



Tryptase values in anaphylaxis and insect allergy

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Purpose of review

To recognize the relevance of serum tryptase measurement as a useful tool for the diagnosis of allergic diseases and mast cell disorders.

Recent findings

Recent data on the role of mast cells and tryptase in allergic and other diseases provide new understanding into the mechanisms and causes of anaphylaxis.

Summary

Measurement of transiently elevated tryptase levels shortly after a severe reaction can help elucidate mechanism behind the reaction in identifying mast cell activation. Hymenoptera venom allergy represents an important cause of morbidity and mortality worldwide. Venom allergy is a typical IgE-mediated reaction because of sensitization to one or more allergens of the venom, and accounts for 1.5–34% of all cases of anaphylaxis. There is a preferential association between insect venom allergy and mastocytosis. The diagnosis of a clonal mast cell disease leads to therapeutic consequences concerning the treatment of venom allergy. In conclusion, baseline tryptase levels support the clinical diagnosis of anaphylaxis and mast cell disorders, determine venom immunotherapy treatment and are relevant in deciding on lifelong treatment.

Keywords

anaphylaxis, clonal mast cell disorders, insect venom allergy, mast cell, mastocytosis, tryptase

INTRODUCTION

Serum tryptase represents a useful biomarker of mast cell activation. Transient elevation is observed during anaphylactic reactions to allergic stimuli, thus its measurement provides unique proof of mast cell degranulation and confirms clinical diagnosis. Recent data highlight different applications of serum tryptase detection showing possible different roles underlying allergic reactions.

Moreover, elevation of baseline serum tryptase is present in individuals affected by mast cell diseases and is one of the laboratory criteria for the diagnosis.

TRYPTASE

Tryptase is the predominant serine protease expressed in human mast cells. It is produced almost exclusively by mast cells, and to a lesser extent by basophils, thus it is considered the marker of mast cell/basophil activation. It is stored in mast cell secretory granules as a heparin-stabilized active tetramer and has a trypsin-like activity. There are four tryptase isoforms but only α and β are considered biologically important. Only β -tryptases appear to be activated intracellularly and stored in granules. After mast cell activation, β -tryptases are secreted

bound to heparin that stabilize the active tetramer and provides the highest biological activity [1^a].

Human mast cells play a central role in allergic, inflammatory and autoimmune processes. Mature mast cells usually express KIT and FC ϵ RI. Mast cell activation leads to the release of substances stored in granules (histamines, chymotrypsin-related serine proteases, such as tryptase, chymase, carboxypeptidase), other products from phospholipid membrane metabolism (platelet-activating factor, leukotrienes, prostaglandins) and synthesized de novo cytokines and chemokines [2].

Mature human mast cells are classified into subpopulations. MC_{TCs} predominate in nonmucosal sites (connective tissue and skin) and express

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KEY POINTS

- Transient elevation of serum tryptase levels is useful in defining anaphylactic reactions to allergic stimuli whereas constant elevation of basal tryptase correlates with mast cell disorders.
- Hymenoptera venom allergy represents the most common trigger of anaphylaxis in individuals with mastocytosis.
- Anaphylaxis manifestations in hymenoptera venom allergy and clonal mast cell disease is characterized by absence of angioedema and erythema and the predominance of cardiovascular symptoms.
- The characteristics of severe hymenoptera venom allergy episodes, with hypotension in the absence of urticaria and/or angioedema, may represent the most relevant factor to identify those patients with hymenoptera venom allergy and clonal mast cell disease, regardless of the baseline levels of tryptase.
- In case of systemic reaction after hymenoptera sting in patients with clonal mast cell disease, it is mandatory to perform in-vivo and in-vitro tests to identify the culprit insect venom and then to start a correct immunotherapy, which must be continued long-life.

tryptase, chymase, carboxypeptidase and chatepsine; MC_{Ts} predominate in mucosal sites (nose, lung and gut mucosa) and express tryptase but no other serine proteases; MC_{Cs} are rare mast cells expressing only chymase. The cytokine environment can modulate the conversion between the subpopulations [1[•],3].

Plasticity of mast cell genes is witnessed by the diversification of mast cell function including innate host defence function against helminths and snake envenomation and regulation of mucosal and skin functions. They act as active connections, interlinking innate and adaptive immunity [3,4].

A low quantity of immature monomeric form of tryptase is constitutively secreted by resting mast cells, whereas the active tetrameric form of mature β -tryptase is only released by activated mast cells [2].

Mast cell tryptase is involved in the pathogenesis of various conditions including severe anaphylaxis with hypotension, cardiovascular, respiratory, digestive, reproductive, and nervous system diseases and others [1[•]].

