

S. PLATZGUMMER <sup>1a</sup>, N. BIZZARO <sup>2a</sup>, M. B. BILO <sup>3b</sup>, V. PRAVETTONI <sup>4b</sup>, L. CECCHI <sup>5b</sup>,  
V. SARGENTINI <sup>6ab</sup>, L. CAPONI <sup>7a</sup>, D. VISENTINI <sup>8a</sup>, I. BRUSCA <sup>9ab</sup>, G. PESCE <sup>10a</sup>, M. BAGNASCO <sup>11a</sup>,  
A. ANTICO <sup>12a</sup>, M. C. MONTERA <sup>13b</sup>, O. QUERCIA <sup>14b</sup>, A. MUSARRA <sup>15b</sup>, L. BONAZZA <sup>16b</sup>,  
P. BORRELLI <sup>17b</sup>, G. CORTELLINI <sup>18b</sup>, B. R. POLILLO <sup>19b</sup>, G. VALENTI <sup>20b</sup>, M. T. ZEDDA <sup>21b</sup>,  
R. ASERO <sup>22b</sup>, D. VILLALTA <sup>23ab</sup>

## Recommendations for the use of tryptase in the diagnosis of anaphylaxis and clonal mastcell disorders

<sup>1</sup> Laboratorio di Patologia Clinica, Ospedale Franz Tappeiner, Merano, Bolzano

<sup>2</sup> Laboratorio di Patologia Clinica, Ospedale San Antonio, Tolmezzo, Azienda Sanitaria Universitaria Integrata, Udine

<sup>3</sup> Unità di Allergologia, Dipartimento di Scienze Cliniche e Molecolari, Università Politecnica delle Marche, Ancona

<sup>4</sup> Dipartimento di Medicina Generale, Immunologia e Allergologia, IRCCS Foundation Ca' Granda, Ospedale Maggiore Policlinico, Milano

<sup>5</sup> SOS Allergologia e Immunologia Prato, USL Toscana Centro, Prato

<sup>6</sup> UOC Patologia Clinica, Ospedale San Filippo Neri ASL Roma 1, Roma

<sup>7</sup> Laboratorio di Patologia Clinica, Azienda Ospedaliero-Universitaria Pisana, Università di Pisa, Pisa

<sup>8</sup> SOS Laboratorio di Immunopatologia e Allergologia, Azienda Sanitaria Universitaria Integrata, Udine

<sup>9</sup> UOC Patologia Clinica, Ospedale Buccheri La Ferla Fatebenefratelli, Palermo

<sup>10</sup> Laboratorio Diagnostico di Autoimmunologia IRCCS, Ospedale Policlinico S. Martino, Università di Genova, Dipartimento di Medicina Interna e Specialità mediche (DIMI), Genova

<sup>11</sup> Dipartimento di Medicina Interna e Specialità Mediche (DIMI), Università degli studi di Genova, Genova

<sup>12</sup> UOC Servizio Medicina di Laboratorio, AULSS 7 Regione Veneto, Santorso, Vicenza

<sup>13</sup> Allergologia e Immunologia Clinica, Ospedale G. Fuscito, Mercato S. Severino, Az. Ospedaliero-Universitaria Ruggi D'Aragona, Salerno

<sup>14</sup> S.S. Interdipartimentale di Allergologia, Ospedale di Faenza, Ravenna

<sup>15</sup> Servizio di Allergologia, Casa delal Salute di Scilla, Scilla, Reggio Calabria

<sup>16</sup> Divisione di Pneumologia, Ospedale di Bolzano, Bolzano

<sup>17</sup> Ambulatorio di Allergologia e Immunologia Clinica, Ospedale Beauregard, Aosta

<sup>18</sup> SSID di Allergologia della Romagna, Rimini

<sup>19</sup> UOS Allergologia, PTP Nuovo Regina Margherita, Roma

<sup>20</sup> Allergologia e Pneumologia, PTA Biondo, ASP, Palermo

<sup>21</sup> Libero professionista in Allergologia, Cagliari

<sup>22</sup> Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano, Milano

<sup>23</sup> SSD Immunologia e Allergologia, Presidio Ospedaliero S. Maria degli Angeli, Pordenone

<sup>a</sup> Gruppo di Studio in Allergologia della Società Italiana di Patologia Clinica e Medicina di Laboratorio

<sup>b</sup> Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri

**KEY WORDS**

*anaphylaxis; tryptase; mastocytosis; hymenoptera sting; post-mortem tryptase*

**Corresponding author**

Danilo Villalta  
SSD Immunologia e Allergologia  
Ospedale S. Maria degli Angeli  
Via Montereale 24  
33170 Pordenone  
Phone: +39 0434-399647  
E-mail: danilo.villalta@asfo.sanita.fvg.it

**Doi**

10.23822/EurAnnACI.1764-1489.133

**Introduction**

Tryptase in its mature form is a neutral serine protease with a molecular weight of 134 kDa. It is present in the secretory granules of mast cells and to a lesser extent in basophils and consists of four beta-tryptase subunits joined by non-covalent bonds and stabilized by proteoglycans. Tryptase is produced in the form of monomer and specifically in the form of alpha, beta, gamma and epsilon subunits. There are two isoforms of alpha-tryptase (alpha I and alpha II) and three isoforms of beta-tryptase (beta I, beta II and beta III) which high structural identity (around 90%). While the gamma subunit remains bound to the membrane of the secretory granule, alpha and beta monomers are continuously released into the circulation without a specific stimulus and constitute part of the tryptase present in serum (1- 4) (**figure 1**). The mature tryptase is released by the secretory granules as a tetramer composed mainly by beta isoform II. While the monomeric tryptase subunits are practically completely inactive, the mature tetrameric molecule is the active enzyme.

Mast cells, discovered in 1879 by Paul Ehrlich (5), contain many mediators (histamine, serotonin, chymase, carboxypeptidase, cathepsin G, proteoglycans, hydrolases and chemotactic factors) in their cytoplasmic granules, but tryptase is the most produced protein and is considered their specific marker (6). The mast cells of the lungs and of the intestinal submucosa contain a higher concentration of tryptase than the mast cells of the skin and of the intestinal mucosa.

Tryptase is also present in basophilic granulocytes, albeit at a much lower concentration (500 times less) than in mast cells, and in very low amounts also in the basophilic precursor cells of the bone marrow (7-9).

