Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: The importance of baseline serum tryptase†

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Background: Severe side effects during venom immunotherapy (VIT) are associated with a variety of risk factors.
Objective: Our aim was to evaluate the association of baseline serum tryptase concentration (BTC) and of other parameters, which are routinely recorded during patient evaluation, with the frequency of severe reactions requiring an emergency intervention during the buildup phase of VIT.
Methods: In this observational prospective multicenter study, we enrolled 680 patients with established honeybee or vespid venom allergy who underwent VIT. Data were collected on tryptase concentration, age, sex, culprit insect, cardiovascular medication, degree of preceding sting reaction, preventive antiallergic medication before therapy, time between last preceding sting reaction and VIT, venom specific IgE concentration, and type of buildup procedure. Relative rates were calculated with generalized additive models.
Results: Fifty-seven patients (8.4%) required an emergency intervention during buildup because of a severe systemic reaction. The frequency of interventions increased significantly with higher BTC (log-linear association; adjusted odds ratio, 1.56; 95% CI, 1.15-2.11; $P < .005$). The predictive power of BTC was markedly greater when VIT was performed for vespid venom allergy than for bee venom (for bee VIT, no significant association; for vespid VIT, log-linear association; adjusted odds ratio, 2.33; 95% CI, 1.28-4.26; $P = .005$). The most important other factor significantly associated with severe reactions during the buildup phase of VIT was bee venom allergy.
Conclusion: Before vespid VIT, measurement of baseline serum tryptase concentration should be used to identify patients with a high risk for side effects. Patients with bee venom allergy require a particularly high degree of surveillance during VIT.
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Key words: *Hymenoptera venom, allergy, venom immunotherapy, tryptase, risk factors, ACE inhibitor, age, sex*

During venom immunotherapy (VIT), severe systemic reactions may occur that involve respiratory and/or cardiovascular function requiring emergency interventions and possibly an early treatment stop. With the exception of honeybee venom allergy,[1-3] risk factors for such severe systemic side effects during the buildup phase of VIT are poorly defined. The recent prospective study of the Interest Group Insect Venom Allergy of the European Academy of Allergy and Clinical Immunology (EAACI) sought to identify independent predictors for a higher complication rate during VIT.[3] However, this study did not perform a separate analysis of those variables, which could have predicted side effects occurring exclusively during buildup. It was also not possible to analyze mild and severe systemic side effects separately, and no adjustments were made for the potentially confounding effect of specific variables such as baseline tryptase concentration (BTC).

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Abbreviations used
ACE: Angiotensin-converting enzyme
BTC: Baseline tryptase concentration
EAACI: European Academy of Allergy and Clinical Immunology
GAM: Generalized additive model
ln: Natural logarithm
ROC: Receiver operating characteristic
VIT: Venom immunotherapy

Baseline tryptase concentration is believed to represent the individual mast cell burden of a patient, and elevated concentrations were found to be associated with severe anaphylactic reactions after a field sting in patients with mastocytosis,[4] but also in unselected individuals.[5-10] It was the aim of the current prospective international multicenter study to determine the importance of BTC and of other suspected risk factors for severe reactions during the buildup phase of VIT, which was performed in unselected patients with Hymenoptera venom allergy. The first part of that study, which examined risk factors for severe anaphylactic reactions after a field sting, was published recently.[5]

METHODS

Study design

The Tryptase in Hymenoptera Venom Allergy study of the Interest Group on Insect Venom Hypersensitivity of the EAACI was a prospective observational cohort study performed in 13 European clinics specializing in the diagnosis and treatment of allergic diseases. The study consisted of several parts and evaluated patients with Hymenoptera venom allergy who were enrolled consecutively and prospectively. In part II of the study, we present data on a patient subgroup that underwent subsequent VIT. Design of the Tryptase in Hymenoptera Venom Allergy study, patient enrollment, diagnostic procedures, and characteristics of the core population have been published recently.[5] Additional information particularly relevant to the current study is presented in detail in this article's Online Repository (Methods section) at www.jacionline.org.

VIT

Indications and contraindications for VIT were made according to international guidelines.[11] Allergen immunotherapy was administered in a setting that allowed prompt recognition and treatment of anaphylaxis. Depending on the type of allergy, specific standardized allergen extracts of various manufacturers containing *Apis mellifera*, *Vespula* spp, *Vespa* spp, or *Polistes* venom were used for therapy (Pharmalgen, Alutard, or Aquagen from ALK-Abello, Hørsholm, Denmark; or venom extract from Anallergo, Florence, Italy; or VENOMENHAL from HAL Allergy, Leiden, Netherlands; or Venomil from Allergy Therapeutics, Worthing, United Kingdom; or Alyostal from Stallergenes, Antony Cedex, France). Allergen immunotherapy extract preparation (including dilution) and application were done by experienced and trained personnel. For all subjects, the maintenance dose was 100 μg. During the buildup phase of VIT, treating physicians were not aware of an individual patient's BTC.

Antiallergic premedication, discontinuation of antihypertensive medication before therapy, and the type of schedule used for the buildup phase of immunotherapy were left to the discretion of the treating study center. Minor modifications within each schedule were allowed. Diagnosis and management of side effects occurring during buildup followed established recommendations.[12]

Baseline and test variables

Besides age and sex, the severity grade (according to Ring and Meßmer[13]; see this article's Table E1 in the Online Repository at www.jacionlin.oerg) of the most severe sting reaction before VIT, the interval between the most recent sting reaction and VIT, the type of antihypertensive medication, which was continued throughout immunotherapy, and the insect specific IgE antibody status were evaluated. Furthermore, we collected information on the use of an antiallergic premedication (none or antihistamines/corticosteroids), schedule of buildup (conventional, rush, ultrarush; see this article's Table E2 in the Online Repository at www.jacionline.org), and type of venom used for therapy. We also recorded the nature of systemic side effects occurring during buildup and the frequency of emergency interventions provided during buildup. The test variable was the serum BTC.

