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The Tryptase Test
Clinical Use in Dermatology and Allergy

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List of Abbreviations

ASM	Aggressive Systemic Mastocytosis
BST	Baseline serum tryptase
ISM	Indolent Systemic Mastocytosis
MC	Mast Cell
MCA	Mast Cell Activation
MCAS	Mast Cell Activation Syndrome
MMCAS	Monoclonal Mast Cell Activation Syndrome
MPCM /UP	Maculopapular cutaneous mastocytosis / urticaria pigmentosa
SM	Systemic Mastocytosis
SM-AHN	SM with associated hematologic neoplasm
SSM	Smoldering systemic mastocytosis
WHO	World Health Organization

Introduction

α/β-Tryptases are trypsin-like serine proteases, abundantly and preferentially produced by, stored in and secreted from all human mast cells (MCs) [1]. Mature tryptase tetramers are stored in secretory granules, accounting for 10–20% of all the protein in this cell type [2]. Basophils also produce, store and secrete tryptase, but contain about 500-fold, on average, lower amounts than mast cells and typically do not contribute a substantial portion of the cell-free tryptase found in blood [3, 4]. However, in neoplastic hematologic conditions, immature basophils can produce and release larger quantities of tryptase, especially in CML with basophilia [5].

Mature β-tryptase tetramers show a wide range of biological activities, at least in vitro, including degradation of fibrinogen, generation of kinins from kininogens, promoting vascular permeability, degradation of the extracellular matrix, facilitating tissue remodeling and affecting cellular migration [6]. Tryptase thereby enhances leukocyte recruitment and angioedema, with chemotactic effects on neutrophils and eosinophils, thereby contributing to the inflammatory late phase of allergy-triggered events. Tryptase also leads to monocyte and macrophage activation and stimulates cell proliferation and growth, processing of (pro)hormones, activation of fibrinolytic enzymes, and degradation of plasma and matrix molecules [7]. Tryptase is a potent mitogen for diverse mesenchymal cells including fibroblasts and endothelial cells. It degrades fibrinogen and activates pro-urokinase. It cleaves vasoactive peptides and hormones, such as vasoactive intestinal peptide (VIP) and pre-atrial natriuretic factor (ANF) into its active form. In the MC granules, the enzymatically active (tetrameric) beta-form of the enzyme is kept stable by heparin.

α/β-Tryptases are derived from two genes located on human chromosome 16, TPSB2, encoding only β-tryptases, and TPSAB1, encoding either α or β tryptases, allowing tryptase α:β genotypes of 0:4, 1:3 and 2:2. These tryptases are expressed first as pretryptases that are rapidly converted to protryptases and then to the tetrameric mature tryptases that are stored in secretory granules, waiting to be released when mast cells are activated to degranulate, and serving as a serum biomarker of systemic anaphylaxis [8]. Conversion of pro to mature tryptase requires cathepsins B or L, or autocatalysis and cathepsin C (dipeptidyl peptidase 1), and heparin in an acidic pH microenvironment. Portions of α and β protryptases are not processed to their mature forms, but instead are constitutively secreted by mast cells at rest, representing the form of tryptase present at baseline in serum or plasma, primarily reflecting genetic factors based on twin studies, but also reflecting the total bodyburden of mast cells. As a biomarker for the mast cell burden, baseline protryptase levels facilitate the diagnosis of systemic mastocytosis. Two additional so-called tryptases, δ and γ, should be mentioned – δ-tryptase, likely a pseudogene as it is missing 40 amino acids from the C-terminus due to a premature stop codon, leading to a defective substrate binding pocket; and γ-tryptase, a transmembrane protease whose expression by different cell types, primary amino acid sequence, biochemical character and immunoreactivity differ substantially from the α/β-tryptases [9].