The release of tryptase and other mediators from the mast cells is commonly due to IgE mediated immunological stimuli, typ-

**Summary**

*Tryptase is a serin-protease produced and released by mast cells after IgE-mediated or non-IgE mediated stimuli. We here review the various aspects related to the molecular characteristics of the enzyme and its biological effects, the genetic basis of its production and the release kinetics. Recommendations for the clinical use of tryptase measurement developed by a task force of Società Italiana di Patologia Clinica e Medicina di Laboratorio and Associazione Allergologi Immunologi Italiani Territoriali e Ospedalieri are given on the best procedure for a correct definition of the reference values in relation to the inter-individual variability and to the correct determination of tryptase in blood and other biological liquids, in the diagnosis of anaphylaxis (from drugs, food, insect sting, or idiopathic), death from anaphylaxis (post mortem assessment) and cutaneous or clonal mast-cell disorders.*

ical of allergic reactions. In addition to immunological stimuli, physical phenomena such as heat and chemicals (toxins, poisons and drugs, dyes, etc.) can also cause the release of tryptase into the bloodstream.

**Genetics**

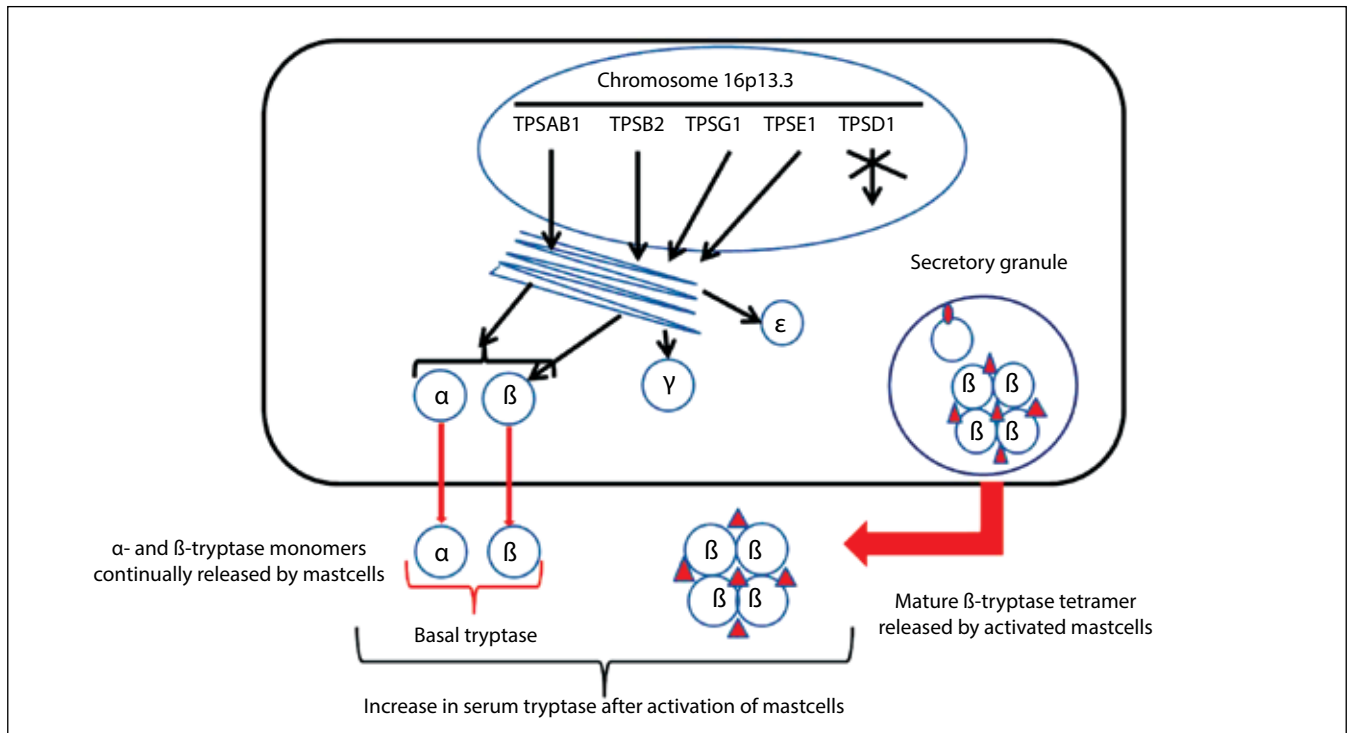
The genes that code for tryptase are located on chromosome 16p13.3. (10-13) and comprise five loci. The tryptase alpha/beta 1 gene (*TPSAB1*) encodes for both alpha and beta I-tryptase, while the *TPSB2* gene encodes for beta-tryptase II and III. The *TPSG1* gene encodes gamma-tryptase and the *TPSE1* gene epsilon-tryptase, a form that is biochemically and immunologically different from alpha and beta tryptase. The *TPSD1* gene, encoding delta-tryptase, is inactive in primates.

The gene that encodes alpha-tryptase is often subject to mutations, which can lead to a transcription deficit or alterations of the catalytic sites, and sometimes a complete deletion (up to 30-57% of the population) (14,15). Also, the polymorphisms of the genes coding for alpha-tryptase and beta-tryptase are high and the number of functional tryptase alleles an individual may carry varies from 2 to 4 (16). Although the absolute deficit of tryptase has never been reported, the number and type of functional alleles carried by an individual may alter the baseline systemic tryptase levels. The frequency of the haplotypes of the two loci of chromosome 16 are 50% for  $\beta\beta/\beta\alpha$ , 25-29% for  $\beta\beta/\beta\beta$  and 21-25% for  $\beta\alpha/\beta\alpha$  (14,17). Although basal tryptase level is considered to be correlated with the amount of mast cells, genetic variations can partly influence the basal value of the enzyme (18-20).

**The biological effects of tryptase**

Tryptase acts as a vasoactive, proinflammatory, chemotactic molecule, as well as in repairing tissue damage (21-25).

**Figure 1** -Tryptase production and intracellular trafficking. Tryptase basal level is due to a continuous release of the alpha and beta monomeric subunits into the bloodstream. The tetrameric mature tryptase, stabilized by proteoglycans (especially heparin) is released only after mast cells activation. Modified from Vitte J (4).



In particular, through the production of bradikinin, tryptase promotes vascular permeability and has a chemotactic action on neutrophils and eosinophils, cells involved in the late phase inflammatory allergic reaction. It also stimulates the proliferation of fibroblasts and collagen, contributing to tissue repair and to *restitutio ad integrum* (26,27), and stimulates the proliferation of the smooth muscles of the bronchi. More recently, a role of tryptase in the genesis of pain, such as post-operative pain, has been demonstrated by stimulating protease-activated nociceptors (28).

### Tryptase measurement

Several monoclonal antibodies have been developed to measure serum tryptase. The first antibody, defined G5 (29), was able to recognize a linear epitope of the beta isoform with a sensitivity of 2.5 µg/L. Later, other monoclonal antibodies were developed, such as G4 and B12, capable of recognizing both the alpha and beta subunits (30).

The only commercial assay to measure tryptase currently available is the fluoroimmunoenzymatic test (FEIA) (ImmunoCAP, ThermoFisher, Uppsala, Sweden), which measures both the im-

mature monomeric forms of the alpha and beta-tryptase and the mature tetrameric form.

