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# Mast cell Tryptase

For diagnosis and prediction

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for Thermo Fischer Scientific

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# Index

<b>Introduction</b> .....	<b>3</b>	<b>Chapter 5</b> .....	<b>19</b>
<b>Chapter 1:</b> .....	<b>4</b>	Measuring Tryptase.....	19
Tryptase - the enzyme:.....	4	Test method for measuring	
Summary of structure and function of Tryptase ...	5	circulating Tryptase levels.....	19
Roles in anaphylaxis .....	6	Normal Tryptase Range .....	19
Normal level of Tryptase.....	6	When to measure .....	20
<b>Chapter 2:</b> .....	<b>7</b>	Tryptase testing in insect venom allergy.....	20
Mast cells – the factory.....	7	Tryptase testing in allergy to drugs.....	20
FcεRI-dependent activation by allergen.....	10	Tryptase testing in mastocytosis.....	20
Mast cell activation in the absence of allergen.....	11	Comparing to individual baseline Tryptase level..	21
List of important Mast cell functions .....	11	Things to consider with tryptase	
Mast cells interacts with many other cell		and transient rise in tryptase.....	21
types in the immune system .....	11	<b>Chapter 6</b> .....	<b>23</b>
Basophil granulocytes.....	11	Practical cases for the utility of serum	
<b>Chapter 3:</b> .....	<b>13</b>	Tryptase in clinical practice.....	23
The pathological role of mast cells.....	13	General points about practical use of Tryptase.....	23
Mastocytosis .....	13	Case 1 - Tryptase in the perioperative setting.....	23
WHO classification of mastocytosis 2016 .....	14	Case 2 - Tryptase in the emergency room .....	24
Major criterion .....	15	Case 3 - Tryptase as a diagnostic marker	
Minor criteria .....	15	when the diagnosis is uncertain .....	24
Mast cell mediator-induced symptoms.....	15	Case 4 – Tryptase in Insect venom anaphylaxis...24	
<b>Chapter 4</b> .....	<b>16</b>	Case 5 – Tryptase in mastocytosis.....	25
Anaphylaxis.....	16	Final remarks.....	26
Elicitors of anaphylaxis.....	17	References.....	26
Anaphylaxis in patients with Mastocytosis.....	17		
Anaphylaxis caused by drug allergy and in			
the perioperative setting.....	17		
Anaphylaxis caused by allergy to insect venom...17			

# Introduction

A subtype of granulocytes found in peripheral tissue, plays a central role in allergic and other inflammatory reactions. These cells called “mast cells” release a substantial number of mediators among which Tryptase is a neutral serine protease and the most abundant mediator stored in mast cell granules. The significant release of Tryptase from the secretory granules is a characteristic feature of mast cell degranulation. Mast cell Tryptase plays an important role in inflammation although the biological function has not been fully clarified. Tryptase is involved in airway homeostasis, vascular relaxation and contraction, gastrointestinal smooth muscle activity and intestinal transport and coagulation <sup>(1)</sup>. During an anaphylactic reaction serum mast cell Tryptase concentration is increased, and increased levels of Tryptase is detected in different haematological diseases and in patients with systemic mastocytosis. The significant release of Tryptase from mast

cells gives a tool, useful to distinguish mast cell-dependent systemic reactions from other systemic reactions, which can present with similar clinical manifestations. Increased Tryptase levels are highly suggestive of an immunologically mediated reaction, but may also occur following direct mast cell activation. Because of the central role of mast cells in the cascade of allergic reactions, patients with increased mast cell Tryptase levels in serum must be investigated for potential IgE mediated allergy. However, also patients without increased mast cell Tryptase levels should be investigated if the clinical picture suggests severe anaphylaxis <sup>(1)</sup>. This paper, summarising important aspect of the function related biological mechanisms and utility of serum Tryptase as a diagnostic marker is based on information from original articles, as well as recent excellent reviews about Tryptase, function of mast cells, mastocytosis, anaphylaxis and other related issues.



## Tryptase – the enzyme:

Endopeptidases also called endoproteinases are proteolytic peptidases that break peptide bonds of nonterminal amino acids (i.e. within the molecule). This is in contrast to so called exopeptidases, which break peptide bonds from end-pieces of terminal amino acids. Endopeptidases usually do not break down peptides into monomers as is the case with some exopeptidases. An example of an endopeptidase is the oligopeptidase, for which the substrates are oligopeptides and not intact protein.

The so-called “PA clan” (Proteases of mixed nucleophile, superfamily A) is the largest group of proteases with common ancestry as identified by structural homology. Members have a chymotrypsin-like fold and similar proteolysis mechanisms but sequence identity of <10%. The clan contains both cysteine and serine proteases (different nucleophiles) <sup>(2,3)</sup>.

The PA clan represents an example of convergent evolution to a molecular structure based on a catalytic triad for hydrolysis <sup>(4)</sup>.

Serine endopeptidases are enzymes that cleave peptide bonds in proteins, in which serine serves as the nucleophilic amino acid at the enzyme’s active site. They are found in both eukaryotes and prokaryotes <sup>(5)</sup>. Serine proteases falls into two broad categories based on their structure: chymotrypsin-like (trypsin-like) or subtilisin-like. As mentioned, they are in humans responsible for co-ordinating various physiological functions, including digestion, immune response, blood coagulation and reproduction <sup>(5)</sup>.

Endopeptidases, like the serine proteases, are usually very specific for certain amino acids. Examples of different endopeptidases include:

- Trypsin – cuts after Arg or Lys, unless followed by Pro. Very strict.
- Chymotrypsin – cuts after Phe, Trp, or Tyr, unless followed by Pro.
- Elastase – cuts after Ala, Gly, Ser, or Val, unless followed by Pro.
- Thermolysin – cuts before Ile, Met, Phe, Trp, Tyr, or Val, unless preceded by Pro
- Pepsin – cuts before Leu, Phe, Trp or Tyr, unless preceded by Pro.

This specificity that Trypsin-like proteases cleave peptide bonds following a positively charged amino acid (lysine or arginine) is driven by the residue which lies at the base of the enzyme’s S1 pocket (generally a negatively charged aspartic acid or glutamic acid).

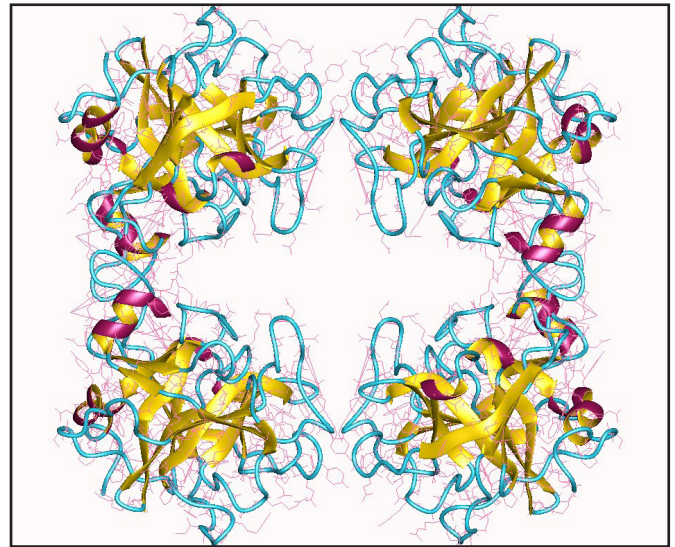


Fig 1. Tryptase alpha/beta 1 – tetramer structure

The family of trypsin-like serine proteases primarily produced and stored in the mast cells and to a lower extent in immature circulating blood basophils are called Tryptase <sup>(6,7)</sup>. The tryptase enzyme is produced and released independently of the location, maturation stage, or subtype of the mast cells <sup>(6)</sup>. The 2 most abundant sub-types of Tryptase produced in the mast cells are named alpha-tryptase and beta-tryptase <sup>(7-9)</sup>. Whereas mast cells produce and release the alpha form constantly, the beta-form is primarily stored in mast cell granules and released upon stimulation, e.g. during an anaphylactic reaction <sup>(10)</sup>.

The 2 forms of Tryptase, are encoded by 2 separate genes. Both are expressed as inactive proenzymes. Alpha-protryptase and beta-protryptase are spontaneously released from resting mast cells. The levels of the protrypsases do reflect the total number of mass cells within the body, but are not an indication of mast cell activation. Beta-protryptase is processed to a mature form, which is stored in granules and released as an active tetramer <sup>(fig 1)</sup> that is bound



to heparin or chondroitin sulfate proteoglycans. In contrast, an amino acid change in alpha-protryptase prevents this for processing to a mature form. Upon mast cell activation, degranulation releases mature Tryptase, which is almost exclusively in the form of beta-Tryptase.

Relatively minor differences in amino acid sequence between alpha-Tryptase and various beta-Tryptases cause major differences in activation, secretion, and activity. Alpha-Tryptase appears as a propeptide form that hinders removal and activation. This has several consequences, including failure to adopt an active conformation and to accumulate in secretory granules. Instead, inactive monomeric proenzyme diverts to a constitutive secretion pathway. This is also the case with unprocessed pro-beta Tryptase, which can be a large fraction of the total Tryptase produced. Thus baseline immunoreactive Tryptase in serum is consisting mainly of pro-alpha and pro-beta Tryptase<sup>(11)</sup>. The situation can be expected to be similar in anaphylaxis. Because alpha-Tryptase is not stored, it will not contribute to an increase in Tryptase levels with acute mast cell degranulation. Alpha-Tryptase has an additional defect in its catalytic domain which limits activity in preparations of enzyme in which the propeptide is artificially removed. In summary, alpha-Tryptase is not stored and seems to be doubly disadvantaged by defects in activation and catalytic domains. Therefore, alpha-Tryptase alone is not a useful marker of mast cell burden or acute activation and is less likely than beta-Tryptases to play a causative role in the pathophysiology of anaphylaxis<sup>(11,12)</sup>.

