

ORIGINAL ARTICLE

Serum concentration of baseline mast cell tryptase: evidence for a decline during long-term immunotherapy for Hymenoptera venom allergy

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Summary

Background Baseline serum mast cell tryptase concentration (BTC) is thought to reflect the constitutive mast cell load or activity of an individual patient. Little is known about the individual stability of BTC during long-term venom immunotherapy (VIT).

Objective To investigate the intra-individual stability of BTC over time in patients with Hymenoptera venom allergy.

Methods Three hundred and two patients were studied. BTC was measured before and at least twice during VIT. At least 4 weeks lay between BTC measurements and the most recent field sting, in-hospital sting, or preceding venom injection. Multifactorial mixed linear models were used to analyse BTC changes over time.

Results Median observation time was 4.2 years (range 2–12 years). Before VIT, the median BTC was 6.8 µg/L (range 1.14–177 µg/L). The median coefficient of variation (CV) over time was 15.3% (range 1.9–63.8%). The median CV was significantly smaller in patients presenting with an elevated BTC (> 11.4 µg/L) than in patients with a normal BTC (11.4%, range 2.6–39.5%; vs. 17.6%, range 1.9–63.8%; $P < 0.001$). During VIT and after adjusting for age and gender, we found a slight but significant decrease of BTC over time (2.5% per year, 95% confidence interval 2.0–3.0%, $P < 0.001$).

Conclusion Individual variation of BTC during VIT does not rise when BTC is increased before therapy. VIT is associated with a small, but continuous decrease of BTC over time possibly indicating a dampened mast cell function or a decline in mast cell burden.

Keywords age, Hymenoptera venom allergy, mastocytosis, serum mast cell tryptase concentration, venom immunotherapy

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Introduction

Tryptase is a neutral protease from human mast cells, and the corresponding serum concentrations indicate mast cell involvement in various diseases [1]. Several subtypes of human mast cell tryptase are known, which are either predominantly released by resting mast cells or upon stimulation. β -Tryptase appears to be the main form of tryptase stored in mast cell granules and is released during systemic anaphylaxis in proportion to the severity of hypotension [2]. Inactive α - and β -protryptases are secreted constitutively from mast cells and form the major component of serum baseline tryptase, which can be

measured by standard assays and which is thought to be independent from mast cell activation [3].

It is assumed that baseline serum tryptase concentration (BTC) reflects the individual mast cell burden of the body. Most likely, this is also true for those individuals who lack a specific gene involved in α -protryptase production and in whom BTC may be only modestly lower than in healthy subjects who are not deficient [3–5]. In addition to the baseline secretion of inactive protryptases, active β -tryptase is released during a systemic anaphylactic reaction. Serum levels peak at 15–120 min after the beginning of symptoms [6] and return to normal usually within 24 h [7].

Measurements of BTC are used to examine various diseases. BTC also serves as a useful molecular marker for mast cell burden and pathologies involving mast cells. All types of systemic mastocytosis, as well as myelodysplastic or myeloproliferative diseases, the hypereosinophilic syndrome associated with FIP1L1-PDGFRA mutation, end-stage renal failure, and the treatment for onchocerciasis may be associated with an elevated BTC of $>20 \mu\text{g/L}$ [1, 7–10]. Measurement of BTC is also helpful in the context of Hymenoptera venom allergy, allowing there to appreciate the risk for a severe reaction after a field sting in untreated patients [11–13], or during a sting challenge in patients on venom immunotherapy (VIT) [6, 12].

Although BTC measurement is used as a diagnostic tool in a variety of conditions, little is known about long-term changes of this variable. In particular, it is uncertain whether immunotherapy in patients with Hymenoptera venom allergy is associated with alterations of BTC. The latter patients are ideal candidates to study BTC during immunotherapy because a significant portion of these patients present with an elevated BTC before treatment.

