Acta Derm Venereol 2012; 92: 484–489

Included in the theme issue:
ATOPIC DERMATITIS, URTICARIA AND ITCH
Acta Derm Venereol 2012; 92: 449–581

INVESTIGATIVE REPORT

Impact of Age and Heterophilic Interference on the Basal Serum Tryptase, a Risk Indication for Anaphylaxis, in 1,092 Dermatology Patients

Sibylle SCHLIEMANN, Florian SEYFARTH, Uta-Christina HIPLER and Peter ELSNER
Department of Dermatology and Allergology, University Hospital Jena, Friedrich-Schiller-University, Jena, Germany

A raised baseline serum tryptase is a risk indicator for anaphylactic reactions, especially in patients with hymenoptera venom allergy. Borderline elevations (> 11.4 µg/l) occur frequently and may necessitate invasive diagnostic procedures to rule out systemic mastocytosis. We retrospectively analysed 1,092 non-mastocytotic patients from our general dermatology clinic with respect to age- and gender-associated effects and investigated the impact of heterophilic antibody interference on the tryptase assay. The results were stratified by gender and five age classes. Sera with raised tryptase ($n = 106$) were re-tested after pre-incubation with Heterophilic Blocking Tubes (HBT®, Scantibodies Laboratory; Santee, CA, USA). A significant increase in baseline tryptase was observed with increasing age. Incubation with HBT® caused a decline of more than 50% in only one case. In conclusion, older patients showed significantly higher serum tryptase levels and heterophilic interference was of subordinate relevance. ***Key words: baseline serum tryptase; mastocytosis; heterophilic antibody; heterophilic blocking tubes; age-related effects; hymenoptera venom allergy.***

(Accepted August 1, 2011.)

Acta Derm Venereol 2012; 92: 484–489.

Sibylle Schliemann, Department of Dermatology, University Hospital Jena, Friedrich Schiller University Jena, Erfurter Straße 35, DE-07734 Jena, Germany. E-mail: schliemann@derma-jena.de

The baseline serum level of the enzyme tryptase, a serine endoprotease found in mast cells, is used as a diagnostic marker of mastocytosis and is considered an important risk indicator for severe anaphylactic reactions, particularly in the context of hymenoptera venom allergy (1–9). Thus, measurement of serum tryptase (ST) levels is recommended for patients with insect venom allergies prior to a planned venom immunotherapy in order to estimate their risk for severe anaphylaxis and to determine whether lifelong immunotherapy should be considered (4, 10, 11). Analysing ST levels may also be relevant in the context of other immediate-type reactions (12, 13).

From the subtypes known, inactive pro-beta tryptase is now believed to be secreted constitutively and is thus thought to be the main determinant of the total baseline concentration of the ST, which correlates with the total mast cell burden. In contrast, mature beta tryptase can increase transiently in severe anaphylaxis (3, 14). The commercial test kit (UniCAP 100® Tryptase, Phadia, Uppsala, Sweden) measures the total tryptase (protryptases and mature beta tryptase). A second determination 4–6 weeks later is recommended to either affirm or exclude a constant elevation of the enzyme. Careful skin examination is mandatory in order to recognize cutaneous mastocytosis (CM) in cases of repeatedly raised values, and an extensive diagnostic procedure of systemic mastocytosis (SM) is indicated. Total ST levels exceeding 20 µg/l are regarded as a minor criterion for SM (15). However, with respect to hymenoptera venom immunotherapy, in cases with constant ST elevation, lifelong therapy is recommended in Germany even if the diagnosis cannot be established (6, 10, 11, 16). Since the procedures involved in the diagnosis of SM are invasive (e.g. skin-, gastrointestinal-, and bone marrow biopsy) and may be associated with adverse events, a considerable number of patients with mildly raised ST will not comply and will forgo complete work-up. Therefore, elevation of the ST levels not only affects the individual patient, but increases the burden of medical care costs. Validated threshold values of ST levels are lacking (10). According to the manufacturer (UniCAP 100® Tryptase, Phadia, Uppsala, Sweden), values exceeding 11.4 µg/l should be regarded as elevated, based on a study with 126 healthy male and female test persons showing a geometric mean of 3.8 µg/l and a 95th percentile of 11.4 µg/l, after re-evaluation of the former threshold of 13.5 µg/l. However, since single cases with CM and SM have been observed in association with ST levels lower than 11.4 µg/l and 13.5 µg/l, respectively, in patients presenting with severe anaphylactic reactions due to hymenoptera stings (17–19); in Germany it has been proposed to consider a diagnosis of mastocytosis if the ST level is only 8–10 µg/l (10). Given the fact that a considerable number of patients do show mildly elevated ST levels in their daily routine, the allergist is frequently confronted with the dilemma of whether or not to perform the complete mastocytosis work-up.

