

An increase in serum tryptase even below 11.4 ng/mL may indicate a mast cell-mediated hypersensitivity reaction: a prospective study in Hymenoptera venom allergic patients

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Summary

Background During a systemic hypersensitivity reaction (SR), an increase in serum tryptase compared to the baseline value is an indicator of mast cell activation, most often due to an IgE-mediated mechanism.

Objective To study the relevance of an increase in serum tryptase below the upper normal value of 11.4 ng/mL.

Methods Serum tryptase levels were measured in 35 patients with Hymenoptera venom hypersensitivity before and during venom exposure. Of these, 20 developed SR to stings or following venom injections during immunotherapy (reactors), while 15 tolerated re-exposure to stings or venom injections during immunotherapy without SR (non-reactors). Serum tryptase was estimated at 2, 5 and 24 h after exposure and was compared to a baseline value obtained before or at least 72 h after exposure.

Results Considering circadian variation of serum tryptase, a relative increase to $\geq 135\%$ of the baseline value (relative delta bound) was defined to indicate mast cell activation. Such an increase was observed in 17 of 20 reactors (85%), but none of 15 non-reactors. A serum tryptase of ≥ 11.4 ng/mL following venom exposure was observed in eight of the 20 reactors (40%) and 2 (13.3%) of the 15 non-reactors. Both these non-reactors also had an elevated baseline serum tryptase.

Conclusions and Clinical Relevance Serum tryptase values obtained during a suspected hypersensitivity reaction must always be compared to a baseline value. A relative tryptase increase to $\geq 135\%$ of the baseline value during a suspected hypersensitivity reaction indicates mast cell activation even below 11.4 ng/mL.

Keywords anaphylaxis, Hymenoptera venom allergy, hypersensitivity reaction, mast cell, tryptase, venom immunotherapy

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Introduction

Tryptase is highly specific for mast cells as only trace amounts of $<1\%$ are detected in basophils and none in other human cells [1, 2]. Protryptases, α - and β -tryptase are produced in mast cells. Protryptases are secreted continuously by non-activated mast cells, β -tryptase is stored in mast cell granula and is only released after mast cell activation in its active form, together with other mediators, during hypersensitivity reactions [3].

The currently available commercial test to measure serum tryptase (sT) detects both protryptases and

mature forms of α -tryptase and β -tryptase. The manufacturer has validated the baseline sT value threshold in 126 patients without evidence of mast cell stimulation and found a geometric mean of 3.8 ng/mL and a 95% upper percentile of 11.4 ng/mL [4].

An elevated baseline sT in the absence of a hypersensitivity reaction is due to continuously secreted protryptases and indicates an increased wholebody mast cell load, in levels above 20 ng/mL most likely due to systemic mastocytosis [5, 6]. An elevated baseline sT is associated with an increased risk of severe anaphylaxis, especially to Hymenoptera stings [7–9].

An increased sT during a systemic hypersensitivity reaction (SR), on the other hand, indicates mast cell activation with release of β -tryptase, most often due to interaction of allergens with mast cell fixed specific IgE-antibodies [3, 5]. The highest increase of sT is usually observed within 30–120 min but a marked increase may be observed up to 6 h [10, 11] after allergen exposure. The sT values ≥ 11.4 ng/mL during a SR as anaphylaxis, but also during acute systemic skin reactions like urticaria, angioedema or flushing, are considered to indicate mast cell activation [4]. However, this interpretation may miss a significant increase of sT due to mast cell activation below 11.4 ng/mL and on the other hand, wrongly indicate mast cell activation in a person with a high baseline sT due to increased wholebody mast cell load. With the total tryptase immunoassay, an isolated sT value can therefore not be used as an indication of mast cell activation. A baseline sT value must be obtained for comparison [12].

The goal of our study was to analyse the course of sT in patients with a history of systemic reactions to Hymenoptera stings with and without hypersensitivity reactions following venom exposure.