Material and methods

Patients

For this retrospective study, we used a local in-hospital database, which included all consecutive patients who presented for the first time in our allergy outpatient clinic between 1997 and 2003. A search was started for patients who had been diagnosed with Hymenoptera venom allergy, who had undergone VIT, and in whom routine measurements of BTC had been performed during clinical visits. To include a patient, corresponding blood samples for BTC measurement had to be taken before VIT and at least twice thereafter during yearly routine follow-up examinations. The minimum follow-up period during the maintenance phase of VIT had to be 2 years. Patients were excluded, if BTC measurements had been performed within 4 weeks after venom exposure (VIT, field sting, in-hospital sting challenge). At the time of BTC measurement, all patients had to be free from current anaphylactic reactions.

Routine measurement of serum tryptase

Blood samples were drawn from a peripheral vein, centrifuged, and stored as serum at -20°C . BTC measurements were usually performed within 2–3 days after sampling. BTC was measured with a fluoroenzyme immunoassay (UniCAP Tryptase; Phadia, Freiburg, Germany), which detects pro-forms and mature forms of α -tryptase and β -tryptase. Duplicate measurements were performed if the concentration at the first assessment was $>10 \mu\text{g/L}$; in this case, the mean value was recorded. The upper 95th percentile for healthy non-allergic individuals

is $11.4 \mu\text{g/L}$. A BTC of $>11.4 \mu\text{g/L}$ (95th percentile, as determined by the manufacturer) was considered elevated.

According to the manufacturer, the inter-assay variability for tryptase levels between >1 and $100 \mu\text{g/L}$ is $\leq 5\%$ (relative difference between multiple measurements). For in-hospital quality control, we performed repeated measurements in 110 random samples taken from patients with a BTC $>10 \mu\text{g/L}$. The first measurement was performed right after blood sampling in fresh serum, the second measurement 1–4 days later. During this time, samples were stored at -20°C . On average, the relative difference between the first and the second measurement was 4.3%.

Statistical analysis

To analyse changes of BTC during VIT, we registered the minimum and maximum BTC ($\text{BTC}_{\min}$, $\text{BTC}_{\max}$) for each patient during follow-up (irrespective of the time of measurement) and compared these values with the BTC before VIT ($\text{BTC}_{\text{preVIT}}$). To calculate the absolute difference (ΔBTC) between $\text{BTC}_{\text{preVIT}}$ and $\text{BTC}_{\min}$ or $\text{BTC}_{\max}$, we always used that value of $\text{BTC}_{\min}$ or $\text{BTC}_{\max}$, which showed the greatest absolute deviation from $\text{BTC}_{\text{preVIT}}$. ΔBTC was then calculated as $|\text{BTC}_{\text{preVIT}} - \text{BTC}_{\min \text{ or } \max}|$. The relative difference ($\Delta\%\text{BTC}$) was calculated as $\Delta\text{BTC} / \text{BTC}_{\text{preVIT}}$. ΔBTC and $\Delta\%\text{BTC}$ are presented as frequency distributions and are described by standard descriptive statistical methods (median, mean, and range).

Furthermore, we calculated for each individual patient the corresponding standard deviation (SD) for all results of BTC over time. To assess the relative variation, we calculated the individual coefficient of variation (CV) on the basis of the relative standard deviation for results of BTC. For the whole cohort, the distribution of individual CVs was described by its median and range. To describe the relationship between individual CVs and the duration of follow-up, we used the Pearson's correlation coefficient.

To account for the magnitude of $\text{BTC}_{\text{preVIT}}$, all calculations were performed separately for patient subgroups with a $\text{BTC}_{\text{preVIT}}$ of more or less than $11.4 \mu\text{g/L}$. To compare ΔBTC , $\Delta\%\text{BTC}$, SD, and CV between these subgroups, we used Wilcoxon's rank sum test for independent samples.

A multiple linear mixed model was used to analyse changes of BTC (after logarithmic transformation with base 10) during the follow-up time, including the variables 'time elapsed after the first measurement', 'gender', and 'age before VIT' as fixed effects. Because the data were not equidistant and because there were only a few observations per person, we included a random intercept for every patient in the model to account for repeated measures. To check for a possible effect of the initial BTC and a possible regression to the mean effect, we fitted an additional mixed linear model. For this additional model, we used all BTC levels except the initial value as an outcome variable and we included the variables 'initial

BTC level' and 'time elapsed after the first measurement' as the fixed effects. Like in the main model, a random intercept for every patient was part of the model. Statistical analyses were carried out using SPSS 17.0 (from SPSS Inc., Chicago, IL, USA).