Endpoint

The endpoint of the current analysis was an emergency intervention during the buildup phase of VIT. Emergency intervention included any type of measure or medication felt to be necessary by the treating physician to control a systemic side effect associated with VIT.

Statistics

Categorical variables were expressed as percentages. Selective comparisons between patient groups were made by the Fisher exact test for binary variables and by the Kruskal-Wallis test for continuous variables. Generalized additive models (GAMs) were estimated by using an R package.[14] Interactions between venom type and the other confounder variables were analyzed by Bayesian logistic regression models.[15,16] Maximum likelihood ratio tests were used to compare different models. Classification performance was described by receiver operating characteristic (ROC) curves. Details of the statistical methods are presented in the Online Repository (Methods section).

RESULTS

Clinical characteristics of patients who underwent immunotherapy

Six hundred eighty patients had VIT. The majority of patients who received immunotherapy were male (57.1%) and had vespid venom allergy (69.6%). Sixty-eight (10.0%) of the patients had a BTC >11.4 μg/L, and 18 (2.6%) >20 μg/L. In 89.0% of the patients, IgE antibodies specific to the causative venom could be detected in serum. Seventy-one patients (10.7%) had no specific IgE antibodies but demonstrated a skin test reaction to the venom administered during VIT. In 8 patients (1.3%) who had specific IgE antibodies, skin test reactions to the culprit venom were absent. In 4 patients, sensitization to the culprit venom was demonstrable only by cellular tests (basophil activation test).

Various types of antihypertensive therapies were continued throughout therapy in 10.0% of the patients. Of the patients, 2.2% remained on β-blocker therapy, and 2.6% on angiotensin-converting enzyme (ACE) inhibitor therapy. The remainder of the patients received calcium inhibitors (4.0%), diuretics (3.4%), angiotensin II receptor antagonists (2.8%), or other drugs (0.4%). A few patients were on 2 (n = 24), 3 (n = 5), or 4 (n = 1) antihypertensive drugs. Of the patients, 27.5% had had a grade III or IV reaction at the preceding index field sting. Before therapy, 24.9% of the patients received a prophylactic antiallergic therapy. During therapy, the venom dose was increased in a conventional way in 10.3% of the patients. A rush protocol was used in 55.0% of the patients, and an ultrarush protocol in 34.7%. An emergency intervention because of severe systemic side effects was necessary in 8.4% of the patients. Clinical characteristics of the patients receiving emergency intervention are presented in this article's Table E3 in the Online Repository at www.jacionline.org.

TABLE I. Distribution of the side effects during the buildup phase of VIT with respect to baseline parameters

Variable		Side effects during buildup phase: No (n = 623) n (%)	Yes (n = 57) n (%)
Preventive antiallergic medication before therapy	Yes	151 (89.3)	18 (10.7)
	No	472 (92.4)	39 (7.6)
β-Blocker medication during therapy	Yes	14 (93.3)	1 (6.7)
	No	609 (91.6)	56 (8.4)
ACE inhibitor medication during therapy	Yes	16 (88.9)	2 (11.1)
	No	607 (91.7)	55 (8.3)
Any antihypertensive medication during therapy	Yes	60 (88.2)	8 (11.8)
	No	563 (92.0)	49 (8.0)
Sex	Male	357 (92.0)	31 (8.0)
	Female	266 (91.1)	26 (8.9)
Highest degree of preceding index sting reaction	I or II	456 (92.5)	37 (7.5)
	III or IV	167 (89.3)	20 (10.7)
Type of venom	Honeybee	174 (84.1)	33 (15.9)
	Vespid	449 (94.9)	24 (5.1)
Type of dose increase during therapy	Conventional	67 (95.7)	3 (4.3)
	Rush	347 (92.8)	27 (7.2)
	Ultrarush	209 (88.6)	27 (11.4)
Age (y) at therapy according to median	<41	301 (91.5)	28 (8.5)
	≥41	322 (91.8)	29 (8.2)
Interval (mo) between last sting reaction and VIT according to first sextile	<2	57 (95.0)	3 (5.0)
	≥2	566 (91.3)	54 (8.7)
Venom-specific IgE	Absent	74 (98.7)	1 (1.3)
	Present	549 (90.7)	56 (9.3)

Associations are shown between clinical, demographic, and therapeutic parameters and the need for an emergency intervention. Percentages indicate the frequency of an emergency intervention among the total number of patients presenting with a specific parameter value.

Risk factors for an emergency intervention during immunotherapy

Emergency interventions had to be performed more often in patients receiving a honeybee VIT or having a positive finding on venom-specific IgE measurements (Table I). There was also a tendency for more interventions in patients who were treated according to a rush or ultrarush protocol.

A random study center effect could not be identified. Corresponding estimates of variation yielded a value of 0. Consequently, the final GAM was not adjusted for such an effect. After adjustment for the other confounders, we observed a significant log-linear association between BTC and the need for an emergency intervention during therapy (Table II). Other significant independent predictors for an emergency intervention were therapy with honeybee venom, younger age (linear association), the type of dose increase (ultrarush, rush) during therapy, a long interval between the most recent sting reaction and VIT (log-linear association), an accompanying antihypertensive therapy, and the presence of venom-specific IgE in serum. Two other confounder variables (grade III or IV reaction after the preceding index sting, female sex) were also retained in the final model. However, they were of minor importance with respect to risk prediction because their P value was clearly above .05. Finally, antiallergic pretreatment and medication with β-blockers or ACE inhibitors were not selected for the final model by the algorithm on the basis of the Akaike information criterion, indicating the prognostic unimportance of these variables.