Methods

Patients

We included 35 adult patients with a history of SR to Hymenoptera stings referred in the years 2007–2009. Of these, 20 developed SR to Hymenoptera venoms, in six following field stings and in 14 following venom injections during venom immunotherapy (VIT) (reactors, Table 1), whereas 15 did not develop SR following venom reexposure by either sting challenge (5) or venom injections during VIT (10) (non-reactors, Table 2). The severity of SR was graded according to HL Mueller [13, 14].

Blood sampling

Blood samples from all study participants were obtained at 2, 5 and 24 h after allergen exposure and sera stored at -20°C . In patients reacting to field stings, the time interval from sting to hospital entry was sometimes difficult to determine exactly and was varied from 2 to 5 h (Table 1). For estimation of baseline sT, blood was taken before either the ultra-rush protocol, maintenance VIT or a sting challenge. In patients referred because of an acute SR following a field sting blood for baseline sT was obtained ≥ 72 h after allergen exposure and at least 24 h after all symptoms of anaphylaxis had disappeared. The venom dose resulting in SR during VIT is mentioned in Table 1. In non-reactors, the cumulative dose of venom applied during an ultra-rush protocol

over 3.5 h is 111.1 μg [15]. Following an SR during VIT, the treatment was continued 1 week later. The average venom dose after a sting is estimated to be 50 μg in honey bees and 3–5 μg in *Vespula* [16].

Diagnostic tests

Specific IgE to Hymenoptera venoms as well as sT were measured by UNICAP (Phadia, Uppsala, Sweden). A specific IgE value of ≥ 0.35 kU/L was regarded as positive.

Intracutaneous skin tests with Hymenoptera venoms at concentrations of 10^{-8} , 10^{-6} , 10^{-4} and 10^{-3} g/L were performed with Pharmalgen venoms (ALK-Abello, Horsholm, Denmark). A weal formation of ≥ 5 mm in diameter with surrounding erythema at a concentration of $\leq 10^{-3}$ g/L was considered as positive.

In patients exposed by venom injections during VIT or by a sting challenge, data on sensitization were obtained before the venom reexposure. In patients who reacted to a field sting, they were obtained within 1 month after the event.

All reactors and non-reactors agreed for participation in the study by a written informed consent. The protocol of the study was accepted by the local ethical committee (Kantonale Ethikkommission, University of Bern, Bern, Switzerland).

Statistics

For statistical analysis, the 20 reactors and the 15 non-reactors were compared with regard to the course of sT during venom exposure. We calculated the relative increase of the highest sT 2–5 h after allergen exposure in % of the baseline sT, in all reactors and non-reactors (Tables 1 and 2).

A value of sT above the absolute bound of 11.4 ng/mL during a suspected SR is often used in clinical practice to decide whether a patient had an anaphylactic reaction or not, although in international guidelines, it is recommended to always compare the actual value with a baseline sT [11, 17, 18]. This absolute bound is an estimator of the 95 upper percentile based on a study by the manufacturer of an *in vitro* test system for the quantitative measurement of tryptase in human serum [4]. Our goal was to find an estimate of the 95% quantile for the relative maximum of sT in non-reactors. The QQ-plot and the Shapiro–Francis test for normality ($W = 0.900$, $P = 0.096$) indicated no major violation. Based on normality assumption, the minimum variance unbiased estimator of the 95 upper percentile [19] was calculated. This led to a bound for the relative increase of sT to 118.3%. Bearing in mind that the sT values have a diurnal variation of about 15% [20, 21], a rather conservative relative delta bound of 135% was chosen for the analysis.

Table 1. Data of 20 patients with a history of anaphylaxis to Hymenoptera stings who reacted during venom exposure (reactors)