Both serum and plasma can be used for the measurement of tryptase (29). The molecule at room temperature is stable for two days (48 hours) and for five days if serum or plasma is stored at 8 °C.

### Reference value of serum tryptase

In the last few years the upper threshold of the reference value indicated by the manufacturer for the FEIA test has been lowered several times starting from an initial value of 15 µg/L, then moving to 13.5 µg/L and finally to a value of 11.4 µg/L. This latter cutoff was obtained by the manufacturer evaluating 126 healthy people, in whom the 95<sup>th</sup> percentile was 11.4 µg/L and the geometric mean 3.8 µg/L.

Schliemann et al (31) in 1092 patients referred to their dermatological service for an allergic / anaphylactic reaction in whom mastocytosis had been excluded, found an average tryptase value of 5.13 ± 3.05 µg/L, a median of 4.46 µg/L and a 95<sup>th</sup> percentile of 10.8 µg/L. Of these patients, 106 had concentrations >8.75 µg/L and 45 >11.4 µg/L. However, these authors

indicated a slight increase in the value of the tryptase threshold with age progression (95<sup>th</sup> percentile in subjects between 15-34 years = 9.23 µg/L; between 35-64 years = 10.76 µg/L; >64 years = 12.25 µg/L).

Considering that severe anaphylactic reactions can occur in patients with mastocytosis even with basal tryptase values below 11.4 µg/L (32,33), some authors advise to consider with caution the interval between 8-11 µg/L and to perform also in these cases further investigation to exclude or confirm an underlying mastocytosis.

It is worth mentioning that the reference levels of mature tryptase are <1 µg/L (16), although this has only theoretical importance as we do not have commercial methods able to distinguish tryptase monomers by the mature form.

A very important feature of tryptase is the low intra-individual variability. In fact, the basal value varies very little over time within the same individual and is determined by the genetic background and not by environmental factors (19). This information is useful in the evaluation of anaphylaxis, as even minimal variations in tryptase concentration in a single individual can already be indicative of the presence of an anaphylactic event, even if values fall within the normal range.

### Interference in the measurement of tryptase

Blood samples may be taken in EDTA, heparin or plain tubes without an anticoagulant and preferably analyzed within 5–7

days. Serum tryptase is stable *in vitro*. However, if there is going to be an anticipated delay in analysis, samples must be frozen at -20 °C (34).

Hemolysis, jaundice and lipemia do not appear to interfere with the measurement of serum tryptase. Heterophile antibodies or rheumatoid factors may instead interfere in the tryptase assay (35,36). If an interference by heterophile antibodies is suspected, the latter should be previously removed (31).

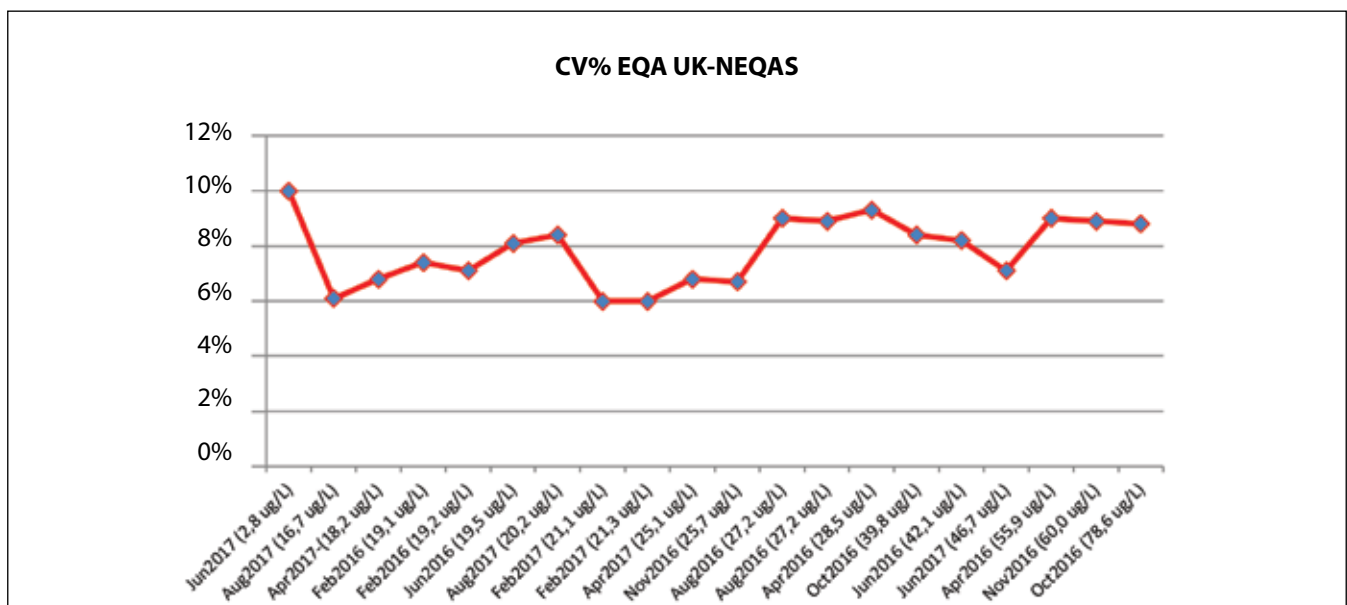
### Reproducibility of the test

The currently commercially available assay to determine tryptase has good reproducibility, with low intra and inter-assay coefficient of variation (CV) values, as confirmed by Schliemann et al (31) which found an intra-assay variability of 1.7% at 8.26 µg/L and of 1.1% at the value of 44.5 µg/L, and an inter-assay variability of 7.1% at 9.85 µg/L and of 5.5% at the value 33.16 µg/L.

The good reproducibility of the test is also confirmed by results of the UK-NEQAS external quality assessment. The global variability within the 20 control sera with values in the range of 2.8 µg/L - 78.6 µg/L, distributed in the period February 2016 - August 2017 (about 200 participants) showed CVs between 6 and 10%, with an average of 7.8% (**figure 2**).

These data were also confirmed by a study of Davson et al (37) in which 28 samples with tryptase values between 3.3 - 127 µg/L were sent to 25 different laboratories. The average CV of all samples was 8% (range 4.4-12.7%).

**Figure 2** - Variation coefficients (CV) obtained in the UK-NEQAS external quality assessment (EQA) for different tryptase values (period February 2016-August 2017; about 200 participants).



**When is it indicated to test for tryptase?**

Tryptase measurement is indicated in the diagnosis of following conditions:

1. idiopathic anaphylaxis or anaphylaxis caused by drugs, food, insect sting;
2. fatal anaphylaxis (*post-mortem* assessment);
3. mastocytosis and mast cell activation syndromes.

**Tryptase and anaphylaxis**

*General considerations*

Tryptase is useful for a correct diagnosis of anaphylaxis since similar symptoms may also be present in the vaso-vagal reaction, in septic and cardiogenic shock as well as in the carcinoid and benign flush.

