

Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy

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Summary

Background Mastocytosis and/or elevated basal serum tryptase may be associated with severe anaphylaxis.

Objective To analyse Hymenoptera venom-allergic patients with regard to basal tryptase in relation to the severity of sting reactions and the safety and efficacy of venom immunotherapy.

Methods Basal serum tryptase was measured in 259 Hymenoptera venom-allergic patients (158 honey bee, 101 *Vespula*). In 161 of these (104 honey bee, 57 *Vespula*), a sting challenge was performed during venom immunotherapy.

Results Nineteen of the 259 patients had an elevated basal serum tryptase. Evidence of cutaneous mastocytosis as documented by skin biopsy was present in 3 of 16 patients (18.8%). There was a clear correlation of basal serum tryptase to the grade of the initial allergic reaction ($P < 0.0005$). Forty-one of the 161 sting challenged patients reacted to the challenge, 34 to a bee sting and 7 to a *Vespula* sting. Thereof, 10 had an elevated basal serum tryptase, i.e. 1 (2.9%) of the reacting and 2 (2.9%) of the non-reacting bee venom (BV) allergic individuals, as compared to 3 (42.9%) of the reacting and 4 (8%) of the non-reacting *Vespula* venom-allergic patients. Thus, there was a significant association between a reaction to the sting challenge and an elevated basal serum tryptase in *Vespula* ($\chi^2 = 6.926$, $P < 0.01$), but not in BV-allergic patients. Systemic allergic side-effects to venom immunotherapy were observed in 13.9% of patients with normal and in 10% of those with elevated basal serum tryptase.

Conclusions An elevated basal serum tryptase as well as mastocytosis are risk factors for severe or even fatal shock reactions to Hymenoptera stings. Although the efficacy of venom immunotherapy in these patients is slightly reduced, most of them can be treated successfully. Based on currently available data, lifelong treatment has to be discussed in this situation.

Keywords hymenoptera venom allergy, immunotherapy, mastocytosis, sting challenge, tryptase

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Introduction

For many years, the association between systemic anaphylactic reactions to Hymenoptera stings and mastocytosis [1–6] has been described occasionally [7–12]. This association was claimed to be observed more often than expected from the individual frequencies of the two conditions.

Traditionally, mastocytosis was most often diagnosed based on typical cutaneous lesions such as urticaria pigmentosa or less frequently teleangiectasia macularis eruptiva perstans. The introduction of a method to determine the mast cell-specific enzyme tryptase in serum offered a new approach to the diagnosis of mastocytosis [13–15]. According to these studies, tryptase in serum has been recommended for the diagnosis of systemic mastocytosis: values above 20 µg/L

taken outside an allergic reaction should raise concern for this disorder, while a normal serum level (< 13.5 µg/L) does not exclude it [6].

In 1994, Schwartz et al. [14] and Schwartz [15] showed that Hymenoptera venom-allergic patients who developed severe anaphylaxis to a sting challenge did not develop only a strong increase of serum tryptase during the reaction. These severe reactors had on the average higher serum tryptase levels already before the challenge, when compared to those who did not react to the sting or developed only mild allergic symptoms [16]. More recently, Ludolph-Hauser et al. [17] reported the frequent occurrence of a constitutively elevated serum tryptase in patients with a history of severe systemic Hymenoptera sting reactions and based on conventional histology concluded the presence of cutaneous mastocytosis in most of them.

We present in our study additional data in this context based on clinical and laboratory examination of 259 patients with systemic Hymenoptera sting reactions. In addition, we

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will analyse safety and efficacy of venom immunotherapy (VIT) in relation to the basal serum tryptase level (bT) in 161 of these patients who have been re-exposed by a sting challenge (CH) during this treatment.

Materials and methods

Patients

Of the 259 patients included in this study (Group A), 158 were allergic to bee venom (BV) and 101 were allergic to *Vespula* venom (VV) (Table 1). All patients gave a history of systemic allergic reactions to Hymenoptera stings and had positive intracutaneous skin tests and/or specific serum IgE-antibodies to the respective venom. Based on clinical symptoms, the reactions of the patients were divided into 4 grades of severity according to Mueller [18].

In a subgroup (B) of 161 patients, a CH had been performed during VIT. One hundred-four were conducted in BV and 57 in VV allergic patients with the respective insects, between 1986 and 2000. From this period, we included all 41 patients who reacted to the CH and also a group of 120 successfully treated patients who did not react to a CH. Overall, 703 patients had been re-exposed by a CH between 1986 and 2000.