Patient (gender/age)	Allergen group (dose of venom)	Symptoms (grading according to HL Mueller)	Blood sample after starting symptoms (h)	Intradermal test with venom of hb or yj (g/L)	UNICAP sIgE with venom of hb or yj (kU/L)	Baseline sT (ng/mL)	sT max 2–5 h (ng/mL)	Relative value of sTmax in % of baseline (%)
1 (m/58)	yj-sting	Generalized pruritus, flush, urticaria, angioedema, dyspnoe, stridor (3)	2	yj 10^{-6}	3.6	4.89	11.2	229.04
2 (f/43)	yj-sting	Generalized pruritus, dyspnoe, wheezing, mortal fear, hypotension (4)	5	yj 10^{-6}	4.1	1.30	4.90	376.92
3 (f/58)	hb UR (cum. 11.1 µg)	Itching throat, flush, nausea, stomach ache, stool-urge, hypotension (4)	2	hb 10^{-4}	>100	2.98	6.10	204.70
4 (f/39)	hb UR (cum. 61.1 µg)	After injection: flush, unease, congested nose, drop of PF 350 → 270 (3)	2	hb 10^{-4}	7.58	7.05	10.0	141.84
5 (m/66)	hb md (100 µg)	Itching palms and tongue, rhinoconjunctivitis, flush (1)	2	hb 10^{-8}	6.81	5.19	12.0	231.21
6 (f/33)	hb md (100 µg)	Flush, unease, cough, dyspnoe, drop of PF 420 → 290 (3)	2	hb 10^{-8}	86.3	2.22	4.90	220.72
7 (m/24)	hb UR (cum. 31.1 µg)	Itching, conjunctivitis, cough, throat-irritation, wheezing, drop of PF 520 → 430 (3)	2	hb 10^{-4}	12.3	2.25	4.40	195.56
8 (m/74)	hb UR (cum. 31.1 µg)	Flush, chest compression, drop of PF 510 → 400, hypotension (4)	2	hb 10^{-4}	2.55	4.85	15.0	309.28
9 (m/53)	hb UR (cum. 100 µg)	Flush, feeling of chest compression, dizziness, hypotension (4)	2	hb 10^{-3}	0.47	97.00	1570	1618.56
10 (m/18)	hb md (100 µg)	Flush, itchy throat, cough, drop of PF 570 → 460 (3)	2	hb 10^{-8}	59.7	5.89	13.0	220.71
11 (f/46)	hb md (80 µg)	Flush, unease, nausea, stomach ache (2)	2	hb 10^{-8}	29.0	8.26	12.0	145.28
12 (f/43)	hb UR (cum. 50 µg)	Dry mouth, flush, tickling palms, blocked nose (1)	2	hb 10^{-6}	18.7	4.30	4.60	106.98
13 (m/27)	hb md (cum. 100 µg)	Generalized exanthema, difficulty to breathe, drop of PF 640 → 550 (3)	2	hb 10^{-4}	3.42	11.50	15.0	130.43
14 (f/29)	hb md (cum. 100 µg)	Flush, abdominal cramping, dyspnoea (3)	2	hb 10^{-6}	9.31	1.54	2.30	149.35
15 (m/42)	yj-sting	Generalized pruritus, nausea, unconsciousness (4)	5	yj 10^{-8}	4.2	9.22	60.0	650.76
16 (f/17)	hb-sting	Exanthema, swollen lips, dyspnoe, unconsciousness (4)	5	hb 10^{-8}	23.1	3.02	11.2	370.86
17 (m/38)	hb UR (cum. 61.1 µg)	Discomfort, stomach ache, drop of PF 800 → 690 (3)	2	hb 10^{-4}	39.2	2.10	4.40	209.52
18 (m/53)	3 yj-stings	Generalized pruritus, urticaria (1)	2	yj 10^{-6}	74.7	4.62	6.25	135.28
19 (m/54)	hb or yj-sting	Generalized pruritus, flush, intestinal cramps (2)	2	hb 10^{-6}	24.7	5.84	13.0	222.60
20 (f/21)	hb UR (cum. 21.1 µg)	Flush, wheezing, drop of PF 460 → 390 (3)	2	yj neg hb 10^{-6}	<0.35 63.6	3.90	5.10	130.77

hb, honey bee; yj, yellow jacket (*Vespula spp*); UR, ultra-rush phase of venom immunotherapy; md, maintenance dose of venom immunotherapy; cum, cumulative dose; PF, peak-flow.

