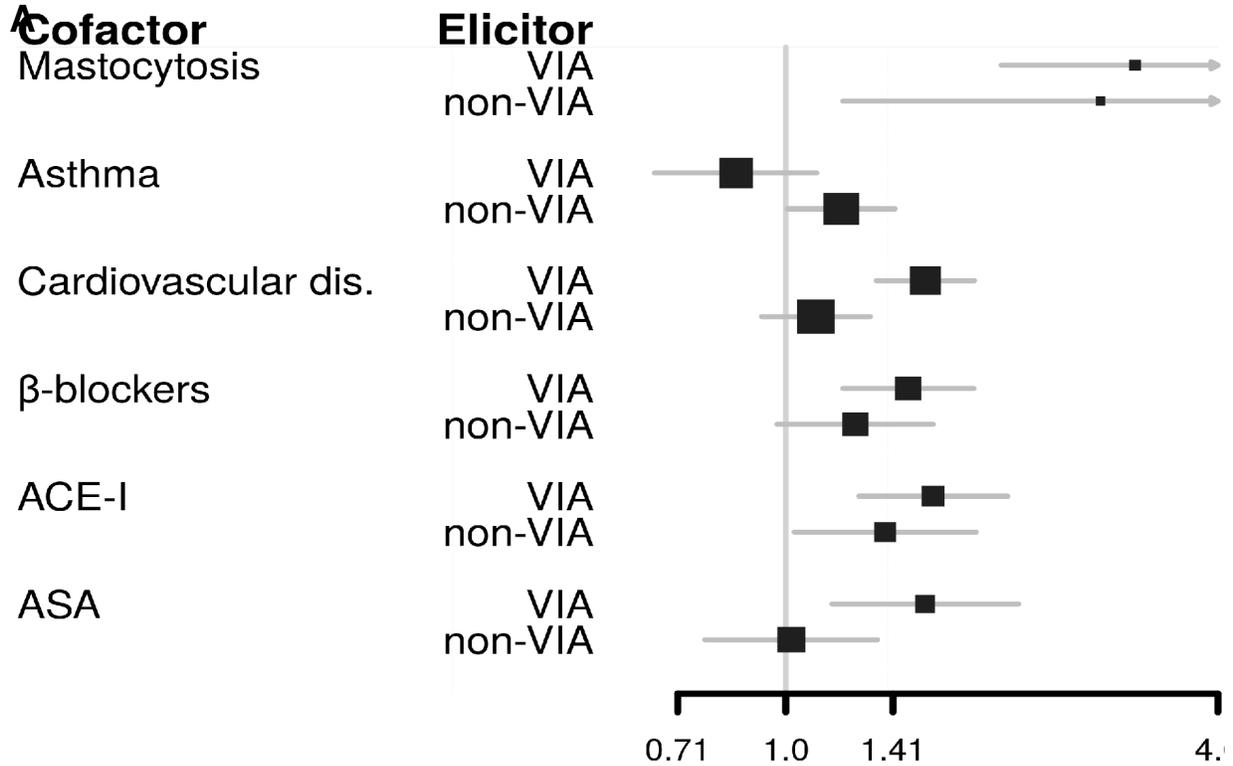
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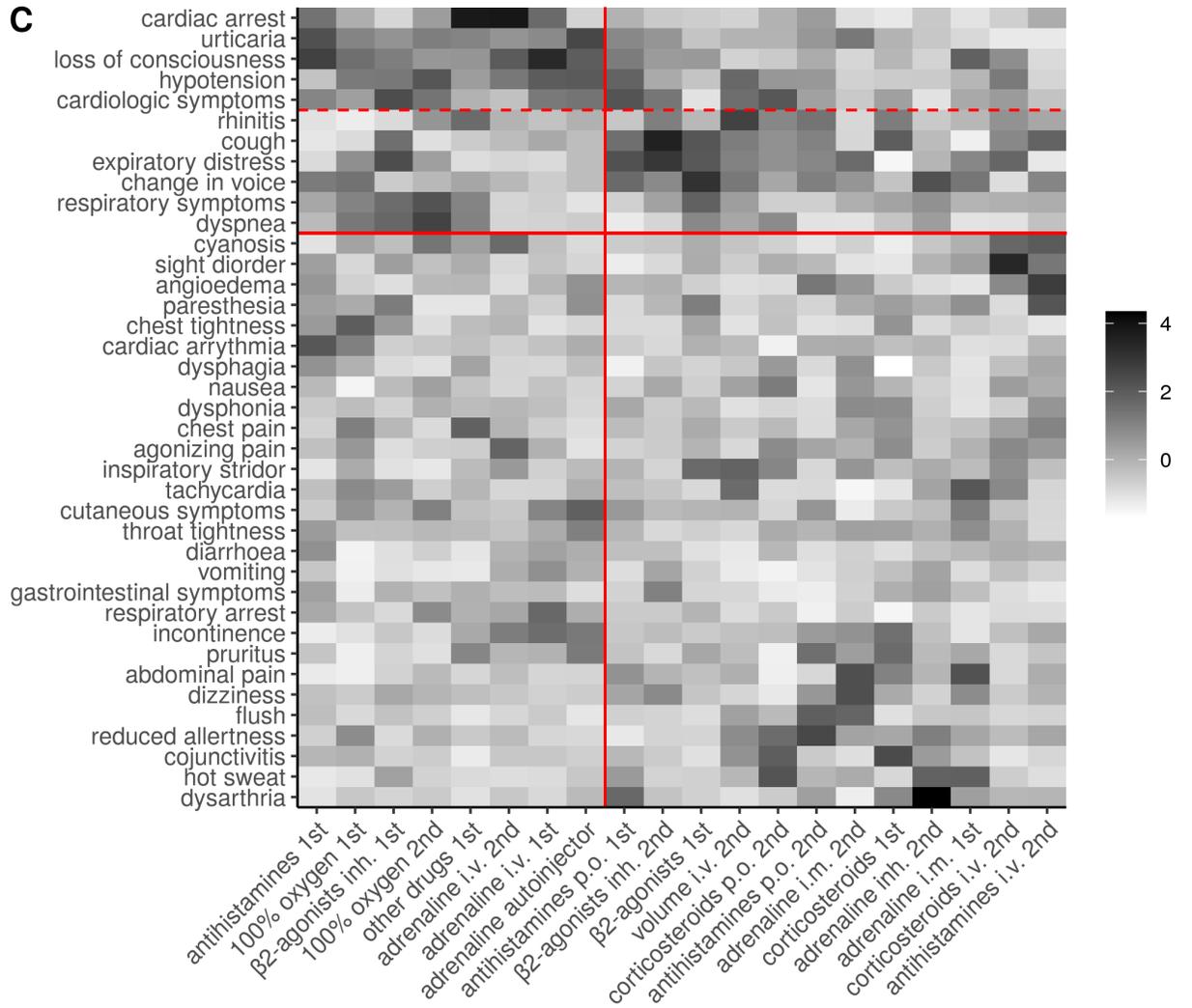
