

Short communication

Possible circadian variation of serum mast cell tryptase concentration

Background: A temporarily elevated level of serum mast cell tryptase (ST) indicates mast cell activation and occurs in systemic anaphylactic reactions (SAR). We measured ST following a sting challenge in vespid venom-allergic patients treated with venom immunotherapy (VIT) and in healthy controls, respectively.

Aim of the study: To assess changes of ST over time in vespid venom-allergic patients at the occasion of a re-sting and in healthy controls.

Methods: A sting challenge was performed in 20 patients on vespid VIT to monitor efficacy of VIT. ST was measured between 9.00 and 10.00 a.m. (baseline). Sting challenge was performed at 2.00 p.m., and ST was determined again 20 min, 90 min and 18 h later. Measurements at corresponding times of the day were done in nine healthy controls.

Results: One patient developed a mild SAR to the sting challenge which was associated with a temporary increase of ST. In the other 19 patients who tolerated the sting challenge without SAR ST decreased significantly by 18.0% (median, range 8.3–36.7%). Twenty minutes after the sting when compared with baseline levels ($P < 0.001$), a significant decrease of ST was still present after 90 min (median 13.7%) ($P < 0.001$), but not after 18 h ($P = 0.57$). A comparably significant temporary decline was found in controls.

Conclusions: The temporary decline of ST in patients and in controls suggests a circadian variation of ST concentration. A normal diurnal pattern of ST concentration after sting challenge is associated with successful treatment.

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Systemic anaphylactic reactions (SAR) due to Hymenoptera stings are initiated by the IgE-mediated release of an array of mediators from mast cells and basophils upon exposure to venom allergens (1). Demonstration of such a mediator release can be found *in vitro* after allergen-specific stimulation of peripheral blood cells (2). *In vivo* an increase of mast cell tryptase (ST) in body fluids has been found to be associated with SAR thereby helping to diagnose SAR (3, 4).

Venom immunotherapy (VIT) is highly effective to protect patients with prior SAR against such reactions after subsequent stings (5). To verify an efficient therapy, a sting challenge with a living insect has to be performed and the subsequent clinical reactions are monitored (6). However, it is not uncommon that patients develop unspecific psycho-vegetative ailments after a challenge test despite a successful therapy. These unspecific symptoms can be hardly distinguished from minor anaphylactic symptoms which, however, would require further therapy (7). Thus, with doubtful clinical reactions an additional determination of a sensitive laboratory parameter would be helpful to definitively proof mediator release and postchallenge SAR.

A possible candidate for such a laboratory parameter would be ST concentration to be determined after challenge (8). However, in the latter study (8) sensitivity of the tryptase assay (Tryptase RI-ACT) was rather low and it was not possible to detect relevant post-sting changes of ST in patients with true SAR or in those found to be protected. Furthermore, a challenge test could also induce a minor mediator release which is too weak to induce clinical symptoms. Recently, a more sensitive tryptase assay became available by which ST can be detected and measured in the circulation of nearly every individual. In the following study we wanted to examine variations of ST concentration over time following a sting challenge and to compare these values with those measured in healthy controls.

Material and methods

Patients

Twenty patients (10 males, 10 females; aged 13–70 years) on immunotherapy with vespid (wasp) venom (ALK lyophilisiert SQ or ALK Depot SQ; ALK-Scherax, Hamburg, Germany) were

studied. All had a history of a SAR to a wasp sting, and sensitization to vespid venom was demonstrated by a positive skin test reaction and/or by identifying IgE antibodies specific to vespid venom in the serum. Patients had been treated for 10–36 months with maintenance doses of 100 or 200 µg of vespid venom given every 4 weeks. The control group comprised nine healthy persons (two males, seven females; aged 22–57 years) who were not treated with VIT and who were not exposed to any known allergens.

Sting challenge test

Sting challenge tests were performed with a living wasp as described previously (6): in brief, the patients were hospitalized and were stung by a living wasp while being closely monitored in an emergency room. Reactions were documented, and treated if necessary.