Table 2. Data of 15 patients with a history of anaphylaxis to Hymenoptera stings who did not react to venom reexposure (non-reactors)

Patient	Gender	Age	Allergen exposure	Intradermal test with venom of hb or yj	UNICAP sIgE hb or yj	Baseline sT	sTmax (2–5 h)	Relative value of sTmax in % of baseline
Units				g/L	kU/L	ng/mL	ng/mL	%
A	m	43	UR yj	10 ⁻⁴	0.65	5.41	4.56	84.28
B	m	28	UR yj	10 ⁻⁴	0	3.06	3.18	103.92
C	m	41	UR hb	10 ⁻⁴	4.30	14.50	13.50	93.10
D	m	28	UR yj	10 ⁻⁴	0	1.00	1.00	100.00
E	f	50	UR yj	neg	0	17.3	15.2	87.8
F	m	46	UR hb	10 ⁻⁴	0/BAT pos	4.14	4.87	117.63
G	f	41	UR hb	10 ⁻⁴	23.40	5.13	4.99	97.27
H	m	48	Sting challenge yj	10 ⁻⁴	0.63	7.98	7.16	89.72
I	f	45	Sting challenge yj	10 ⁻⁴	1.10	3.49	4.03	115.47
J	f	65	Sting challenge yj	10 ⁻⁴	4.08	3.61	3.49	96.67
K	m	42	Sting challenge hb	10 ⁻³	0.46	3.24	3.99	123.14
L	f	31	Sting challenge yj	10 ⁻⁶	9.34	5.25	4.67	88.95
M	m	60	UR hb	10 ⁻⁴	48.20	2.53	2.38	94.07
N	f	38	UR hb	10 ⁻⁸	63.20	4.59	4.54	98.91
O	m	34	UR hb	10 ⁻⁸	38.50	4.47	4.35	97.31

BAT pos, basophil activation test positive; hb, honey bee; UR, ultra-rush; sT, serum tryptase; yj, yellow jacket wasp.

The difference between the absolute peak bound of 11.4 ng/mL and our relative delta bound of 135% of baseline sT to indicate mast cell activation was analysed by the exact test based on McNemars χ^2 statistic [22]. The discriminatory power of the 11.4 ng/mL peak bound and our relative delta bound between index and control patients was analysed by Fishers exact test.

Results

Allergic background of reactors and non-reactors to Hymenoptera venom exposure

All the 20 reactors with a history of SR to Hymenoptera stings had positive skin tests and sIgE to venoms of the causal insect. The 15 non-reactors during allergen exposure all had a positive history of systemic sting reactions and all except one (non-reactor E) also had positive diagnostic tests to venoms. Non-reactor E had both negative skin tests and sIgE despite a history of sting induced anaphylaxis. He was treated by ultra-rush VIT because of a strongly elevated baseline sT, which is known to increase the risk of severe reactions [8, 9] (Tables 1 and 2).

Of the 20 reactors, six were exposed to Hymenoptera field stings and 14 to venom injections during VIT, eight during the first day of an ultra-rush protocol and six during maintenance immunotherapy. Of the 15 non-reactors, five were exposed by sting challenge tests during maintenance VIT and 10 by injections during the first day of an ultra-rush protocol.

Clinical reactions to venom exposure in 20 reactors

Following venom exposure, five of the 20 reactors developed only cutaneous and/or gastrointestinal symptoms (grade I and II), nine also developed respiratory symptoms (grade III) and six in addition, developed cardiovascular symptoms (grade IV) (Table 1).

Course of sT following allergen exposure in reactors and non-reactors

According to the peak bound of 11.4 ng/mL, only 8 (40%) of the 20 reactors had a sT level of ≥ 11.4 ng/mL during exposure, of whom two had also a baseline sT of >11.4 ng/mL. None of the non-reactors had an increase of the sT to ≥ 11.4 ng/mL but two had an elevated baseline sT, while sT did not increase during venom exposure (patients C and E) (Tables 1 and 2, Figs 1a and b).

Of the 20 Hymenoptera venom allergic reactors, 17 (85%) had a relative increase to $\geq 135\%$ of baseline sT, two of them with an elevated baseline sT (patients 9 and 13). Two of the three patients who had no increase of sT to $\geq 135\%$ were very near to this bound with increases over 130% (pts 13 and 20) and had grade III reactions, while patient 12 who showed only a slight increase had a mild grade I reaction. None of the 15 non-reactors had a relative increase to $\geq 135\%$ of baseline sT.