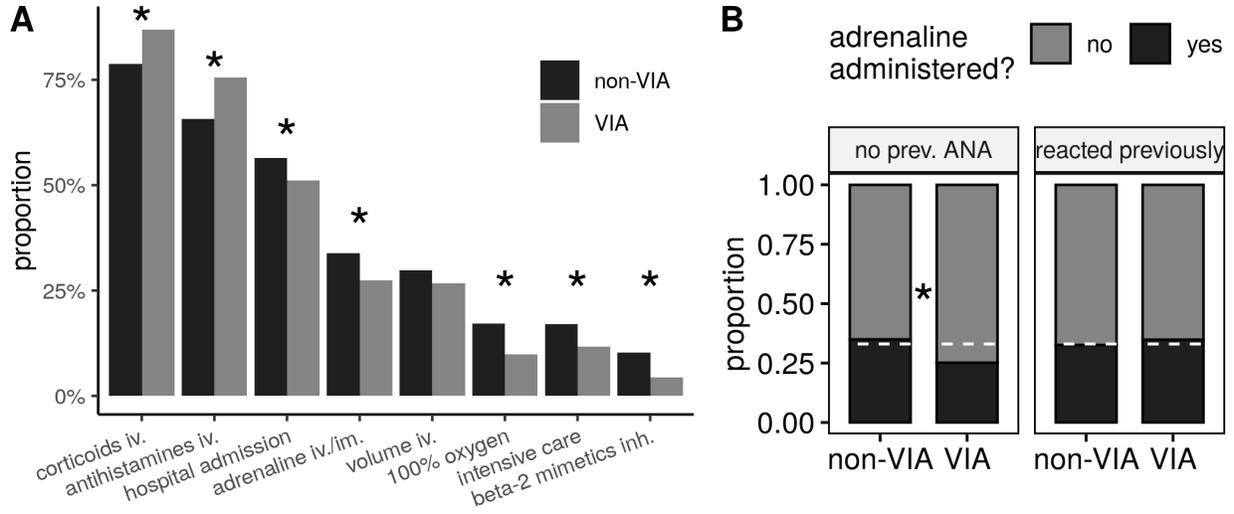
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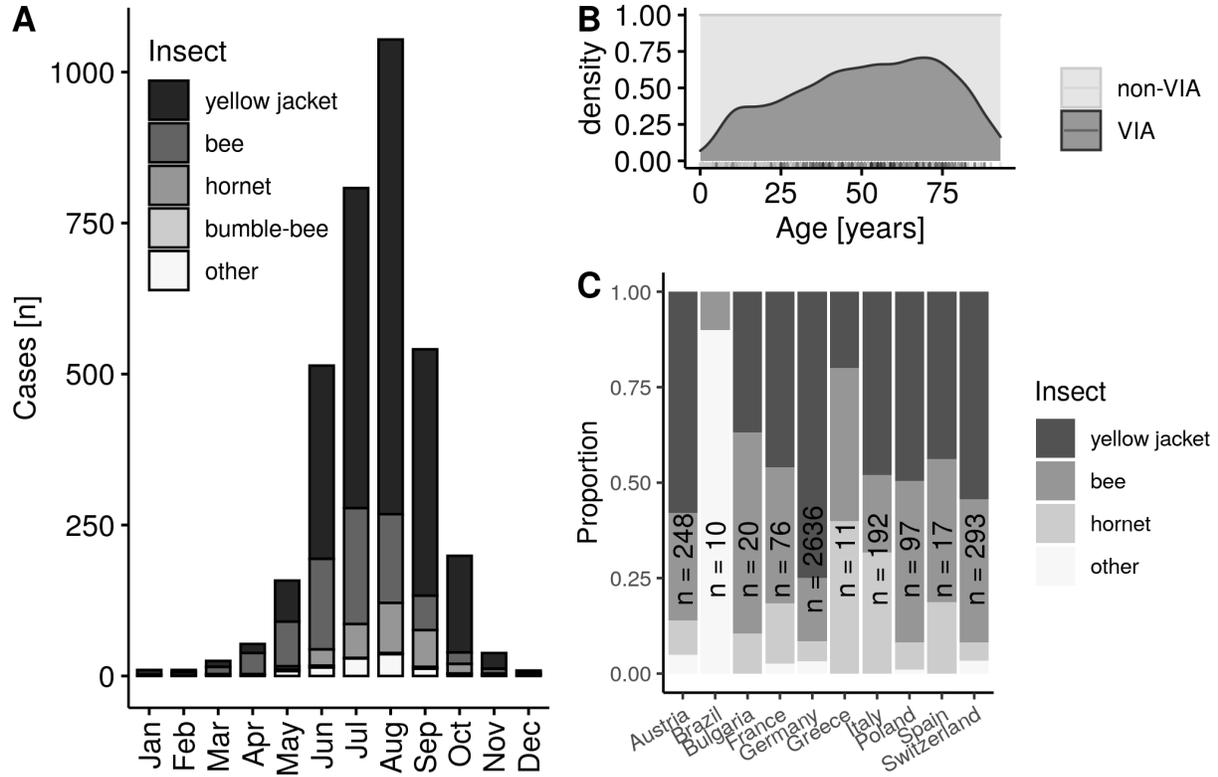
