

Original article

Influence of total IgE levels on the severity of sting reactions in Hymenoptera venom allergy

Background: Detection of specific IgE for Hymenoptera venoms and skin tests are well established diagnostic tools for the diagnosis of insect venom hypersensitivity. The aim of our study was to analyze the effect of total IgE levels on the outcome of generalized anaphylactic reactions after a Hymenoptera sting.

Methods: Two hundred and twenty patients allergic to bee, wasp, or European hornet venom were included in the study. Their specific and total IgE levels, serum tryptase levels, skin tests, and sting history were analyzed.

Results: In patients with mild reactions (grade I, generalized skin symptoms) we observed higher total IgE levels (248.0 kU/l) compared to patients with moderate reactions (grade II, moderate pulmonary, cardiovascular, or gastrointestinal symptoms; 75.2 kU/l) and severe reactions (grade III, bronchoconstriction, emesis, anaphylactic shock, or loss of consciousness; 56.5 kU/l; $P < 0.001$). Accordingly, 25% of the patients with low levels of total IgE (< 50 kU/l), but no individual with total IgE levels > 250 kU/l, developed loss of consciousness ($P = 0.001$). Additionally, specific IgE levels were related to total IgE levels: Specific IgE levels increased from 1.6 to 7.1 kU/l in patients with low (< 50 kU/l) and high (> 250 kU/l) total IgE levels, respectively ($P < 0.001$). Specific IgE levels correlated inversely to the clinical reaction grades, however, this trend was not statistically significant ($P = 0.083$).

Conclusion: Patients with Hymenoptera venom allergy and high levels (> 250 kU/l) of total IgE, predominantly develop grade I and grade II reactions and appear to be protected from grade III reactions. However, this hypothesis should be confirmed by extended studies with sting challenges.

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The incidence of Hymenoptera stings in the general population ranges from 56.6% to 94.5% (1). Up to 7.5% of the general population is reported to have experienced systemic anaphylactic reactions to Hymenoptera stings (2). Immunotherapy is an established mode of treatment for this condition, and offers long-term protection from further generalized reactions in 85–95% (3, 4). Detection of allergen-specific IgE serum antibodies (RAST, CAP-FEIA, Phadia, Sweden), skin-testing (skin prick test, intradermal test) and personal history are the mainstay of the diagnostic procedure in cases of insect venom hypersensitivity. Recently, the basophil activation test was suggested as an additional method to detect Hymenoptera venom allergy (5–7).

The determination of serum total IgE (CAP-FEIA) is mainly used as a global marker for atopy. It is employed to roughly assess the atopic disposition of

individual patients. Furthermore, several attempts have been made to determine total IgE in cord blood to predict atopic disease (8, 9). In addition to allergic diseases, total IgE plays an important role in the diagnosis of parasitic diseases and immune deficiencies. Total IgE is currently not used as an additional parameter to assess the pattern of sting reactions during the diagnostic procedure confirming Hymenoptera venom allergy.

Several attempts have also been made to identify risk factors for the development of systemic sting reaction (SSR) and to predict their severity. Regarding the onset of SSR, a short interval between stings has been reported to be a risk factor (10). On the other hand, bee-keepers who are stung frequently develop significantly fewer sting reactions compared with bee-keepers who experience occasional stings (11). Several other risk factors for SSR have been proposed: patients with a previous severe sting reaction (12), elderly patients (13–16), patients with cardiovascular diseases (15), and individuals with elevated serum tryptase levels (17, 18).

Abbreviations: SSR, systemic sting reaction; WV, wasp venom; HBV, Honey bee venom.