Determination of ST levels is indicated in patients with a history of anaphylaxis and insect venom allergy. In our institution, ST values above 8.75 µg/l would be followed

by a confirmatory blood sampling after 4–6 weeks. Values greater than this involve total skin inspection with special consideration of CM, eventual skin biopsy, and detailed case history focused on mastocytosis, e.g. asking for unexplained anaphylactic symptoms, drug and food intolerance reactions, spontaneous fractures (20), as well as gastrointestinal and cardiac complaints (21, 22). Further diagnostic steps according to recommendations are initiated depending on the history (15, 23).

We have observed a high number of mildly to moderately raised ST values in patients without mastocytosis, especially in older individuals. Therefore, the aim of this retrospective analysis was to investigate a potential association of ST levels and age in a large sample of non-mastocytotic patients. In addition, we intended to investigate the influence of interfering circulating antibodies as potential causes of elevated ST levels, most often referred to as heterophilic antibodies, based on their ability to bind different antigens. The origin of heterophilic antibodies can either be non-iatrogenic (e.g. rheumatoid factors, autoimmune diseases) or result from animal exposures (anti-animal antibodies). They are able to interfere with two-site (sandwich) immunoassays. Therefore, they may cause false-positive results (24, 25). Recently, evidence has emerged that falsely elevated ST levels, e.g. in patients where mastocytosis was ruled out, might be attributed to heterophilic interference (26, 27). Principally, two different procedures can reduce heterophilic interference. Serum samples can either be pre-incubated with animal serum (non-specific blocking) or, as used in our study, with specific antibodies against heterophilic antibodies, e.g. HBT®, (25) which blocks human anti-mouse, anti-goat, anti-sheep, anti-rabbit antibodies and rheumatoid factor.

MATERIALS AND METHODS

Test population and procedure

Data were derived from a patient population attending a general dermatology clinic at the University Hospital Jena, which supplies approximately 2.7 million inhabitants in the area with dermatology services, including in-patient treatment as well as services of a general dermatology and allergy ambulance with approximately 7,000 patients seen per year. Since the majority of patients seeking help for allergic diseases in Germany is seen by dermatologists, the patient population includes a percentage of at least 25% with conditions related to allergic diseases, such as different types of eczema, food-, drug,- and insect venom allergy, and urticaria. In this clinic, the ST levels are routinely determined for patients with a history of immediate-type allergies and unexplained or suspected anaphylaxis in the attached biochemistry laboratory results. Including referral material, 3,131 single ST routine determinations (UniCAP 100® Tryptase, Phadia, Uppsala, Sweden, single measurements) were performed in our laboratory between February 2001 and October 2007, corresponding to 2,394 individuals, some with repeated determinations over time. Since the aim of this retrospective analysis was to investigate a potential association of ST levels and age in a large sample of non-mastocytotic patients, we excluded data from all referral patients ($n = 1{,}271$) with unknown case histories as well as data from patients with established mastocytosis ($n = 22$), either cutaneous or systemic. We further excluded data from patients with borderline-elevated ST levels ($n = 9$) that did not undergo any type of biopsy, including skin biopsy in the case of suspected cutaneous mastocytosis, or bone-marrow and/or gastrointestinal biopsy in the case of suspected systemic mastocytosis. In patients with repeated determinations over time, calculated means of ST levels were used for further analysis, since the variability of values within the respective subjects was low. Finally, ST data of 1,092 patients, characterized by a known clinical history and exclusion of CM and SM in cases of borderline or raised ST levels according to the above-mentioned criteria, were included for statistical analysis. The institutional ethics committee of the University Hospital Jena approved the study protocol. The subjects were classified into five age classes (0–4, 5–14, 15–34, 35–64, and ≥ 64 years), a classification derived from asthma surveillance (28). Those sera showing ST levels above 8.75 µg/l (a critical value that was pragmatically chosen based on the arithmetic mean (± 3.1 µg/l) of ST levels obtained from 100 healthy individuals in Jena) underwent re-testing, using Heterophilic Blocking Tube® (HBT®, Scantibodies Laboratory, Santee, USA, batch H554D).