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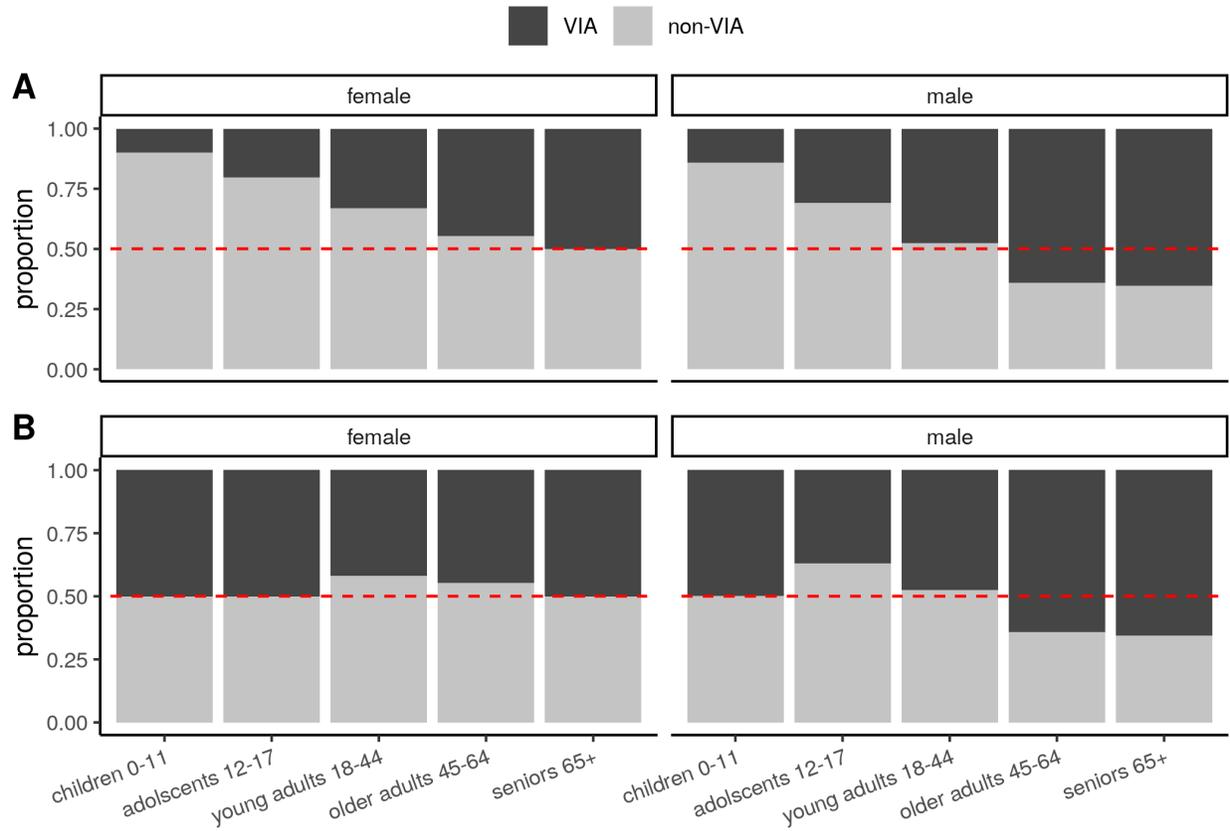


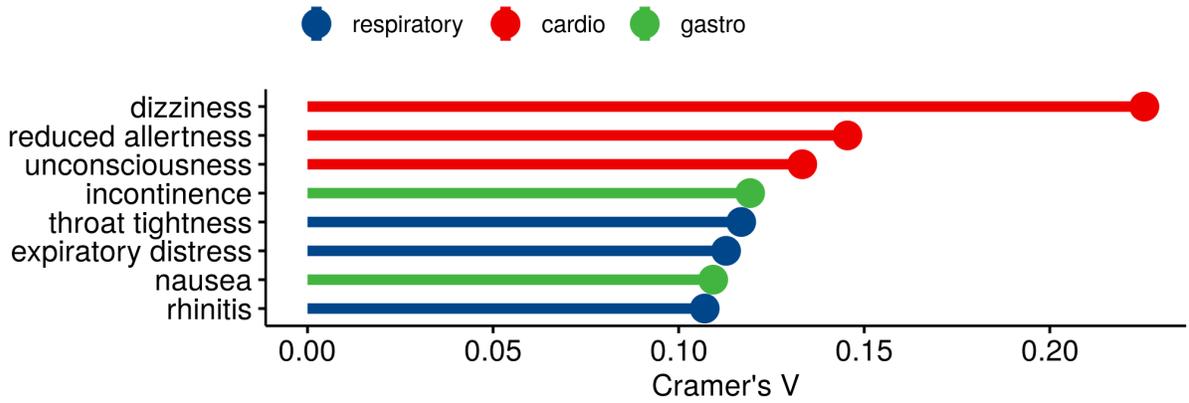