Different assays measuring tryptase have been developed during the last three decades [10]. Whereas the first assay measured only mature tryptase, which is normally undetectable at baseline (<1 ng/mL), the current commercial tryptase assay, offered from ThermoFisher on the Phadia platform, measures both pro and mature forms of α/β-tryptases in body fluids [11]. The assay is certified for serum and plasma. This tryptase assay is simple, reliable and highly reproducible. There are no pre-analytical caveats and the serum samples can be shipped frozen or on ice or stored frozen before measurements [12]. Prior reports of false positives, particularly for patients exposed to chimeric antibodies such as infliximab, presumably due to heterotypic human anti-mouse antibody, have been largely abrogated by including an agent in the commercial assay that suppresses heterotypic antibody activity [13, 14].

Under nonanaphylactic conditions, tryptase levels reflect the total body MC burden, which is used to diagnose and monitor mast cell diseases, such as mastocytosis [15]. In severe systemic reactions, mature β-tryptase is released within minutes and tryptase levels increase above baseline, peaking 30–90 min after onset of the reaction [8,16]. After peaking, the net increase of tryptase declines with a half-life of about 2 hours, returning to baseline by 8 to 10 hours. Therefore, comparing the basal serum tryptase level measured in the symptom-free interval with that measured during a severe reaction-event can confirm the presence of a mast cell activation syndrome [17].

In dermatology, high tryptase levels in skin blister fluid implicate mast cell activation in bullous pemphigoid, mastocytosis or urticaria [18, 19]. In allergy, high tryptase levels has been used to diagnose or confirm conditions with mast cell activation, e.g., systemic anaphylaxis using serum or plasma, or local IgE-mediated allergic reactions, e.g., allergic conjunctivitis using tear fluid, allergic rhinitis using nasal fluid or allergic asthma using airway fluid [17, 20].

Tryptase Levels in Healthy Individuals

Under nonanaphylactic conditions in healthy adult subjects, median serum tryptase levels range about 3.5–5 ng/mL [11, 21–23]. According to Phadia AB, the manufacturer of the commercially available assay, the median serum baseline tryptase is 3.8 ng/mL, with 95% of 126 apparently healthy adult donors having values <11.4 ng/mL (http://www.phadia.com/en-GB/5/Products/ImmunoCAP-Assays/ImmunoCAP-Tryptase/). In more than 99% of healthy controls serum tryptase levels were ≤15 ng/mL [21, 22]. In a given healthy individual without anaphylaxis, tryptase values are remarkably stable over time. Between individuals, only slight variations (≤0.5 ng/mL) occur according to the haplotype of the tryptase genotype, gender or body mass index [21, 23]. Age appears to have a slight, but significant influence on basal serum tryptase levels [21, 22, 24]. In infants during the first three months of life, higher tryptase values have been reported with a median of 6.1 ng/mL [25]. In children aged six months to 18 years, an inverse correlation with age has been described with a median of around 3.5 ng/mL [26, 27]. In contrast, in adults, baseline tryptase concentrations increase with age. In one study, a median of 4.4 ng/mL in donors aged 18–30 years compared to 6.6 ng/mL in those older than 80 years [21]. Genetic factors based on twin studies account for 87% of the variance of baseline total tryptase levels [28]. Heterophilic antibody-interference in the assay has resulted in falsely increased tryptase values for a few patient with the original assay [14]. However, an update of the tryptase assay has almost completely eliminated this interference [29].

Are there Internal Diseases Which Influence Tryptase Levels?