Tryptase has a wide range of biological activities but the activity is limited to several known natural substrates. Tryptase is involved in the regulation of cell proliferation and growth, processing of (pro) hormones, activation of fibrinolytic enzymes, and degradation of plasma and matrix molecules, and Tryptase appears to be a most potent mitogen for diverse mesenchymal cells including fibroblasts and endothelial cells<sup>(13-15)</sup>. In addition, Tryptase degrades fibrinogen and activates pro-urokinase<sup>(16,17)</sup>. Other biologic effects include cleavage of vasoactive intestinal peptide<sup>(18)</sup>. The enzymatic activity of beta-Tryptase depends on environmental factors. Optimal conditions and factors appear to be present within

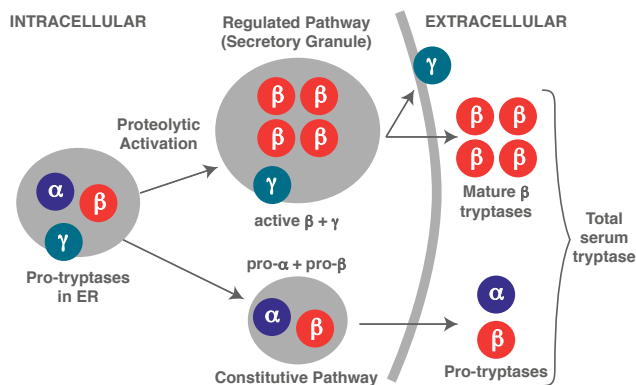


Fig 2. Activation and secretion of  $\alpha$ ,  $\beta$  and  $\gamma$  tryptases<sup>(11)</sup>.

## Summary of structure and function of Tryptase

Structure	Biological function
<ul style="list-style-type: none"> <li>tetrameric neutral serine protease</li> <li>molecular weight of 134 kDa</li> <li>alfa-tryptase and beta-tryptase</li> <li>beta-tryptase is stored in the secretory granules of mast cells</li> <li>alfa-protryptase is secreted constitutively as inactive proenzyme and is the major form</li> <li>Regulation unknown</li> </ul>	<ul style="list-style-type: none"> <li>Cleave <ul style="list-style-type: none"> <li>vasoactive intestinal peptides</li> <li>calcitonin gene-related peptides</li> <li>fibronectin</li> <li>kinonogens</li> </ul> </li> <li>Stimulate IL-8</li> <li>Up-regulate ICAM-1 expression</li> <li>Recruitment of inflammatory cells</li> <li>Stimulate other mast cells</li> <li>Amplifying mast cell degranulation</li> </ul>

the mast cell secretory granules, being the primary site of enzyme expression and storage. The formation of the tetramer structure is optimal at low pH (6.0), an ionic strength equivalent of 160 mM NaCl, and a temperature-range of 22-37°C<sup>(19)</sup>.

During an anaphylactic reaction, mast cell granules release large quantities of Tryptase and a significant amount of the enzyme can be detected in peripheral blood, normally 15 minutes after initiation of the anaphylactic cascade. The levels decline under first-order kinetics with half-life of approximately 2 hours. By comparison, histamine (another immunologic mediator released by activated mast cells) is cleared from blood within minutes. Increased serum levels may also occur after allergen challenge or in pa-

tients with systemic mastocytosis or mast cell activation syndrome. In case of an anaphylactic reaction – allergy driven or not - the role of Tryptase is important.

### **Roles in anaphylaxis** <sup>(68)</sup>

Anaphylaxis is mediated predominantly by mast cells tryptase

- $\alpha$ -tryptase
  - Released by mast cells constitutively
  - Increased baseline release in mastocytosis
- $\beta$ -tryptase
  - Stored in mast cells granules
  - Released after IgE-dependent activation
  - >more specific marker than total tryptase
  - BEST marker of systemic mast cell activation in anaphylaxis

### **Normal level of Tryptase**

- In healthy individuals, the Tryptase baseline levels have been reported to range approximately between 1–15  $\mu\text{g/l}$ . <sup>(70)</sup>
- Each individual has its own unique baseline level, which usually is stable over time. Some individuals with elevated baseline levels of tryptase, approximately  $>10 \mu\text{g/l}$ , are considered to be at increased risk for severe anaphylactic reaction.
- Elevated levels of tryptase can usually be detected for up to 3 to 6 hours after the anaphylactic reaction. The return to baseline levels can generally be verified approximately 24 hours after the reaction. <sup>(69,70)</sup>
- To ensure that peak values are measured samples should preferably be collected between 15 minutes and 3 hours after the suspected event causing mast cell activation. <sup>(69,70)</sup>

Elevated baseline tryptase levels are also found in subgroups of patients with mastocytosis, myelodysplastic syndrome (CMML), myeloproliferative neoplasm, acute myeloid leukemia AML, chronic myeloid leukemia (CML) and chronic eosinophilic leukemia (CEL).

# Mast cells – the factory

Mast cells are found within all layers of the skin and mucosa of the airways and the gastrointestinal tract. Degranulation from these cells is directly involved in the pathogenesis of immune mediated diseases such as inhalational allergy, food allergy, venom allergy, drug allergy and mastocytosis. Mast cells also play a role in several other diseases, not related to allergy and/or anaphylaxis and those are beyond the scope of this paper.

Mast cells are the major source for production, storage and release of circulating Tryptase. Since Paul Ehrlich more than 130 years ago discovered mast cells, (or mastzellen) intensive research have improved our knowledge about and the understanding of the importance of mast cells in biology and medicine. The discovery of immunoglobulin E <sup>(20,21)</sup>

and later, the high affinity receptors for IgE and IgG on mast cells lead into a deeper understanding of the acute allergic mechanisms. Mast cells express a range of inflammatory mediators including Tryptase, histamine, cytokines, chemokines, and growth factors. They also play a role in many varying diseases, from allergic diseases, parasitic infections, haematological malignancies, arthritis and osteoporosis.

Since the first description in 1878 an exciting history of classical research activities have been applied and generated important knowledge about immunological cell activities and communication between the different cells in the human organism <sup>(22)</sup>. Figure 3 highlights some of the important historical findings. For further details please see original paper <sup>(23)</sup>

Year(s)	
1878	Paul Ehrlich describes mast cells in his doctoral thesis at the University of Leipzig and coins the term “mastzellen” derived from German word “mast” (breast)
1891	Water solubility of mast cells demonstrated by Paul Ehrlich
1891	Description of cells with basophilic granules in leukemia by Paul Ehrlich
1898	Paul Ehrlich describes two types of cells with basophilic granules. One localized to tissue (tissue mast cells) and another derived from bone marrow and localized to blood (blood mast cell, basophil, mast leukocyte)
1900	Demonstration of bone marrow origin of mast cells by Jolly
1900	Paul Ehrlich describes antibody formation theory
1902	Description of “anaphylaxis” is made by Paul Portier and Charles Richet
1906	The term “allergy” is coined by Clemens von Pirquet
1908	Paul Ehrlich receives the Nobel Prize in Physiology or Medicine along with Ilya Ilyich Mechnikov for discoveries in immunology
1913	Nobel Prize awarded to Charles Richet for discovery of “anaphylaxis”
1921	Description of passive transfer of hypersensitivity with serum by Prausnitz, popularly known as the Prausnitz-Kustner reaction (P-K reaction)
1952	Discovery of “histamine” in mast cells by James Riley and Geoffrey West
1961	Mast cells identified in the bronchial tissue in asthma
1973	Putative receptor on mast cells for IgE recognized
1979	Slow-reacting substance of anaphylaxis (SRS-A) identified in mast cells as a “leukotriene”
1967	Kimishige Ishizaka recognizes immunoglobulin E as reaginic antibody
1989	Plaut and Paul show mast cells are capable of secreting multiple lymphokines
1990	Steel locus kit ligand (KL) is identified as ligand for c-kit and reported to be involved in mast cell proliferation
1991	Cloning of human mast cell chymase is reported
1994	Ability of mast cells to phagocytose bacteria is shown
1994	Murine mast cells are shown to present antigen to T cells
1996	Mast cells are shown to be important in defense against E. coli infection
2001–2003	Toll-like receptors are described on mast cells
2003	Toll-like receptor and high-affinity IgE signaling shown to induce distinct gene profiles in mast cells
2004	Expression of nitric oxide synthase and nitric oxide in human mast cells demonstrated
2009–2010	Molecular involvement of mast cells in diverse gastrointestinal tract diseases such as Crohn’s disease and eosinophilic esophagitis demonstrated
2012	Mast cells may link eczema to airway reactivity in a mouse model

Fig 3. Selected historical highlights on mast cell discoveries – modified from <sup>(23)</sup>



Mast cells in connective tissue and mucosal surfaces, are particularly numerous at the interface between the external and internal environment. Being important in the environmental interface of the body and close to potential sites of physical damage they are placed at the site needed for action. They are evolutionarily conserved and primary immunodeficiency of mast cells has never been described, and they are likely to be important in normal development and maintenance of healthy tissue. Mast cells take part in the coordination of the inflammatory response and the repair responses to tissue damage <sup>(25)</sup>.

The function and pathologic contribution of mast cells is evident in allergic respiratory as well as skin diseases such as rhinitis, allergic asthma and IgE mediated exacerbations of atopic dermatitis. Also in pulmonary fibrosis, and pulmonary hypertension mast cells seems to be involved. Mast cell activation may also be important in acute respiratory distress syndrome, chronic obstructive pulmonary disease (COPD), and lung neoplasia <sup>(25)</sup>.

Mast cells develop along a granulocyte/monocyte Q3 lineage from pluripotent CD341 and CD171 hematopoietic stem cells, which originate from the bone marrow <sup>(26)</sup>. In practice, mononuclear progenitors enter the systemic circulation and then migrate into various tissues. The exact mechanism for this site directed migration is not well described, but because numerous chemoattractants for human mast cells have been identified, tissue homing is thought to be primarily driven by a process of active recruitment. This has been reviewed in details elsewhere <sup>(27)</sup>. Mast cell progenitors originate from haematopoietic stem cells in the bone marrow via the myeloid lineage. Progenitors then circulate in the blood stream and eventually migrate into tissues, whereupon interaction with structural cells and their cytokine environment determines their differentiation and maturation into distinct phenotypes. A change in the mast cell's environment in pathological conditions also contributes to mast cell heterogeneity.