Table 3 shows that the mean relative bound of sT increases with the severity of the hypersensitivity reaction to venom exposure.

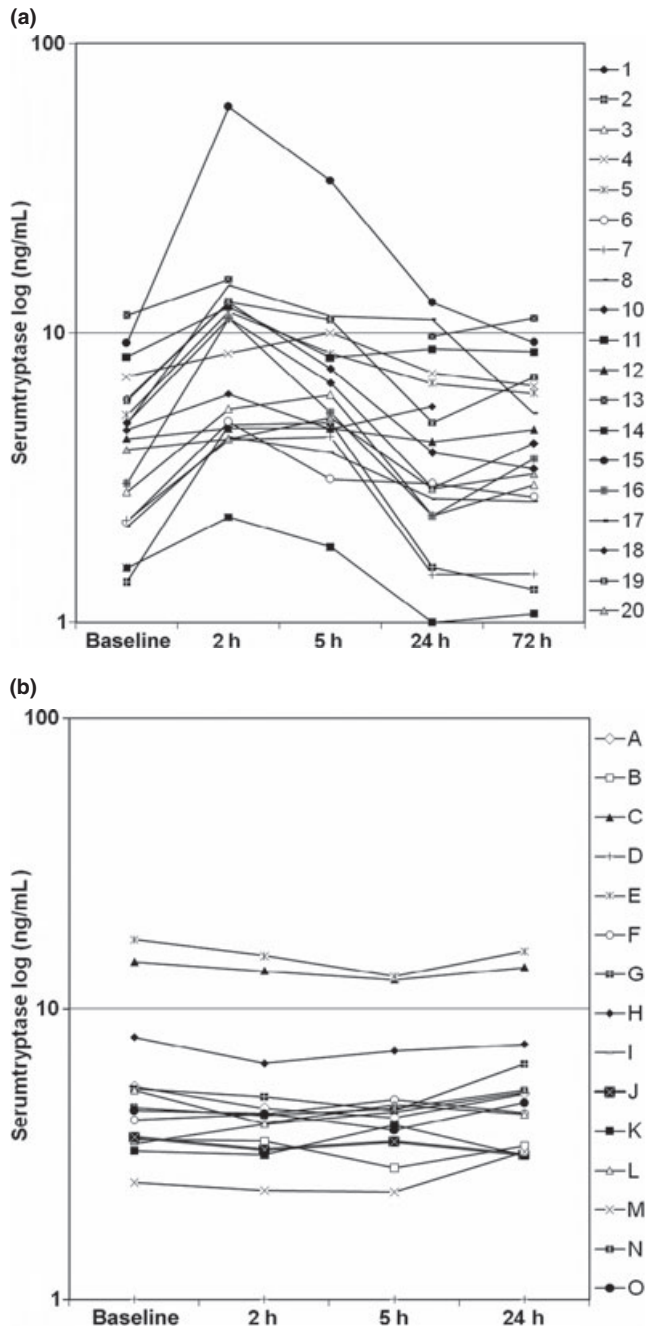


Fig. 1. (a) Evolution of sT in 20 patients with a history of anaphylaxis to Hymenoptera stings who reacted during venom exposure (reactors). (b) Evolution of sT in 15 patients with a history of anaphylaxis to Hymenoptera stings who did not react to venom reexposure (non-reactors). For better comparison of reactors and non-reactors, patient 9 of reactors with mastocytosis and extremely high tryptase values up to 1570 ng/mL was not included in a).