Elevated serum tryptase levels (STLs) can be detected also in other conditions, such as mast cell disorders that will be elucidated below, haematologic malignancies of myeloid origin, or in nonneoplastic conditions, such as end-stage chronic renal failure, onchocercosis and chronic urticaria [5]. Familial tryptasemia is a recently described disease in which members of the same family, without mastocytosis,

present elevated baseline STLs because of a greater number of the β -tryptase gene *TPSAB1* [3].

MEANING AND RELEVANCE OF SERUM TRYPTASE MEASUREMENT DURING ALLERGIC REACTION

European Academy of Allergy and Clinical Immunology has recently proposed a simplification of the diagnostic criteria of acute allergic reactions, dividing them into local (grade 1) and systemic (grades 2 and 3) [6].

Different anaphylaxis phenotypes have been described: type I reactions; cytokine-release reactions; mixed reactions; bradykinin-like and complement-like reactions. The endotypes underlying these phenotypes correspond to the following processes, respectively: IgE-mediated and non-IgE-mediated mechanism; cytokine-mediated mechanisms; mixed mechanisms; and direct activation by complement or bradykinin [7^{••}]. Biomarkers are important in allowing endotyping of patients [8].

Mast cell acute activation occurs in patients with allergic reactions, with consecutive massive release of vasoactive and proinflammatory substances from mast cells. Degranulation of mast cells develops in the first seconds after mast cell activation with binding of IgE to the high-affinity receptor FC ϵ RI [1[•]]. The ideal biomarker for mast cells and basophil activation has not been identified yet. Such biomarker should satisfy several criteria: to be specific for mast cells/basophils; detectable in blood or biological fluids; it should be a true indicator of mast cells/basophil activation *in vivo*; it also should be stable and not influenced by metabolism or by contamination from other sources; and finally, it should be easily and reproducibly measured and have acceptable costs [2,8].

Measurement of STLs supports the diagnostic workup of systemic anaphylaxis, although diagnosis based on clinical signs and symptoms remains essential [9]. During anaphylaxis, serum tryptase peaks 60–90 min after the onset of the reaction and, in general, starts to decrease after 120 min and return to normal levels within 24–48 h. Therefore, for the diagnosis of anaphylaxis, blood samples should be collected within 1–2 h of the reaction and after 24 h in order to detect this decrease. An increase of over 11.4 ng/ml is indicative of acute mast cell/basophil activation [10].

The allergic triggers correspond to different tryptase rises. High peak levels are observed in anaesthesia-related immediate reactions, intravenous drugs and venoms [11^{••}]. Moreover, tryptase increase correlates with an increasing reaction severity: Dua *et al.* [11^{••}] reported tryptase rise of 148% above

the baseline in anaphylaxis group vs. 24% in nonanaphylaxis group.

However, sensitivity is not absolute and normal STLs do not exclude anaphylaxis [12]. Sensitivity of absolute STL is low, remaining in the normal range in up to 40% of anaphylaxes. This condition is probably affected by the fact that 27% of population lack α -tryptase genes, thereby limiting peak plasma levels [8].

Conflicting data on usefulness of STLs during anaphylaxis depend probably on the fact that previous studies only used the cut-off of 11.4 ng/ml to identify a peak. The sensitivity of the test is enhanced by valuing the relative increase of acute tryptase compared with baseline levels. In fact, the relative increase from baseline regardless of the absolute peak value in an individual patient has shown higher sensitivity. There is no consensus on the minimal increase in serum tryptase suggestive of a mast cell activation-related reaction. Several studies agree in using the equation where transient elevation of tryptase is confirmed if the increase in tryptase is at least 20% above baseline plus 2 ng/ml within 4 h of the allergic reaction [2,8]. This algorithm is a valid tool for perioperative hypersensitivity reactions with sensitivity, specificity, positive predictive value and negative predictive values of 75, 86, 94 and 53%, respectively [13].

Finally, in forensic medicine, postmortem STL is considered a promising diagnostic indicator of death because of anaphylactic shock, especially when higher than 30.4 ng/ml [14].

TRYPTASE AS MARKER OF MASTOCYTOSIS

Among the mediators, tryptase is actually considered the most specific parameter of mast cell activation (MCA) [15–17]. Its specificity derives from the preeminent production by mast cells compared with other cells [18]. In fact, low amounts of tryptase are produced by immature leukemic basophils and myeloblasts [19,20]. Accordingly, STLs can be raised both in systemic mastocytosis, in FIP1L1/PDGFR α + chronic eosinophilic leukemia and in nonmast cell-lineage myeloid neoplasms [20,21[¶]]. Conversely, tryptase is not able to distinguish normal and neoplastic mast cells, so reactive conditions (such as allergic diseases, chronic urticaria, chronic inflammatory diseases, chronic helminth infections) or other conditions (in particular, hereditary α -tryptasemia) might be the cause of elevated STLs [21[¶]].