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Currently, there is no evidence of a higher risk of severe reactions in patients with atopic constitution (19, 20). Published data concerning the correlation between age and total IgE are varying; total IgE levels have been reported to be stable in mid-adulthood (21); however, a large study revealed an overall decrease in total IgE over a 10-year period (22). Yet other authors established a reduction in total IgE with increasing age in women only (23). Preliminary clinical observations in our allergy outpatient clinic showed differences in the severity of sting reactions, depending on total IgE levels. Most of the severe anaphylactic reactions occurred in patients with low levels of total IgE. Conversely, atopic patients with high levels of total IgE predominantly developed generalized skin symptoms. In 1991, Przybilla et al. (13) observed, in a retrospective study, that patients with total IgE ≥ 100 kU/l had less severe reactions to Hymenoptera stings than did patients with total IgE < 100 kU/l. As patients with elevated total IgE were also significantly younger, the authors presumed that age might be a critical factor in determining the severity of the clinical symptoms after a sting. Given this background, we initiated a study to clarify the association between the severity of sting reactions and total IgE levels. Additionally, we analyzed the effect of age on total IgE in our study population as well as the correlation between total IgE and Hymenoptera venom-specific IgE levels.

Material and methods

Patients

Two hundred and twenty patients, who had been admitted to our outpatient clinic from January 2004 until December 2004 because of systemic allergic reactions with at least generalized skin symptoms after a Hymenoptera sting, were screened. Their personal history was taken, and the usual diagnostic procedures were performed. Determination of specific and total IgE was performed 12 weeks (± 1 week) after the initial sting reaction. As wasp and European hornet belong to the family of Vespidae and their venoms contain the same major antigens (24), we did not differentiate between these genera. In order to eliminate established risk factors for severe sting reactions, patients with a history of cardiovascular disease (such as coronary heart disease or cardiac arrhythmia and patients on beta-blockers and ACE inhibitors) were primarily excluded. Data of patients with elevated serum tryptase levels (> 11.4 $\mu\text{g/l}$) were recorded, but not used for the entire statistical analysis. As age was also reported to be a risk factor, patients in the clinical reaction groups (grade I–III SSR, see below) have been age-matched to keep age differences between these groups nonsignificant and thus establish a meaningful correlation between the severity of SSR and total IgE for several statistical analyses.

Personal history

According to the modified classification of Ring and Messmer (2), generalized skin symptoms, such as flush, urticaria and angioedema were classified as grade I SSR. Mild to moderate pulmonary, cardiovascular or gastrointestinal symptoms were rated as grade II

SSR. Bronchoconstriction, emesis, anaphylactic shock and loss of consciousness were classified as grade III SSR. For further analysis, urticaria was rated as a clear sign of generalized skin reactions (grade I SSR), and loss of consciousness as a severe affliction of the cardiovascular system (grade III SSR).

Skin tests

The nature of sensitization was confirmed by standardized end-point titration intradermal tests (0.02 ml of 0.001, 0.01, 0.1, and 1 $\mu\text{g/ml}$ solution) using purified bee and wasp venom extracts (ALK wässrig; ALK-Abello, Austria). Intradermal tests were considered to be positive in the presence of a wheal ≥ 5 mm in diameter and erythema.

Determination of specific IgE and total IgE

Specific and total IgE antibody levels in the patients' serum were measured using CAP-FEIA according to the manufacturer's instructions. Patients were grouped according to the total IgE levels: group 1 < 50 kU/l, group 2 50–250 kU/l, and group 3 > 250 kU/l.

Tryptase determination

Serum tryptase was measured with ImmunoCAP Tryptase (Phadia, Uppsala, Sweden) according to the manufacturer's instructions. Serum tryptase levels > 11.4 $\mu\text{g/l}$ (95th percentile of the general population) were considered to be elevated.

Atopy score

The Erlangen Atopy Score proposed by Diepgen et al. (25), a diagnostic scoring system based on anamnestic and clinical criteria of atopic symptoms and skin signs, was used to detect an atopic disposition. Individuals with > 10 points were considered being atopic.