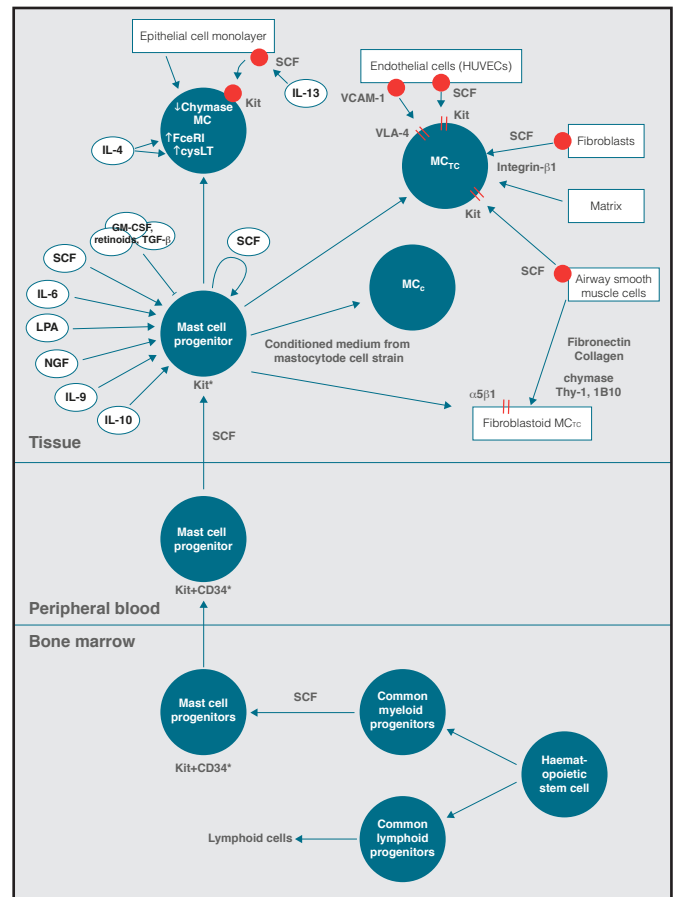


Fig 4. Recruitment of mast cell progenitors and differentiation of mast cell phenotypes <sup>(27)</sup>.

Mature differentiated human mast cells are capable of proliferating, so increase in mast cell numbers in various diseases may occur as a result of both recruitment of new cells and proliferation of local cells <sup>(28,29)</sup>.

The morphological characteristic of mast cells is the presence of dense, membrane-bound intracellular granules. In human mast cells, these granules contain an acidic proteoglycan matrix consisting of heparin sulphate, to which preformed mediators, such as histamine, serine proteases (tryptase, chymase, cathepsin G, and carboxypeptidase A), and some cytokines are attached. The acidic granule environment conserves mediators in an inactive state until the granules and their contents are released from the cells via exocytosis. Granule fusion and exocytosis in response to FcεRI-dependent activation is also termed “compound exocytosis” or “anaphylactic degranulation.” The process is not cytotoxic and mast cells are able to recover and replenish their granules.

Although FcεRI-triggered exocytosis in diseases such as asthma and pulmonary fibrosis can result in almost complete degranulation of mast cells, these cells also often exhibit only partly degranulation and the cell and granule membranes remain intact<sup>(25,30,31)</sup>.

Mast cells are sources of many multifunctional cytokines and chemokines regulated by external stimuli based on the immunological response and figure 8 lists the most important proteases and mediators from mast cells<sup>(27)</sup>.

Mature human mast cells can be divided in two large subsets based on their protease content. The mast cell Tryptase/Chymase (MCTC) subset of cells store Tryptases, Chymases, and carboxypeptidases in their granules, whereas MCT contain only Tryptases.

Mast cell phenotypic heterogeneity, reflected in their extensive range of sensitivity to activation, and the variations in stored and released mediators, underlies the array of responses mast cells are able to generate<sup>(24)</sup>.

Mediator	Stored or newly synthesized	Biological effects
Histamine	Stored	Induces bronchoconstriction and tissue oedema; increases vascular permeability, mucus secretion, fibroblast proliferation, collagen synthesis and endothelial cell proliferation; activates dendritic cells.
Heparin	Stored	Anticoagulant effects; provides proteoglycan-based storage matrix for mediators in MC granules; sequesters growth factors to prevent their degradation; activates fibroblasts; induces endothelial cell migration.
Tryptase	Stored	Degrades respiratory allergens, cross-linked IgE and neuropeptides; generates C3a and bradykinin; enhances bronchial hyperresponsiveness and ASM contractility; indirectly activates collagenase; increases fibroblast proliferation and collagen synthesis; activates epithelial cells and TGF-β molecules; enhances MC histamine release; recruits neutrophils, cleaves and inactivates CCL 5 and – 11.
Chymase	Stored	Increases mucus secretion; degrades ECM and several cytokines including IL-4; facilitates processing of type-1 pro-collagen molecules; induces synthesis of angiotensin II; reduces adhesion of T cells to ASM; activates IL-1β; releases membrane-bound SCF; enhances IL-33 activity through proteolytic cleavage.
PGD <sub>2</sub>	Newly synthesized	Induces bronchoconstriction and tissue oedema; increases mucus secretion; activates dendritic cells; acts as chemoattractant for eosinophils, Th2 cells and basophils via the CRTh2 receptor, activates ILC2 cells.
LTC <sub>4</sub> (and extracellular derived D <sub>4</sub> and E <sub>4</sub> )	Newly synthesized	Induces bronchoconstriction and tissue oedema; increases mucus secretion; IL-13-dependent ASMC proliferation and tissue fibrosis; promotes maturation and recruitment of dendritic cells; induces IL-4 secretion from eosinophils, and IL-5, IL-8 and TNF-α release from MC; chemo-attractant for MCs; induces IL-4, IL-5 and IL-13 production by mouse ILC2 cells.

Fig 5. List of proteases and mediators of mast cells<sup>(27)</sup>.

Mast cells containing only Tryptase (MCT), are typically found within mucosal surfaces such as the bronchial epithelium in asthma, whereas mast cells containing Tryptase, Chymase, cathepsin G, and CPA (MCTC) tend to reside within connective tissues<sup>(27,32)</sup>.

Mast cell proteases are stored within mast cell granules as active enzymes and constitute approximately 25% of mast cell protein content. Chymases, Tryptases, and carboxypeptidase A are primarily expressed by mast cells and as mentioned above, mast cell proteases play an important modulatory role during allergic reactions. Added to this, beta

-Tryptase acts to limit allergic inflammation through the cleavage of IgE after being released by activated mast cells<sup>(24,33)</sup>.

Activation of mast cell responses can happen in different ways. They can be activated by stimuli from neuropeptides, cytokines, growth factors, toxins, basic compounds, complement, immune complexes, certain drugs, and by physical stimuli. In the allergic reaction, an adaptive immune response mediated by a specific allergen binding to the high affinity IgE receptor on the mast cell surface is responsible for the activation.

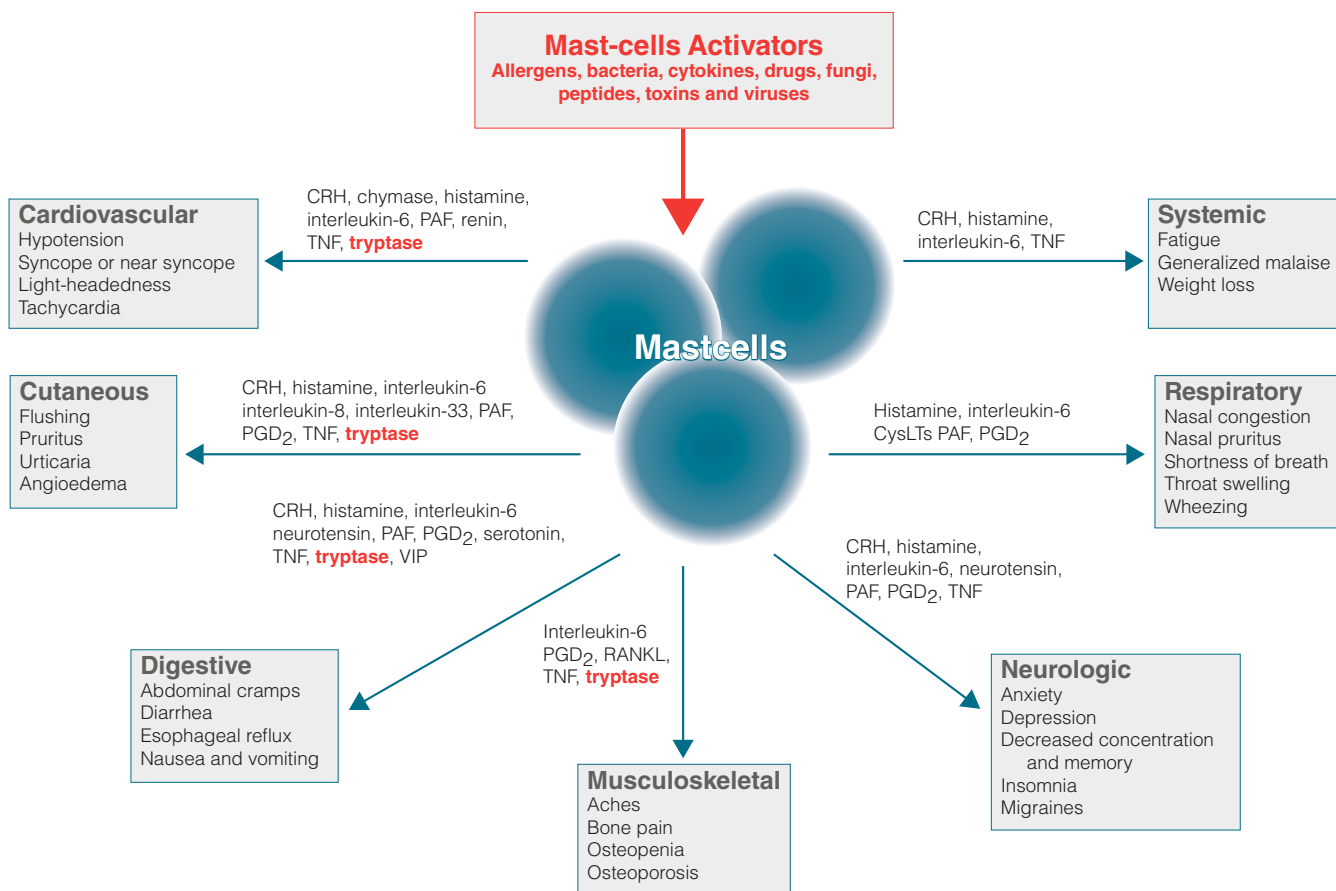


Fig 6. Clinically Relevant Mediators Released from Mast Cells and Putative Effects. The most clinically relevant mediators released from mast cells, the corresponding organs and systems, and potential associated symptoms are shown (adapted from <sup>(34)</sup>).