Our relative delta bound detects an allergic reaction significantly better than the absolute bound ($\chi^2 = 8.64$, $P < 0.01$) in reactors. For the non-reactors, as expected, no significant difference was found ($\chi^2 = 2.14$; $P > 0.1$, not significant). However, in the non-reactors, two patients had baseline sT levels above the absolute

Table 3. Maximal relative increase of serum tryptase 2–5 h after allergen exposure in relation to severity of the systemic reaction

Severity grade after HL	Number of patients	Relative increase in % of tryptase after 2–5 h	
		Mean value	SD
Non-reactors	15	99.2	11.4
Grade 1 and 2	5	162.4	55.3
Grade 3	9	258.1	160.6
Grade 4	6	382.5* (493.5)	165.2* (558.1)

*Mean value and standard deviation (SD) without patient 9 with mastocytosis, in brackets with patient 9.

bound, while none had increases above the relative delta bound, which means that it is unlikely that the relative bound was chosen too low. Seventeen of the 20 reactors and none of the 15 non-reactors had values above the relative bound of 135%. According to Fisher's exact test, there is a significant connection between the group membership of a patient and the fact that the relative maximum lies above 135% ($\chi^2 = 25.74$, $P = 0.0001$). However, there is no significant connection between the group membership of a patient and the fact that the absolute maximum lies above 11.4 ng/mL ($\chi^2 = 2.552$, $P = 0.1422$, not significant) (Fig. 2).

Discussion

Diagnosing an allergic cause of an acute SR is sometimes difficult, for example, during a perioperative acute reaction with arterial hypotension, which may be

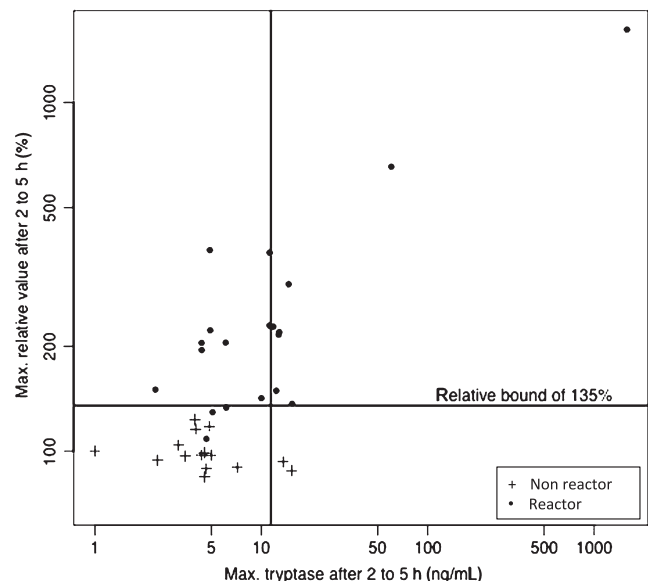


Fig. 2. Comparison of the absolute bound of 11.4 ng/mL with the relative bound of $\geq 135\%$ of the baseline sT in 35 patients with a history of anaphylaxis to Hymenoptera stings, 20 reactors and 15 non-reactors during venom exposure.

Table 4. Identification of anaphylaxis by serum tryptase determination: Comparison of different bounds based on the 20 reactors and 15 non-reactors after allergen exposure of this study

Bound	20 reactors positive	Positive predictive value	15 non-reactors positive	Negative predictive value
Peak bound of 11.4 ng/mL	8 (40%)	0.80	2 (13%)	0.520
Peak bound of 8.23 ng/mL Enrique [24]	11 (55%)	0.846	2 (13%)	0.590
Absolute delta bound of ≥ 2 ng/mL compared to baseline tryptase Brown [12]	16 (80%)	1.0	0	0.789
Relative delta bound of $\geq 135\%$ compared to baseline tryptase Present study	17 (85%)	1.0	0	0.833
Comparison of the relative delta bound of $\geq 135\%$ for sensitivity by χ^2 -test vs.			χ^2	<i>P</i>
Absolute peak bound of 11.4 ng/mL			8.23	<0.005
Absolute peak bound of 8.24 ng/mL			4.29	<0.05
Absolute delta bound of ≥ 2 ng/mL			0.17	>0.25

The lower specificity of the two peak bounds did not reach significance.

due to bleeding, fluid loss, unintended drug effects, including overdosing, toxic effects and allergy to drugs or natural rubber latex. Arterial hypotension and unconsciousness following Hymenoptera stings, with no typical allergic symptoms, like urticaria or angioedema, may be caused not only by venom allergy, but also by a vasovagal reaction due to anxiety and pain. Estimation of sT within 0.5–5 h may be helpful in such a situation because a relevant increase of sT due to mast cell activation will indicate an SR, most often due to IgE-mediated allergy. Such an increase is especially frequent in SR to hymenoptera stings, is also observed in anaphylaxis to drugs, but less often in SR to foods [12, 18, 23, 24].