Results

Patients

Three hundred and two patients fulfilled the inclusion criteria and the corresponding tryptase concentrations could be analysed. The majority of the patients were female ($n = 172$, 57%), suffering from vespid venom allergy ($n = 257$, 85.8%), and presented with a grade I or II maximum systemic allergic reaction, according to Ring and Messner [14] after a field sting before VIT ($n = 206$, 68.2%). A significant portion of patients were suffering from mastocytosis ($n = 56$, 18.5%). The median age was 47.0 years (range 6–75 years). In a univariate analysis, patients with a BTC of $> 11.4 \mu\text{g/L}$ before VIT were significantly more likely to be older, to suffer from mastocytosis or to have had a more severe maximum systemic reaction after a field sting (Table 1).

Baseline serum mast cell tryptase concentration before and during venom immunotherapy

Follow-up during VIT was 2–12 years (median 4.2, mean $\pm$ SD: 4.7 ± 2.1 years). During VIT, BTC was measured twice in 77, three times in 81, four times in 70, and more than five times (up to 11 times) in 74 patients. In total, the results of 1467 measurements before and during VIT were available for analysis.

BTC before VIT ($\text{BTC}_{\text{preVIT}}$) ranged from 1.14 to $177 \mu\text{g/L}$ (median $6.83 \mu\text{g/L}$; mean $\pm$ SD: $11.39 \pm 17.57 \mu\text{g/L}$). In 78 of the 302 subjects, $\text{BTC}_{\text{preVIT}}$ was $> 11.4 \mu\text{g/L}$ and was associated with mastocytosis in 46 patients. Ten patients had mastocytosis without an elevated $\text{BTC}_{\text{preVIT}}$.

Individual variation of baseline tryptase concentration during venom immunotherapy

For all patients, the median of the *absolute* difference (ΔBTC) between $\text{BTC}_{\text{preVIT}}$ and the subsequent maximum deviation from $\text{BTC}_{\text{preVIT}}$ was $2.07 \mu\text{g/L}$ (range 0.18–51.0 $\mu\text{g/L}$, mean $\pm$ SD: $3.54 \pm 6.12 \mu\text{g/L}$). In patients with a $\text{BTC}_{\text{preVIT}} \leq 11.4 \mu\text{g/L}$, the median of ΔBTC was $1.57 \mu\text{g/L}$ (range 0.18–10.80 $\mu\text{g/L}$; mean $\pm$ SD: $1.97 \pm 1.48 \mu\text{g/L}$), whereas in the subgroup with a $\text{BTC}_{\text{preVIT}} > 11.4 \mu\text{g/L}$, the median of ΔBTC was $4.40 \mu\text{g/L}$ (range 0.45–51; mean $8.04 \pm 10.60 \mu\text{g/L}$). Differences between subgroups were significant ($P < 0.001$, Fig. 1a).

The median of the *relative* difference ($\Delta\%\text{BTC}$) between $\text{BTC}_{\text{preVIT}}$ and the subsequent maximum deviation from

Table 1. Distribution of baseline variables with respect to the baseline serum tryptase concentration (BTC) at first visit

	BTC $\leq 11.4 \mu\text{g/L}$	BTC $> 11.4 \mu\text{g/L}$	P-value
Gender			
Male	93 (71.5%)	37 (28.5%)	0.426
Female	131 (76.2%)	41 (23.8%)	
Age (mean $\pm$ SD)	43.37 ± 16.01	50.23 ± 13.54	0.001
Maximum severity of previous sting reaction*			
Grade I or II	176 (85.0%)	31 (15.0%)	< 0.001
Grade III or IV	46 (50.5%)	45 (49.5%)	
Honeybee venom allergy [†]	70 (72.9%)	26 (27.1%)	0.98
Vespid venom allergy [†]	191 (74.5%)	68 (25.5%)	
Mastocytosis (total)	10 (17.9%)	46 (82.1%)	< 0.001
Type of mastocytosis			
CM	2	2	
CM, bone marrow biopsy n.d.	2	14	
CM, SM? [‡]	4	8	
SM	2	22	

P-values refer to a univariate analysis.