Data analysis

All data are expressed as median (25%; 75% percentiles) on the raw scale, unless otherwise indicated. Data were tested for normality using the Kolmogorov–Smirnov test. Parametric tests were carried out after logarithmic data transformation of specific and total IgE. Pearson's correlation was calculated to determine the degree of linear relationship between two continuous variables. Categorical variables were compared by the chi-square test or Fisher's exact test. For comparison of groups with continuous variables ANOVA was used. The level of significance was set at $P < 0.05$. The SPSS 14.0 software (SPSS Inc, Chicago, IL, USA) was used for statistical analysis.

Results

History

One hundred and fifty patients with a clearly identified insect sting reaction and conclusive diagnostic tests were included in the study. In detail, 25 patients have been excluded because of elevated tryptase levels and 45 have been lost due to the age-matching of the three reaction groups after performing ordinal regression analysis.

Table 1. Mean age, sex and culprit insect of patients with a history of a systemic sting reaction

	Grade I (n = 23)	Grade II (n = 73)	Grade III (n = 54)	Total (n = 150)	P-value
Age: years, mean (SD)	33.9 (±7.5)	36.0 (±7.9)	38.1 (±7.6)	36.4 (±7.8)	0.072
Females: n (%)	14 (61)	36 (49)	22 (41)	72 (48)	0.257
Reaction to WV: n (%)	17 (74)	49 (67)	43 (80)	109 (73)	0.291
Reaction to HBV: n (%)	6 (26)	24 (33)	11 (20)	41 (27)	0.291

Patients with grade I, II, and III reactions did not differ significantly with regard to age, sex and culprit insects.

Twenty-three patients (15.3% of the total test population) had a history of grade I SSR, while 73 patients (48.7%) had experienced grade II SSR and 54 patients (36.0%) grade III SSR. The systemic allergic sting reaction was caused by wasp or European hornet stings in 109 patients (72.7%), and by honeybee stings in 41 patients (27.3%). We observed an atopic disposition (clinical atopy score >10) in 37 of 150 (24.7%) of patients. The three groups (grade I–III) did not differ significantly with respect to age ($P = 0.072$) and sex ($P = 0.257$; Table 1).

Total IgE, specific IgE

Total IgE was only slightly influenced by age in our study group, as the shared variance of total IgE and age was 3.2% (Fig. 1). In contrast, there was a clear positive correlation between total IgE and specific IgE, with a shared variance of 27.5% (Fig. 2). Median specific IgE levels in patients with total IgE levels < 50 kU/l were 1.6 (0.7; 3.4) kU/l, in patients with total IgE levels from 50 to 250 kU/l 4.9 (2.1; 13.3) kU/l, and 7.1 (4.2; 23.8) kU/l in

patients with a total IgE > 250 kU/l ($P < 0.001$). On average, specific IgE levels were approximately 4.4-fold higher in patients with total IgE levels > 250 kU/l as compared with those with total IgE levels < 50 kU/l. Additionally, specific IgE levels were increasing from patients with grade III SSR to those with grade II or grade I SSR, but the differences between the reaction grades showed only a statistical trend and did not reach statistical significance [grade III 3.2 (0.9; 8.6) kU/l; grade II 4.4 (1.7; 8.6) kU/l; grade I 5.0 (3.4; 16.3) kU/l; $P = 0.083$].

Severity of sting reaction and total IgE

First, an ordinal regression analysis on the entire dataset ($n = 220$) was performed: total IgE levels ($P < 0.01$) and age ($P < 0.01$), but not tryptase levels ($P = 0.275$) and the clinical atopy score ($P = 0.213$) influenced the severity of the sting reaction. Because of a couple of published studies describing elevated tryptase levels as risk factor, these patients have been still excluded for further analysis. After age-matching and eliminating patients with coexisting risk factors 150 patients were further analyzed: we observed in patients with grade I SSR higher median total IgE levels [248.0 (100.0; 373.0)] compared to patients with grade II SSR [75.2 (37.7; 278.5)] and grade III SSR [56.5 (30.3; 122.5)] kU/l. Grade I and III ($P < 0.001$) as well as grade I and II ($P = 0.022$), but not grade II and III ($P = 0.113$) differed significantly regarding their total IgE levels.