An sT value above the peak bound of 11.4 ng/mL during a suspected SR is often considered to indicate mast cell activation. Such an absolute bound is, however, not optimal for two reasons: (1) in our study, not only two reactors (10%) but also two non-reactors (13.3%) had a baseline sT over 11.4 ng/mL. While the two reactors developed increases of sT after allergen exposure, the two non-reactors did not; (2) an increase below 11.4 ng/mL may also indicate an SR, but this will be missed if using the absolute bound.

Several possibilities to improve the value of sT for the identification of acute allergic/anaphylactic reactions have been proposed: Enrique compared 17 patients with mostly food or drug-induced anaphylaxis with 13 who developed only cutaneous reactions, like urticaria or angioedema, most often of unknown causes, and found that a peak bound of 8.23 instead of 11.4 ng/mL distinguished the two groups much better. A control group with no symptoms was, however, not included and baseline sT was only measured in part of the patients [25]. Lin proposed an even higher peak bound of 15 ng/mL in an emergency department study on acute allergic reactions, but could not show an advantage of this approach [26].

In our opinion, it is much more important to always compare the sT during a suspected SR with the baseline sT, in blood taken at least 3 days later and at least 24 h after disappearance of any symptoms of hypersensitivity [12, 17, 18]. By this approach, relevant increases also below 11.4 ng/mL and in patients with elevated baseline sT will be identified. Brown *et al.* analysed 64 patients allergic to the venom of the Australian ant *Myrmecia pilosula* during immunotherapy with venom or placebo by a sting challenge and compared 11 with severe anaphylaxis to 41 with no SR and to 11 with only milder SR. An absolute delta bound of 2 ng/mL between baseline sT and sT during 60 min after allergen exposure was definitely more sensitive in a receiver-operator characteristics curve analysis ($P = 0.0172$) than the conventional peak bound [12].

We chose a somewhat different approach. We considered that in patients with a baseline sT over 10 ng/mL the specificity of an absolute delta bound of 2 ng/mL will decrease, while in sT levels below the mean value of 3.8 ng/mL [4] the sensitivity will be lower. We therefore calculated the 95% quantile for the relative increase of sT in non-reactors and also considered the circadian variation of baseline sT [20, 21], resulting in the choice of a relative bound of 135% of baseline sT. In Table 4, we compare the absolute bounds of 11.4 and 8.23 ng/mL [25] with the absolute delta bound of Brown [12] and with our relative delta bound for 20 reactors and 15 non-reactors. It is obvious that the delta bounds are highly superior to the absolute bounds, while there is no significant difference between the two delta bounds, both with regard to sensitivity and to specificity. Our relative delta bound may, however, be more reliable in patients with an elevated bT – four of the 35 patients (11.4%) in our study. The absolute delta bound of 2 ng/mL will be less specific in patients with baseline sT values over 20 ng/mL, for example, in our reactor 9 with a baseline sT of

97 ng/mL, an increase to 99 ng/mL could definitely not be considered to indicate anaphylaxis.

For our relative delta bound, an increase of less than 135%, for example, of $\geq 120\%$ could theoretically even be relevant and indicate mast cell activation, if the baseline sT would be estimated at the same time of the day at least 3 days after a suspected SR.

However, more data on the circadian variation of sT, with and without allergen exposure, will be needed to confirm this. Also for the statistical analysis of the observed increase of the relative delta bound with severity of the SR (Table 3), considering the large variability within severity grades, data from more patients will be necessary.

In conclusion, we observed that the diagnostic value of sT during a suspected allergic/anaphylactic hypersensitivity reaction can be improved significantly by always comparing the peak value with the baseline sT of the patient, and instead of a peak bound using a relative delta bound.

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