When anaphylaxis occurs, serum tryptase values begin to rise about 5 - 30 minutes after the event, reach the peak after 1-3 hours and return to the basal value within 16-24 hours from the end of the event. The half-life of tryptase is about 1.5-2.5 hours. In **figure 3** the kinetic of tryptase is simulated in three patients with an anaphylactic reaction and with different basal values of tryptase. Even in patients with peak values >100 µg/L, tryptase returns to basal values within 24 hours after the end of the event. However, we must bear in mind that in anaphylactic reactions in which the main involved effector cell is not the mast cell,

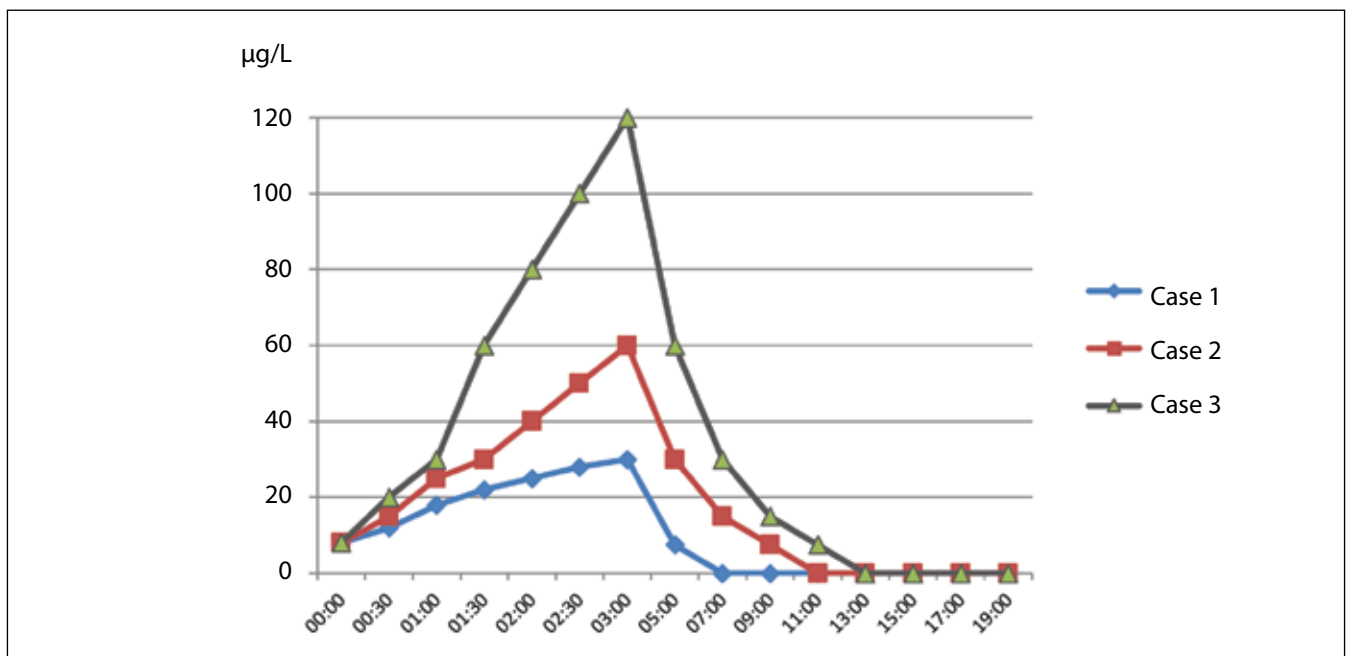
tryptase may not rise. Moreover, the method to measure tryptase is not an immediate procedure, so results will not be available during the acute episode of anaphylaxis.

In a study in which 30 patients with anaphylaxis were evaluated, Enrique et al (38) showed that using an initial cutoff of 13.5 µg/L proposed by the manufacturer, only 35% (= sensitivity) of the patients had values above this threshold, with a specificity of 92.3%. The sensitivity increased to 94.2% using a cutoff value of 8.2 µg/L, without losing specificity. Similar data were also obtained from other authors (39,40). This indicates that both the value of 13.5 µg/L and the one currently proposed by the manufacturer (11.4 µg/L) are too high to guarantee a sufficient sensitivity of the test in case of anaphylaxis. Various authors have come to the conclusion that the individual increase from the basal tryptase value is more sensitive than the absolute value and this is also supported by the fact that the basal values of an individual remain stable over time.

In consideration of what previously reported, Valent et al (41) suggest that values above 120% of the baseline value + 2 (baseline value x 1.2 + 2) should be considered significant for an anaphylactic event. This International consensus equation has been recently validated (42,43), and it is somehow innovative as it relativizes the concept of a fixed reference value for tryptase.

In order to be able to attribute significance to a value, however, given the speed with which tryptase values fall within the norm after a triggering event, the timing of blood withdrawals is ab-

**Figure 3** - Kinetics of tryptase in three patients with different maximum values.



solutely important and the training of the Emergency Room personnel is fundamental.

Brown et al (40) have shown that in patients referred to the emergency department for suspected anaphylaxis of whom no previous basal tryptase value was available, a change in value of 2.0 µg/L between two samples spaced one hour apart from the other had a sensitivity and specificity for the diagnosis of anaphylaxis of 73% and 91%, respectively.

Persistently elevated tryptase values after an anaphylactic reaction justify the expansion of the diagnosis for the search for mastocytosis, as indicated by the recent guidelines (44).

#### *Tryptase characteristics in different types of anaphylaxis*

##### *a. Drug and anesthetic -induced anaphylaxis*

Drugs and anesthetics (especially muscle relaxants) are among the main causes of anaphylaxis. In these cases the highest levels of tryptase are often observed, especially when the drugs are administered intravenously (45,46).

Particular attention must be paid to patients who have experienced a drop in blood pressure during general anesthesia, and acute tryptase levels varies as a function of the clinical severity of anaphylaxis (46). However, these events need to be differentiated from non immunological anaphylaxis caused by an isolated histamine release due to a rapid injection of anesthetic drugs (especially morphine derivatives).

##### *b. Food anaphylaxis*

In food anaphylaxis, tryptase levels are usually lower than those observed in drug-induced anaphylaxis (47,48). Food anaphylaxis has a slower development and mast cell degranulation is often limited to the intestinal mucosa. In food anaphylaxis, besides release from mast cells, other pathogenetic mechanisms can also be involved, such as a release of basophilic granulocyte mediators or activation of complement factors (C3a and C5a) and kinins. In food anaphylaxis, therefore, the basal value formula  $x \cdot 1.2 + 2$  can be particularly useful, since tryptase values are often not very high.