Estimation of basal serum tryptase and venom-specific IgE antibodies

Levels of the mast cell specific enzyme tryptase (α - and β -tryptase) and of venom-specific IgE (sIgE) were estimated by UniCAP (Pharmacia & Upjohn, Uppsala, Sweden) [19].

Table 1. Demographic, clinical and laboratory data on 259 Hymenoptera venom-allergic patients

Group of patients	A	B
<i>N</i>	259	161
Age (mean value, range)	38.4 (4–77)	40.4 (9–75)
Sex (male/female)	156/103	101/60
Responsible insect:		
honey bee/ <i>Vespula</i>	158/101	104/57
Grade of pretreatment reaction [18]		
I	10 (3.9%)	3 (1.9%)
II	43 (16.6%)	25 (15.5%)
III	100 (38.6%)	66 (41%)
IV	106 (40.9%)	67 (41.6%)
Skin test endpoint concentration (g/L)*		
10^{-3}	Seven patients	Three patients
10^{-4} – 10^{-8}	250 patients	156 patients
Venom-specific IgE (kU/L)*		
<0.35	Seven patients	Four patients
≥0.35	251 patients	157 patients
Basal serum tryptase		
Normal/elevated (%)	240/19 (92.7/7.3)	151/10 (93.8/6.2)
≥13.5 µg/L mean value/SD	20.25/7.16	21.4/9.03
Range	14.1–44.4	14.5–44.4
<13.5 µg/L mean value/SD	5.85/2.19	5.77/2.31
Range	1.0–12.6	1.07–12.6

Group A: Hymenoptera venom-allergic patients.

Group B: Subgroup of A, including those patients who underwent a sting challenge during venom immunotherapy.

*Before starting venom immunotherapy.

A sIgE value of ≥0.35 kU/L was regarded as positive. In 1 of the 259 patients, no pretreatment sIgE was available.

Basal serum tryptase (bT) was estimated in blood samples obtained between two weeks and one year after the systemic allergic reaction to a Hymenoptera sting. In the sting-challenged patients, (subgroup B) the blood samples were taken within 10 months before, usually immediately before the CH. A bT of ≥13.5 µg/L was considered to be elevated. In a study with 129 unselected, apparently healthy children and adult subjects (64 male, 65 female) the geometric mean value was 5.6 µg/L, and the upper 95% confidence limit was 13.5 µg/L (data supplied from Pharmacia & Upjohn, Uppsala, Sweden, 1998).

Skin tests

Intracutaneous tests were performed with serial 10-fold dilutions of BV and VV (Pharmalgen ALK, Horsholm, Denmark) with concentrations ranging from 10^{-8} to 10^{-3} g/L (0.00001–1.0 µg/mL) as described previously [20]. A weal of 5 mm or more in diameter with erythema after 20 min at a concentration of 10^{-3} g/L or less was regarded as a positive reaction. The lowest concentration causing such a reaction is called the skin test endpoint concentration (STPC). In two of the 259 patients, no pretreatment skin test was available.

Venom immunotherapy (VIT)

VIT was started by ultrarush, rush or conventional protocols [21] with Pharmalgen venoms (ALK, Denmark). After reaching the maintenance dose of 100 µg, the interval between the injections was increased to 4 weeks in the first and 6 weeks in subsequent years.

Sting challenge (CH)

The sting CH was performed as described previously [22]. All patients were challenged with the insect to which they had reacted before treatment and with the venom of which they had been treated. Most of them had a VIT duration of 3–5 years before the CH. Twenty-one of the 104 (20%) BV allergic patients had a CH already within the first 6 months, mostly because they were heavily exposed (e.g. beekeepers, gardeners), none of these had an elevated bT. In all patients who underwent a CH informed consent was obtained.

The reaction to the CH was scored from 0 to 5. Score 0: no systemic symptoms; score 1: only subjective systemic symptoms; score 2: any of the above and objective cutaneous symptoms; score 3: any of the above and gastrointestinal symptoms; score 4: any of the above and respiratory symptoms; score 5: any of the above and cardiovascular symptoms (such as arterial hypotension, collapse, loss of consciousness, cyanosis, incontinence).