Moreover, the frequency of grade III SSR was decreasing in patients with higher total IgE ($P < 0.001$), whereas the increase of grade I SSR in these patients failed to show statistical significance (Table 2).

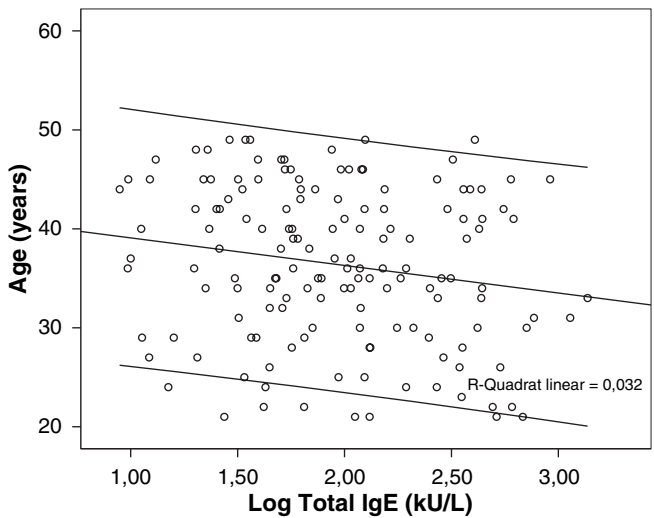


Figure 1. Correlation between total IgE and age. Negative correlation between total IgE levels and age with 95% confidence intervals, $n = 150$, $r = -0.179$, $r^2 = 0.032$, $P = 0.029$. Data of total IgE were log-transformed.

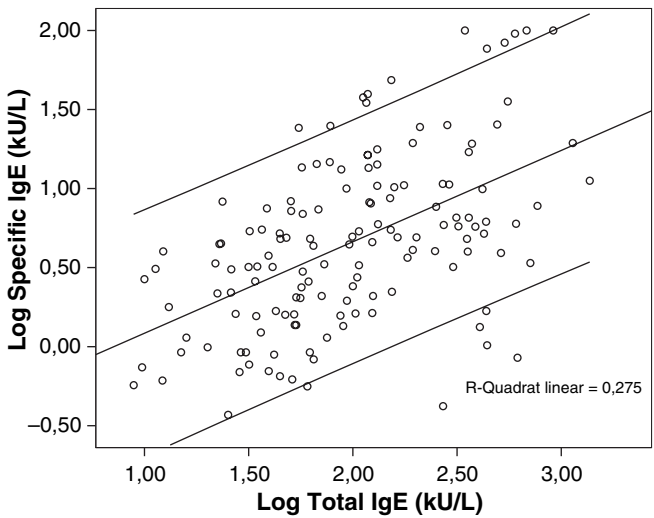


Figure 2. Correlation between total IgE and specific IgE. Positive correlation between total IgE and specific IgE with 95% confidence intervals, $n = 150$, $r = 0.525$, $r^2 = 0.275$, $P < 0.001$. Logarithmic data of specific and total IgE were used.

Table 2. Frequency of clinical reaction grades related to total IgE levels

Reaction grade	Total: <i>n</i>	Total IgE <50	Total IgE 50–250	Total IgE >250
I <i>n</i> (rel %)*	23	3 (6)	9 (14)	11 (31)
II <i>n</i> (rel %) [†]	73	22 (46)	30 (46)	21 (58)
III <i>n</i> (rel %) [‡]	54	23 (48)	27 (41)	4 (11)
	150	48 (100)	66 (100)	36 (100)

*The higher frequency of grade I reactions in patients with >250 kU/l total IgE was not statistically significant ($P = 0.104$).

[†]Grade II reactions appear to be completely independent from total IgE levels ($P = 0.368$).

[‡]Grade III reactions are less frequent in patients with total IgE levels >250 kU/l ($P < 0.001$).