*Classification of hitherto most severe systemic reaction after a field sting modified according to Ring and Meßmer [14]: grade I, generalized skin symptoms (e.g., flush, generalized urticaria, angioedema); grade II, mild to moderate pulmonary, cardiovascular, and/or gastro-intestinal symptoms; grade III, anaphylactic shock, loss of consciousness; grade IV, cardiac arrest, apnea.

[†]Fifty-four patients were diagnosed as having honeybee and vespid venom allergy.

[‡]CM and ≤ 2 diagnostic minor criteria for SM according to the WHO criteria [15].

CM, cutaneous mastocytosis; SM, systemic mastocytosis.

$\text{BTC}_{\text{preVIT}}$ was 29.43% (range 3.53–165.15%, mean $\pm$ SD $34.33 \pm 22.20\%$). $\Delta\%\text{BTC}$ also differed significantly between patients with (median 24.9%, range 3.7–101.4%) and without (median 30.5%, range 3.5–165.2%) an elevated $\text{BTC}_{\text{preVIT}}$ ($P = 0.002$, Fig. 1b).

For all patients, the median of the CV was 15.3% (range 1.9–63.8%). The median of CV was 11.4% (range 2.6–39.5%) in the patient subgroup that had a $\text{BTC}_{\text{preVIT}} > 11.4 \mu\text{g/L}$. Patients with a $\text{BTC}_{\text{preVIT}} \leq 11.4 \mu\text{g/L}$ had a CV of 17.6% (range 1.9–63.8%). CV was associated with the duration of the observation period i.e. the time of the last observation (correlation coefficient for the whole cohort, $r = 0.250$). This association could also be found in patients with a $\text{BTC}_{\text{preVIT}} \leq 11.4 \mu\text{g/L}$ ($r = 0.153$) or $\text{BTC}_{\text{preVIT}} > 11.4 \mu\text{g/L}$ ($r = 0.241$).

Age and gender-adjusted changes of baseline tryptase concentration during venom immunotherapy

The results of the main multiple, linear mixed model are presented in Table 2. We found a significant decrease of BTC during VIT, whereas BTC rose linearly with increasing

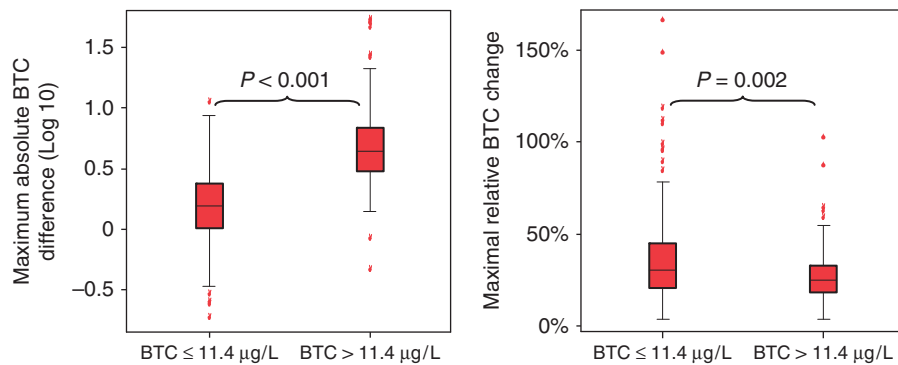


Fig. 1. Maximal absolute difference ($\mu\text{g/L}$) (a) and maximal relative change (%) (b) between baseline tryptase concentration (BTC) measured before and during venom immunotherapy. The upper line of the boxplot indicates the 75th percentile, the middle line the median, and the lower line the 25th percentile. Stars indicate outliers. For definition of terms, see Methods. Concentration differences are given after a logarithmic transformation ($\log 10$). Data are stratified according to normal ($\leq 11.4 \mu\text{g/L}$) or increased ($> 11.4 \mu\text{g/L}$) BTC before immunotherapy.