The clinical response is determined by the location of the mast cell and a complex interaction with other structural cells and the presence of an allergen and allergen specific IgE antibodies.

### FcεRI-dependent activation by allergen.

FcεRI is the high affinity receptor for IgE. FcεRI-dependent activation of mast cells is relevant to allergic diseases, including allergic rhinitis, allergic asthma and food allergy and is the most extensively studied mechanism of activation. The FcεRI receptor is expressed on the mast cell plasma membrane and comprises a tetramer with four subunits <sup>(35)</sup>. Cross binding of a specific allergen between two specific IgE molecules signals the activation and degranulation of the mast cell as shown in figure 7.

### Subsequent contact with allergen

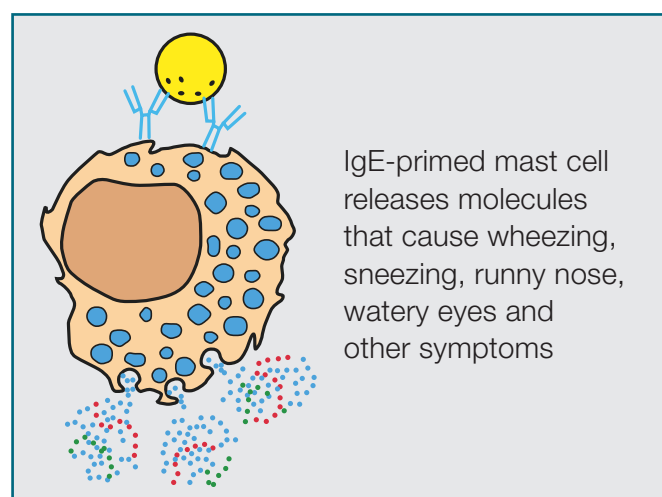


Fig 7. Reference: Immunology, Janis Kuby, Third Edition



## **Mast cell activation in the absence of allergen.**

The binding of IgE to FcεRI in the absence of allergen can influence mast cell development, survival, and function, and this includes the initiation of intracellular signalling events leading to mediator release. Mast cells express a number of receptors via which they can detect pathogens and pathogen-related products <sup>(25,36)</sup>.

In addition to the cross-linking of FcεRI-bound IgE molecules by allergen, the binding of IgE alone to FcεRI can initiate intracellular signalling events with protein phosphorylation, Ca<sup>2+</sup> influx and subsequent mediator release. Mast cells express numerous classes of receptors which mediate their activation by diverse stimuli independently of IgE <sup>(27)</sup>.

Mast cells also express receptors for a number of inflammatory mediators that are able to induce further mast cell activation and production of various mediators/cytokines and/or degranulation. These include receptors for tumor necrosis factor alpha (TNF-α), proteases, PAF, complement, SCF, IL-33, TSLP, IL-1b, and IFNγ <sup>(27)</sup> and there is evidence that mast cell mediators are responsible for the bronchoconstriction observed in both the early and late asthmatic reaction after bronchial provocation <sup>(25,27)</sup>.

So, although mast cells, being a major effector cell of allergic reactions, are activated through exposure to antigens (allergens), mast cells can also be activated by other triggers, including anaphylatoxins, aggregated IgG, certain drugs, venoms, and physical stimuli (pressure and temperature changes), as well as cytokines and neuropeptides such as corticotropin-releasing hormone, neurotensin, stem-cell factor, and substance P.

Once released, the effects of preformed mediators often remain localized. Histamine activity is short-lived in vivo due to degradation by Histamine-N-methyltransferase (HMT), while the active tetramer of Tryptase rapidly dissociates into inactive monomers in the absence of heparin.

## **List of important Mast cell functions**

- mast cells secrete heparin, Tryptase and t-plasminogen activator (tPA) thereby regulating fibrinolytic mechanisms providing the appropriate perfusion and nutrition necessary for repair
- vasoactive amines, Tryptase, IL-4, and NGF contribute to the regeneration of damaged nerve fibers
- Tryptase can specifically activate the protease activated receptor-2 (PAR-2), which inhibits osteoclast differentiation
- angiogenesis
- mast cell proteases contribute to immune tolerance in that they reduce antigenicity and leukocyte recruitment through cleavage of antigens, toxic peptides, cytokines and chemotactic factors

## **Mast cells interacts with many other cell types in the immune system**

Interaction with B-cells, T-cells and eosinophils seems to be an important aspect of allergic and anaphylactic clinical response to foreign allergens. This has been carefully reviewed specifically related to respiratory asthmatic disease by Bradding and Arthur <sup>(27)</sup>.

## **Basophil granulocytes**

Basophils is a distinct cell type, but with some overlapping effector functions with mast cells. Both cell types can develop from a common precursor cell. The main function of both cell types is as effector cells, rather than as participants in the initiation of adaptive immune responses. Mast cells and basophils cooperate to protect the host against secondary infections from ticks and to contribute, to various degrees, to immune responses against helminths. A major difference between the two cell types is that basophils only produce and release minor amounts of Tryptase and related enzymes <sup>(37)</sup>. Figure 11 lists selected characteristics for mast cells and basophils respectively.

Characteristic	Mast cells	Basophils	Both mast cells and basophils
Size	5-10µm	5-7µm	NA
Nuclear morphology	Round	Indented or segmented	NA
Lifespan	Weeks to months	60 hours	NA
Proteases expressed	Chymases and tryptases	mMCP8 and mMCP11	CPA, granzymes and cathepsins
Proteoglycans expressed	Heparin	NA	Chondroitin sulphates
Cytokines produced	IL-8, IL-10, IL-25, TNF and VEGF	NA	IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-13, IL-15, TSLP and others
Activating receptors expressed	KIT, FcγRI and FcγRIIA	2B4, LIR7 leptin receptor	FcεRI, FcγRIIIA, CD200R3, C3aR, C5aR, LTB4R1, IL-3R, IL-18R, IL-33R and TSLPR
Inhibitory receptors express	NA	LIR3	CD200R1, CD300a (also known as LMIR1 in mice), FcγRIIB, GP49B1 and LIR2
Survival factors expressed	BCL-X	PIM1 kinase	MCL1 and BCL-2
Transcription factors expressed	MITF	P1-RUNX1 and C/EBPα	GATA factors and STAT5
Immunomodulatory mediators produced	PGD2 and LTB4	NA	LTC4, PAF, histamine and serotonin
Other associated molecular markers	NA	CD49b, basogranulin and CD203c	CD40L and CRTH2

Fig 11. Selected characteristics for mast cells and basophils<sup>(37)</sup>.

# The pathological role of mast cells

The mast cells interact with the microenvironment and the release of the bioactive mediators can lead into an inadequate (unintended) regulation of their functions which can have serious pathogenic consequences to the organism. Examples of diseases which are related to uncontrolled proliferation of mast cells and release of bioactive substances are: <sup>(24)</sup>

### Allergic reactions

#### Crohn's disease

- Mast cells are redistributed and found in the muscle layers of strictures in the bowel, which has led to the suggestion that mast cells and their mediators may play a role in stricture formation

#### Autoimmune diseases

- Examples of mast cell association with autoimmune diseases include: Type I diabetes, Guillain-Barré syndrome, bullous pemphigoid, Sjögren syndrome, chronic urticaria and experimental vasculitis

#### Cardiovascular diseases

- Both chymase and tryptase released by mast cells induce proteolytic changes in high-density lipoprotein (HDL) particles, which interfere with cholesterol efflux by macrophages leading to the formation of foam cells that constitute atheroma that forms plaque in blood vessels

#### Mastocytosis

- When measuring mast cell released Tryptase, mast cell disorders like mastocytosis plays an important role in the subsequent interpretations of Tryptase level as they may cause baseline Tryptase level to be elevated

### Mastocytosis

Mastocytosis is a biological disorder that is characterized by an increased numbers of mast cells in the skin, bone marrow, gastrointestinal tract, liver, spleen, and lymph nodes. It is not known how many people are suffering from mastocytosis, but the incidence has been conservatively proposed to be from three to seven new patients per million per year. Most cases of mastocytosis have a benign cause over a lifetime. Some cases occur during infancy and early childhood and others appear in adulthood. Mastocytosis in childhood may resolve spontaneously and usually only involves the skin; whereas the course in patients with adult-onset disease is normally chronic and includes not only the skin but also systemic symptoms from other organ systems <sup>(38)</sup>.

Mastocytosis represents a very heterogeneous group of disorders, in all cases characterized by abnormal growth and accumulation of mast cells. The abnormal growth can appear just in one or in several organs simultaneously. In cutaneous as well as systemic mastocytosis the diseases are associated with the proliferation and accumulation of clonal mast cells. In the case of cutaneous mastocytosis this is defined so, that the respiratory system and mucosal surface is not involved. In systemic mastocytosis, mast cells accumulate in several organs; however, involvement of the lungs and kidneys is usually not observed, and respiratory symptoms are not significant <sup>(39)</sup>.