TABLE II. Results of the final generalized additive model for the risk to need an emergency intervention during buildup phase of immunotherapy

Variable	*P* value	Odds ratio	95% CI	
Therapy with honeybee venom	<.001	3.60	2.36	5.50
Presence of venom specific IgE in serum	.013	6.243	1.435	27.159
Index sting reaction grade III or IV	.149	1.421	0.874	2.310
Female sex	.157	1.349	0.883	2.061
Any antihypertensive medication during therapy	.032	2.144	1.051	4.374
Age at therapy (per year)	.034	0.984	0.970	0.999
Type of dose increase during therapy: conventional	.044	0.397	0.158	0.995
Type of dose increase during therapy: ultrarush	.008	1.787	1.153	2.770
Interval between most recent sting reaction and VIT*	.039	1.199	1.006	1.429
BTC at first office visit†	.004	1.556	1.149	2.108

Those variables are shown that were selected according to the modeling procedure.
Index sting, A field sting that caused the most severe reaction before the first office visit.
*After a logarithmic transformation. *Odds ratio*, a rise of the natural logarithm of interval (mo) by 1.
†After a logarithmic transformation. *Odds ratio*, a rise of the natural logarithm of tryptase concentration by 1.

There was evidence that the effect of predictive variables (including tryptase concentration) varied between vespid and honeybee VIT. When including all interactions between venom type and different predictive variables into an extended model, the specific association between venom type and BTC was almost significant ($P = .066$). According to the maximum likelihood ratio test, the combined effect of all predictive variables depended significantly on the venom type. The extended model, which considered corresponding interactions, differed significantly from a model, which did not include the interactions ($P = .030$). Further analysis showed that there was a significant association between risk and BTC (after logarithmic transformation) for vespid VIT patients (odds ratio, 2.337; 95% CI, 1.279-4.260; $P = .005$; Fig 1), whereas no significant BTC effect was estimated for patients who were treated for honeybee venom allergy ($P = .785$).

According to these associations, it can be estimated for the whole cohort that, regardless of the actual level, in a patient with a BTC that is 2-fold higher than in another patient with otherwise identical risk factors, the risk for an emergency intervention will simultaneously increase by a factor of approximately 1.36, whereas in patients receiving vespid VIT, this factor will be 1.80.

We also examined possible interactions between IgE status and BTC or type of venom. There was no evidence that BTC depended on IgE status. Patients with bee venom allergy in whom IgE was absent appeared to have a lower BTC (median BTC, 3.27 ng/mL; 25% quartile, 2.50; 75% quartile, 5.74) than patients with IgE concentrations above the detection limit (median BTC, 4.46; 25% quartile, 3.09; 75% quartile, 6.91). A slight difference was also found for patients allergic to vespid venom (IgE negative, median BTC, 4.22 ng/mL; 25% quartile, 3.14; 75% quartile, 6.03; IgE positive, median BTC, 4.30; 25% quartile, 2.53; 75% quartile, 6.23). However, the groups were not significantly different for BTC ($P = .230$; Kruskal-Wallis test).

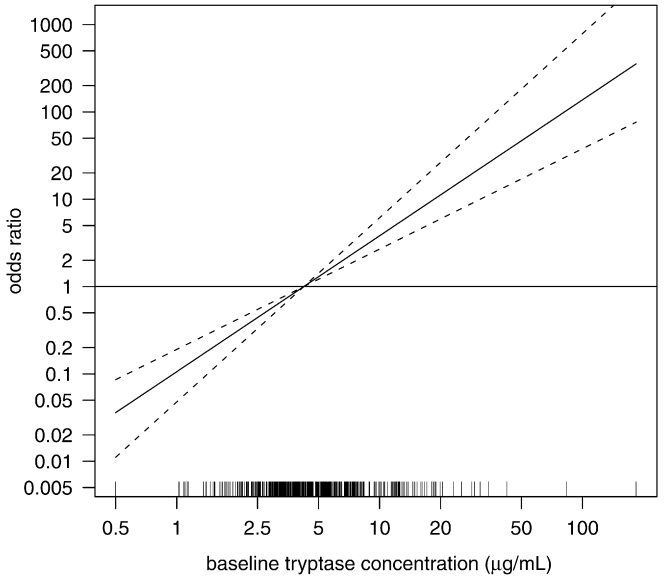

FIG 1. Linear function and 95% confidence band *(dashed lines)* for the effect of BTC on the risk to need an emergency intervention during the buildup phase of vespid immunotherapy (final multivariate generalized additive model). Odds ratios are referred to those of the median of tryptase concentration. The odds ratio of the latter has been set at 1.

There was some evidence that IgE status correlated with the type of venom. Among patients without venom-specific IgE, 78.7% had vespid venom allergy, whereas in patients with venom-specific IgE, the corresponding frequency was lower (68.4%; $P = .069$; Fisher's exact test). However, the number of patients who required an emergency intervention and were also negative for IgE was too small (1 patient among those with vespid venom allergy and no patient among those with bee venom allergy) to allow estimation of interaction effects among IgE status, venom type, and BTC.