age. Males had a higher BTC than females. Compared with $\text{BTC}_{\text{preVIT}}$, BTC decreased significantly by 2.5% per year during VIT (95% confidence interval 2.0–3.0%). The additional model, which included the first BTC value as a predictor and the other BTC values as an outcome variable, also indicated a significant decrease (2.6% per year, 95% confidence interval 2.0–2.9%). The interaction between the first BTC value and trend was not significant (parameter = 0.004263, $P = 0.157$) showing that the relative decrease over time had no significant relationship to the initial value. The results were confirmed by analysing the relative differences between $\text{BTC}_{\text{preVIT}}$ and the last measured individual BTC value during VIT. We found a slight decrease over time (median change – 10% $\mu\text{g/L}$, range – 65–165%, mean $\pm$ SD: $-6.9 \pm 1.7\%$).

Discussion

In this study, we report an analysis of long-term changes of BTC, which was determined repeatedly during a mean observation period of 4.7 years in a heterogeneous group of patients with Hymenoptera venom allergy. During this time, all patients had VIT. Before VIT, a significant portion (25.8%) of our patients presented with an elevated BTC ($> 11.4 \mu\text{g/L}$). This frequency is higher than that reported in other cohorts of patients with Hymenoptera venom allergy [13, 16]. An explanation for this difference may be found in the comparatively high percentage of patients with mastocytosis (18.5%) who were included in our study, and of whom many had an elevated BTC. Patients with mastocytosis or with elevated BTC were likely candidates for inclusion because recommendations of the German Association of Allergology and Clinical Immunology suggest to perform lifelong VIT in patients with mastocytosis or $\text{BTC} > 20.0 \mu\text{g/L}$ [17].

One central aspect of our study was to examine the variability of BTC over time. It should be noted, however,

Table 2. Results of the multiple linear mixed model

Variable	Estimate (%)	95% confidence interval (%)		P-value
		Lower limit	Upper limit	
Male gender	31	9.1	58	0.004
Observation time (per year)	–2.6	–3.0	–2.0	< 0.001
Age (per year)	1.4	0.7	1.8	< 0.001

The outcome variable is the logarithmic (base 10) value of BTC.

In the table, the estimated percentage change for the fixed effects are displayed. The estimated standard deviations are 0.09 (SE = 0.021) for the residual and 0.34 (SE = 0.015) for the random individual intercept.

that the interpretation of our results is subject to some limitations. First, individual CVs were derived from serial measurements in individual patients and were taken as a surrogate measure of individual BTC variability over time. In the context of our study, CV only represents the crude intra-individual variance, and the corresponding values are purely descriptive. Therefore, results regarding CVs must be interpreted with caution. Second, our statistical analysis was only exploratory because of the variable number of observations per patient and because of the possibility of autocorrelation.

We found that, when referred to BTC before VIT, BTC during VIT showed an average individual CV of 15.3%. A small portion of this variation was most likely due to the intrinsic variability of the tryptase assay and/or to the short-term storage of the samples. It was remarkable that the CV was lower (11.4%) in that patient subgroup, which presented with an abnormal BTC before VIT ($> 11.4 \mu\text{g/L}$), and which simultaneously included a large number of patients suffering from mastocytosis (81%). The average CV increased slightly with an increasing length of the observation period and with lower BTC before VIT. For

extreme values, we observed a maximum deviation of BTC from pre-VIT values of 29.4%.

When analysing only patients who had a normal BTC before VIT ($\leq 11.4 \mu\text{g/L}$), we found a CV (17.6%), which was larger than that expected from established operating figures of the tryptase assay in healthy individuals. In healthy subjects, the mean CV during a 4-week period is 7.2% (range 3.4–11.2%) [18]. Schwartz et al. [6] and Jones et al. [18] also studied healthy individuals and found that BTC showed little variation throughout a period of 15 months (five subjects) and 4 years (14 subjects), respectively.