##### *c. Anaphylaxis caused by hymenoptera stings*

The literature underlines that there is a preferential association between venom anaphylaxis and elevated baseline serum tryptase level as well as clonal mastcell disorders (49). Rueff et al (50,51) found a close correlation between basal tryptase values and the risk of developing serious systemic reactions after a hymenoptera sting, as well as in the induction phase of venom immunotherapy. These authors have shown that even values  $>5$  µg/L are related to a greater risk of anaphylaxis, and that the risk increases with the increasing value of basal tryptase, especially in the older age (52).

It is noteworthy that mastocytosis in most cases is only discovered after a person has experienced an adverse reaction following

a sting by hymenoptera. Indeed, Bonadonna et al (53) showed that, if the basal tryptase value is  $>11.4$  µg/L and anaphylaxis occurs after a hymenopteran sting, in 88% of the cases we are faced with mastocytosis and that, in the presence of basal tryptase  $>11.4$  µg/L and a negative specific IgE assay for hymenoptera (bee and vespids), the probability of an underlying mastocytosis is 100%.

On the other side, even in the presence of normal tryptase level, patients with severe anaphylaxis (and absence of urticaria or angioedema) due to sting may suffer from clonal mastcell disorders (so called “bone marrow mastocytosis”, a subvariant of systemic mastocytosis with a lower burden of clonal mastcells) (33).

Accordingly, a recent consensus by the Italian allergy societies on the management of hymenoptera venom allergy states that, in case of systemic reactions, tryptase determination should be always performed in the diagnostic work up (54). In the presence of persistently high tryptase values, life-long venom immunotherapy is recommended, even if the diagnosis of mastocytosis has not been confirmed (55-57).

##### *d. Idiopathic anaphylaxis*

Idiopathic anaphylaxis or spontaneous anaphylaxis is a diagnosis of exclusion and mandates careful consideration of all recognizable and rare causes of anaphylaxis (58).

Idiopathic anaphylaxis represents an opportunity for identification of hidden allergens, cofactors, previously unrecognized novel triggers and also for identification of mastocytosis or clonal mast cell disorders. Other differential diagnoses include “allergy-mimics” such as asthma masquerading as anaphylaxis, undifferentiated somatoform disorder, panic attacks, globus hystericus, vocal cord dysfunction, scombroid poisoning, vasoactive amine intolerance, carcinoid syndrome and pheochromocytoma (58). Acute serum tryptase measurements are invaluable in patients reporting recurrent episodes and for differential diagnoses.

#### **Fatal anaphylaxis and use of post-mortem tryptase**

*Post-mortem* measurement of tryptase have been reported since 1991 (48), suggesting the possibility to perform a *post-mortem* diagnosis of anaphylaxis, as well as to classify some unexplained deaths as due to anaphylaxis, including some sudden deaths in the pediatric age (59-61). Although this concept may still find its validity, however, it should be borne in mind that *post-mortem* levels of tryptase have been found to be high also in people who died of severe trauma, myocardial infarction, asphyxia, or lung disease. Over time, therefore, the *post-mortem* tryptase cutoff value considered indicative of anaphylaxis as a possible cause of death increased from 44.5 µg/L (61) to 110 mg/L proposed by McLean-Tooke et al (62) in 2014. These authors have evidenced that increased values of tryptase in *post-mortem* sera are quite

frequent even if the cause of death is different from anaphylaxis and only values  $>110 \mu\text{g/L}$  have a high diagnostic efficiency for anaphylaxis. However, subsequent studies have again proposed lower thresholds and to date a definitive consensus has not yet been reached (63-65).

Given that data regarding utility of tryptase measurement largely come from case studies or case series (with small sample sizes) and multiple variables increase the uncertainty of measurement when serum samples are obtained from cadavers, to date there is no standardized international reference range for post mortem tryptase (34).

Summarizing all the aspects related to tryptase mentioned above, the intra- and inter-individual characteristics, the kinetics during anaphylaxis, the stability of the basal values, the analytical variability, in the suspicion of anaphylaxis the following is recommended:

#### Recommendations for the determination of tryptase in anaphylaxis

1. Make the first blood withdrawal preferably 30 minutes – 3 hours after the event.
  - Although the increase in tryptase may already be present after 5-20 minutes after the event, it is advisable to carry out the test after at least 30 minutes to avoid false negative results.
  - In patients with known basal value (not a frequent event), compare the value obtained with the basal value. If the value is 120% higher than the baseline value +  $2 \mu\text{g/L}$  anaphylaxis is confirmed.
  - Consider that values above the cutoff may already be indicative for an anaphylactic event.
2. If possible, take a second sample 1-6 hours after the event to evaluate the kinetics.
3. Make another blood withdrawal at least 24 hours after the event (better after 42-78 hours). This is considered as a baseline value and serves to compare the data to that obtained within 3 hours of the event.
4. Always perform tryptase measurement in subjects who have experienced an anaphylactic reaction following a hymenoptera sting even if the event occurred 24 hours before. High basal values should suggest a mastocytosis.
5. Tryptase can also be determined in *post-mortem sera* when the cause of death is uncertain. In this case it should be remembered that many variables have to be carefully factored into the process of interpretation, including that causes of death other than anaphylaxis can determine an increase in tryptase and only very high values can be indicative of anaphylaxis as the cause of death.

#### The role of tryptase in clonal mastcell disorders

In maculopapular cutaneous mastocytosis and in mastocytoma the levels of tryptase are usually not increased. Therefore, when a persistent increase in tryptase is observed, the presence of systemic mastocytosis must always be evaluated. In cases of cutaneous mastocytosis of the pediatric age, if a high value of tryptase ( $>20 \mu\text{g/L}$ ) is present in the absence of systemic involvement, it can be attributed to the release of tryptase from mast cells of the skin. However, it is strongly recommended to monitor the level of tryptase over time. If the level decreases during puberty it is not necessary to perform a bone marrow evaluation.

In diffuse cutaneous mastocytosis, persistently high values, however, must lead to a more in depth diagnostic work up in order to rule out systemic mastocytosis.

Serum tryptase is the most specific laboratory marker for the diagnosis of systemic mastocytosis. As already reported above, the test was included among the minor criteria for diagnosis. Since patients with systemic mastocytosis usually have basal values of tryptase  $>20 \mu\text{g/L}$ , it is essential to measure the protein away from events caused by the release of mediators from the mast cells and it is also necessary to check the concentration of serum tryptase at least a second time to confirm a persistent rise of the enzyme.

It should be noted that the absolute value of serum tryptase does not indicate the type of mastocytosis (49). Mastocytosis associated with a hematological disease and aggressive forms of mastocytosis may have values similar to the indolent form (66). In mastocytic leukemia, however, the values are usually extremely high and can reach levels as high as  $>1000 \mu\text{g/L}$ .

In the mastocytosis with associated hematologic disease subtype, the criterion of tryptase is not applied since the high value of the enzyme can also derive from the precursor cells of the bone marrow.