Inflammatory diseases including viral or bacterial infections generally have no influence on serum baseline tryptase levels, which remain remarkably stable [30]. Individuals with chronic active helminth infection, dengue shock or hemorrhagic fever syndrome or renal failure may have increased serum tryptase concentrations [30][31–33]. Having homozygous α-tryptase alleles in TPSAB1 is a significant risk factor for these more aggressive forms of Dengue [34]. Additionally, a familial autosomal dominant condition called alpha-tryptasemia, due to α-tryptase copy number variations, has been reported in which affected individuals exhibit vibratory urticaria along with gastrointestinal, musculoskeletal, autonomic dysfunction and atopic conditions. Another disorder of vibratory urticaria, again autosomal dominant, occurs with a gain of function mutation in adhesion G protein coupled receptor E2, preventing this adhesion molecule to bind to and inhibit the β-chain of this G protein coupled receptor. Autosomal dominant cold urticaria can occur due to a gain of function mutation in phospholipase Cγ2 associated with low levels of IgA and IgM, switch-memory B cells and circulating NK cells [35], while cold-induced autosomal familial cold autoinflammatory syndrome due to a gain of function mutation in Nlrp3 presents with cold-induced pruritic/burning rash due in part to activation of mast cells, along with IL-1-dependent fever [36]. Elevated tryptase levels also are found in patients with non-hematologic malignancies [37, 38]. Among patients with hematologic malignancies, elevated levels of serum tryptase >15 ng/mL 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 [30].

Tryptase Testing in Dermatological Diseases

The initial development of the tryptase assay focused mainly on acute allergic reactions and mastocytosis [10, 39]. In addition to this more restricted use, tryptase is also used to detect mast cell activation or an increased mast cell load in dermatological, allergological and hematological disorders. In dermatology, mast cells are reportedly involved in several diseases, which make these a possible target for tryptase determination.

More than 1000 patients in a general dermatology clinic were retrospectively analyzed for their baseline serum tryptase value [29]. In this population, where patients with mastocytosis, but not other potential reasons for increased tryptase values, such as hymenoptera venom allergy or hematological malignancies were excluded, borderline elevated levels ≥11.4 ng/mL were found in 4.1% of patients. Only two non-mastocytosis patients displayed values above 20 ng/mL (25.0 and 27.2 ng/mL). Thus, slightly elevated tryptase values are not uncommon in a dermatology clinic also caring for allergic patients, particularly if the patients also suffer from hymenoptera venom allergy. Another study analyzed 3374 patients and found 96 patients (3%) with BST >15 ng/mL [40]. Of those, 16 patients were having acute reactions, such as urticaria or anaphylaxis. The most frequent diagnoses of those 80 patients with no acute symptoms were mastocytosis, history of anaphylaxis, or chronic urticaria. Whether any had alpha-tryptasemia was not known.

Urticaria is caused by mast cell activation in the skin, mostly without a significant increase of mast cell numbers [41]. Acute urticaria may be a manifestation of an immediate-type allergic reaction and of anaphylaxis. However, in the majority of cases, urticaria is a distinct

disease, where the occurrence is sporadic and not associated with any known triggers. Sometimes physical and inducible factors, such as mechanical friction, cold, heat, exertion, pressure, light or vibration may lead to wheals. More seldom, infection or inflammation may trigger the immune system. Urticaria is divided into acute or chronic urticaria according to the duration of disease of shorter or longer than 6 weeks, respectively. A significant increase in tryptase values has been demonstrated in patients with acute and chronic urticaria and in associated angioedema compared with controls, though in most studies the total tryptase levels remained in the normal range [40, 42, 43]. Chronic urticaria subjects with tryptase elevated ≥13.5 ng/mL were reported to be older and may have a more severe disease course as indirectly measured by a trend towards greater use of oral steroids for symptom control [42]. Mast cells may be involved in dermatological inflammatory diseases, such as psoriasis or atopic eczema. In both diseases, an accumulation of MC has been described. However, in serum there was no correlation between tryptase and either the severity of psoriasis or the severity of atopic eczema, if measured by PASI and SCORAD, respectively [44]. Also in the autoimmune diseases scleroderma, bullous pemphigoid, pemphigus vulgaris and vasculitis as well as in benign or malignant skin neoplasia, such as hemangioma, malignant melanoma, basal cell carcinoma or squamous cell carcinoma an accumulation and possible involvement of MC has been described [45, 46]. Significant increases in BST in these patient groups as compared to healthy controls, however, have not been reported.