A persistently high STL (>20 ng/ml) is one of the minor criteria for the diagnosis of systemic mastocytosis according the WHO [22]. However, it can have wide variations across all WHO subgroups:

marked STLs (>200 ng/ml) are often shown by advanced systemic mastocytosis, such as smoldering systemic mastocytosis (SSM, of which is a B-symptom criterion), aggressive systemic mastocytosis (ASM) and systemic mastocytosis with associated haematological neoplasm (systemic mastocytosis-AHN) [23], whereas bone marrow mastocytosis (BMM), a sub-variant of systemic mastocytosis with a lower burden of clonal mast cells, could have STLs under that diagnostic cut-off values [24].

Elevated STLs can be associated with clinical symptoms of MCA, often as anaphylaxis, but only in some cases they are an expression of the so-called MCA syndrome (MCAS). To diagnose this nosological entity, specific criteria are required, including STLs. An increase in STLs of more than 20% + 2 ng/ml from the individual's baseline levels is considered a requirement for the diagnosis of MCAS [21[¶]]. That increase, to be considered related to the clinical event, should be measured 1–4 h after the event. Instead, baseline STLs should be evaluated at least 24–48 h apart from any sign or symptom [15–17].

TRYPTASE AND HYMENOPTERA VENOM ALLERGY

Hymenoptera venom allergy (HVA) is responsible for 7–59% of anaphylaxis, being the first cause of allergic anaphylaxis [25]. HVA is a typical IgE-mediated disease, whose clinical manifestations are the result of mast cell degranulation, which is triggered by the binding of the venom allergens to specific IgE. Severity can vary from large local reactions (LLR) to systemic anaphylaxis. Prevalence rates of systemic reaction to hymenoptera sting are estimated to reach up to 7.5% in the adult European population, and up to 3.3% in the United States [26,27].

Tryptase measurements may be used as a reliable method for stratifying risk among HVA patients. Elevated baseline STL was present in about 5% of patients with HVA and mild–moderate sting reactions, and in 25% of those who had hypotensive shock [28[¶]]. Farioli *et al.* classify patients into a low risk of severe reaction when tryptase values are less than 4 ng/ml and those defined as an intermediate risk who have tryptase values ranging from at least 4–7.5 ng/ml, and finally, those with a high risk as having tryptase values greater than 7.5 ng/ml [29].

Up to 7% of adult patients with HVA are affected by clonal mast cell disease (CMD). Moreover, up to 75% of patients with severe HVA are at risk of further severe reactions if re-stung. The literature confirms that there is a preferential association between HVA and mastocytosis [30] for several reasons:

- (1) The prevalence of HVA in systemic mastocytosis patients (20–30%) is higher than in the general population (0.3–8.9% in the European adult population) [5,31].
- (2) Hymenoptera venom sting represents the most common trigger of anaphylaxis in adult mastocytosis patients (22–60% of cases) [32,33]; on the contrary, in children with mastocytosis, hymenoptera stings play no role in eliciting anaphylaxis [32].
- (3) The association between HVA and mastocytosis is also confirmed by the higher prevalence of CMD in patients with systemic HVA (1–7.9%) than in the general population (1–1.3 cases per 10 000) [34,35].

CLINICAL FEATURES OF PATIENTS WITH HYMENOPTERA VENOM ALLERGY AND CLONAL MAST CELL DISORDERS

Allergic/anaphylactic symptoms after hymenoptera sting are mostly present in patients with an indolent variant of systemic mastocytosis without skin lesions where they represented the initial clinical manifestation and the reason for bone marrow biopsy in the majority of cases [30,33].

The anaphylactic reactions of patients with CMD and HVA are characterized in most cases by the absence of angioedema and erythema and the predominance of cardiovascular symptoms, such as hypotension leading to loss of consciousness [24,36].

CMDs associated with HVA are represented, apart from systemic mastocytosis, by monoclonal mast cell activation syndromes (MMAS), which are characterized by the absence of skin lesions and the demonstration of bone marrow mast cell clonality by detection of KIT D816V mutation and/or abnormal expression of Cd25 and/or CD2 on mast cell, but lack sufficient criteria for systemic mastocytosis [28^{''}].

Increased serum baseline tryptase appears to be a useful criterion for selecting patients with HVA eligible for bone marrow evaluation when systemic mastocytosis is suspected [16,27,28^{''}]. Nevertheless, a CMD cannot be excluded in individuals with systemic severe HVA but with normal STL [24].