Risk prediction for requirement of an emergency intervention during vespid VIT

According to our data analysis, tryptase cutoff concentrations depend on confounders (eg, IgE status, type of insect) when predicting an emergency intervention in patients with vespid venom allergy. Therefore, we used the venom-specific final multivariate model, which included all confounders, for risk prediction in this subgroup. This model was able to distinguish a vespid VIT without side effects from that requiring an emergency intervention with a classification performance of area under the curve of 0.79. On an individual basis, the following procedure, based on the venom-specific final model, can be used to calculate a patient's risk score and to predict the risk of requiring an emergency intervention:

$$\begin{aligned}\text{score} = {} & -5.96 + 1.04 \cdot x_{\text{IgE}} + 0.27 \cdot x_{\text{Sting}} \\ & + 0.94 \cdot x_{\text{Hypertens}} - 0.022 \cdot x_{\text{Age}} + 1.19 \cdot x_{\text{Female}} \\ & - 0.13 \cdot x_{\text{Severity}} - 0.36 \cdot x_{\text{Dosage:conventional}} \\ & + 0.93 \cdot x_{\text{Dosage:UltraRush}} + 0.85 \cdot x_{\text{BTC,}}\end{aligned}$$

with

- x_{IgE}: no detection of IgE = 0, detection of IgE = 1
- x_{Sting}: natural logarithm (ln; interval [months] between last sting reaction and VIT)
- $x_{\text{Hypertens}}$: no antihypertensive medication during VIT = 0, antihypertensive medication during VIT = 1
- x_{Age}: age (years)
- x_{Female}: male sex = 0, female sex = 1
- x_{Severity}: highest degree of preceding index sting reaction I or II = 0, highest degree of preceding index sting reaction II or IV = 1
- $x_{\text{Dosage: conventional}}$: type of dose increase not conventional = 0, type of dose increase conventional = 1
- $x_{\text{Dosage: ultra-rush}}$: type of dose increase not ultrarush = 0, type of dose increase ultrarush = 1
- x_{BTC}: ln (BTC, in μg/L).

According to the score value, a specific predicted risk could be attributed to each patient. With regard to sensitivity, the following cutoff values for the predicted risk score could be derived from the ROC of the vespid venom–specific final multivariate model: sensitivity ≈ 80 %: –2.82; sensitivity ≈ 90 %: –3.40; sensitivity ≈ 95 %: –3.92. However, corresponding specificity was consistently low (55%, 40%, and 25%).

DISCUSSION

Our study is the first to evaluate the importance of BTC in the serum and of a variety of other suspected risk factors for severe systemic reactions during the buildup phase of VIT. When risk factors are studied for systemic reactions during VIT, a bias may occur because early pharmacologic interventions can artificially reduce the true degree of severe systemic reactions. Therefore, we decided not to use the actual degree of a systemic reaction as the endpoint of our study, but rather the requirement for an unplanned intervention to control such a VIT-related reaction. We found that emergency interventions during buildup were necessary in 8.4% of the patients.

There are only 3 other large epidemiologic studies to which the results of our study may be compared.[1,3,17] Albeit of a subjective nature, the frequency of emergency interventions in our study corresponds closely to that reported by Lockey et al,[1] indicating a reproducible judgment of potentially vital threats during VIT. Several epidemiologic findings in our cohort are also in line with findings from the other studies such as the predominance of male subjects[3] or of those with vespid venom allergy.[1,3,17] In some studies, however, the criteria for defining systemic side effects may have been different from ours, and the reported frequencies of systemic side effects during the buildup phase of VIT were clearly higher (20%[3] or 21.2%[17]).

The key finding of our study is that, for the whole cohort, BTC correlates significantly with the frequency of severe side effects during the buildup phase of VIT. These results expand our recent observations in which we found a comparable, independent association between tryptase concentration and the severity of severe allergic reactions after a field sting.[5]

However, in contrast with reactions after a field sting, the association between BTC and the severity of a secondary systemic reaction during VIT depended on the type of venom and was evident only in patients receiving vespid venom. For patients receiving vespid venom, it could be calculated that a rise of BTC from a value of 4.21 μg/L (median of the whole cohort) to 20 μg/L will simultaneously increase the risk for an emergency intervention by a factor of approximately 3.75 (with all other risk factors remaining the same). The association

between BTC and severe side effects during vespid VIT was of such a dimension that even for the whole cohort (after including patients receiving honeybee VIT), corresponding associations could still be identified.

Because 2.6% of our patients had a BTC >20 μg/L, it is highly likely that a small number of our patients had systemic mastocytosis or monoclonal mast cell activation syndrome.[18,19] Presumably, the true proportion of patients with mastocytosis was even somewhat higher because an apparently normal BTC does not exclude mastocytosis in all patients with insect venom allergy.[20] Unfortunately, we were unable to include a secondary work-up for mastocytosis into the study.

The findings of the current study provide further evidence for the concept that the individual mast cell burden is an important predictor for the severity of secondary reactions after venom exposure.[21] The importance of that concept is also emphasized by the particularly high risk for VIT-associated severe side effects commonly observed in patients with mastocytosis. In patients with mastocytosis, the frequency of systemic side effects during VIT averaged about 24%, and 7.6% of the patients with mastocytosis needed adrenaline to control severe reactions.[22]

Our results suggest that, besides being an independent prognostic variable, even a low elevation of BTC within the 95th percentile may be relevant for the severity of a systemic reaction during vespid VIT. We therefore recommend measuring BTC especially in patients who undergo vespid VIT. Furthermore, BTC affected the risk for a systemic allergic reaction to vespid VIT at all tryptase concentrations, predicting an increasing risk as values increase from the lower limit of detection (1 μg/L), irrespective of whether BTC was within the normal range for healthy subjects or above that range. Fig 1 reveals that the slope of the odds ratio graph is linear. Therefore, any BTC above the lower detection limit should be part of the decision-making process about whether to provide a particularly intensive surveillance to an individual patient during vespid VIT. However, individual prediction of an emergency intervention during the buildup phase of VIT requires consideration of other confounders such as venom-specific IgE or type of dose increase. Consequently, only cutoff values from the vespid venom final multivariate model should be used to predict an individual patient's risk.