Heterophilic Blocking Tube

In total, 106 samples (lowest ST: 8.82 µg/l) were tested using HBT® (Scantibodies Laboratory, Santee, USA, batch H554D). The frozen samples (–20ºC) were thawed at room temperature, and 500 µl of each probe incubated with HBT® for one hour. The samples were then administered to the UniCAP 100® and the ST levels were determined as described above. In cases in which the current ST level was lower than the preceding value, heterophilic interference was considered (29). However, due to potential ageing of the samples and storage and test kit modifications over time, some degree of deviation in the tryptase values is expected. Thus, it is possible for heterophilic interference to be mimicked by these deterioration effects. In order to eliminate this potential bias of over-interpretation of heterophilic interference, the respective samples were additionally re-tested without preceding heterophilic blocking (19). The differences between former and actual values were averaged and served as a correction factor that was added to the individual values obtained with heterophilic blocking. Only these corrected values were considered for the final evaluation of heterophilic interference.

Internal assay quality control

Intra- and inter-assay measurements were conducted for internal quality control of the assay and to determine the coefficient of variation (CV). To establish the intra-assay variability, 10 repeated tryptase measurements per serum sample were performed on a single day. Two serum samples from two subjects were used in total, revealing a mean value of 8.26 µg/l (CV 1.7%, sample 1), and mean value of 44.5 µg/l (CV 1.1% sample 2). Subsequently, the same procedure was repeated after incubation with HBT® according to the manufacturer's directions, and again the tryptase was repeatedly determined 10 times, resulting in mean values of 8.47 µg/l (CV 1.8%) and in 45.1 µg/l (CV 2.6%), respectively.

To determine the inter-assay variability, the CV was calculated based on five tryptase measurements on five consecutive days. Two serum samples from subjects different than used for the intra-assay survey, resulted in mean values of 9.85 µg/l (CV 7.1%) and of 33.16 µg/l (CV 5.5%), respectively. Subsequently, aliquots of the samples were incubated with HBT® and underwent the same test procedure, resulting in mean values of 9.53

µg/l (CV 8.7%) and 33.26 µg/l (CV 1.2%). We were not able to validate the heterophilic antibody assay, which would require the use of specimens with true positive or negative interference, which are not provided by the manufacturer.

Statistical analysis (SPSS, version 13)

Descriptive data analysis of the whole population as well as within age classes and sexes included calculation of arithmetic means and medians, standard deviations (SD), 95th percentiles and 95% confidence intervals (95% CI) of the ST levels. Univariate variance analysis served for trend analysis of potential age-associated variations of the mean baseline ST level. The Mann–Whitney *U* test was used for further investigation of differences between age classes and between genders, since this test does not require normally distributed data and is used to assess observations from groups independent of each other. The level for statistical significance was $p < 0.05$, with Holm-Bonferroni corrections for multiple comparisons (30). Since we were interested in the prevalence of raised ST levels with increasing age, data were dichotomized into two categories of either normal or raised values in a first step. Due to the lack of a truly validated published threshold value in the literature, the definition of either "normal" (< 8.75 and < 11.4 µg/l, respectively) or "raised" values (≥ 8.75 and ≥ 11.4 µg/l, respectively) was based both on the pragmatically chosen mean value (8.75 ± 3.1 µg/l) used in our department between 2001 and 2007 (result obtained from 100 healthy individuals) as well as on the released value of 11.4 µg/l. The latter refers to the current product information of the manufacturer (UniCAP 100® Tryptase) and reflects the 95% percentile derived from 126 healthy individuals. Correlation between age classes and the numbers of patients with elevated ST levels was analysed using the χ^2 test.