However, it is evident that these results cannot be compared with our observations. A major difference in the latter series is the fact that we studied diseased subjects who may have been susceptible to an entirely different spectrum of BTC modifiers than healthy subjects. A large portion of BTC variation in our patients must be attributed to specific mechanisms acting during the observation period or to intrinsic disease-related variations of BTC. It can be excluded that circadian variations were responsible for the variability of BTC measurements in our institution. Routine blood samples were usually taken always during the morning, and the maximal difference between morning and afternoon BTC was found to be $< 6\%$ [19].

In general, the large variability of BTC during the observation period might be explained by temporary concentration changes associated with certain concurrent diseases. Baseline tryptase is released from mast cells, which are involved in tissue remodelling, inflammation, homeostasis, and immune defence [20, 21]. Several diseases such as chronic urticaria [22, 23], asthma [24], onchocerciasis [25], nummular eczema [26], or cardiovascular pathologies [27] are known to be associated with temporary mast cell activation or hyperplasia and, compared with controls, with a higher BTC. Because we do not know whether our patients had been suffering from such diseases (in addition to Hymenoptera venom allergy) during the observation period and whether they had been treated or not, we also cannot exclude a role of these confounding events for the observed variability of BTC. However, because some of these diseases represent contraindications for VIT, it is unlikely that these variables were the main causes for the observed variability of BTC during the observation period.

It is more likely that the other determinants of BTC were more important. One potential determinant for BTC is the age of the patient. After adjusting for confounders including gender and observation time, we found that BTC increased linearly by about 1.3% per year. Our results confirm the recent findings by Kucharewicz et al. [13] who also identified a direct correlation between patient age and BTC. In the study by Jones et al. [18], the number of subjects was presumably too small to allow a detection of the comparatively small effect of age on BTC.

The causes for the age effect are unknown. One may speculate whether mast cell activity increases with higher age. Mast cells are involved in degenerative vessel diseases [28, 29], and it has been shown that BTC is elevated in patients with arteriosclerosis [27]. Besides the vascular system, age may also affect skin. Ageing skin undergoes progressive degenerative change. Structural and physiological changes that occur as a natural consequence of intrinsic ageing combined with the effects of a lifetime of ongoing cumulative extrinsic damage and environment insult (e.g. overexposure to solar radiation) may lead to a small, but chronic rise of cutaneous mast cell activity and to a consecutive increase of BTC [30].

The second and most interesting determinant for BTC changes during VIT was the time that had passed between the first measurement before VIT and subsequent measurements during VIT. After adjusting for age and gender, we found a significant decrease of BTC by 2.5% per year. This decrease is so much more remarkable because patients grew older during the years of follow-up, and should, therefore, have actually experienced an increase of BTC.

We can only speculate on the reasons for this continuous counter change in BTC. The only therapeutic measure, to which all our patients were exposed continuously throughout the observation time, was VIT. We cannot exclude the possibility that this ongoing therapy also led to a continuous reduction of BTC. The precise immunological mechanisms related to the protective long-term effects of VIT are poorly understood. The essential step in VIT, however, is known and consists in the induction of a tolerant state in peripheral T cells. The latter is initiated by the autocrine action of IL-10 and other cytokines, which are increasingly produced by the antigen-specific T cell. As a side-effect, IL-10 may also reduce mast cell density and growth, thereby possibly also reducing BTC during VIT [31, 32].

It should be noted, however, that we did not study a control group of patients with Hymenoptera venom allergy, who did not receive VIT. Therefore, the natural history of BTC in those patients is unknown. On the other hand, such an untreated control group will be hardly acceptable within the scientific community. Considering distinct guidelines for immunotherapy in patients with Hymenoptera venom allergy [33, 34], it would be considered unethical by most of the professionals to withhold an established therapy just for scientific reasons over a very long period of time.

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ALK-Abelló and HAL. All other authors have no such interests to declare.

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