Histopathology

Sixteen of the 19 patients with elevated bT underwent a dermatological examination with histological assessment of a skin biopsy sample. Two patients refused to participate and one had died before from cardiovascular disease unrelated to his venom allergy. Histopathological analyses were performed by stains with H&E, toluidine blue (pH 4.0,

incubation time 30 min) and Giemsa. The histological diagnosis of cutaneous mastocytosis required a typical morphology (hyperpigmentation, teleangiectases) as well as an increased number of mast cells per high power field (HPF, 400 $\times$). A number of more than 10 mast cells per HPF, counted over several fields in the upper dermis, are generally considered to be diagnostic. Mast cells were only counted when the nucleus and the metachromatic granules were visible at the same time, i.e. 'shadows' of cells (no nucleus, only a cytoplasm with granules) were not counted. An improved histological analysis including immunohistochemical staining of mast cells with antitrypsin antibodies and a special stereological counting technique will be reported as a methodological paper.

Statistics

χ^2 contingency table analysis was used to compare patients with regard to sex, grade of the allergic reaction, responsible insect and the presence or absence of an elevated bT.

Spearman's non-parametric rank correlation test was used to analyse correlations between age, grade of the allergic reaction, reaction score after sting challenge, bT, STEPc and sIgE.

The non-parametric Mann-Whitney *U*-test was used to compare reaction scores at the CH in patients with normal or elevated bT and according to the insect used for the CH. It was also used to compare sIgE and STEPc before the CH in reactors and non-reactors.

Results

Clinical and laboratory data of 259 Hymenoptera venom-allergic patients

An elevated bT was found in 19 (7.3%) of the 259 Hymenoptera venom-allergic patients. Histological evidence of skin mastocytosis was present in 3 of the 16 examined patients with elevated bT (18.8%) (Table 1). Elevated bT was significantly correlated to the grade of the systemic allergic reaction ($\chi^2 = 13.349$, $P = 0.0039$) and most often associated with a history of a severe shock reaction (Table 2, Fig. 1). Elevated bT was observed significantly more often in *Vespula*

Table 2. Grade of allergic reaction [18] and basal serum tryptase levels (bT) in 259 Hymenoptera venom-allergic patients

	Grade of allergic reaction				Total
	I	II	III	IV	
Number of patients (%)	10 (3.9)	43 (16.6)	100 (38.6)	106 (40.9)	259
Elevated bT ($\geq 13.5 \mu\text{g/L}$)	0 (0)	0 (0)	4 (4)	15 (14.2)	19 (7.3)
bT (normal and elevated)	5.09	5.06	5.8	6.61	5.95
Geometry mean value range	1.8–11.9	1.0–12.6	2.17–23.9	1.1–44.4	1.0–44.4

$\chi^2 = 13.349$, $P = 0.0039$.

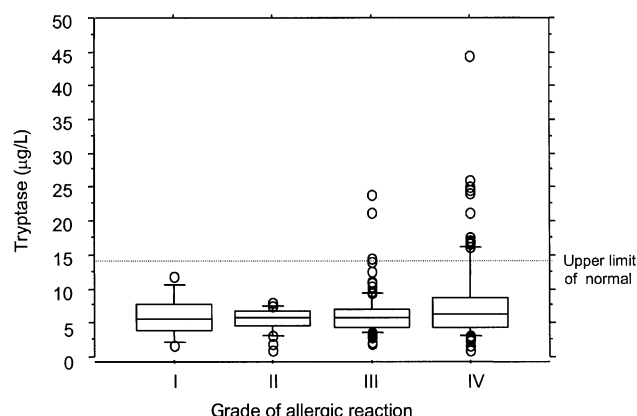


Fig. 1. Basal serum tryptase and grade of allergic reaction [18] in 259 Hymenoptera venom-allergic patients.

Table 3. Basal serum tryptase levels in relation to the responsible insect in 259 Hymenoptera venom-allergic patients

Insect	Basal serum tryptase ($\mu\text{g/L}$)		Total
	≥ 13.5	< 13.5	
Honey bee (%)	7 (4.4)	151 (95.6)	158
<i>Vespula</i> (%)	12 (11.9)	89 (88.1)	101
Total (% of all patients)	19 (7.3)	240 (92.7)	259

$\chi^2 = 5.032$, $P = 0.025$.

than in BV-allergic individuals ($\chi^2 = 5.032$, $P = 0.025$; Table 3). No association of bT with age, sex and STEPc was found. No sIgE were detected in 3 of 19 patients (15.8%) with elevated bT, but were detected in only 4 of 239 (1.7%) with normal bT ($\chi^2 = 13.287$; $P < 0.01$). In 19 patients, a double estimation of bT was carried out before and during VIT 1–7 years apart. The mean coefficient of variation was 0.115, the relative co-efficient of variation was 2.704%. Serum levels of bT thus seem to be astonishingly stable over years and not influenced by VIT.