RESULTS

Description of the test population

The patient population was composed of 672 (61.5%) females (age range 3–90 years, mean age 44 years) and 420 (38.5%) males (age 1–92 years, mean age 44 years), with the majority of patients being in the age range 35–64 years (56.0%), followed by those 15–34 years (26.4%), and ≥ 65 years (13.4%). Children from 0 to 4 years and from 5 to 14 years were under-represented ($n = 9$, 0.82% and $n = 37$, 3.4%, respectively).

Serum tryptase levels in patients with mastocytosis

Twenty-two patients (0.92% of 2394) with confirmed diagnosis of CM or SM who were not included in the data-set of 1,092 analysed patients mentioned above were clinically characterized as follows: SM was diagnosed in 13 individuals, based on detection of mast cell infiltrates in biopsies (e.g. ileum) by the use of mast cell staining and elevated ST level (< 20 µg/l (16.3 µg/l): $n = 1$; 20–50 µg/l: $n = 4$; > 50 µg/l: $n = 8$). Six patients with SM displayed an additional cutaneous manifestation, e.g. urticaria pigmentosa. Three out of 9 patients with isolated CM displayed unsuspicious ST levels of 4.08 µg/l, 4.72 µg/l, and 6.90 µg/l, respectively. Altogether, 10 out of 22 patients with confirmed mastocytosis were affected by IgE-mediated hymenoptera venom-allergy.

Serum tryptase levels in the non-mastocytotic population

The mean ST level in the total test population ($n = 1{,}092$) was 5.13 ± 3.05 µg/l, the median 4.46 µg/l (95th percentile: 10.8 µg/l, 95% CI: 4.95–5.31 µg/l). Only two subjects displayed ST values exceeding 20 µg/l (25.02 and 27.15 µg/l); however, the diagnosis of mastocytosis was not confirmed in these patients.

Serum tryptase levels related to age

The mean ST level increased steadily with age (Fig. 1). Univariate variance analysis (tryptase vs. age class) supported the hypothesis of an age-associated trend ($p < 0.001$). Pairwise comparison between the age classes confirmed significant differences in the mean ST levels in all pair comparisons between age classes III, IV, and V after α-adjustment according to Holm-Bonferroni. In contrast, the ST levels in age class I (0–4 years) did not differ significantly from any other class, while subjects from 5 to 14 years (class II) showed significantly lower concentrations only compared with the ≥ 64 year-old patients (Table I). The number of patients younger than 14 years was under-represented in our test population.

In total, 106 subjects showed ST values exceeding 8.75 µg/l (9.71%), and 45 subjects showed values exceeding 11.4 µg/l (4.12%). Older subjects were over-represented with respect to increased ST levels, which was most obvious in subjects over 64 years of age (Table II). The higher prevalence in older patients

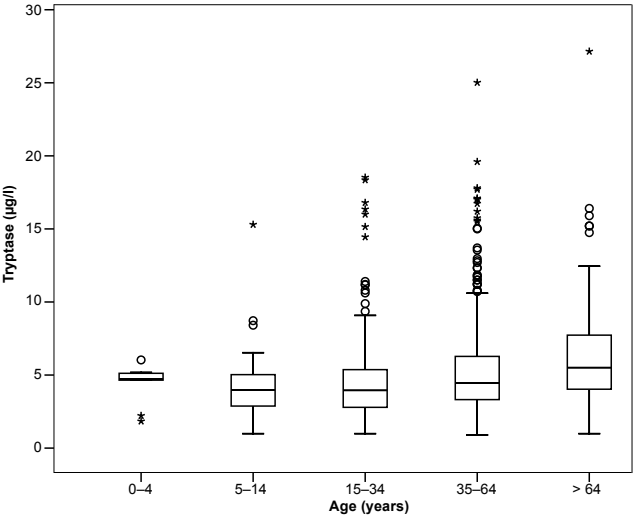

Fig. 1. Basal serum tryptase levels according to age (circles indicate values ≥1.5 inter-quartile and asterisks indicate values ≥3 inter-quartile ranges from the end of the box).