Systemic Mastocytosis is more severe and more common in adults than in children. Many different symptoms may appear and these often include skin lesions. Diffuse and not well defined symptoms such as, pain in inner organs, bone pain, diarrhoea and vomiting, weight loss and cardiovascular symptoms may occur.

In systemic mastocytosis, Tryptase levels are most often increased, but may be normal, while in cutaneous mastocytosis with symptoms only from the skin, Tryptase levels are usually within normal range. However, it is important to implement regular follow-up of cutaneous mastocytosis since there is a small risk of development into the more severe systemic forms of mastocytosis <sup>(39–42)</sup>.





The many variants of mastocytosis has been categorized by WHO and reviewed in details<sup>(40)</sup>. Mastocytosis appears as a very complex and variable set of clinical signs and disorders.

Below is shown the detailed WHO classification of cutaneous and systemic mastocytosis as well major and minor criteria for systemic mastocytosis<sup>(43)</sup>

Fig 9. Typical childhood polymorphic maculopapular cutaneous mastocytosis/ urticaria pigmentosa presenting with red-brown macules and slightly elevated plaques on the trunk and extremities<sup>(43)</sup>. (Knut Brockow)

## WHO classification of mastocytosis 2016

CM (cutaneous mastocytosis)	MPCM = UP (maculopapular cutaneous mastocytosis = urticaria pigmentosa)
	DCM (diffuse cutaneous mastocytosis)
	Mastocytoma of skin (cutaneous mastocytoma)
SM (systemic mastocytosis)	ISM (indolent systemic mastocytosis)
	SSM (smoldering systemic mastocytosis)
	SM with AHN (systemic mastocytosis with associated hematologic non-mast cell-lineage disease)
	ASM (aggressive systemic mastocytosis)
	MCL (mast cell leukemia)
MC sarcom	

\* Valent P et. al. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. Blood. 2017; 129(11):1420-1427.

## Major criterion

The presence of multifocal dense aggregates of >15 mast cells as detected with Tryptase or other special stains in bone marrow or other extracutaneous organs.

## Minor criteria

- 1) Atypical morphology or spindle shapes in >25 percent of the mast cells in bone marrow sections, bone marrow aspirate, or other extracutaneous tissues
- 2) Mutational analysis of KIT showing a codon 816 mutation (eg, Asp816Val) in bone marrow, blood, or extracutaneous organs (see later in this paper)
- 3) Bone marrow or other extracutaneous mast cells expressing the surface markers CD2, CD25, or both
- 4) Baseline serum tryptase levels >20 ng/mL. (This criterion does not apply to patients with AHNMD)

(WAO [http://www.worldallergy.org/professional/allergic\\_diseases\\_center/mastocytosis/#table1](http://www.worldallergy.org/professional/allergic_diseases_center/mastocytosis/#table1))

As an example of the complexity of mastocytosis, patients with systemic mastocytosis may have associated osteopenia or osteoporosis. Pathologic fractures or osteoporosis may in some cases be symptoms indicating manifestation of mastocytosis. Also musculoskeletal pain is a common presenting symptom. In a case series of 362 patients with mastocytosis, it is reported that 54% had some pains and 18% felt it caused intolerable disability<sup>(40)</sup>.

Also the liver, spleen and lymph nodes are common organs affected in systemic mastocytosis especially in cases where the disease appears in its aggressive form. In a study of 41 patients with mastocytosis, 61% had indications of liver disease. Twenty-four percent had hepatomegaly with the majority having elevation in liver enzymes<sup>(40)</sup>.

## Mast cell mediator-induced symptoms

Mast cells have abundant secretory granule proteases, which make up most of the proteins released from mast cells of which Tryptase is the major endopeptidase. Total amount of Tryptase in serum is comprised of mature Tryptase stored in granules and released only upon activation and immature (pro) Tryptase, which is constitutively secreted by the mast cell. Patients with mastocytosis generally have elevated baseline serum Tryptase as well as elevated level of histamine. Together with the typical symptoms mentioned above, abdominal pain is the most frequent symptom, followed by nausea, diarrhoea, and vomiting. Diarrhoea is not generally related to gastric hypersecretion and has been attributed to altered intestinal secretion, structural mucosal abnormalities, and hypermotility<sup>(40)</sup>. These symptoms are believed to be the consequence of the abnormal high release of mast cell mediators.

Some patients report recurrent spontaneous episodes with a single symptom or a combination of symptoms, and where findings sometimes resemble the classical prodromal symptoms of anaphylaxis. The presence, frequency and severity of these symptoms cannot be predicted by the different organ involvement, although such symptoms are considerably more frequent with systemic disease. Severe or protracted anaphylaxis may occur in patients with extensive disease. Those episodes are expected to be IgE-mediated, as following a bee sting in a sensitized individual or patients sensitised to foods or drugs<sup>(38)</sup>.

# Anaphylaxis

The first description and definitions of anaphylaxis was made by Richet and Portier in 1902, when they by reinjection of a foreign substance into dogs provoked a systemic reaction leading to death within minutes. Richet and Portier found that a second injection from the nematocysts of *Physalia* induced a violent reaction in dogs, including the death of “a fine big dog by the name of Neptunus.” Remarkably, dogs experienced no apparent ill effects when they received only a single injection<sup>(44)</sup>.

Anaphylaxis is defined as a systemic reaction involving multiple organ systems. It is related to and influenced by cellular events in mast cells and basophils resulting in the release of and systemic distribution of mediators. Understanding the mechanisms of mast cell activation and degranulation is critical to understanding the mechanisms of anaphylaxis. Recent reports have identified important regulatory components of the signalling cascade and, consequently, potential targets for therapeutic intervention<sup>(45)</sup>.

Although all details - especially with focus on the rapid transformation from local reaction to systemic reaction - of the mechanism of anaphylaxis is not understood, a detailed overview of the process and mechanisms is nicely reviewed<sup>(45)</sup> and recently the WAO published a consensus paper with guidelines on diagnosis and treatment of anaphylaxis. It is concluded that intense research is needed to explore and fully understand the details and mechanisms of the anaphylactic reaction<sup>(46)</sup>.

For the understanding of the mechanisms of anaphylaxis the regulation of intracellular events that result in the release of anaphylaxis mediators is important. The rapid degranulation of the mast cells bioactive substances and generation of arachidonic acid metabolites (lipid mediators), and production of cytokines and chemokines is activated by a very effective intracellular signalling cascades. The mechanisms for mast cell activation and mediator release are usually dependent on the binding of IgE to the high affinity receptor, FcεRI. Upon antigen binding to IgE, these receptors aggregate and initiate the signalling cascade.

Also changes in calcium concentration plays a central role in mast cell degranulation, and increased activity in calcium channels do contribute to initiation

and maintenance of the threshold for the degranulation<sup>(45)</sup>. Mediator of anaphylaxis like histamine, Tryptase, leukotrienes, prostaglandins, TNF-α, and PAF, generated by mast cell or basophil activation, have been long shown to trigger the major physiological manifestations of anaphylaxis<sup>(47)</sup>.

In “non-allergic” anaphylaxis, the clinical features are a result of direct, pharmacological or ‘toxic’ stimulation of mast cells and basophils, causing these cells to release their inflammatory mediators. Non-allergic anaphylaxis does not involve an immunological mechanism and, therefore, previous contact with the substance is not necessary<sup>(48)</sup>.

Anaphylaxis is characterised by one or more of the following conditions...

- Systemic, potentially life threatening condition
- Allergic reaction or non-allergic pathogenesis
- Fast, unexpected, generalized immune reaction
- Release of potent vasoactive mediators from mast cells and basophils
- It can occur within seconds or minutes of exposure to the trigger

<b>Signs and symptoms of anaphylaxis</b>	
Swelling of the conjunctiva	
Runny nose	
Swelling of lips, tongue and/or throat	
Central nervous system	<ul style="list-style-type: none"> <li>• lightheadedness</li> <li>• loss of consciousness</li> <li>• confusion</li> <li>• headache</li> <li>• anxiety</li> </ul>
Heart and vasculature	<ul style="list-style-type: none"> <li>• fast or slow heart rate</li> <li>• low blood pressure</li> </ul>
Respiratory	<ul style="list-style-type: none"> <li>• shortness of breath</li> <li>• wheezes or stridor</li> <li>• hoarseness</li> <li>• pain with swallowing</li> <li>• cough</li> </ul>
Skin	<ul style="list-style-type: none"> <li>• hives</li> <li>• itchiness</li> <li>• flushing</li> </ul>
Gastrointestinal	<ul style="list-style-type: none"> <li>• crampy abdominal pain</li> <li>• diarrhea</li> <li>• vomiting</li> </ul>
Pelvic pain	
Loss of bladder control	

Fig 10. Signs and symptoms of anaphylaxis



...and in practical terms anaphylaxis can appear by the following symptoms.

- When any one of the following three occurs within minutes or an hour of exposure there is a risk of anaphylaxis
  - 1) Involvement of the skin or mucosal tissue plus either respiratory difficulty or a low blood pressure causing symptoms
  - 2) Two or more of the following symptoms after contact with a likely allergen:
    - a. Involvement of the skin or mucosa
    - b. Respiratory difficulties
    - c. Low blood pressure
    - d. Gastrointestinal symptoms
  - 3) Low blood pressure after exposure to a known allergen
- Skin involvement may include: hives, itchiness or a swollen tongue among others.
- Respiratory difficulties may include: shortness of breath, stridor, or low oxygen levels among others.
- Low blood pressure is defined as a greater than 30% decrease from a person's usual blood pressure. In adults, a systolic blood pressure of less than 90 mmHg is often used<sup>(49)</sup>

### Elicitors of anaphylaxis

The most frequently reported elicitors of anaphylaxis are insect venoms, drugs and foods.

Common anaphylaxis triggers are certain medications, especially penicillin, foods, such as peanuts, tree nuts (walnuts, pecans, almonds, cashews), wheat (in children), fish, shellfish, milk and eggs

Insect stings from bees, yellow jackets, wasps, hornets and fire ants and less common causes of anaphylaxis are Latex and medications used in anaesthesia and NSAID medication<sup>(46,51)</sup>.