#### *Other pathologies that can determine an increase in tryptase*

High tryptase values were detected in patients with acute myeloid leukemia (67), myelodysplastic syndrome (68), hypereosinophilic syndrome (associated with the FIP1L1 PDGFRA mutation) (69), terminal renal failure (70), in abdominal aortic aneurysm (71), in some forms of infestation with helminths (72) and, in rare cases, a genetic increase in the family has been described (73,74). In particular, familial tryptasemia is a recently described disease in which members of the same family present elevated baseline tryptase levels due to hereditary alpha-tryptaseaemia (autosomal dominant) due to increased germline copies of alpha-tryptase gene (TPSAB1) (73,75). These patients have an elevated baseline tryptase with or without non-specific multisystem symptoms. This condition has not yet been well-characterized (75).

### Recommendations for the determination of tryptase in suspected mastocytosis

1. *Determination of tryptase in suspected infantile mastocytosis.*
  - Normal tryptase values do not exclude mastocytosis.
  - When tryptase values are <20 µg/L, with absence of mediator release symptoms and of hepatosplenomegaly or enlarged lymph nodes or changes in blood count, it is not necessary to proceed with bone marrow biopsy.
  - Values >20 µ/L and/or mediator release symptoms and/or hepatosplenomegaly, are indications to proceed with bone marrow biopsy.
2. *Determination of tryptase in suspected clonal mastcell disorders in adults.*
  - In the suspicion of an adult mastocytosis it is recommended to always measure tryptase, since the value is often increased. However, it should be noted that the value is not indicative of a specific clinical form of mastocytosis, with the exception of mastocytic leukemia which has very high levels.
  - In anaphylactic reactions due to insect sting it is recommended to always measure tryptase, because of a possible underlying indolent systemic mastocytosis. In all cases, a high tryptase value should be confirmed with a second test after at least 3-4 days.

In acute myeloid leukemia, high levels of tryptase, produced by precursor cells, were observed in 40% of patients. In myelodysplastic syndromes, tryptase is synthesized specifically by atypical mast cells. In hypereosinophilic syndromes with FIP1L1 PDGFRA mutation, mast cell hyperplasia is the cause of increased tryptase.

#### *Tryptase measurement on fluids other than blood*

Besides serum, tryptase can be measured in other biological fluids, such as the broncho-alveolar lavage fluid, the intestinal fluid, the nasal and the lacrimal secretion. In basal conditions, its determination in the nasal fluid can be particularly useful for monitoring inflammation *in situ* and high concentrations are characteristic of allergic rhinitis.

During the provocation tests for allergic diseases, high levels of tryptase can be found in all the aforementioned materials. After

nasal challenge the mast cells release histamine and preform tryptases and this determines an increase in their concentration in these secretions (76), together with that of prostaglandin (PGD2) and cysteinyl leukotrienes (Cys-LT). The level of mediators released after nasal challenge is extremely variable from individual to individual. In particular, in atopic responders, tryptase levels can increase up to seven times the baseline value, a much higher rate than for histamine (77). Furthermore, it has been shown that the increase in tryptase in the nasal secretion appears to be particularly significant in patients with perennial allergy to *Dermatophagoides pteronyssinus* (78).

Tryptase measurement, both in basal conditions and after allergenic challenges, can be performed using the nasal microaspiration technique that allows a quantitative measurement of mediators in secretions even with small volumes of appropriately diluted materials (79). Nasal sticks are especially useful in pediatric age (80). The collected material can be processed, with or without any dilution (after washing the solid phase with NaCl 0.9% and Tween 0.05% 3 times for 5 minutes) with the fluoroimmunoenzymatic method (FEIA) similarly to what is done for serum dosage.

Less used, but equally possible, are measurements in the lacrimal secretion, saliva and bronchial and intestinal lavage fluids. The presence of tryptase in tears is due to the release by conjunctival mast cells in patients with allergic inflammation. In particular, the level of tryptase is high in the acute phase of the reaction, but not in the late one. Finally, in subjects in whom the presence of a food allergy is suspected, it can be useful to measure tryptase in the saliva before and after a challenge test with the offending food (81).

### Conclusions

Measurement of blood tryptase is a simple test, with a good reproducibility, within the reach of all laboratories, and it is very useful for the diagnosis of all forms of mastocyte activation. Its greatest usefulness is in the diagnosis of anaphylaxis, when the clinic it is uncertain or may be compatible with other causes, as well as in the diagnosis of mastocytosis. Therefore, this tool should be available in all laboratories. Thanks to tryptase dosage, often performed after an anaphylactic episode following an hymenoptera sting, many forms of mastocytosis, especially in the indolent form, can now be identified. This highlights the need for integrated work among allergists, hematologists and clinical pathologists in the diagnosis of these diseases.

### Conflict of interests

The authors declare that they have no conflict of interests.