Measurement of serum tryptase

ST was measured with a fluoroenzyme immunoassay (UNICAP Tryptase; Pharmacia Diagnostics, Freiburg, Germany) which detects both α - and β -tryptase. Duplicate measurements were performed, if the concentration was > 10 µg/l; in this case, the mean value was recorded. ST concentrations of more than 11.4 µg/l (95th percentile in control subjects, as determined by the manufacturer) were considered elevated.

Experimental protocol

Baseline blood samples were taken in controls and in patients between 9.00 a.m. and 10.00 a.m. at the day of the study. Sting challenge tests were performed between 2.00 and 3.00 p.m. in the patients. No intervention was performed in controls. Postchallenge blood samples were taken 20 min, 90 min and about 18 h later (8.00–9.00 a.m. the following day). Blood from control subjects was obtained at corresponding times.

Statistical analysis

Values are presented as median with corresponding range. For comparison of baseline ST between patients and controls the Mann–Whitney *U*-test was used. Statistical analysis within a group was performed with the Wilcoxon test. A *P*-value < 0.05 was considered significant.

Results

Nineteen of the 20 patients tolerated the sting challenge without SAR. A 64-year-old female patient developed a mild SAR with flushing and discomfort.

In patients, the baseline ST concentration was 2.0–56.3 µg/l (median: 6.3 µg/l; Table 1). Two of the four patients with baseline ST > 11.4 µg/l, agreed to be examined for systemic mastocytosis including bone marrow biopsy (according to 9). In one patient cutaneous mastocytosis, and in another patient also systemic involvement of mastocytosis was found. The baseline ST in controls was 3.2–85.3 µg/l (median: 9.7 µg/l;

Table 1. Serum levels of mast cell tryptase in patients

Gender/age (yrs)	Severity‡ of sting reaction (history)	Mast cell tryptase (µg/l)			
		Baseline	20 min after	90 min after	18 h after
		Sting challenge			
Systemic anaphylactic reaction to the sting challenge					
w/64	III	10.4	13.7	13.3	11.3
No systemic reaction to the sting challenge					
m/61	II	2.0	1.4	1.4	1.9
f/64	II	4.1	3.0	3.4	4.2
m/53	II	4.3	3.7	3.6	4.6
f/63	II	5.3	3.3	3.9	4.2
f/44	II	5.7	4.7	6.0	7.8
m/70	II	5.8	4.9	5.2	5.6
f/47	II	5.9	4.6	5.1	6.4
f/25	II	5.9	4.8	5.9	6.9
f/64	II	6.1	4.8	4.7	5.7
m/13	II	6.3	4.6	4.6	4.8
m/24	I	6.4	5.3	5.4	6.6
f/35	III	7.8	6.7	6.7	8.0
m/59	II	8.2	7.3	8.0	9.8
m/36	II	8.3	6.8	7.8	7.7
f/58	II	10.2	8.0	8.5	9.3
m/44	II	19.3	16.2	17.3	19.7
f/69	III	19.3	17.7	18.6	21.2
m/57*	III	33.3	28.5	29.5	35.7
m/32†	III	56.3	44.8	48.6	52.3

m, male; f, female.

*Cutaneous involvement of mastocytosis (no investigation for systemic involvement).

†Systemic mastocytosis.

‡Severity of sting reactions (after 4).

I, systemic skin symptoms; II, mild to moderate respiratory and/or cardiovascular symptoms; III, full shock.

Table 2. Serum levels of mast cell tryptase in controls

Gender/age (yrs)	Mast cell tryptase (µg/l)			
	Blood sampling			
	0	I	II	III
w/43	3.2	3.1	3.3	3.5
w/36	4.3	3.8	3.6	4.3
w/31	5.4	5.3	5.4	5.7
w/38	6.2	5.9	6.1	6.5
m/37	9.7	9.8	9.8	11.1
w/30	10.2	9.5	9.3	10.9
w/57	10.9	10.6	10.0	11.9
w/38*	14.7	13.2	13.2	14.9
m/22*	85.3	85.1	85.1	88.7

m, male; f, female.