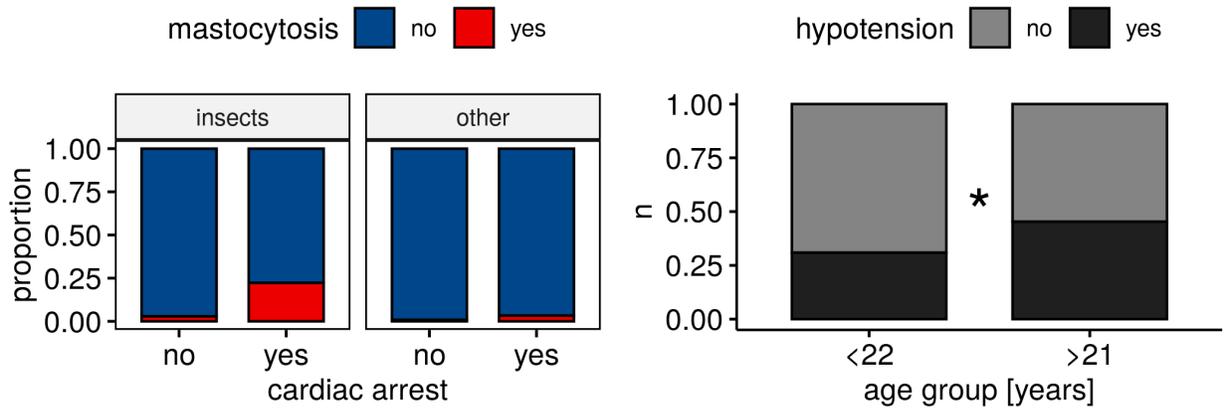




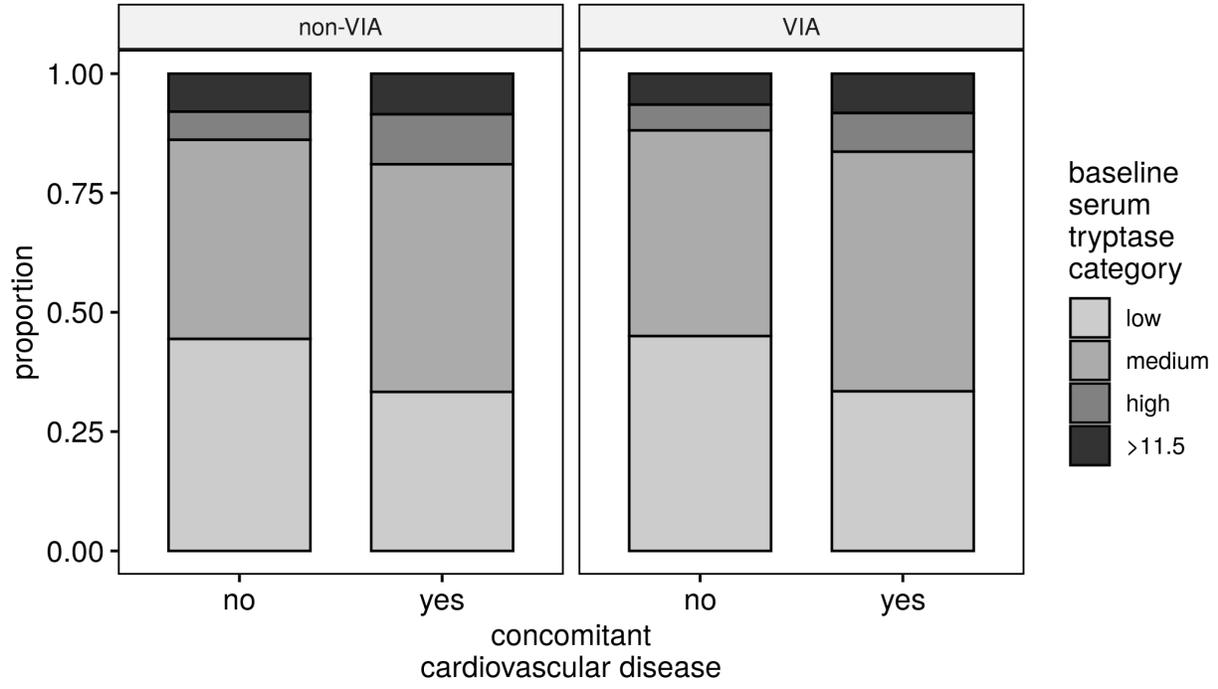


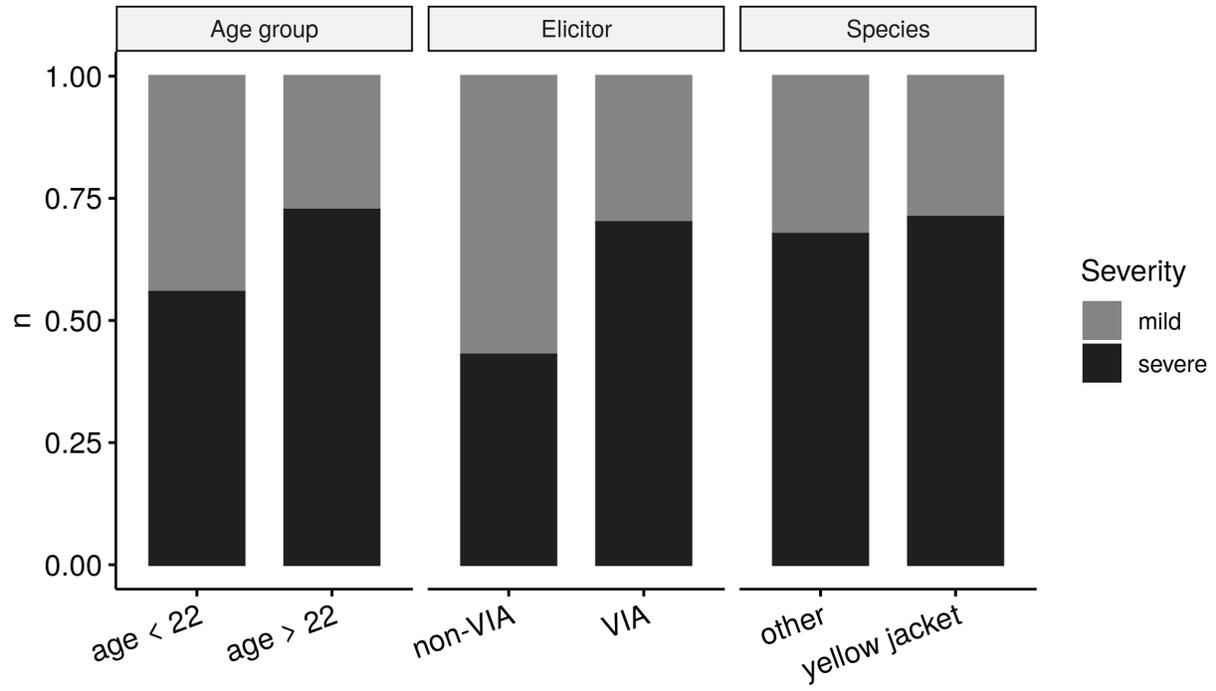