The following other correlations between clinical and laboratory parameters were observed in Spearman's rank: the grade of the allergic reaction was positively correlated with the age (tied *Z*-value 3.445, $P < 0.0001$), while sIgE (tied *Z* = -4.592 , $P < 0.0001$) and to a lesser extent STEPc (tied *Z* = -2.057 , $P = 0.0397$) were negatively correlated with age. As expected, there was also a close correlation between STEPc and sIgE (tied *Z* = -7.035 , $P < 0.0001$), but none between reaction grade and STEPc as well as sIgE.

Clinical and laboratory data of 161 patients in relation to efficacy and tolerance of venom immunotherapy

Of the 161 patients who underwent a sting challenge (CH) during venom immunotherapy (VIT), 104 (64.6%) were allergic to BV and 57 (35.4%) to VV (Table 4). Forty-one (25.5%) of them developed a systemic allergic reaction to the CH, 34 were allergic to BV, 7 to VV ($\chi^2 = 7.049$, $P = 0.0045$). The reaction score was significantly higher in BV- than in VV-allergic individuals (Mann-Whitney *U*-test: tied

Table 4. Reaction to sting challenge during venom immunotherapy in relation to basal tryptase in 161 Hymenoptera venom-allergic patients

	Sting challenge BV	Sting challenge VV	Total
<i>Total number of patients</i>	104	57	161
Elevated bT ($\geq 13.5 \mu\text{g/L}$)	3 (2.9%)	7 (12.3%)	10 (6.2%)
<i>No SR to sting challenge</i>	70 (67.3%)	50 (87.9%)	120
Elevated bT	2 (2.9%)	4 (8%)	6 (5%)
<i>SR to sting challenge</i>	34 (32.7%)	7 (12.3%)	41
Elevated bT	1 (2.9%)	3 (42.9%)	4 (9.8%)

SR: systemic reaction, BV: bee venom, VV: *Vespula* venom.
 BV: $\chi^2 = 0.001$, not significant; VV: $\chi^2 = 6.926$, $P < 0.01$; BV+VV: $\chi^2 = 1.187$, not significant.

$Z = -3.335$, $P = 0.0009$). In the 10 patients with elevated bT the pretreatment grade of severity was 3.9, compared with 3.26 in those with normal bT. Systemic allergic side-effects to VIT injections were reported by 22 (13.7%), 18 to BV and 4 to VV. Side-effects occurred in 1/10 (10%) patients with elevated bT and in 21/151 (13.9%) with normal bT. Objective reactions to the CH were seen in 24.5% with a normal bT and in 40% with an elevated basal value. However, due to the low number of patients with elevated bT the differences did not reach statistical significance.

Elevated bT was more frequently observed in patients who reacted the CH than in successfully treated patients, but this finding reached statistical significance only for VV-allergic patients ($\chi^2 = 6.926$, $P < 0.01$). The CH reaction score was higher in allergic patients with elevated than with normal bT (3.5 vs. 2.73), but this difference was only significant for VV-allergic patients (Mann–Whitney U -test: tied $Z = -2.238$, $P = 0.025$).

With the non-parametric Mann–Whitney U -test, significantly higher sIgE (tied $Z = -2.692$, $P = 0.0071$) and STEPc (tied $Z = -3.466$, $P = 0.0005$) were found before the CH in patients who reacted to this re-exposure than in successfully treated patients. The outcome and the score of the CH were, however, not correlated with the grade of the pretreatment reaction. In Spearman's rank, the score at re-exposure correlated with both STEPc (tied $Z = 2.988$, $P = 0.0028$) and sIgE (tied $Z = 2.613$, $P = 0.009$) before re-exposure and also with the STEPc (tied $Z = 2.284$, $P = 0.022$), but not the sIgE before starting VIT.

Discussion

Tryptase is a mast cell-specific enzyme that exists in three forms: α -tryptase and pro- β -tryptase, enzymatically inactive monomers, are secreted continuously by mast cells and indicate the total body mast cell load [23]. β -tryptase, the enzymatically active tetramer is stabilized through binding on heparin and stored in mast cell granula. It is liberated during acute allergic reactions. The serum concentration of β -tryptase is thus a measure for mast cell activation [23]. The commercially available test measures α -, pro- β - and β -tryptase [19]. If an elevated level of tryptase is observed outside an acute allergic reaction, it thus indicates an increased total body mast cell load, while within the context of an allergic reaction it suggests mast cell activation.