### Anaphylaxis in patients with Mastocytosis

The review from Brockow and Metcalfe<sup>(38)</sup> report that in adults with the diagnosis of mastocytosis the prevalence of anaphylaxis, being reported as high as 49%, is significantly higher than expected in the general population. In children with cutaneous mastocytosis, the prevalence seems significantly lower (6% to 9%). In adults, those with systemic

mastocytosis had an increased risk for anaphylaxis as compared to patients with cutaneous mastocytosis only. In children, the risk to develop anaphylaxis is restricted to those with extensive skin symptoms and also a high baseline serum level of Tryptase. The most frequent and most severe symptoms of anaphylaxis in patients with mastocytosis are decreased blood pressure and tachycardia. Symptoms such as dizziness, dyspnea, flushing, nausea and diarrhoea and in general severe reactions are typical for patients with mastocytosis. The elicitors for anaphylaxis in mastocytosis are the same as for anaphylaxis in general. Risk factors for anaphylaxis include increased serum Tryptase levels and indolent variant of systemic mastocytosis<sup>(38,50)</sup>.

### Anaphylaxis caused by drug allergy and in the perioperative setting

Anaphylaxis occurring in connection to surgery and use of anaesthesia may present differently from other causes of anaphylaxis. Symptoms and signs are often the same as for anaphylaxis in general, but may be masked by either the anaesthesia or extensive regional blockade. Cutaneous symptoms, such as flushing, urticaria and oedema, are common, but, during anaesthesia, these are usually hidden by surgical draping's<sup>(48)</sup>.

Hypotension and tachycardia are typical cardiovascular symptoms which can rapidly progress into severe arrhythmias and cardiovascular collapse. They are the most common and serious symptoms and, in some cases, cardiovascular collapse may be the only presenting symptom. Respiratory symptoms, such as bronchospasm, after the induction of anaesthesia are less common, but may predominate in asthmatic patients. There are many differential diagnoses to anaphylaxis in the perioperative setting and a marker for anaphylaxis such as Tryptase is particularly useful in this setting.

### Anaphylaxis caused by allergy to insect venom

In the general population, stinging insect allergy is responsible for more than 10% of all cases of anaphylaxis. Mast cell disorders like mastocytosis are associated with severe anaphylaxis from insect stings and should be considered in affected subjects<sup>(52)</sup>.

In patients with hymenoptera venom allergy, elevated baseline Tryptase is strongly associated with severe anaphylaxis. Fatal sting reactions were reported in patients with mastocytosis, notably after stopping venom immunotherapy. During allergen specific venom immunotherapy most patients with mastocytosis are protected from further severe sting reactions. Based on these observations immunotherapy for life can be recommended for patients with mastocytosis and venom allergy<sup>(46,53)</sup>. In all patients with anaphylaxis following hymenoptera stings, baseline serum Tryptase should be determined. A value above 11.4 microg/l is often due to mastocytosis and indicates a high risk of very severe anaphylaxis following re-stings<sup>(39,54)</sup>.

Key points from the research on insect sting and anaphylaxis are the following<sup>(55)</sup>

- Anaphylaxis to insect stings occurs in 3% of adults and less than 1% of children.
- Anaphylaxis to insect stings is generally more benign in children, but severe reactions can be associated with sustained risk for decades.
- Diagnostic tests can identify the presence of sensitization to insect venom but are poor predictors of sting anaphylaxis.
- There is a specific association between insect-sting anaphylaxis and mastocytosis, particularly when there is hypotension during the reaction.
- Venom immunotherapy is highly effective in preventing sting anaphylaxis, and leads to lasting tolerance in most patients who are treated for 5 years.

Elevated tryptase levels are found in subgroups of patients with myelodysplastic syndrome, chronic myelomonocytic leukemia, myeloproliferative neoplasm, acute myeloid leukemia, chronic myeloid leukemia and chronic eosinophilic leukemia.

Among patients with hematologic malignancies, reactive leucocytosis/thrombocytosis or idiopathic cytopenia, elevated levels of serum Tryptase >15 ng/ml (> 99th percentile of healthy controls) cluster almost exclusively in myeloid neoplasm like SM, MDS, MPN, AML, CML and CEL. In patients with lymphoid neoplasms, including lymphomas and multiple myeloma, Tryptase levels are usually within normal range<sup>(56)</sup>.

Normal Tryptase levels are usually also found in patients with non-hematologic malignancies including reactive leucocytosis/thrombocytosis or idiopathic cytopenia including patients with idiopathic cytopenia of unknown significance<sup>(57)</sup>. The myeloid disorders in which a high percentage of patients presented with elevated Tryptase, include SM, acute myeloid leukaemia's (AML), myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML), chronic myeloid leukaemia (CML), and chronic eosinophilic leukaemia (CEL) It is of note that not all patients in these groups exhibit elevated Tryptase levels and that the percentage of cases with elevated Tryptase varies depending on the type of disease. In particular, >90% of the patients with SM have a markedly elevation of serum Tryptase levels. About 30-40% of all patients with AML and 30- 35% of all patients with CML present at diagnosis with an increase in serum Tryptase >15 ng/ml<sup>(56)</sup>.

# Measuring Tryptase

## Test method for measuring circulating Tryptase levels.

- Anti-tryptase, covalently coupled to the solid phase, reacts with the tryptase in the patient serum sample.
- After washing, enzyme-labelled antibodies against tryptase are added to form a complex.
- After incubation, unbound enzyme-anti-tryptase is washed away, and the bound complex is then incubated with a developing agent.
- After stopping the reaction, the fluorescence of the eluate is measured. The fluorescence is directly proportional to the concentration of tryptase in the serum sample.

ImmunoCAP Tryptase is an in vitro test system that measures the total level of Tryptase in human serum.

The measure includes all inactive proforms of alpha-tryptase and beta-tryptase, as well as the enzymatically active mature beta-tryptase. The ImmunoCAP Tryptase assay is for use in the instruments Phadia 100, Phadia 250 and Phadia 1000.

Tryptase proforms are continuously released from the mast cells into the bloodstream and therefore it also reflects the number of mast cells. The lower detection limit is 1 microg/l and the reproducibility within as well as between assays is very high. In the healthy adults population, the normal average level of Tryptase in serum is estimated to 3,4 microg/l (geometric mean) with an upper 95% percentile corresponding to 11.4 microg/l. In patients with systemic mastocytosis is above 20 microg/l and it is estimated that the range from 10-20 microg/l reflects an increase in mast cell concentration and activity indicating increased risk for an anaphylactic reaction if triggered by environmental factors (allergy). Healthy individuals may have baseline levels up to approximately 20 µg/l.

The reference values for children aged 6 months to 18 years appear to be similar to adult values with a median median range of 3.5 ng/mL while Infants younger than 6 months shows a median value of 6.1 ng/mL.<sup>(39)</sup>

## Normal Tryptase Range

A study with 124 self-reported healthy individuals (56 males and 68 females) was performed with Phadia 250. The age range was 3-67 years for males and 4-63 years for females.

The following ImmunoCAP Tryptase results were obtained:

*Geometric mean*      3.4 µg/l  
*95 upper percentile*    11.0 µg/l

In patients with systemic mastocytosis levels of tryptase are, in general, persistently elevated above 20 µg/l.

Baseline tryptase levels in the range of approximately 10-20 µg/l reflect an increased mast cell burden indicating an increased risk in patients with history of severe anaphylactic reaction.

In severe cases the triggering agent causing a transiently elevation of tryptase should be identified.

Measuring elevated levels of Tryptase is generally indicating increased mast cell proliferation and mediator release - and if transient, an ongoing allergic reaction or anaphylactic reaction. Persistent elevated baseline level of Tryptase is an indication of possible mastocytosis.

Mast cells	Total Tryptase in health and disease		Tryptase levels
Resting	Proforms α-tryptas β-tryptase	Baseline levels in healthy individuals	1-15 µg/l
<b>Risk patients</b>	<b>Elevated baseline levels Underlying mastocytosis</b>		<b>&gt;10 µg/l</b>
Increased levels	Proforms α-tryptas β-tryptase	Systemic mastocytosis  Haematological neoplasms	>20 µg/l  >> 200 µg/l
Activated	Mature β-tryptase  Proforms α-tryptas β-tryptase	<b>Anaphylactic reaction</b> Severe or fatal systemic reactions	Transiently elevated from baseline

Fig 11. Total Tryptase levels in healthy subjects and different diseases.<sup>(70, 71,72)</sup>

## When to measure

Tryptase levels are transiently elevated in subjects with systemic anaphylaxis and since it diffuses slowly and slower than the histamine released the Tryptase level peaks between 15 and 120 minutes after the anaphylactic event with a degradation half-time of 2 hr<sup>(69)</sup>. Usually the elevated levels of Tryptase can be detected for up to 3 to 6 hours after the anaphylactic reaction and it returns to pre-event baseline normal level within 12-14 hours but in some severe cases more than 24 hours after the reaction<sup>(69)</sup>. If levels are still above normal range after 24 hours, another sample should be taken after 1-2 weeks to establish baseline levels. Sequential measure during anaphylaxis and comparison to individual baseline levels has also been suggested – see below.

## Tryptase testing in insect venom allergy

In patients with insect venom allergy, an elevated baseline serum Tryptase level is associated with an increased risk of severe anaphylactic reactions.<sup>(58)</sup> On average the prevalence of systemic anaphylactic sting reactions is around 3 % in the general population. Up to 25 % of patients with severe venom reactions have an elevated baseline Tryptase level. The importance of identifying these patients has been emphasized as they are predisposed for severe anaphylactic reactions. Increased levels may be, but need not necessarily be, due to an underlying mastocytosis<sup>(59,60)</sup>.