Serum levels of mature tryptase are typically below the lower limit of the assay, i.e., <1 ng/mL, in the absence of systemic anaphylaxis. Even with conditions involving local mast cell activation, including most cases of urticaria or angioedema, due to the slow diffusion of tryptase into the circulation and its dilution once in the blood, mature tryptase is not detected in serum or plasma, even though local levels are elevated in tissue fluids, such as skin blister fluid, nasal fluid, tears or sputum. In older studies, when total tryptase levels could not yet be measured in the blister fluid from patients with bullous skin conditions or in suction blister fluid, elevated mature tryptase levels have been reported [18, 19, 47]. Highly increased levels were detected in patients with bullous pemphigoid and allergic contact dermatitis, whereas concentrations in those with bullous erysipelas, insect sting reactions and pemphigus vulgaris were barely detectable [18]. In patients with cheiropodopompholyx [dyshidrotic hand eczema], burned skin, toxic epidermal necrolysis and epidermolysis bullosa no mature tryptase was detected in serum. Another study confirmed these findings in patients with bullous pemphigoid [48]. In suction blister fluid, elevated mature tryptase levels were increased in patients with urticaria, but undetectable in those with prurigo of unknown origin, eczema, psoriasis, or atopic dermatitis [19]. In a more recent study measuring total tryptase levels in suction blister fluid from patients with systemic mastocytosis, atopic dermatitis and controls, levels were elevated in patients with SM compared to those with AD and to healthy controls [47]. Blister fluid tryptase highly correlated with dermal mast cell numbers. Thus, tryptase determination can be used to confirm a mast cell contribution to the mechanism of a disease and may aid in the differential diagnosis of a bullous skin condition, e.g., between toxic epidermal necrolysis and bullous pemphigoid.

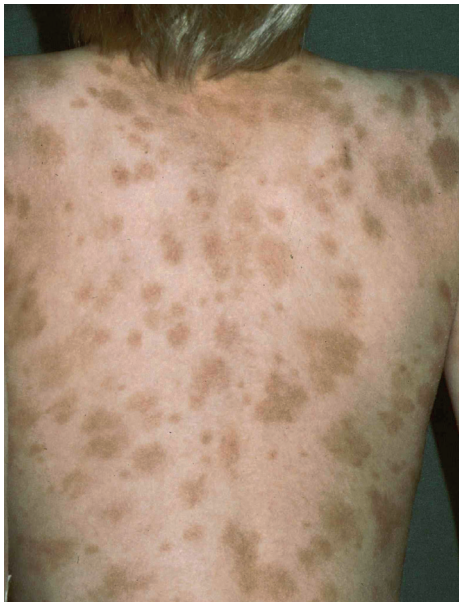

Figure 1. Typical childhood polymorphic maculopapular cutaneous mastocytosis / urticaria pigmentosa presenting with red-brown macules and slightly elevated plaques on the trunk and the extremities.

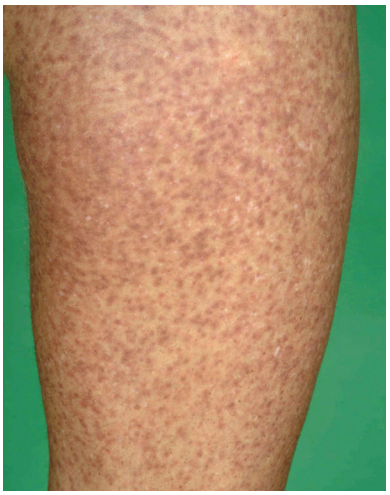

Figure 2. Adult-type monomorphic maculopapular cutaneous mastocytosis / urticaria pigmentosa on the thigh characterized by smaller less well demarcated red-brown macules and slightly elevated papules, which may merge with one another to form patches in areas of greatest density.