In this paper, we could confirm the previous observations [14, 17, 24] of a strongly increased incidence of elevated basal tryptase serum levels in patients with a history of severe shock reactions to Hymenoptera stings. We found a clear association of bT with the severity of the allergic sting reaction: None of the 43 patients with mild reactions of grade I and II, but 14.2% of the 106 patients with shock reactions presented with elevated bT. An elevated bT must therefore be considered a risk factor for severe, potentially fatal allergic reactions to Hymenoptera stings – and possibly also other allergens. This is sustained by observations of almost exclusively severe shock reactions in patients with urticaria pigmentosa [8] and of two patients with mastocytosis, who developed fatal anaphylaxis to a *Vespula* sting some years after discontinuation of VIT [25]. In patients with generalized reactions to Hymenoptera stings, venom-specific sensitization can usually be detected by either skin or serological tests. In the literature, however, cases of severe systemic reaction have been reported [8], where neither in the serum nor in the skin test an IgE-mediated mechanism could be verified. In our study population, all patients with elevated bT had at least an STEPc of 10^{-3} g/L , while in 15% of them – as compared to only 1.6% of those with normal bT – no specific IgE could be found. This confirms previous observations of often low total and specific serum IgE in patients with mastocytosis [26]. This phenomenon is most likely due to increased absorption of circulating IgE to abundant tissue mast cells. The determination of bT is therefore especially indicated in patients with a history of severe Hymenoptera sting reaction, but negative sIgE.

Our observations differ in two aspects from those reported by Ludolph-Hauser et al. [17]: (1) Evidence of cutaneous mastocytosis was only found in part of our patients with elevated bT, whereas Ludolph-Hauser reported clinical and/or routine histological evidence of urticaria pigmentosa or teleangiectasia eruptiva macularis perstans in 11 of 12 patients with elevated bT. While the clinical diagnosis of urticaria pigmentosa is most often obvious, that of teleangiectasia macularis eruptiva perstans can be rather difficult and the histologic criteria for this diagnosis are not yet clearly elaborated (methodological paper in preparation). (2) The percentage of Hymenoptera venom-allergic patients with elevated bT was somewhat higher in Ludolph-Hausers [17] report than in ours 10.5% vs. 7.3%. This difference is even more pronounced when we look at severe shock reactions where they found 24.3% with elevated bT, while we detect only 14.2%. However, this difference is reduced in the population of the *Vespula*-sensitive patients, of whom overall 11.9% and of those with severe shock reaction 19% had an elevated bT. These results are similar to those of Ludolph-Hauser and co-workers, who examined mostly *Vespula*-allergic patients, while the majority (61%) of our patients were BV allergic.

To our surprise, we found among our 259 patients significantly more VV- than BV-allergic individuals with an elevated bT – 11.9% vs. 4.4%. The reason for this finding is not quite clear. One possible explanation could be that VV contains stronger directly mast cell-activating peptides than BV and could therefore amplify an IgE mediated reaction more easily or even provoke anaphylaxis by direct mediator release from mast cells. This is, however, not documented so

far. A documented difference between the two species is that the honey bee with its sting deposits a more than ten times higher amount of venom than *Vespa* does [27]. An increased total body load of mast cells as a factor favouring IgE-mediated anaphylaxis could thus be of minor relevance in honey BV allergy.

The distribution according to the responsible insect was similar in the 161 sting-challenged patients and in the total population, roughly two-thirds being allergic to BV and one third to VV. As previously observed [22], significantly more BV- than VV-allergic patients developed systemic allergic side-effects during VIT and also reacted to the CH. A systemic allergic reaction to the CH, indicating incomplete protection, was observed more often in patients with elevated bT, but this finding reached significance only for the VV-allergic individuals. Allergic side-effects during VIT were not increased in patients with elevated bT. The outcome of the CH was significantly associated with venom sensitivity according to skin testing and serum-specific IgE before the CH. No such association was observed between pretreatment sIgE and the severity of the pretreatment reaction. This discrepancy may be explained by the fact that the interval between the pretreatment reaction and initial diagnostic testing was highly variable, while pre-challenge testing was usually conducted immediately before the CH. The outcome and the score of the CH was, however, not correlated to the grade of the pretreatment reaction.

In conclusion, our results confirm the elevation of basal serum tryptase as a risk factor for severe systemic allergic reactions to Hymenoptera stings. They indicate that there is some reduction of efficacy and maybe also of tolerance of VIT in these patients, especially in VV-allergic individuals. Taken together with previous observations, these results suggest that VIT may have to be continued lifelong in patients with elevated bT and/or clinical evidence of mastocytosis.

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