Table I. *Pairwise comparisons of tryptase levels between age classes*

		Age class				
		I	II	III	IV	V
Age class	I	–	0.446	0.487	0.816	0.1
	II	0.446	–	0.967	0.104	**<0.001**
	III	0.487	0.967	–	**<0.001**	**<0.001**
	IV	0.816	0.104	**<0.001**	–	**<0.001**
	V	0.1	**<0.001**	**<0.001**	**<0.001**	–

Significant differences (Mann–Whitney test) are in bold type ($p<0.05$ after adjustment of α according to Holm-Bonferroni). Age classes: I: 0–4 years, II: 5–14 years, III: 15–34 years, IV: 35–64 years, V: >64 years.

was significant with respect to values exceeding 8.75 µg/l in the χ^2 test ($p=0.011$); however, this was not the case for values exceeding 11.4 µg/l ($p=0.323$).

Heterophilic interference

In only 12 cases, incubation with HBT® resulted in a decline in the ST level from formerly elevated values to those < 8.75 µg/l or < 11.4 µg/l, respectively. In just a single sample, the previous value of 12.35 µg/l was reduced to 5.10 µg/l after incubation with HBT®, thus suggesting interfering heterophilic antibodies. In all other samples ($n=11$), the declines observed were much lower and thus not significant (–0.2 µg/l to –2.6 µg/l mean deviation: –1.0 µg/l).

Serum tryptase levels related to gender

The mean ST level was significantly higher in males (5.61 ± 3.40 µg/l) compared with females (4.83 ± 2.77 µg/l, $p<0.001$). While there was no significant difference between gender with respect to the prevalence of values exceeding 8.75 µg/l ($n_{♂}=49$ (11.7%), $n_{♀}=57$ (8.5%)), values exceeding 11.4 µg/l occurred more often in males ($n_{♂}=24$ (5.71%), $n_{♀}=21$ (3.13%), $p<0.005$).

DISCUSSION

Borderline-increased values of baseline ST levels are observed frequently in clinical dermatology, not only in hymenoptera venom-allergic patients. These increased levels necessitate re-testing and further diagnostic procedures in many patients (10, 31). In our population, 4.12% were affected, with ≥ 11.4 µg/l considered as a raised level, corresponding to percentages from a study that analysed 758 insect venom-allergic patients, with 5.8% of subjects affected by elevated ST levels (1). In a different study, this applied to 11% (12 out of 109 patients) (32). The most important finding from our analysis was the significant and continuous increase in ST levels within three age classes from young adults (15–34 years) through patients older than 64 years. This finding is in accordance with recent results from studies performed in populations of insect venom-allergic patients (1, 32, 33). In contrast, no age or sex associations were detected in a previous study with 259 hymenoptera venom-allergic patients, when a ST level of ≥ 13.5 µg/l was regarded as raised (6). Increased tryptase levels were associated with age in a random sample study from an adult population in Spain ($n=420$) with a median tryptase level of 6.6 µg/l in persons older than 80 years (34). Ninety-five percentiles calculated from our results suggest age-specific upper thresholds of 9.23 µg/l in young adults (15–34 years), of 10.76 µg/l in middle-aged adults (35–64 years), and of 12.25 µg/l in subjects over 64 years of age. Thus, it might be reasonable to consider upper limits higher than the reported 11.4 µg/l in individuals older than 64 years.

Table II. *Subjects with raised serum tryptase levels related to age*

	Serum tryptase levels	
Age class	>8.75 µg/l *n* (%)	>11.4 µg/l *n* (%)
I	0 (0)	0 (0)
II	2 (5.4)	1 (2.7)
III	17 (5.9)	8 (2.8)
IV	64 (10.5)	26 (4.2)
V	23 (15.8)	10 (6.8)

Age classes [years]: I: 0–4 years, II: 5–14 years, III: 15–34 years, IV: 35–64 years, V: >64 years.