Tryptase Testing in Childhood Mastocytosis

Mastocytosis (MC) is a proliferative disorder of hematopoietic mast cell progenitors, leading to abnormal accumulation of mast cells in the tissue of one or more organs, such as skin and bone marrow, but also in the gastrointestinal tract, lymph nodes, liver and spleen [49, 50]. The molecular basis of the disease in most adults and children is an activating mutation, generally somatic, in mast cell DNA encoding KIT, a protein tyrosine kinase receptor [50, 51]. More than 80% of all adult patients with systemic mastocytosis [SM] and about 30% of pediatric cases of mastocytosis in the skin carry a point mutation causing aspartic acid to be replaced by valine in codon 816 (D816V) [52]. Other children and a few adults have distinct mutations affecting the extracellular, juxtamembrane or catalytic regions [53].

In the majority of children with mastocytosis, only the skin appears to be involved (cutaneous MC), i.e., the bone marrow biopsy lacks mast cell aggregates and other features needed to diagnose systemic mastocytosis (SM). The most common cutaneous variants are maculopapular cutaneous mastocytosis (urticaria pigmentosa; MPCM/UP; Fig. 1) characterized by red-brown macules or slightly elevated papules of various sizes and elevations [54, 55]. Also plaque-types and nodular types of MPCM/UP have been described. Blisters may form in the first three years of life on heavily infiltrated mast cell lesions. Solitary mastocytomas are also common in children. They are typically mildly elevated, well-demarcated macules, plaques, or nodules that are reddish-brown in color and several centimeters in diameter. Diffuse cutaneous mastocytosis (DCM) is a rare condition with diffuse mast cell infiltration of the entire skin leading to red-brown thickening of the skin, accentuation of the skin folds and a peau d'orange-like aspect without any islands of normal skin. When mechanically irritated (stroked bluntly with a spatula], cutaneous mastocytosis lesions develop a wheal-like edematous erythema referred to as Darier's sign and in more infiltrated lesions flushing and blister formation may develop. The diagnosis of childhood mastocytosis is based on clinical grounds and is established by a positive Darier's sign or skin biopsy. The majority of children with CM have a baseline serum tryptase concentration <11.4 ng/mL, within the normal range. Increased BST levels may indicate an extensive cutaneous involvement, such as DCM, mast cell mediator release or systemic disease.

Mast cell mediator release and infiltration of tissues with mast cells may lead to flushing, blistering, pruritus, and diarrhea in some patients with childhood mastocytosis, whereas anaphylaxis is uncommon in this age group and appears to be limited to those with severe skin involvement and increased BST levels. There is a significant association between the risk for anaphylaxis and BST levels in children with mastocytosis [56]. A study of 111 children with mastocytosis showed that BST was significantly higher [1] in patients with extensive cutaneous disease vs those with <90% of BSA involved (45.5 vs 5.2 ng/mL, respectively), as well as [2] in children with severe mastocytosis-related symptoms resembling anaphylaxis, requiring emergency therapy and hospitalization vs those without (46.2 vs 5.2 ng/mL, respectively) [57]. A receiver operating characteristics curve was calculated for the definition of BST cutoffs to predict the need for daily antimediator therapy, hospitalization, and the management in an intensive care unit. The respective values were 6.6, 15.5, and 30.8 ng/mL, with a sensitivity and specificity of 77% and 79%, 100% and 95%, and 100% and 96%, respectively. The results of that study indicate that increased serum baseline tryptase levels in association with extensive cutaneous involvement identifies patients at risk for severe MC activation events in pediatric mastocytosis. Bullous lesions were another marker for severe complications [58].

The literature reports resolution percentages of 30 to 65 for pediatric-onset mastocytosis by early adulthood and improvement or stable disease in most other cases [59]. Presence of the D816V mutation together with hepatomegaly and/or splenomegaly is associated with SM and persistence of disease into adulthood [59]. Baseline serum tryptase levels are a good reassuring correlate of clinical improvement into resolution and remain the same or trend downward.