The characteristics of patients with HVA and indolent systemic mastocytosis without skin involvement [ISMs(-)] are the prevalence of the male sex, a significantly lower mast cell burden, lower STLs and lower frequency of dense compact mast cell aggregates in bone marrow sections than in ISM skin positive [ISMs(+)] patients. They also frequently show coexisting populations of phenotypically normal and aberrant mast cell in bone marrow

and a lower frequency of multilineage KIT mutation [37,38].

The majority of patients do not report mast cell activation symptoms between acute episodes; therefore, most of these patients may have HVA severe reactions as the unique clinical manifestations of mastocytosis [37,39].

Progression to aggressive mastocytosis has not been yet reported in systemic mastocytosis patients with HVA [40] and, on the contrary, HVA seems to be very rare in patients with aggressive subtypes of systemic mastocytosis, who harbour the highest mast cell load [41].

One important explanation of some non Ig-E-mediated reactions is the presence of clonal mast cell disorder [42^{''}]. In a study of 379 patients with a history of sting anaphylaxis, skin test results were positive in 100% of those with normal tryptase levels, but negative in 10% of those with elevated tryptase values [28^{''}].

IMPLICATIONS OF ELEVATION OF TRYPTASE LEVEL ON MANAGEMENT OF HYMENOPTERA VENOM ALLERGY

Skin tests with venoms are generally well tolerated, even in patients with mastocytosis [43,44] even when tests are carried out simultaneously at different concentrations, but a preliminary step has been recommended. Only after reading the reactions to this first set, should a higher concentration be used. This caution is to be especially maintained in patients with a history of severe anaphylactic reaction or suffering from mast cells disorders [45^{''}].

Venom immuno-therapy (VIT) represents a well tolerated and effective treatment, which decreases the risk of subsequent systemic reactions and reduces morbidity and mortality. VIT in the general population is reported to be effective in 77–84% of patients treated with honeybee venom and in 91–96% of patients receiving vespid venom [26].

After some debate, which was mainly because of safety concerns, it is now generally accepted that VIT is clinically justified in those patients with severe HVA and documented mastocytosis [30], even though elevated baseline STL is also associated with more frequent and more severe reactions to VIT injections and a higher risk of treatment failure (systemic reaction to a sting during VIT) [42^{''}].

On the basis of the data of literature available up to now, VIT conferred full protection in the majority (86%) of re-stung mastocytosis patients, although this percentage is slightly smaller than that reported in patients without systemic mastocytosis [46]. These patients have a higher chance of relapse if they stop VIT including fatal reaction to stings [42^{''}].

According to the published case series, conventional, cluster and rush protocols are well tolerated and effective in patients with systemic mastocytosis associated with anaphylaxis to hymenoptera venom-induced anaphylaxis [44,47,48].

Moreover, the probability of having mastocytosis is quite high when VIT protection is lost after treatment. This would suggest that patients with HVA-induced anaphylaxis who lose protection after a proper course of VIT should be investigated for mastocytosis. When a diagnosis of mastocytosis is established, these patients should continue life-long VIT [49[■]]; therefore, from a practical point of view, regardless of the STL, it has been suggested that an accurate haematological work-up be performed before stopping immunotherapy in those patients with very severe reactions with hypotension and without urticaria and angioedema in order to exclude CMD.

Patients with mastocytosis who have experienced severe systemic reactions should carry two or more epinephrine self-injectors. This is also advised for all mastocytosis patients treated with VIT, even if they had reached the maintenance dose [50].

TRYPTASE VALUE AND NONHYMENOPTERA INSECT ALLERGY

Allergic IgE-mediated reactions to mosquito bites are not uncommon and may rarely cause systemic reactions in patients affected by mastocytosis [51].

Information about allergies to insect bites from different species is extremely limited with consequent lack of diagnostic tools and immunotherapy [52[■]] and the field of insects as novel dietary ingredients has been scarcely investigated [53].

CONCLUSION

Patients with CMD and HVA represent a population requiring specific management. The diagnosis of a CMD leads to therapeutic consequences concerning the treatment of HVA: in particular, these patients have to undergo long-life VIT, in order to prevent further, potentially fatal severe reactions [30].

It is recommended that STL should be measured in patients with severe sting anaphylaxis, those with hypotensive reactions, those with no urticaria, those with negative venom-IgE test results, as well as patients who have repeated systemic reactions during VIT or reactions to stings during VIT. It is also recommended to check the STL before discontinuing VIT because an abnormal result would be a contraindication to stopping treatment [27,42[■]].

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