*Cutaneous mastocytosis (no investigation for systemic involvement);

0, baseline sample: 9.30–11.00 a.m.

I: 2.30 p.m.; II: 4.00 p.m.

III, sample taken the following morning: 8.00–9.00 a.m.

Table 2) and did not differ significantly from that in patients. ST > 11.4 µg/l was found in two subjects who both turned out to have cutaneous mastocytosis.

In those 19 patients who tolerated the challenge test without SAR, the median ST following the sting was 4.9 $\mu\text{g/l}$ (1.4–44.8) after 20 min, 5.9 $\mu\text{g/l}$ (1.4–48.6) after 90 min, and 6.9 $\mu\text{g/l}$ (1.9–52.3) after 18 h. Evaluation of changes from baseline revealed a significant drop of ST concentration 20 and 90 min after the sting challenge (20 min: median change -18.0% , range -8.3% to -36.7% , $P < 0.001$ vs baseline; 90 min: median change -30.4% , range $+5.1$ to -30.4 , $P < 0.001$ vs baseline), 18 h after sting challenge ST concentrations had increased again to baseline values ($P = 0.57$) (Fig. 1). This temporary decrease over time was seen regardless whether the baseline ST was elevated or not (Table 1).

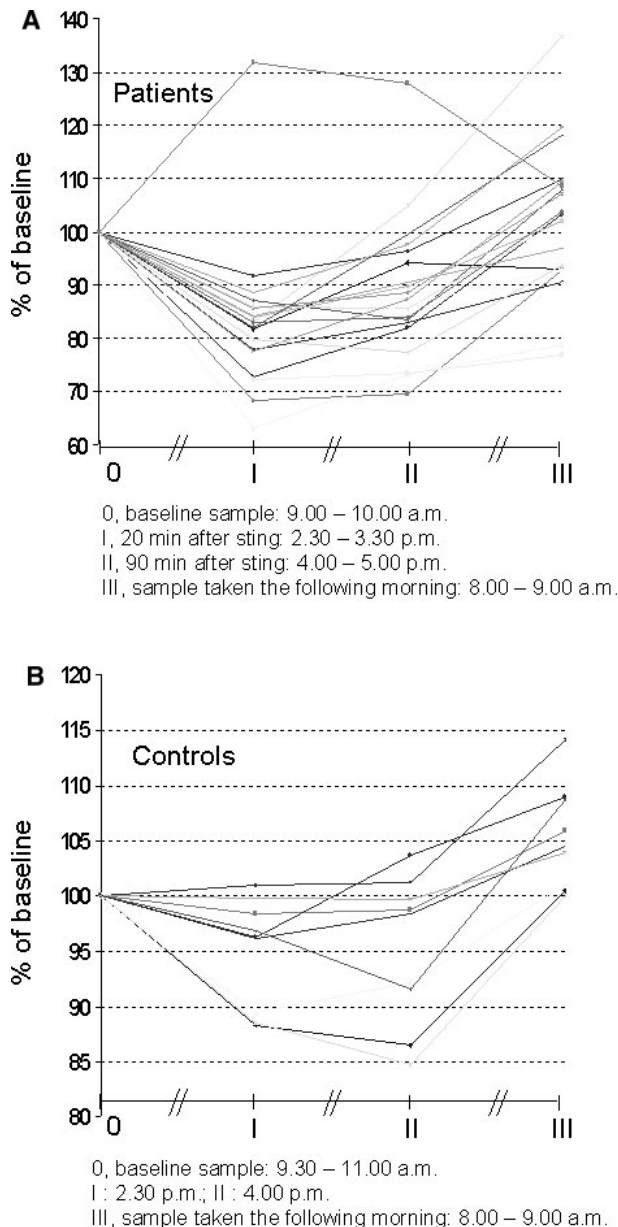


Figure 1. Change of serum levels of mast cell tryptase in patients (A) and controls (B).

The one patient with a mild post-sting SAR showed a slight increase of ST concentration in comparison with the baseline value (Table 1, Fig. 1).