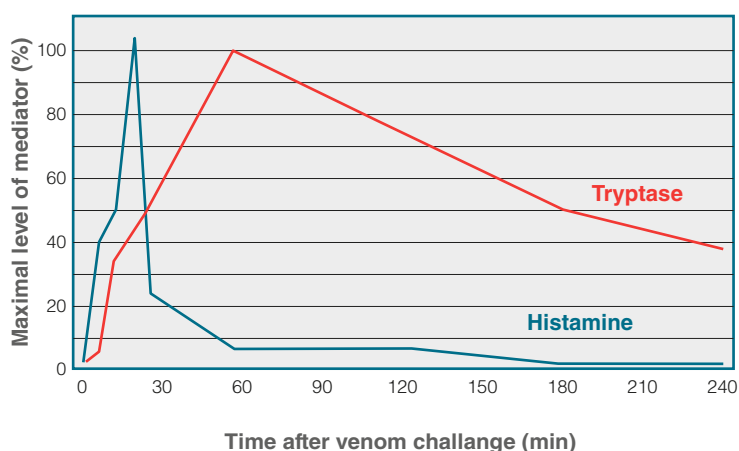


Fig 12. Serum level of Tryptase and Histamine up to 4 hours after a venom challenge<sup>(69)</sup>.

## Tryptase testing in allergy to drugs

Severe reactions during anaesthesia and surgery are rare. However, when they occur it is important to identify the mechanism and underlying cause to be able to initiate preventive actions. An allergy investigation should always be performed as a follow up together with baseline Tryptase measurements. Mast cell Tryptase is a useful investigation after a possible anaphylactic reaction during anaesthesia. Increased mast cell Tryptase levels are highly suggestive of an immune mediated reaction. Patients with increased mast cell Tryptase levels must be investigated for allergy, and patients without increased mast cell Tryptase levels should be investigated if the clinical picture suggests severe anaphylaxis.

Elevated baseline Tryptase levels indicate an increased mast cell burden and may serve as a risk factor for severe reactions during surgery. To confirm an anaphylactic reaction, the importance of measuring the transient increase of Tryptase during the perioperative phase is well established.

Measurements of transiently elevated Tryptase level immediately after the reaction can help to identify if the reaction was due to mast cell activation<sup>(48,61-63)</sup>. If positive, further allergy investigations e.g. specific IgE tests, skin testing or provocation tests should be performed to find the likely trigger of the reaction<sup>(46)</sup>.

## Tryptase testing in mastocytosis

Serum concentrations of Tryptase can be dramatically increased in mastocytosis<sup>(64)</sup>.

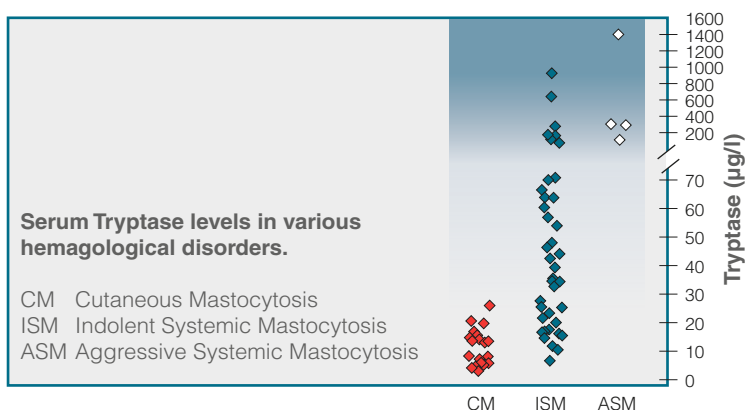
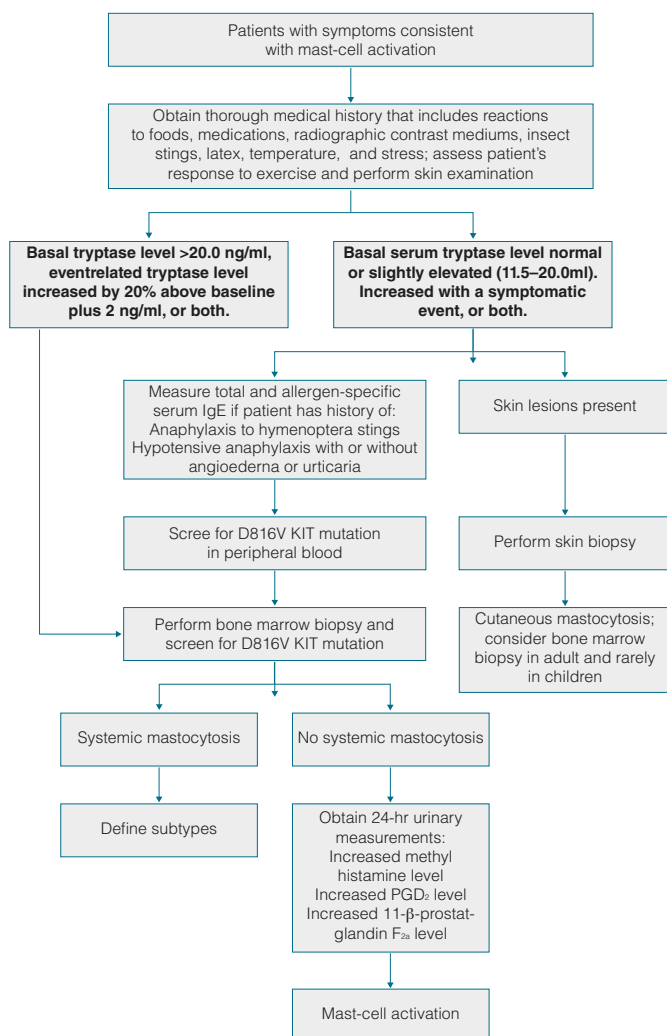


Fig 13. Relative levels of Tryptase in different forms for mastocytosis (adapted from (64) and EAACI training course)

If the Tryptase level is elevated during a symptomatic event, it should be measured again at least 24 hours after symptoms resolve. In the case of anaphylaxis, the serum beta fraction Tryptase level increases during the event and returns to the baseline level after the event. If the baseline Tryptase level is also elevated, the likelihood of systemic mastocytosis should be considered. In addition, measurement of total and allergen-specific serum IgE to rule out the presence of an allergic disease should be performed if a patient's history is suggestive of an allergic trigger (such as hymenoptera stings), regardless of the presence or absence of systemic mastocytosis<sup>(34)</sup>.

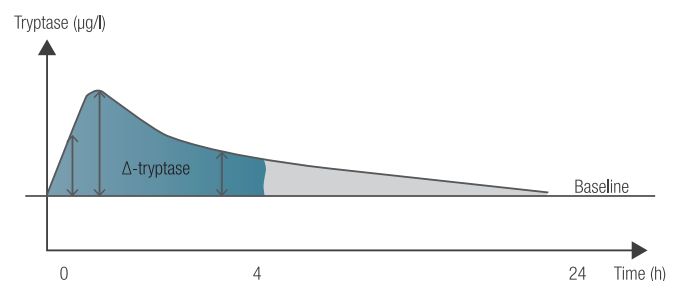
The following algorithm for evaluation and diagnosis of mastocytosis is recommended<sup>(34)</sup>



## Comparing to individual baseline Tryptase level

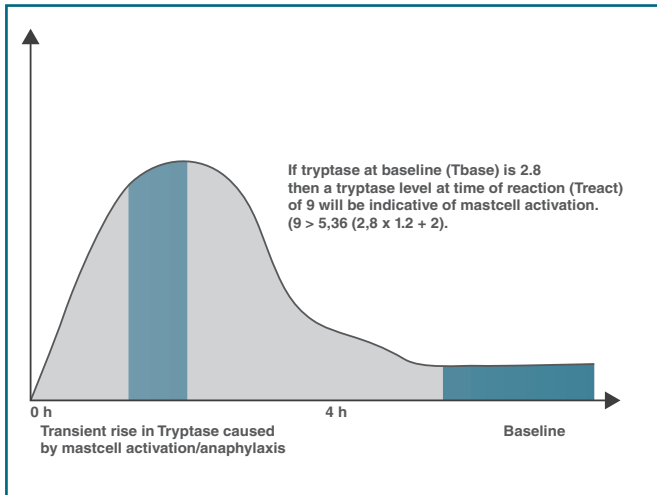
The impact of measuring the individual changes in the level of Tryptase during and after systemic allergic reactions or anaphylaxis is an effective way to confirm potential mast cell activation. The variation in baseline level in the normal population as well as in patients with risk for anaphylaxis is varying. It is demonstrated that peak Tryptase levels were significantly higher in patients with drug induced anaphylaxis versus food-induced anaphylaxis<sup>(22)</sup>. It was also shown that peak levels of Tryptase varied and depended on the severity grade of the anaphylactic reaction<sup>(22)</sup>.

In order to support the diagnosis “anaphylaxis”, the increase of acute levels over baseline levels provides robust evidence for mast cell activation, and is more important than a single absolute acute level. Elevated basal tryptase levels  $\geq 11.4$  ng/mL per se are not indicative of mast cell activation and a normal baseline serum tryptase level does not exclude anaphylaxis<sup>(25)</sup>. In primary MC disease (mastocytosis and MCAS), baseline tryptase levels are usually elevated<sup>(26)</sup>. In a consensus conference, after review of key data, the members proposed that the minimal increase of acute serum total tryptase level, to be indicative of mast cell activation, should be at least 20% plus 2 ng/mL over the baseline serum tryptase level<sup>(9)</sup>. For example, if a patient has a baseline serum tryptase level of 10 ng/mL, the acute peak tryptase level should be  $\geq 14$  ng/mL ( $10 + 0.2 \cdot 10 + 2$  ng/mL). Acute peak levels of tryptase versus a baseline serum tryptase level can also occur below 11.4 ng/mL.





## Things to consider with tryptase and transient rise in tryptase



- A transient increase in tryptase concentration shortly (within 15 minutes to 3 hours) after a severe reaction e.g. anaphylaxis, is a marker of mast cell activation.
- The tryptase level normally returns to baseline at approx. 24-48 hours after complete resolution of all clinical symptoms.
- The baseline tryptase level in each individual is normally very stable over time.