In a study at the National Institutes of Health, clinical features of 105 children with mastocytosis were evaluated [59]. BST and at least 1 subsequent tryptase measurement were performed in 84 and 37 patients, respectively. Children with MPCM/UP had a median, 25th, and 75th percentile BST levels of 5.9, 4.7, and 12.0 ng/mL, respectively, which was higher as compared with the control population (3.5, 2.3, and 4.7 ng/mL; $P \leq 0.0001$). Only six children had levels higher than the WHO minor diagnostic cutoff of 20 ng/m set primarily for adults. Patients with DCM (median, 25th, and 75th percentile tryptase levels of 67.0, 24.9, and 154.0 ng/mL, respectively) and children with systemic disease (median, 25th, and 75th percentile tryptase levels 111.5, 42.0, and 187.0 ng/mL, respectively] had significantly greater BST levels than those with cutaneous mastocytosis and MPCM/UP ($P < 0.0001$ for both). No child in this study with a tryptase level ≤11.4 ng/mL had a final diagnosis of SM. A major resolution was more common in patients with UP or DCM and less common

in patients with SM. Patients with bone marrow mast cell infiltrates consistent with SM had increased serum tryptase levels, which correlated with the percentage of mast cell cellularity within the bone marrow ($P < 0.0001$). The mean percentage decrease in serum tryptase levels was associated with greater disease resolution ($P < .0014$). In children with all variants of mastocytosis, serum tryptase levels decreased or remained stable with time. None of the 9 patients with serum tryptase levels ≥20 ng/mL along with severe symptoms, likely due to mast cell mediators, but no organomegaly, showed SM on bone marrow evaluation.

Thus, serum tryptase levels generally decrease with time in pediatric patients with mastocytosis and is a good biomarker for resolution. Whereas BST levels ≥20 ng/mL together with severe mediator symptoms alone are no indication for a bone marrow biopsy, organomegaly or demonstration of the D816V mutation may indicate SM and also an increase of BST over time suggest the need for monitoring over concern about disease progression [59].

Tryptase Testing in Adult Patients with Mastocytosis

In about half of the adults with mastocytosis, the diagnosis is made because of their characteristic skin lesions of MPCM/UP [54, 55]. In contrast to pediatric mastocytosis, where lesions may vary between different children in terms of size, pigmentation, induration and elevation, having been termed “polymorphic”, in adults lesions are typically small 3–4 mm red-brown macules or slightly elevated monomorphic papules (Fig. 2) [55]. They occur in a symmetric distribution, initially on the sides of the trunk and thighs. In extensive disease, they spread to other areas of the skin and may form clusters or become confluent to form mottled skin areas or diffuse patches. MPCM/UP lesions in adults may be erythematous and telangiectatic rather than hyperpigmented, a picture which has then be termed Telangiectasia Macularis Eruptiva Perstans, a term no longer recommended for cutaneous mastocytosis lesions [55]. However, the level of pigmentation of lesions differs in individual patients with mastocytosis in the skin and telangiectatic lesions may reflect the depigmented end of the MPCM/UP spectrum rather than as a separate entity [60]. The diagnosis of mastocytosis in the skin is made by its typical clinical appearance, Darier’s sign and/or skin biopsy.

The overall prevalence of the systemic type of mastocytosis in adults with mastocytosis reportedly is >95% [52], with bone marrow involvement in the majority of the patients. Additional organs may be affected in more aggressive disease. To diagnose systemic mastocytosis, according to the World Health Organization (WHO), several criteria have to be fulfilled [61]. Among the one major and four minor criteria for systemic mastocytosis (Table 1), having one major and one minor criteria or three minor criteria are required for classifying mastocytosis as systemic [61]. Basally secreted tryptase from mast cells, resulting in a baseline serum tryptase level >20 ng/mL, is a minor diagnostic criterion for systemic mastocytosis. Basal serum tryptase levels are a widely used to screen patients with suspected systemic mastocytosis [62], especially in patients with typical mastocytosis skin lesions or a history of a systemic anaphylactic reaction to an insect sting or a pathologic fracture [63]. In these patients, diagnostic procedures should include an analysis of the D816V KIT mutation in the peripheral blood and/or a bone marrow examination searching for further WHO serum tryptase levels reflect in part the total burden mastocytosis criteria (Table1). In mastocytosis, basal of mast cells. This marker correlates well with disease variant and