Several other important conclusions may be derived from our results. In accordance with numerous other studies,[1-3,17] bee venom allergy was an independent predictor for a higher risk during VIT. However, this finding is remarkable because we recently found that before VIT, vespid venom allergy was an independent predictor for a higher risk of a severe systemic reaction after a field sting.[5] A possible explanation for this discrepancy may be found in the immunologic mechanisms, which may differ fundamentally depending on whether they are involved in secondary allergic reactions after a field sting or during VIT.

Several observations suggest highly specific immunologic reactions during the buildup phase of VIT. First, the association of BTC with anaphylactic reactions was nonlinear on the log scale when systemic reactions after a field sting before VIT were examined,[5] but it was log-linear with respect to immunotherapy in the present study. Furthermore, in contrast to reactions during the buildup phase of VIT, there was no evidence that the predictive power of BTC depended on the insect species when field sting reactions (before therapy) were analyzed in the population of the current study (see the Online Repository, Results section). Second, we and others[1,3] found that the severity of the preceding field sting reaction did not correlate with the extent of side effects during VIT. Third, risk factors for severe adverse advents during VIT were not those associated with severe field sting reactions. Thus, in accordance with other authors, ACE inhibitor medication[23] and sex appeared to be less important during VIT. Finally, others showed that severe side effects during VIT were fairly equally distributed across the whole dose range when vespid venom was injected, whereas there was a cluster of side effects in the 1-μg to 30-μg dose range during the buildup phase of bee VIT.[1]

A possible clue to the mechanisms causing anaphylaxis during VIT may be found in the schedules used for the buildup phase and in their relation to the amount of venom that would be delivered by an insect field sting. During a honeybee field sting, up to 150 μg (average, 59 μg) venom is presumably emitted, whereas most vespid species inject much less venom (1.7-5 μg; *Polistes*, 3-17 μg[24]). On the other hand, irrespective of the type of buildup or of insect venom, we applied the same starting and maintenance dose during the buildup phase. Therefore, compared with vespid VIT, subjects receiving honeybee venom were exposed to a significantly higher number of injections, which provided subclinical amounts of venom. These comparatively small amounts of antigens may be associated with proallergic reactions occurring within the regulatory mechanisms responsible for mast cell activation via the high-affinity IgE receptor.[25,26]

An alternative although less likely explanation for less severe reactions during vespid VIT may be found in the procedures used to obtain venom for medical purposes. Bee venom is collected by electrostimulation from living insects, whereas vespid venom is obtained by dissection of the venom sac. Corresponding vespid venom extracts contain body proteins including proteases that can degrade vespid venom allergens, thereby making them potentially less allergenic.[2]

Another interesting finding of our study was that the individual type of schedule used for buildup correlated significantly with the frequency of side effects during that phase of therapy. Compared with a rush schedule, an ultrarush schedule increased the risk to require an emergency intervention during buildup by a factor of 1.8. These results are in line with findings from the recent prospective EAACI study, in which a rapid dose increase was also an independent predictor for a higher complication rate during VIT.[3] On the other hand, previous retrospective, single-center studies that compared different types of rush VIT could not identify an increased risk associated with ultrarush schedules.[17,27] However, the clearly superior study design makes it much more likely that the results of the current study and of the older EAACI study reflect the true therapeutic risk. Therefore, we strongly suggest carefully weighing the pros and cons of an ultrarush therapy and providing particular surveillance to those patients actually receiving that type of buildup.

Three other aspects of our results deserve a specific comment. When analyzing the use of an antihypertensive medication throughout VIT, we found a significant association with the need for an emergency intervention. No specific drug could be identified as being associated with the frequency of severe side effects. Because of small numbers, however, associations between single classes of antihypertensive drugs and side effects during buildup cannot be excluded entirely. It can be expected that medication was continued only during buildup in those patients who had severe hypertension. The latter is known to be associated with severe cardiac diseases (myocardial insufficiency, arrhythmias) and decreased efficiency for hemodynamic compensation, thereby possibly explaining the association between medication

and the severity of side effects during VIT. On the other hand, we cannot exclude a certain therapeutic bias because medication status was known to the physician in charge and could have affected the decision to start an emergency therapy. Because severe hypertension requires therapy, it is also questionable whether a discontinuation of such drugs would have reduced the anaphylactic risk during VIT or whether, in fact, cardiovascular risk would have even increased.[28]

We found no evidence that an antiallergic premedication reduced the frequency of severe side effects during buildup. However, the precise relevance of this observation is uncertain because we could analyze neither the role of specific drugs nor protective effects in specific patient subgroups. It cannot be excluded that, for example, antihistamines may be of some value because protective effects (fewer side effects affecting the skin) have been described in patients receiving VIT for a honeybee venom allergy.[29,30]

Finally, there was also a significant association between the frequency of severe side effects and 2 further variables: the time that had passed since the most recent anaphylactic reaction, and the presence of venom-specific IgE antibodies in serum. On the basis of challenge tests with live insects in patients not treated with VIT, an association between a positive IgE titer and the frequency of severe anaphylactic reactions has been suggested before.[31,32] On the other hand, in observational studies examining ultrarush VIT, IgE levels did not correlate with the severity of side effects.[33,34] These negative findings, however, which were retrospective in nature, cannot be compared to the results of our study because of significant differences in patient selection, study design, definition of side effects, and classification of IgE levels. In patients with other allergies such as food or natural rubber latex allergy, IgE levels have been found repeatedly to correlate with the severity of symptoms after a corresponding allergen exposure or challenge, emphasizing the uniform importance of that immunologic variable for anaphylactic reactions.[35-37]