In controls, median ST was 9.7 $\mu\text{g/l}$ (3.2–85.3) at baseline, 9.5 (3.1–85.1) $\mu\text{g/l}$ at 2.30 p.m., 9.3 (3.3–85.0) $\mu\text{g/l}$ at 4.00 p.m., and 10.9 (3.5–88.7) $\mu\text{g/l}$ on the following day. Compared with baseline values, ST concentration (change from baseline) decreased significantly by 3.7% (median, range $+0.9\%$ to -11.7%) in the 20 min samples ($P < 0.05$ vs baseline) and tended to decrease in the 90 min sample (by range 1.6%, median $+3.4$ to -15.3 , $P = 0.07$ vs baseline). In the 22 h sample ST was significantly increased by 4.5% (median, range 0 to $+14.1\%$; $P < 0.05$ vs baseline). Details are given in Table 2 and Fig. 1.

Discussion

We performed sting challenges in patients allergic to vespid venom who were on maintenance VIT. Those patients who tolerated this sting challenge without SAR subsequently demonstrated a temporary slight, but significant decrease of ST concentration. ST concentration returned to pre-sting levels only the morning after. A similar diurnal pattern of ST concentration was found in healthy controls. The one patient who developed a SAR to the sting challenge showed an increase of ST 20 and 90 min after the sting. ST values had normalized the next morning.

Sting challenge is an established diagnostic tool to document treatment success or failure in patients who are on maintenance VIT (5,6). Besides monitoring vital parameters also post-sting ST measurements may be helpful (10). Thereby, an acute mast cell activation might be detected on the one hand, supporting the diagnosis of a post-sting anaphylactic reaction, or, on the other, such an event could be ruled out with high certainty in the case of a normal finding. Our data indicate that in all patients a well-tolerated sting is accompanied even by a post-sting decrease of ST concentration. Therefore, post-sting measurement of ST concentration may add to certitude by which post-sting SAR can be ruled out. However, to do so, it appears necessary to sample blood according to a standardized protocol taking into account possible diurnal variations of ST concentration.

Possibly, the temporary ST decrease after the challenge is not related to the insect sting itself, but may rather represent a natural circadian variation with lower levels in the afternoon and higher levels in the morning. Such a mechanism is suggested by the findings in healthy volunteers showing a time-dependency of ST concentration which did not differ from that in patients. However, it should be noted that our study was not designed to examine possible circadian rhythms of ST concentration precisely. To the best of our knowledge no such study exists up to date. As circadian variations of ST

concentration cannot be excluded, one should always consider the time of the day at which blood samples were taken for ST measurement. This aspect may be particularly important if ST is used to monitor an individual patient over time and if only slight differences are found. Further studies exactly addressing the issue of natural diurnal variations will be required to establish a reference pattern for the time dependency of ST concentration.

When sting challenges were performed in Hymenoptera venom allergic patients in the past, subsequent SAR were found to be associated with an increase of ST concentration, irrespective if the patient was treated with VIT (8) or was still untreated (10). Among our patients there was only one who had a mild SAR to the sting challenge and this patient showed a slight rise of post-sting ST concentration. Based on data of only one patient it is impossible to draw firm conclusions on possible interactions between elevated post-sting ST and associated SAR. Nevertheless, it has been reported that patients with milder symptoms

of SAR exhibited also weaker increases of ST compared with patients with more severe symptoms (10). However, a rise in ST concentration was not found in all patients, even if the anaphylactic reaction had been severe (11, 12). Natural diurnal variations of ST concentration may interfere with a potential increase of ST concentration after SAR, thereby, possibly explaining these contradictory findings. The majority of patients with SAR after sting challenge demonstrate an ST peak which occurs between 15 and 60 min after allergen exposure but which may be already observed after 1–5 min in individual patients (10). Therefore, blood sampling at the 'wrong' time may give 'false negative' results.

Based on our data it is only safe to conclude that slightly decreased or unchanged ST concentrations 20 min, 90 min or 18 h after challenge are associated with a successful therapy. The minor decrease of ST during the day possibly reflects circadian rhythm and is not related to sting challenge.

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