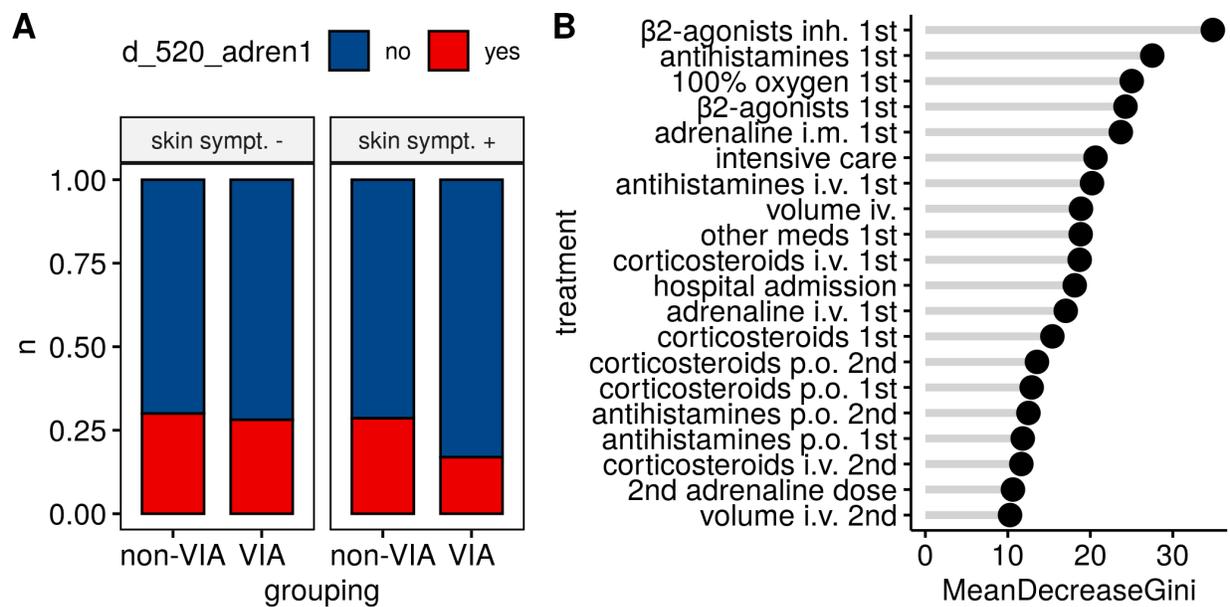


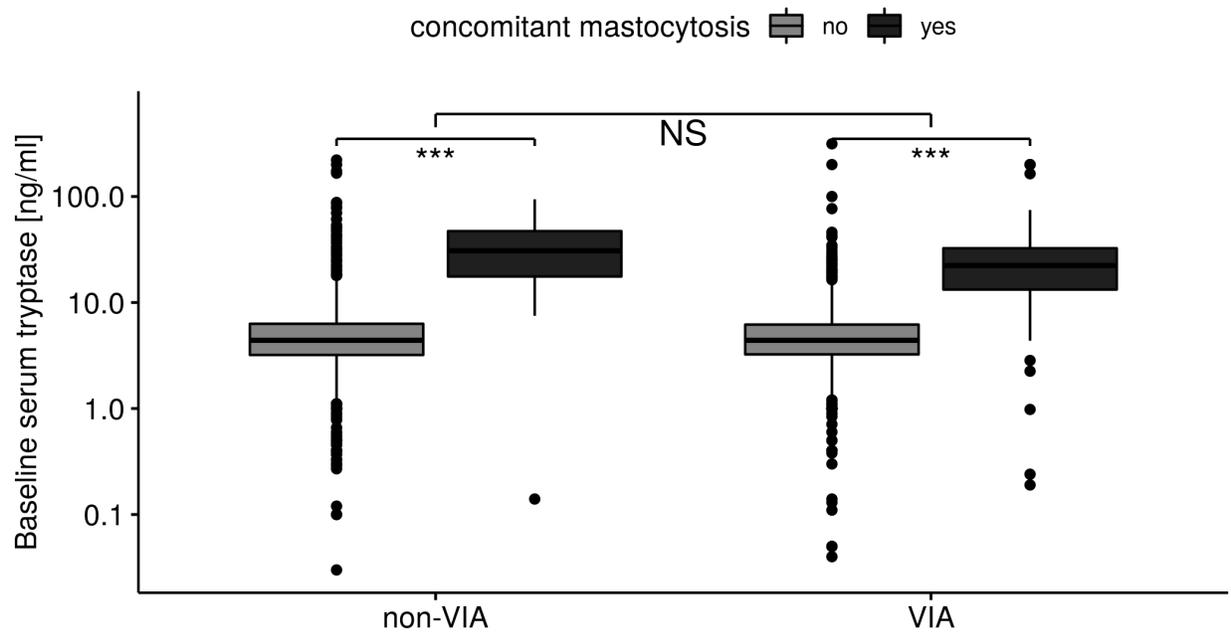


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Supplementary Figures (online-only material)

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 patient 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.

	<i>Dependent variable:</i> 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