Our findings are limited due to the retrospective analysis derived from a selected patient population. The cohort may not be representative of cohorts from specific allergy clinics or other countries. The critical values currently propagated by the test kit manufacturer refer to a healthy control population of 126 unselected, apparently healthy children and adults. Our former pragmatically chosen critical value of 8.75 µg/l was lower than the manufacturer's recommendations, due to German dermatological guidelines and literature which proposes to consider mastocytosis if values exceed only 8 µg/l (9, 10). In addition, we did not systematically evaluate other potential reasons for increased ST levels, such as diverse haematological disorders (e.g. acute myeloid leukaemia) (23, 35), haemodialysis (36), or other allergic conditions, such as chronic urticaria (37) or anaphylactic reactions, which cause transient elevation of β-tryptase (38). The extent of influence of these conditions in our population remains unclear. As a consequence, a controlled prospective population-based study would be necessary to confirm the observed increase in ST levels with age in a general healthy population. Re-evaluation of the currently established threshold values for baseline ST levels according to age and specific patient panels should be discussed in case population-based studies to confirm these results.

It has been stated that otherwise unexplained elevation of the ST levels might be due to heterophilic interference (26). Heterophilic antibodies can be differentiated into

natural antibodies and autoimmune antibodies (e.g. rheumatoid factor) (25). Natural antibodies seem to be the most important subgroup; however, their function is poorly understood. In sandwich enzyme-linked immunoassays (ELISAs), which are widely used in clinical chemistry, heterophilic antibodies might produce false positive results. Healthy older individuals possess autoantibodies that occur in higher percentages with age, and the higher prevalence of autoantibodies (cardiolipin, anti-nuclear) in elderly persons has been explained to be particularly related to natural antibodies (39), which is in accordance with results from a comparison performed in a panel of elderly subjects (67–95 years) with younger patients (25–48 years) (40). Regardless, age dependence of heterophilic interference is still controversial, as not all authors confirmed age-associated increases of natural autoantibodies (41–43). A more than 10-fold reduction in ST levels after incubation with HBT® was described in a young male patient with excluded CM (27). In a subsequent investigation of the ST levels of 50 patients with positive and negative rheumatoid factor, a common agent of heterophilic interference, incubation with HBT® led to an approximately 10-fold decrease in the concentrations measured (initial values >20 μg/l) in three out of 30 IgM-rheumatoid-factor-positive subjects (27). In a recent study 14 (17%) out of 83 samples with positive rheumatoid factor showed a >17% decrease in ST after HBT® blocking and 8 of 14 (57%) reverted from elevated to normal range values, with falls of up to 98% (29). However, our study did not investigate the presence of rheumatoid factor, and heterophilic antibodies were of subordinate relevance in the 106 cases with raised ST levels that were investigated with HBT. Minor variations in the ST test kits used over time are assumed, and usage of aged sera perhaps influenced our results.

We also observed differences in ST levels associated with gender. Males showed significantly higher ST values compared with females, although neither sex differed significantly with respect to their mean ages. We cannot explain this observation, which contrasts with previous observations of higher ST levels in females from a population of healthy adults ($n = 106$ and 109, respectively) (14, 44). This finding requires further study.

In summary, mild increases in the ST levels that were neither related to mastocytosis nor heterophilic interference were more common in older patients. Furthermore, older patients showed significantly higher ST levels in a German general dermatology population. These findings should be substantiated in a controlled prospective study in the general population.

The authors declare no conflicts of interest.