*Algorithm: Tryptase at time of reaction ( $T_{react}$ ) > baseline tryptase ( $T_{base}$ )  $\times$  1.2 + 2.*

Important: Blood should be drawn for tryptase level measurement between 1-3 after start of reaction. A baseline should be established minimum 24 hours after by a second bloodtest.

# Practical cases for the utility of serum Tryptase in clinical practice

## General points about practical use of Tryptase

- Can be analysed in both serum and plasma
- Is very stable over time
- Does not need special handling prior to sending to lab
- Reproducible values in the same patient
- Result not available in the acute setting

## Case 1 – Tryptase in the perioperative setting

A fifty-six-year old male patient was scheduled for laparoscopic surgery for a hernia. The anaesthetic was induced without complications. Fifteen minutes after a urinary catheter was placed and the surgeon had started, the patient developed an increase in pulse and decrease in blood pressure. The surgeon commented that the skin was very red and the anaesthetist noticed generalized flushing of the skin when lifting the drapes. Anaphylaxis was suspected and treated with adrenaline and fluids stabilizing the patient. Surgery was completed and serum Tryptase was measured 1½ hours after symptom onset.

The patient was referred for specialized perioperative allergy investigations and the serum Tryptase level 1½ hours after the reaction was 10.8 µg/l. Serum tryptase measured in a further blood sample 8 hours after the reaction was 3.1 µg/l.

Baseline serum Tryptase in a blood sample measured 5 weeks after the anaphylactic reaction was 2.6 µg/l. Using the absolute upper limit of normal of 11.4 µg/l the Tryptase level at the time of reaction was not elevated. However, even a small increase may be important. Therefore, an algorithm has been introduced to identify minor relevant increases in serum Tryptase. This is based on a comparison with the patient's own baseline serum Tryptase level.

*Algorithm: Tryptase at time of reaction*  
*> baseline Tryptase x 1.2 + 2*

When comparing the Tryptase level measured 1½ hours after the reaction with the baseline using this algorithm a significant and clinically important increase was confirmed:

*Inserting 1½ hours values from case:*  
 $10.8 > 2.6 \times 1.2 + 2 = 5.12$

This discrete elevation is still suggestive of an IgE mediated mast cell degranulation.

## The follow up allergy investigations diagnosed an IgE mediated allergy to chlorhexidine and the patient was warned against future exposure.

If the Tryptase level measured 8 hours after the anaphylactic reaction is compared with the baseline level there is no significant increase.

*Inserting 8 hour values:*  
 $3.1 < 2.6 \times 1.2 = 5.12$

This case also illustrates the importance of sampling blood for serum Tryptase measurement as early as possible following a suspected allergic reaction.

## Learning points

Serum Tryptase is useful for supporting the diagnosis of IgE mediated allergic reactions in the perioperative setting. Elevations within the normal range may be clinically relevant and serum Tryptase measured at the time of a suspected allergic reaction should always be compared to the patient's own baseline value. The baseline value is usually very reproducible in the individual patient. The recommended algorithm for estimation of elevation in serum Tryptase is:

## Tryptase at time of reaction > baseline Tryptase x 1.2 + 2<sup>(41)</sup>

The optimal time for serum Tryptase measurement is in the interval 15 minutes – 3 hours after a reaction and samples taken later may no longer show an elevation. Normal serum Tryptase does not exclude an IgE mediated allergic reaction if the clinical symptoms are convincing. In such cases allergy investigations are recommended.

## Case 2 – Tryptase in the emergency room

A thirty-six year old female patient felt faint and developed generalized itching, urticarial rash and wheezing 20 minutes after a steroid injection. The attending doctor suspected anaphylaxis and treated her with intramuscular adrenaline. The patient was transferred to the emergency department for further observation.

Serum Tryptase measured one hour after onset of symptom was 35.7 µg/l.

The patient was referred for allergy investigations and a baseline serum Tryptase measured 4 weeks later was 4.5 µg/l.

A significant increase in serum Tryptase during the anaphylactic reaction was suggestive of an IgE mediated mast cell degranulation.

**The follow up allergy investigations diagnosed a rare IgE mediated allergy towards carbonylmethylcellulose, an excipient in the steroid injection, which is also present in tablets, cosmetics and household products.**

Following her anaphylactic reaction the patient was anxious and constantly worrying about being re-exposed to the excipient, potentially causing another life-threatening allergic reaction. One morning about 20 minutes after intake of her usual daily medications she felt faint, had palpitations and felt shortness of breath. She called an ambulance and was brought to the emergency department. Here it was noted that she was anxious, but with stable vital signs. She had no rash or wheezing during this reaction. Her symptoms subsided after calm reassurance that she was not having an allergic reaction. The doctors informed her that she most likely experienced an anxiety attack with hyperventilation. Serum Tryptase measured one hour after symptom onset was 4.3 µg/l, ie not elevated from her baseline level of 4,5 µg/l. This helped confirm that there was no significant mast cell degranulation and therefore an allergic mechanism was unlikely.

### Learning points:

Serum Tryptase is useful for confirming IgE mediated mast cell degranulation. It can also be used to show a lack of significant elevation in serum Tryptase in cases where an allergic mechanism is less

likely. It is however important to note that a normal serum Tryptase does not itself rule out an IgE mediated allergic reaction. If the clinical symptoms are convincing, allergy investigations are always recommended.

## Case 3 – Tryptase as a diagnostic marker when the diagnosis is uncertain

A sixty-five year old male patient had an uneventful anaesthetic and operation for a fracture of his left hand. While being transferred to the recovery room – 15 minutes after completed surgery – he developed an acute severe drop in blood pressure and a cardiac arrest. There were no respiratory or skin symptoms. He was resuscitated successfully within a few minutes, and on suspicion of an acute coronary event he was transferred to the cardiology department for further investigations. An allergic reaction was thought to be less likely due to a lack of skin and respiratory symptoms and since no drug was administered immediately prior to the reaction. However, since an allergic mechanism could not be completely excluded, the anaesthetist took a blood sample for serum Tryptase measurement one hour after the reaction. This turned out to be highly elevated at 69.4 µg/l. The patient was referred for allergy investigations and his baseline serum Tryptase was within the normal range at 5.8 µg/l. The significant increase in serum Tryptase during the reaction, was suggestive of IgE mediated mast cell degranulation.

**The follow up allergy investigations proved allergy to a bone cement inserted in his hand 30 minutes before the reaction.**

### Learning points

Serum Tryptase can be a useful diagnostic marker even when the diagnosis of anaphylaxis is uncertain.

## Case 4 – Tryptase in Insect venom anaphylaxis

A sixty-eight year old male felt a sudden sting on his right arm while cutting the hedge. He noticed a wasp and brushed it away. After a few minutes, he felt itching spreading throughout the body and he felt dizzy and had to sit down. He called his wife who found him lying half-conscious on the grass. She called an ambulance which arrived within a few

minutes. By the time the ambulance arrived his face and hands were swollen and he had an urticarial rash all over. The paramedic immediately administered intramuscular adrenaline. After two doses the patient woke up and was transferred to the emergency department for further treatment. In hospital, his blood pressure was low and he was treated with standard anaphylaxis treatment: adrenaline, fluids, antihistamines and steroids. Serum Tryptase was measured two hours after symptom onset. The patient was further stabilized over the next few hours and discharged the following day with an adrenaline auto injector.

Serum Tryptase measured two hours after the reaction was elevated at 78,3 µg/l.

**Follow up allergy investigations confirmed allergy to wasp venom with positive skin testing and specific IgE for wasp venom.**

The baseline serum Tryptase was slightly elevated at 14,6 µg/l. Since an elevated baseline serum Tryptase is considered a risk factor for severe reactions in insect venom allergy he was started on immunotherapy with wasp venom and was advised to always carry an adrenaline auto injector.

#### **Learning points**

In insect venom allergy, a measure of baseline serum Tryptase can be used in predicting the risk of future severe reactions. As serum Tryptase peaks 1-2 hours after a reaction, it should always be taken after the patient is stabilized.

#### **Case 5 – Tryptase in mastocytosis**

A Forty-three year old male patient underwent surgery to remove the gallbladder. He suddenly devel-

oped an acute decrease in blood pressure, increase in pulse and generalized flushing. Anaphylaxis was suspected and treated, and the surgery was completed. A blood sample for serum Tryptase was measured 45 minutes after symptom onset.

The patient was referred for specialized perioperative allergy investigations and tested with all drugs and substances that he was exposed to during surgery, but no causal relation was identified. Serum Tryptase measured 45 minutes after the reaction was elevated at 46.8 µg/l. His baseline serum Tryptase was 11.2 µg/l just below the upper limit of normal. As no allergy-related cause could be identified it was necessary to investigate for potential mastocytosis, due to the slightly elevated baseline Tryptase.

**A blood sample was sent for analysis for KIT 816 mutation, which was positive confirming that the patient suffered from underlying mastocytosis.**

#### **Learning points**

Patients with mastocytosis can develop anaphylaxis without a specific allergen being involved. In this case, where allergy testing was negative, a slightly elevated baseline serum tryptase was suspected to be an indication of mastocytosis. The patient was further investigated and mastocytosis confirmed. Mastocytosis may be present even with a baseline serum tryptase within the normal upper limit of 11.4 µg/l.

# Final remarks

The intention of this white book is, binding together the opportunity of measuring the mast cell release of variants of Tryptase – with a very specific and highly sensitive assay – with the fact that serum level of Tryptase is correlated to different diseases and physiological conditions caused by an unintended release of mediators from human mast cells. Tryptase is a major component of the substantial number of mediators and enzymes that are released from the mast cells during systemic allergic reactions and anaphylaxis, but diseases re-

lated to unintended mast cell activity or abnormal control of the mast cell population (mastocytosis) are of complex nature. Even then, mast cells are responsible for specific severe systemic pathophysiological conditions, which in some cases can lead into a life-threatening situation as a consequence of severe anaphylactic reactions. The recommendation is therefore to implement and routinely use testing for baseline and acute levels of Tryptase for the diagnosis and potential prediction of severe life-threatening disease.

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