INVESTIGATIVE REPORT

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

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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.

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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 (pro-tryptases 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® 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 (−20°C) 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® 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.

Intra 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), 95% 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 test assumptions 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 and 95th percentile 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 95th percentile derived from 126 healthy individuals. Correlation between age classes and the numbers of patients with elevated ST levels was analysed using the χ² 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...
Reduced to 5.10 μg/l after incubation with HBT®®, thus a single sample, the previous value of 12.35 μg/l was suggesting interfering heterophilic antibodies. In all of values exceeding 8.75 μg/l (<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 χ² 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®, 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.

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

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

<table>
<thead>
<tr>
<th>Age class</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>–</td>
<td>0.446</td>
<td>0.487</td>
<td>0.816</td>
<td>0.1</td>
</tr>
<tr>
<td>II</td>
<td>0.446</td>
<td>–</td>
<td>0.967</td>
<td>0.104</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age class</td>
<td>III</td>
<td>0.487</td>
<td>0.967</td>
<td>–</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IV</td>
<td>0.816</td>
<td>0.104</td>
<td>–</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>V</td>
<td>0.1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>–</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Serum tryptase levels</th>
<th>&gt; 8.75 μg/l</th>
<th>&gt; 11.4 μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age class</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>I</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>II</td>
<td>2 (5.4)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>III</td>
<td>17 (5.9)</td>
<td>8 (2.8)</td>
</tr>
<tr>
<td>IV</td>
<td>64 (10.5)</td>
<td>26 (4.2)</td>
</tr>
<tr>
<td>V</td>
<td>23 (15.8)</td>
<td>10 (6.8)</td>
</tr>
</tbody>
</table>

Age classes [years]: I: 0–4 years, II: 5–14 years, III: 15–34 years, IV: 35–64 years, V: >64 years.
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 rheumatoid factor showed a > 17% decrease in ST after incubation with HBT® blocking and 8 of 14 (57%) reverted from elevated rheumatoid factor showed a >17% decrease in ST. 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