Besides IgE, antigen-specific IgG might be important for the frequency of severe side effects during VIT. Surprisingly, we found that such side effects were significantly less common early after the last field sting reaction. The mechanisms behind this association are speculative. An exposure to the elicitor is known to cause a temporary rise of antigen-specific IgG lasting for several weeks.[38] There is evidence that specific IgG can block IgE-mediated anaphylaxis. This mechanism is particularly important in situations in which antigen levels are insufficient to induce IgG-mediated anaphylaxis. With low antigen doses, as are used during buildup, pre-existing high levels of IgG antibodies were found to prevent the development of any anaphylactic response.[39]

In summary, in patients undergoing VIT, even minor elevations of BTC are associated with more frequent severe systemic reactions during buildup, independent of other prognostic variables. Especially in patients who are allergic to vespid venom, the need for emergency interventions during immunotherapy correlates closely with BTC. Future research that clarifies the mechanisms related to venom-specific side effects during VIT is needed.

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Clinical implications: Systemic side effects of vespid VIT are associated with an elevated BTC.

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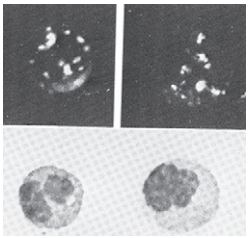
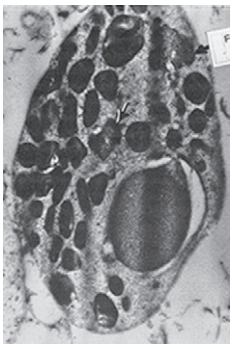


Photomicrograph of peritoneal exudate eosinophils of guinea pig after administration of fluorescein-labeled BSA-antibody complexes showing fluorescence of intracellular soluble immune complexes in cells of upper row corresponding to stained cells in bottom row (reproduced with permission[1]).



Photomicrograph of rabbit lung eosinophil showing phagocytized ferritin-antibody complex (reproduced with permission[2]).

ALLERGY ARCHIVES

THE EOSINOPHIL, A HISTORICAL RETROSPECTIVE: ANTIGEN-ANTIBODY COMPLEXES

To explain the development of eosinophil infiltrations in guinea pig peritoneum after passive sensitization and challenge, Litt suggested an eosinotactic function for immune complexes. In a follow-up investigation with fluorescence microscopy, Litt demonstrated actual uptake of labeled Bovine Serum Albumin-antibody complexes in guinea pig peritoneal exudate.[1] At Massachusetts General Hospital, Sabesin by election microscopy displayed eosinophil phagocytosis of ferritin-antibody complexes in rabbit lung.[2]

In the experimental model of eosinophil infiltrations into politeal lymph nodes induced by reverse passive sensitization and antigen challenge in rabbit foot pads,[3] Cohen and associates studied qualities of immune complexes. Their investigations demonstrated identical eosinophil responses to varying species origin of antisera and sources of protein antigens, soluble antigen-antibody complexes, aggregated and denatured γ-globulins, and number rather than size of immune complexes. Eosinophilia induced by high-molecular-weight polysaccharides and protein-coated polystyrene latex particles favored the concept that antigens simulating the critical physiochemical character of immune complexes could engender immediate eosinophil responses without the immediate formation of antibody.

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METHODS

Study design

The prospective data collection was approved by the institutional review board of each participating center, and each patient or patient's parent consented to an anonymous data analysis.

Enrollment

Eligible patients were consecutively enrolled in the study in each of the participating sites beginning in May 2001; enrollment was complete by August 2003. The study was terminated at the end of 2009 after completion of the follow-up examinations. Eligible patients were those who had experienced a systemic reaction after a preceding field sting, who had a hitherto untreated Hymenoptera venom allergy, and who consented to VIT. Patients who had allergy to both bee and vespid venom were not included in the analysis, nor were patients in whom it was not possible to attribute clinical symptoms to a specific insect unequivocally, because the risk for severe allergic reactions may depend on, and vary with, the culprit insect.

Data collection, diagnosis, and procedures

After enrollment, standardized data collection forms were completed at the sites to provide information about the patient's history, the results of testing, and adverse events during immunotherapy. The Medical Advisory Committee of the study (B. Biló, J. Birnbaum, F. Bonifazi, P. Ewan, M. Jutel, H. Oude Elberink, H. Mosbech, U. Müller, B. Przybilla, F. Ruëff) approved the final version of the forms (developed by F. Ruëff and B. Przybilla). Among a variety of other variables, we recorded the hitherto most severe systemic reaction after a field sting and the interval between the last systemic reaction after a field sting and VIT. Classification of systemic reactions corresponded to that proposed by Ring and Meßmer (Table E1).[E1] In addition, a blood sample was taken at the time of the first patient visit to determine baseline serum tryptase concentration and concentrations of insect specific IgE antibodies. At least 14 days had to have passed between blood sampling and the time of the last allergic reaction. Because sponsoring existed only for data entry and not for further expensive diagnostic work-up (bone marrow biopsy, dermatological examination by a specialist) and because corresponding costs would not have been covered by several national health insurance systems, a secondary work up for mastocytosis was not part of the study.

Serum BTC was determined in blood samples obtained at least 14 days after the last systemic allergic reaction. Serum was frozen at –20°C, and samples were shipped on dry ice to Phadia (Freiburg, Germany) for collective measurements. Serum tryptase was measured by ImmunoCAP Tryptase. According to the manufacturer, the interassay variability for tryptase levels between <1 and 100 μg/L is < 5%. The upper 95th percentile for healthy individuals without allergy was 11.4 μg/L.