REFERENCES

1. Blum S, Gunzinger A, Muller UR, Helbling A. Influence of total and specific IgE, serum tryptase, and age on severity of allergic reactions to Hymenoptera stings. Allergy 2010; 66: 222–228.
2. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. Immunol Allergy Clin North Am 2006; 26: 451–463.
3. Caughey GH. Tryptase genetics and anaphylaxis. J Allergy Clin Immunol 2006; 117: 1411–1414.
4. Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. Allergy 2005; 60: 1339–1349.
5. Yunginger JW, Nelson DR, Squillace DL, Jones RT, Holley KE, Hyma BA, et al. Laboratory investigation of deaths due to anaphylaxis. J Forensic Sci 1991; 36: 857–865.
6. Haeberli G, Bronnimann M, Hunziker T, Muller U. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy 2003; 33: 1216–1220.
7. Payne V, Kam PC. Mast cell tryptase: a review of its physiology and clinical significance. Anaesthesia 2004; 59: 695–703.
8. Ludolph-Hauser D, Rueff F, Fries C, Schopf P, Przybilla B. Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to hymenoptera stings. Lancet 2001; 357: 361–362.
9. Rueff F, Przybilla B, Bilo MB, Muller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with hymenoptera venom allergy: importance of baseline serum tryptase – a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol 2009; 124: 1047–1054.
10. Przybilla B, Müller U, Jarisch R, Rueff F. Erhöhte basale Serumtryptasekonzentration oder Mastozytose als Risikofaktor der Hymenopterenallergie. Allergo J 2004; 13: 440–442.
11. Kleine-Tebbe J. Die spezifische Immuntherapie (Hyposensibilisierung) bei IgE-vermittelten allergischen Erkrankungen. Allergo J 2009; 18: 508–537.
12. Dybendal T, Guttormsen AB, Elsayed S, Askeland B, Harboe T, Florvaag E. Screening for mast cell tryptase and serum IgE antibodies in 18 patients with anaphylactic shock during general anaesthesia. Acta Anaesthesiol Scand 2003; 47: 1211–1218.
13. Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. Allergy 2008; 63: 226–232.
14. Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, et al. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. J Immunol 2003; 170: 5667–5673.
15. Valent P, Akin C, Escribano L, Fodinger M, Hartmann K, Brockow K, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. Eur J Clin Invest 2007; 37: 435–453.
16. Rueff F, Placzek M, Przybilla B. Mastocytosis and hymenoptera venom allergy. Curr Opin Allergy Clin Immunol 2006; 6: 284–288.
17. Fricker M, Helbling A, Schwartz L, Muller U. Hymenoptera sting anaphylaxis and urticaria pigmentosa: clinical findings and results of venom immunotherapy in ten patients. J Allergy Clin Immunol 1997; 100: 11–15.
18. Oude Elberink JN, de Monchy JG, Kors JW, van Doormaal JJ, Dubois AE. Fatal anaphylaxis after a yellow jacket sting,