## References

1. Pereira PJ, Bergner A, Macedo-Riberio S, et al. Human beta tryptase is a ring like tetramer with active sites facing a central pore. *Nature* 1998; 392:306-11.
2. Schwartz LB, Bradford TR. Regulation of tryptase form human lung mast cells by heparin. Stabilization of the active tetramer. *J Biol Chem* 1986; 261:7372-9.
3. Goldstein SM, Leong J, Schwartz LB, et al. Protease composition of exocytosed human skin mast cell protease-proteoglycan complexes. Tryptase resides in a complex distinct from chymase and carboxypeptidase. *J Immunol* 1992; 148:2475-82.
4. Vitte J. Human mast cell tryptase in biology and medicine. *Mol Immunol* 2015; 63:18-24.
5. Ehrlich P. Beiträge zur Kenntnis der granulierten Bindegewebszellen und der eosinophilen Leukozyten. *Arc Ana Physiol* 1879; 3:166-96.
6. Welle M. Development, significance, and heterogeneity of mast cells with particular regard to the mast cell-specific proteases chymase and tryptase. *J Leuko Biol* 1997; 61:233-43.
7. Castells MC, Irani AM, Schwartz LB. Evaluation of human peripheral blood leucocytes for mast cell tryptase. *J Immunol* 1987; 138:2184-89.
8. Jogie-Brahim S, Min HK, Fukuoka Y, et al. Expression of alpha-tryptase and beta-tryptase by human basophils. *J Allergy Clin Immunol* 2004; 113:1086-92.
9. Schwartz LB, Irani A, Koller K, et al. Quantitation of histamine, tryptase, and chymase in dispersed human T and TC mast cells. *J Immunol* 1987; 138:2611-15.
10. Miller J, Westin E, Schwartz L. Cloning and characterization of complementary DNA for human tryptase. *J Clin Invest* 1989; 84:1188-95.
11. Miller J, Moxley G, Schwartz L. Cloning and characterization of a second complementary DNA for human tryptase. *J Clin Invest* 1990; 86:864-70.
12. Vanderslice P, Ballinger SM, Tam Ek, et al. Human mast cell tryptase: multiple cDNAs and genes reveal a multigene serine protease family. *Proc Natl Acad Sci USA* 1990; 87: 3811-15.
13. Pallaoro M, Fejzo MS, Shayesth L, et al. Characterization of genes encoding known and novel human mast cell tryptases on chromosome 16p13.3. *J Biol Chem* 1999; 274:3355-62.
14. Soto D, Malmsten C, Blount JL, et al. Genetic deficiency of human mast cell a triptasi. *Clin Exp Allergy* 2000; 32:1000-6.
15. Abdelmotleb A, Rose-Zerilli M, Barton S, et al. Alpha-tryptase gene variation is associated with levels of circulating Ig E and lung function in asthma. *Clin Exp Allergy* 2013; <http://dx.doi.org/10.1111/cea.12259>
16. Trivedi N, Tamraz B, Chu C, et al. Human subjects are protected from mast cell tryptase deficiency despite frequent inheritance of loss-of-function mutations. *J Allergy Clin Immunol* 2009; 124:1099-105.
17. Schwartz L. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin N Am* 2006; 26:451-63.
18. Min HK, Moxley G, Neale MC, Schwartz LB, et al. Effect of sex and haplotype on plasma tryptase levels in healthy adults. *J Allergy Clin Immunol* 2004; 114:48-51.
19. Sverrild A, van der Sluis S, Kyvik K, et al. Genetic factors account for most of the variation in serum tryptase – a twin study. *Ann Allergy Asthma Immunol* 2013; 111:286-9.
20. Lyons JJ, Sun G, Stone K, et al. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. *J Allergy Clin Immunol* 2014; 133:1471-74.
21. Miller H, Pemperton A. tissue specific expression of mast cell granule serin proteinases and their role in inflammation in the lung and gut. *Immunology* 2002; 105:357-90.
22. Heutinck K, ten Berge I, Hack C, Hamann J, Rowshani A. Serine proteases of the human immune system in health and disease. *Mol Immunol* 2010; 47:1943-55.
23. MacLachan J, Shelburne C, Hart J, et al. Mast cell activators: a new class of highly effective vaccine adjuvants. *Nat Med* 2008; 14:536-41.
24. McNeil H, Adachi R, Stevens R. Mast cell restricted tryptase: structure and function in inflammation and pathogen defense. *J Biol Chem* 2007; 282:20785-9.
25. Van der Linden P, Hack C, Poortman J, et al. Tryptase levels as an indicator of mast cell activation in systemic anaphylaxis and mastocytosis. *J Allergy Clin Immunol* 1992; 90:110-8.
26. Frungieri M, Weidinger S, Meineke V, et al. Proliferative action of mast cell tryptase is mediated by PAR 2, COX2, prostaglandins, and PPARγ: possible relevance to human fibrotic disorders. *Proc Natl Acad Sci USA* 2002; 99:15072-7.
27. Chen L, Schrementi M, Ranzer M, et al. Blockade of mast cell activation reduces cutaneous scar formation. 2014; *PLoS ONE* 9:e85226.
28. Oliveira S, Silva C, Ferreira J. Critical role of protease – activated receptor 2 activation by mast cell tryptase in the development of postoperative pain. *Anesthesiology* 2013; 118:679-90.
29. Schwartz LB, Bradford TR, Rousse C, et al. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. *J Clin Immunol* 1994; 14:190.
30. Enander I, Matsson P, Nystrand J, Anderson AS, Eklund E, Bradford TR, Schwartz LB. A new radioimmunoassay for human mast cell tryptase using monoclonal antibodies. *J Immunol Methods* 1991; 138:39-46.
31. Schliemann S, Seyfarth F, Hipler U, Elsner P. Impact of age and heterophilic interference on the basal serum tryptase, a risk indication for anaphylaxis in 1092 dermatology patients. *Acta Derm Venereol* 2012; 92:484-9.
32. Przybilla B, Müller U, Jarisch R, Rueff F. Erhöhte basale Serumtryptasekonzentrationen oder Mastozytose als Risikofaktor der Hymenopterenallergie. *Allergo J* 2004; 13:440-2.
33. Zanotti R, Lombardo C, Passalacqua G, Caimmi C, Bonifacio M, De Matteis G, et al. Clonal mast cell disorders in patients with severe Hymenoptera venom allergy and normal serum tryptase levels. *J Allergy Clin Immunol* 2015; 136:135-9.
34. Beck SC, Wilding T, Buka RJ, Baretto RL, Huissoon AP, Krishna MT. Biomarkers in human anaphylaxis: a critical appraisal of current evidence and perspectives. *Front Immunol* 2019; 10:494.
35. van Toorenbergen A, van Daele P, Boonstra J. False elevated serum tryptase assay result caused by heterophilic antibodies. *J Allergy Clin Immunol* 2005; 116:1159-60.
36. Sargur R, Cowley D, Murng S, et al. Raised tryptase without anaphylaxis or mastocytosis: heterophilic antibody interference in the serum tryptase assay. *Clin Exp Immunol* 2011; 163:339-45.
37. Davson E, Chapman K, Wienholt L, et al. Inter-laboratory variation in the measurement of serum Tryptase published: 07 September 2017; [https://doi.org/10.1111/imj.1\\_13578](https://doi.org/10.1111/imj.1_13578)
38. Enrique E, Garcia-Ortega P, Sotorra O, Gaig P, Richart C. Usefulness of UniCAP-Tryptase fluoroimmunoassay in the diagnosis of anaphylaxis. *Allergy* 1999; 54:602-6.
39. Payne V, Kam PC. Mast cell tryptase: a review of its physiology and clinical significance. *Anaesthesia* 2004; 59:695-703.