Venom-specific serum IgE was measured with the ImmunoCAP method (Phadia, Uppsala, Sweden) or by using the Allergopharma Specific IgE RV-system (Allergopharma, Reinbek, Germany). Because of a variable, assay-dependent precision of IgE measurement and because of a potential insect dependency of IgE concentration, no attempts were made to classify IgE concentration according to assay-specific scoring systems. Rather, we labeled patients as IgE-positive or IgE-negative depending on whether venom-specific IgE could be detected.

Diagnosis of Hymenoptera venom allergy followed specific guidelines published by the EAACI and the American Academy of Allergy, Asthma & Immunology.[E2-E4] A skin prick test was read positive at a wheal diameter of 3 mm at a venom concentration of 100 μg/mL or less; the intradermal test was positive at a wheal diameter of 5 mm at a concentration of 1 μg/mL or less. Allergy was diagnosed if a patient presented with a conclusive history including entomologic identification and a corresponding venom sensitization (according to an identification of venom-specific serum IgE and/or a positive skin test reaction to venom). Patients with vespid genus allergy (*Vespula*, *Vespa*, or *Polistes*) were combined into 1 group.

Patients who had no specific IgE antibodies and/or positive skin test reaction to the presumed culprit venom were in addition examined by basophil activation test after stimulation with honeybee and vespid venom. The procedure of the test and the interpretation of the results are presented elsewhere.[E5]

Data accuracy

Accuracy of data in the forms was ensured by the specific qualification of research staff. Institutional supervisors monitored local data entries by looking over 5% to 10% of the forms. All patients were assigned a unique code during a single hospital visit, and forms were transmitted to a central depository without identification of the patient. Each patient form was checked for completeness and plausibility by members of the steering committee. Oversight of data collection and analysis, integrity of the data, and research were provided by the EAACI Interest Group.

Statistical methods

Covariate-adjusted effects of BTC on the requirement for an emergency intervention during therapy were evaluated by multiple logistic regression models, which combined separate effects of all individual confounding variables (GAMs). For the dependent variable, we first defined a separate starting model, which contained only the independent variables age and sex. To construct the preliminary confounder model for emergency interventions during therapy, we then analyzed the following variables: type of causative insect, medication with β-blockers, ACE inhibitors or any kind of antihypertensive drug, antiallergic pretreatment (corticosteroids and/or antihistamines), degree of the systemic reaction at field sting (I/II or III/IV according to Ring and Meßmer[E1]; Table E1), time between the last systemic reaction after a field sting and VIT, presence or absence of venom-specific IgE in plasma, and type of protocol used for dose increase (conventional, rush, ultrarush; Table E2). Model selection for a multiple logistic additive model was performed by stepwise selection based on the Akaike information criterion.[E6,E7] To test the tryptase effect, we then added the variable BTC to this preliminary GAM, thereby creating the final GAM with a nonparametric effect for tryptase. If appropriate, the nonparametric tryptase term was simplified to a log-linear term. A random effect was used to adjust for study center.

To examine whether the effect of confounder variables including BTC also depends on the type of venom, we tested interactions between venom type and the other confounder variables. For that purpose, we used Bayesian logistic regression models to stabilize estimation of the (interaction) parameters. However, these models could not account for study center effects.[E8,E9] For the dependent variable, we constructed an extended final GAM, which also included the individual interaction terms. This extended model allowed, on the one hand, an estimation of baseline effects in patients who received bee VIT or had bee venom allergy (when analyzing severe reactions after a field sting before therapy) and, on the other hand, an estimation of the modifying effects of vespid VIT or vespid venom allergy (field sting reaction).

To analyze the combined effect of all interaction terms, and to compare final generalized additive models, which did or did not include the interactions, a maximum likelihood ratio test was used.[E10] Furthermore, to evaluate venom specific effects during immunotherapy, an alternative final generalized additive model was generated by which we could estimate effects of all variables separately depending on venom type.

The performance of the final model, which had been obtained for patients with vespid venom allergy, was evaluated by using ROC analysis. On the basis of this final model, we derived an equation for a patient-specific score predicting the risk for an emergency therapy during the buildup phase of vespid VIT.

RESULTS

Association of BTC with severe reactions after a field sting in patients subsequently undergoing VIT

The current population was a subpopulation of a larger core population, in which we had examined risk factors (including BTC) for severe reactions after a field sting. For the cohort of the current study, we also performed an analysis regarding the association of BTC with severe reactions after a field sting (before VIT). By using data from that previous publication,[E11]

we also found for the current subgroup that BTC was equally important to predict a severe anaphylactic reaction after a field sting in patients with vespid or honeybee venom allergy.

For the current subgroup, we used the published final model (limited model) derived to predict the risk for severe systemic sting reactions (grade III or IV) after a field sting.[E11] According to that limited model, independent risk factors were a nonlinear, continuous term for ln(BTC), vespid venom allergy, 1 or more preceding less severe systemic sting reactions before the index sting, male sex, and therapy with ACE inhibitors. We then constructed an extended model that contained, with respect to the culprit insect and in addition to the other predictors, a nonlinear effect modifier for the nonlinear continuous term for ln(BTC). The fits of the 2 models were virtually indistinguishable (log-likelihoods of the extended model including the effect modifier, –338.6 (approximate degrees of freedom, 9.3); log-likelihoods for the limited model without the effect modifier, –339.0 (approximate degrees of freedom, 8.12); likelihood ratio test, $P = .874$), and neither of the estimated nonlinear terms could be reduced to a log-linear term. On the basis of these findings, there was no evidence that the association between BTC and the frequency of severe systemic reactions after a field sting (before VIT) depended on the type of venom.