Furthermore, we compared the occurrence of loss of consciousness as a severe affliction of the cardiovascular system and urticaria as a typical skin symptom in patients with different total IgE levels: 12/48 patients (25.0%) with a total IgE < 50 kU/l (group 1) had a confirmed history of loss of consciousness, while 13/66 patients (19.7%) whose total IgE ranged from 50 to 250 kU/l (group 2), and none of the 36 patients with a total IgE > 250 kU/l (group 3), had experienced loss of consciousness ($P = 0.001$; Fig. 3A). With respect to total IgE, groups 1 and 3 ($P = 0.001$), as well as group 2 and 3 ($P = 0.004$), but not group 1 and 2 ($P = 0.499$), differed significantly with regard to the relative frequency of loss of consciousness.

Conversely, 22/48 patients (45.8%) with a total IgE value < 50 kU/l had a confirmed history of urticaria, 43/66 patients (65.2%) with total IgE levels of 50–250 kU/l, and 32/36 patients (88.9%) with total IgE levels > 250 kU/l have had generalized urticaria ($P < 0.001$, Fig. 3B). Statistical comparison of the total IgE groups revealed that groups 1 and 2 ($P = 0.04$), group 1 and 3 ($P < 0.001$) as well as group 2 and 3 ($P = 0.01$) differed significantly with regard to the occurrence of urticaria.

Discussion

In the current study, we could demonstrate that the severity of sting reactions was influenced by total IgE levels. Loss of consciousness was only observed in patients with low (< 50 kU/l) and moderate (50–250 kU/l) levels, and not in patients with high levels of total IgE (> 250 kU/l). Comparing groups with low and high total IgE levels, it was found that patients with low total IgE levels generally developed grade III SSR significantly more often than others. In agreement with this observation, median total IgE levels were significantly lower in individuals with grade III SSR. Conversely, for grade I SSR we observed more patients with high total IgE levels. Only grade II reactions appeared to be completely independent from total IgE levels. This might be explained by the wide range of grade II symptoms comprising nausea, dizziness, and laryngeal

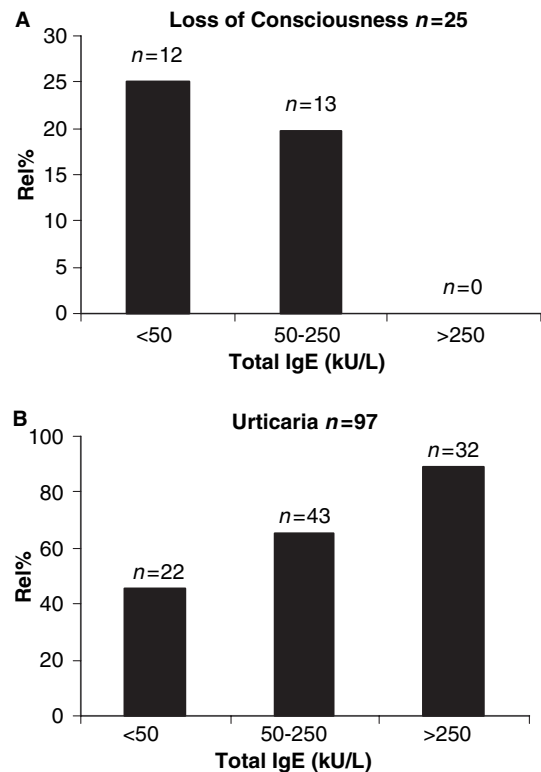


Figure 3. Relative frequencies (%) of loss of consciousness (A) and urticaria (B) in relation to total IgE levels. The groups with total IgE < 50 kU/l, 50–250 kU/l, and > 250 kU/l consisted of 48, 66, and 36 patients, respectively. The frequency of loss of consciousness decreased ($P = 0.007$), while the occurrence of urticaria increased in patients with high IgE levels ($P < 0.001$).

dyspnoea, which are mostly difficult to objectify and mainly depend on the subjective feelings of the patients. We are aware of changes of specific and presumably total IgE titers following a sting reaction. The extent and duration of this phenomenon are individually variable (26, 27). In our study, the median interval from the sting to the analysis of blood samples was 12 weeks (± 1 week), a time point, where IgE levels are supposed to be stabilized after the sting reaction. Nevertheless, our primary aim was to compare total IgE levels, which are probably only slightly influenced by the rise of specific IgE for one allergen.