despite venom immunotherapy, in two patients with mastocytosis. J Allergy Clin Immunol 1997; 99: 153–154.
19. Ludolph-Hauser D, Schopf P, Rueff F, Przybilla B. Okkulte kutane Mastozytose. Hautarzt 2001; 52: 390–393.
20. de Gennes C, Kuntz D, de Vernejoul MC. Bone mastocytosis. A report of nine cases with a bone histomorphometric study. Clin Orthop Relat Res 1992; 279: 281–291.
21. Bredfeldt JE, O'Laughlin JC, Durham JB, Blessing LD. Malabsorption and gastric hyperacidity in systemic mastocytosis. Results of cimetidine therapy. Am J Gastroenterol 1980; 74: 133–137.
22. Thomas D, Dragodanne C, Frank R, Prier A, Chomette G, Grosgogeat Y. [Systemic mastocytosis with myo-pericardial localization and atrioventricular block.] Arch Mal Coeur Vaiss 1981; 74: 215–221 (in French).
23. Sperr WR, Jordan JH, Baghestanian M, Kiener HP, Samorapoompichit P, Semper H, et al. Expression of mast cell tryptase by myeloblasts in a group of patients with acute myeloid leukemia. Blood 2001; 98: 2200–2209.
24. Kricka LJ. Human anti-animal antibody interferences in immunological assays. Clin Chem 1999; 45: 942–956.
25. Levinson SS, Miller JJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. Clin Chim Acta 2002; 325:1–15.
26. van Toorenenbergen AW, Hooijkaas H, Heerenbrink GK, Dufour-van den Goorbergh DM. Heterophilic antibody interference in a tryptase immunoassay. Clin Biochem 2008; 41: 331–334.
27. van Toorenenbergen AW, van Daele PL, Boonstra JG. False-elevated serum tryptase assay result caused by heterophilic antibodies. J Allergy Clin Immunol 2005; 116: 1159–1160.
28. Moorman JE, Rudd RA, Johnson CA, King M, Minor P, Bailey C, et al. National surveillance for asthma – United States, 1980–2004. MMWR Surveill Summ 2007; 56: 1–54.
29. Sargur R, Cowley D, Murng S, Wild G, Green K, Shrimpton A, et al. Raised tryptase without anaphylaxis or mastocytosis: heterophilic antibody interference in the serum tryptase assay. Clin Exp Immunol 2011; 163: 339–345.
30. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat 1979; 6: 65–70.
31. Valent P, Horny HP, Li CY, Longley JB, Metcalfe DD, Parwaresch RM, et al. Mastocytosis (mast cell disease). World Health Organization classification of tumours: pathology and genetics. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Tumours of haematopoietic and lymphoid tissues. Geneva: WHO; 2001, p. 291–302.
32. Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M. Basal serum tryptase level correlates with severity of hymenoptera sting and age. J Investig Allergol Clin Immunol 2007; 17: 65–69.
33. Guenova E, Volz T, Eichner M, Hoetzenecker W, Caroli U, Griesinger G, et al. Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly. Allergy 2010; 65: 919–923.
34. Gonzalez-Quintela A, Vizcaino L, Gude F, Rey J, Meijide L, Fernandez-Merino C, et al. Factors influencing serum total tryptase concentrations in a general adult population. Clin Chem Lab Med 2010; 48: 701–706.
35. Sperr WR, Stehberger B, Wimazal F, Baghestanian M, Schwartz LB, Kundi M, et al. Serum tryptase measurements in patients with myelodysplastic syndromes. Leuk Lymphoma 2002; 43: 1097–1105.
36. Dugas-Breit S, Schopf P, Dugas M, Schiffl H, Rueff F, Przybilla B. Baseline serum levels of mast cell tryptase are raised in hemodialysis patients and associated with severity of pruritus. J Dtsch Dermatol Ges 2005; 3: 343–347.
37. Hidvegi B, Nagy E, Szabo T, Temesvari E, Marschalko M, Karpati S, et al. Correlation between T-cell and mast cell activity in patients with chronic urticaria. Int Arch Allergy Immunol 2003; 132: 177–182.
38. Fukuoka Y, Schwartz LB. Active monomers of human beta-tryptase have expanded substrate specificities. Int Immunopharmacol 2007; 7: 1900–1908.
39. Candore G, Di Lorenzo G, Mansueto P, Melluso M, Frada G, Li Vecchi M, et al. Prevalence of organ-specific and non organ-specific autoantibodies in healthy centenarians. Mech Ageing Dev 1997; 94: 183–190.
40. Manoussakis MN, Tzioufas AG, Silis MP, Pange PJ, Goudevenos J, Moutsopoulos HM. High prevalence of anticardiolipin and other autoantibodies in a healthy elderly population. Clin Exp Immunol 1987; 69: 557–565.
41. Hurez V, Kaveri SV, Kazatchkine MD. Expression and control of the natural autoreactive IgG repertoire in normal human serum. Eur J Immunol 1993; 23: 783–789.
42. Lacroix-Desmazes S, Mouthon L, Coutinho A, Kazatchkine MD. Analysis of the natural human IgG antibody repertoire: life-long stability of reactivities towards self antigens contrasts with age-dependent diversification of reactivities against bacterial antigens. Eur J Immunol 1995; 25: 2598–2604.
43. Lacroix-Desmazes S, Mouthon L, Kaveri SV, Kazatchkine MD, Weksler ME. Stability of natural self-reactive antibody repertoires during aging. J Clin Immunol 1999; 19: 26–34.
44. Min HK, Moxley G, Neale MC, Schwartz LB. Effect of sex and haplotype on plasma tryptase levels in healthy adults. J Allergy Clin Immunol 2004; 114: 48–51.