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TABLE E1. Classification of most severe systemic reactions at index field sting modified according to Ring and Meßmer[E1]

Classification	Symptoms
Grade I	Generalized skin symptoms (eg, flush, generalized urticaria, angioedema)
Grade II	Mild to moderate pulmonary, cardiovascular, and/or gastrointestinal symptoms
Grade III	Anaphylactic shock, loss of consciousness
Grade IV	Cardiac arrest, apnea

TABLE E2. Schedules and venom doses (μg) for the buildup phase of VIT

Rush		Ultrarush		Conventional	
Day	**Dose**	**Day**	**Dose**	**Week**	**Dose**
1	0.02	1	0.1	1	0.02
	0.04		1	2	0.04
	0.08		10	3	0.08
	0.2		20	4	0.2
2	0.4		30	5	0.4
	0.8		40	6	0.8
	2	15	50	7	2
	4		50	8	4
3	8	45	100	9	8
	10			10	10
	20			11	20
	40			12	40
4	60			13	60
	80			14	80
	100			15	100
Total no. of injections	15		9		15
Cumulative dose (μg)	325.5		301.1		325.5

TABLE E3. Clinical characteristics of patients requiring an emergency treatment during the buildup phase of VIT

Patient #	Age (y)	Sex	Venom for treatment	BTC (μg/L)	Sting reaction*	Specific IgE†	Antihypertensive medication	Buildup phase
1	48	M	BV	19.80	III or IV	+	0	Rush
2	52	M	BV	4.50	I or II	+	0	Rush
3	17	M	BV	21.90	I or II	+	0	Rush
4	52	M	BV	29.70	III or IV	+	Angiotensin II receptor antagonist	Rush
5	21	F	BV	<1.0	I or II	+	0	Ultrarush
6	59	M	BV	1.80	I or II	+	0	Rush
7	32	M	BV	3.15	I or II	+	0	Rush
8	46	F	BV	3.53	III or IV	+	0	Ultrarush
9	14	F	BV	3.72	I or II	+	0	Ultrarush
10	46	F	BV	6.07	I or II	+	0	Ultrarush
11	73	M	BV	6.72	III or IV	+	0	Ultrarush
12	52	M	BV	7.10	I or II	+	0	Ultrarush
13	42	F	BV	14.00	III or IV	+	0	Rush
14	44	M	BV	<1.0	III or IV	+	0	Rush
15	4	M	BV	1.38	I or II	+	0	Rush
16	50	M	BV	1.43	I or II	+	Calcium antagonist	Rush
17	44	F	BV	3.64	I or II	+	0	Ultrarush
18	45	F	BV	3.95	III or IV	+	0	Ultrarush
19	53	F	BV	4.20	III or IV	+	0	Ultrarush
20	49	M	BV	4.52	III or IV	+	ACE inhibitor, β-blocker	Ultrarush
21	20	F	BV	9.14	I or II	+	0	Rush
22	46	M	BV	3.23	I or II	+	0	Ultrarush
23	11	M	BV	4.26	I or II	+	0	Rush
24	33	M	BV	4.46	I or II	+	0	Rush
25	13	M	BV	6.64	III or IV	+	0	Ultrarush
26	38	M	BV	9.38	III or IV	+	0	Rush
27	76	M	BV	9.91	III or IV	+	Calcium antagonist, diuretics	Rush
28	29	M	BV	16.70	I or II	+	0	Conventional
29	38	M	BV	4.19	I or II	+	0	Rush
30	35	M	BV	7.43	III or IV	+	0	Ultrarush
31	10	M	BV	3.17	I or II	+	0	Ultrarush
32	32	F	BV	4.97	I or II	+	0	Conventional
33	7	M	BV	3.28	I or II	+	0	Rush
34	46	M	VV	14.90	III or IV	-	0	Rush
35	45	F	VV	3.53	I or II	+	0	Rush
36	17	F	VV	3.72	I or II	+	0	Rush
37	33	F	VV	3.76	III or IV	+	0	Ultrarush
38	35	M	VV	6.25	I or II	+	Calcium antagonist	Conventional
39	65	M	VV	16.00	III or IV	+	Calcium antagonist	Ultrarush
40	60	F	VV	2.39	I or II	+	Angiotensin II receptor antagonist	Rush
41	47	F	VV	4.62	I or II	+	0	Ultrarush
42	23	M	VV	5.91	I or II	+	0	Ultrarush
43	53	M	VV	5.99	III or IV	+	0	Ultrarush
44	51	M	VV	6.04	I or II	+	0	Ultrarush
45	66	M	VV	7.34	I or II	+	ACE inhibitor	Rush
46	34	F	VV	10.00	III or IV	+	0	Ultrarush
47	25	F	VV	10.20	I or II	+	0	Ultrarush
48	31	M	VV	3.21	I or II	+	0	Ultrarush
49	42	F	VV	3.81	I or II	+	0	Ultrarush
50	47	F	VV	5.25	III or IV	+	0	Rush
51	26	F	VV	6.00	I or II	+	0	Ultrarush
52	61	F	VV	12.00	I or II	+	0	Ultrarush
53	32	F	VV	15.30	III or IV	+	0	Rush
54	25	F	VV	3.13	I or II	+	0	Rush
55	41	F	VV	5.29	I or II	+	0	Rush
56	19	F	VV	5.32	I or II	+	0	Ultrarush
57	27	F	VV	10.60	I or II	+	0	Rush

BV, Honeybee venom; *F*, female; *M*, male; *VV*, vespid venom.

*Severity grade of most severe systemic sting reaction before VIT according to Table E2.

†Specific IgE antibodies to the venom used for treatment (–, negative; +, positive).