A correlation between total IgE and specific IgE was also observed in the current study. Higher total IgE levels were associated with high specific IgE, and patients with low total IgE levels showed low, and in some cases even undetectable specific IgE levels. As a consequence, patients with low total IgE need to undergo very careful clinical assessment because the determination of specific IgE antibodies might occasionally yield false-negative results in these individuals. Moreover, we found an inverse correlation between the venom-specific IgE and the severity of sting reaction: patients with grade III reactions had lower venom-specific IgE titers compared

with those with grade I reactions. Nevertheless, this trend was not statistically significant. Previous studies did not observe any correlation between specific IgE and severity of sting reaction (20, 28, 29), but this might be as a result of the different method of IgE determination (RAST) in earlier studies: First, the RAST has a different sensitivity and specificity compared with the CAP-FEIA system. Secondly, only ordinal classes and not the metric IgE titers were used for statistical analysis in these studies (30, 31).

Although the reasons why patients with low total IgE levels are more susceptible to severe SSR is not clear, it might be speculated that multiple-specific IgE antibodies for various allergens interfere with antigen-binding and cross-linking of venom-specific IgE. The allergic response requires efficient cross-linking of high affinity IgE receptors (FcεRI) on mast cells and/or basophils, by the presence of at least two FcεRI-bound IgE antibodies capturing an allergen molecule (bivalent interaction) to induce cell activation and mediator release. If numerous different IgE antibodies saturate IgE receptors and impede the binding of Hymenoptera venom-specific IgE to effector cells, this could reduce the likelihood of cross-linking specific IgE by the given allergen. Consequently, degranulation and immediate hypersensitivity responses to allergens might be delayed, or even partially prevented. This could explain our clinical observation that cardiovascular symptoms after a positive sting challenge usually occur during the first 10 min after the sting, whereas generalized skin symptoms were observed 30–90 min after the sting (data not shown). It is not clear, why generalized skin symptoms are mostly absent in patients with severe cardiovascular symptoms. It might be speculated that if the mediator release takes place rapidly because of a fast simultaneous activation of effector cells, the cardiovascular system could be predominantly affected. Consequently, epinephrine is released to control the

circulation, which leads, among others, to a constriction of the peripheral blood vessels, and, possibly, to an inhibition of skin symptoms.

Alternately, the histamine content of basophils might be higher in patients with low levels of total IgE because of the lack of continuous release of histamine, which might occur in atopic patients reactive to multiple allergens. However, previous studies failed to show that basophils from patients prone to severe anaphylactic reactions were more reactive compared with basophils from sting victims with mild symptoms (5).

Following activation by a ligand, receptors in general become readily unresponsive to the agonist (desensitization) and are down-regulated (internalization) to limit the resulting cellular responses (32). In atopic patients who respond to multiple allergens with a continuous release of histamine and other mediators of anaphylaxis, the corresponding receptors involved in the anaphylactic reaction might be consistently desensitized and down-regulated, which leads to blunted responses to allergens, including Hymenoptera venom. Consequently, it is possible that patients with low total IgE levels and severe reactions exhibit enhanced reactivity to histamine and other mediators of anaphylaxis, as their receptors for these mediators are not being desensitized and down-regulated by other allergens. However, our results have to be confirmed in a larger number of patients with sting challenges as prospective severity-scoring tool. Additionally, future studies addressing the underlying mechanisms are of interest.

In summary, the present investigation revealed that the quantity of total IgE possibly influences the severity of a sting reaction, because patients of our study population with severe sting reactions showed significantly lower total IgE levels. Additionally, high-specific IgE levels were related to high total IgE levels, but not to severe sting reactions.

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