40. Brown SG. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol* 2004; 114:371-6.
41. Valent P, Akin C, Arock M, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *Int Arch Allergy Immunol* 2012; 157:215-25.
42. Baretto RL, Beck S, Heslegrave J, Melchior C, Mohamed O, Ekbote A, et al. Validation of international consensus equation for acute serum total tryptase in mast cell activation: A perioperative perspective. *Allergy* 2017; 72:2031-4.
43. Valent P, Bonadonna P, Hartmann K, Broesby-Olsen S, Brockow K, Butterfield JH, et al. Why the 20% + 2 Tryptase Formula Is a Diagnostic Gold Standard for Severe Systemic Mast Cell Activation and Mast Cell Activation Syndrome. *Int Arch Allergy Immunol* 2019; 180:44-51.
44. Simons F, Arduoso L, Bilò M, et al. World Allergy Organization anaphylaxis guidelines: summary. *J Allergy Clin Immunol* 2011; 127:587-93.
45. Takazawa T, Sabato V, Ebo DG. In vitro diagnostic tests for perioperative hypersensitivity, a narrative review: potential, limitations, and perspectives. *Br J Anaesth* 2019; 123:e117-e125.
46. Vitte J, Amadei L, Gouitea M, et al. Paired acute-baseline serum tryptase levels in perioperative anaphylaxis: an observational study. *Allergy* 2019; 74:1157-65.
47. Samson H, Mendelson L, Rosen J. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992; 327:380-4.
48. Yunginger J, Nelson D, Squillace D, et al. Laboratory investigation of deaths due to anaphylaxis. *J Forensic Sci* 1991; 36:857-65.
49. Bonadonna P, Scaffidi L, Boni E. Tryptase values in anaphylaxis and insect allergy. *Curr Opin Allergy Clin Immunol* 2019; 19:462-7.
50. Rueff F, Przybilla B, Bilò M, 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; 124:1047-54. doi: 10.1016/j.jaci.2009.08.027
51. Rueff F, Przybilla B, Bilò M, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase. *J Allergy Clin Immunol* 2010; 126:105-11.
52. Guenova E, Volz T, Eichner M, et al. Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly. *Allergy* 2010; 65:919-23.
53. Bonadonna P, Perbellini O, Passalacqua G, et al. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. *JACI* 2009; 123:680-6.
54. Bilò MB, Pravettoni V, Bignardi D, et al. Hymenoptera venom allergy: management of children and adults in clinical practice. *J Invest Allergol Immunol* 2019; 29:180-205.
55. Haeberli G, Bronnimann M, Hunziker T, et al. 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; 33:1216-20.
56. Kleine-Tebbe J. Die spezifische Immuntherapie bei Ig E vermittelten allergischen Erkrankungen. *Allergo J* 2009; 18:508-37.
57. Rueff F, Placzek M, Przybilla B. Mastocytosis and hymenoptera venom allergy. *Curr Opin Allergy Clin Immunol* 2006; 6:284-8.
58. Bilò MB, Martini M, Tontini C, Mohamed OE, Krishna MT. Idiopathic anaphylaxis. *Clin Exp Allergy* 2019; 49:942-52.
59. Pumphrey R, Roberts I. Postmortem findings after fatal anaphylactic reactions. *J Clin Pathol* 2000; 53:273-6.
60. Buckley M, Variend S, Walls A. Elevated serum concentrations of  $\beta$  tryptase, but not  $\alpha$  tryptase, in sudden infant death syndrome (SIDS). An investigation of anaphylactic mechanisms. *Clin Exp Allergy* 2001; 31:1696-704.
61. Edston E, Eriksson O, van Hage M. Mast cell tryptase in postmortem serum – reference values and confounders. *Int J Legal Med* 2007; 121:275-80.
62. McLean-Tooke A, Goulding M, Bundell C, et al. Postmortem serum tryptase levels in anaphylactic and non anaphylactic deaths. *J Clin Pathol* 2014; 67:134-8.
63. Tse R, Wong CX, Kesha K, Garland J, Tran Y, Anne S, Elstub H, Cala AD, Palmiere C, Patchett KL. Post mortem tryptase cut-off level for anaphylactic death. *Forensic Sci Int* 2018; 284:5-8.
64. Xiao N, Li DR, Wang Q, et al. Postmortem Serum Tryptase Levels with Special Regard to Acute Cardiac Deaths. *J Forensic Sci* 2017; 62:1336-8.
65. Sun KJ, He JT, Huang HY, et al. Diagnostic role of serum tryptase in anaphylactic deaths in forensic medicine: a systematic review and meta-analysis. *Forensic Sci Med Pathol* 2018; 14:209-15.
66. Schwartz LB, Sakai K, Bradford T, et al. The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. *J Clin Invest* 1995; 96:2702.
67. Sperr W, Jordan H, Baghestanian M, et al. Expression of mast cell tryptase by myeloblasts in a group of patients with acute myeloid leukemia. *Blood* 2001; 98:2000-9.
68. Sperr W, Stehberger B, Wimazal F, et al. Serum tryptase measurements in patients with myelodysplastic syndromes. *Leuk Lymphoma* 2002; 43:1097-105.
69. Klion A, Noel P, Akin C, et al. Elevated serum tryptase levels identify a subset of patients with a myeloproliferative variant of idiopathic hypereosinophilic syndrome associated with tissue fibrosis, poor prognosis, and imatinib responsiveness. *Blood* 2003; 101:4660-6.
70. Sirvent A, Gonzalez C, Enriquez R, et al. Serum tryptase levels and markers of renal dysfunction in a population with chronic kidney disease. *J Nephrol* 2010; 23:282-90.
71. Pastorello EA, Schroeder JW, Veronesi SM, Pravettoni V, De Gasperi A, Cantoni S, et al. Two cases of elevated tryptase in abdominal aortic aneurysm. *Eur Ann Allergy Clin Immunol* 2015; 47:58-61.
72. Cooper P, Schwartz B, Irani A, et al. Association of transient dermal mastocytosis and elevated plasma tryptase levels with development of adverse reactions after treatment of onchocerciasis with ivermectin. *J Infect Dis* 2002; 186:1307-13.
73. Sabato V, Van De Vijver E, Hagendorens M, et al. Familial hypertryptasemia with associated mast cell activation syndrome. *J Allergy Clin Immunol* 2014; 134:1448-50.
74. Sethuraman G, Handa S, Radotra B, et al. Diffuse cutaneous mastocytosis with bullae or bullous mastocytosis: a question of semantics. *Ped Dermatol* 1999; 16:409-11.
75. Lyons JJ. Hereditary alpha tryptasemia: genotyping and associated clinical features. *Immunol Allergy Clin North Am* 2018; 38:483-95.
76. Juliusson S, Holmberg K, Baumgarten CR, et al. Tryptase in nasal lavage fluid after local allergen challenge. Relationship to histamine levels and TAME-esterase activity. *Allergy* 1991; 46:459-65.

77. Castells M, Schwartz L.B. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. *J Allergy Clin Immunol* 1988; 3:6-14.
78. Bellussi L, De Lauretis A, D'Onza M, et al. Specific nasal provocation test in allergic rhinitis diagnosis: reliability and standardization *Acta Otorhinolaryngol Ital* 2002; 22:208-14.
79. Wang D, Clement P, Smitz J, et al. Correlation between complaints, inflammatory cells and mediator concentrations in nasal secretions after nasal allergen challenge and during natural allergen exposure. *Int Arch Allergy Immunol* 1995; 106:278-85.
80. Di Rienzo L, Coen Tirelli G, Marsico C, et al. Nuova metodica RHINOSTICK per la diagnosi di rinite allergica in età pediatrica: nostra esperienza. *Orl Ped* 2001; 12:17-9.
81. Rueff F, Friedl T, Arnold A, et al. Release of mast cell tryptase into saliva: a tool to diagnose food allergy by a mucosal challenge test? *Int Arch Allergy Immunol* 2011; 155:282-8.