Table 1: WHO criteria for the diagnosis of systemic mastocytosis

WHO criteria for the diagnosis of systemic mastocytosis:	
Major criterion	Multifocal dense aggregates of ≥15 mast cells per aggregate in bone marrow and/or other extracutaneous tissues
Minor criteria	Morphologically atypical mast cells, e.g., spindle-shaped, in smears of biopsy sections of bone marrow or other extracutaneous organs Aberrant expression of CD25 and/or CD2 by mast cells in the bone marrow or other tissue sites D816V KIT mutation in bone marrow, blood or other extracutaneous organs Baseline serum tryptase level >20 µg/l

organ involvement in mastocytosis [64], as well as with other biomarkers, such as interleukin-6, soluble Kit (CD117) or soluble interleukin-2 receptor alpha (soluble CD25) levels [64, 65]. It even correlates with the D816V KIT allele burden, i.e., which often indicates cells outside of the mast cell lineage being carriers [66].

Systemic mastocytosis is subdivided into different clinical variants according to organ system impairment and mast cell burden (Table 2) [49, 50]. The most common form [90–95% of adult mastocytosis patients] is indolent systemic mastocytosis (ISM). Patients with ISM shows a wide range of baseline serum tryptase levels, which in

Table 2. Updated WHO Classification of Mastocytosis 2016

Cutaneous mastocytosis (CM)	Maculopapular CM (MPCM) = urticaria pigmentosa (UP) Diffuse CM (DCM) Mastocytoma of skin
Systemic mastocytosis (SM)	Indolent SM (ISM) Smoldering SM (SSM) SM with associated hematologic neoplasm (AHN)* Aggressive SM (ASM) Mast cell leukemia (MCL) Mast cell sarcoma (MCS)

**The previous term SM-AHNMD (SM with clonal hematologic non-mast cell-lineage disease) and the new term AHN can be used synonymously.*

most cases exceed 20 ng/mL, but can be normal [63, 67]. In ISM patients with very high serum tryptase levels (>200 ng/mL), strong mast cell infiltration of the bone marrow and palpable organomegaly (splenomegaly, hepatomegaly or lymphadenopathy), but without signs of organ system impairment, smoldering SM (SSM) is diagnosed [50]. Less common advanced forms include SM with a non-mast cell associated hematologic neoplasm (AHN), aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL) [50]. Most patients with advanced disease variants have markedly elevated baseline serum tryptase levels, often >200 ng/mL [67]. The cumulative probability of disease progression in patients with ISM is low (calculated to be 1.7% in ten years) and ISM patients carry a normal life expectancy [52]. Follow-up baseline serum tryptase levels in patients with ISM and SSM, obtained as clinically appropriate, typically on an annual basis, reflect changes in that patient's mast cell burden. Patients with stable ISM or SSM show only a slight rise or stable levels over the years. Sudden increases or decreases in the baseline tryptase level can indicate a change in disease status [67, 68]. A declining level in patients with ISM are often associated with less frequent and less severe mast cell activation events, while declining levels in a patient with AHN-SM or ASM can be due to a more aggressive AHN such as acute myeloblastic leukemia. A marked increase in the basal serum tryptase level suggests a marked increase in the mast cell burden, raising a concern for mast cell leukemia. Thus, a reproducible change in a patient's baseline serum tryptase level should be considered in the context of other clinical and laboratory parameters.

Most symptoms in patients with mastocytosis are related to the release of mast cell mediators or to an increased number of mast cells in the tissue. Patients may be asymptomatic, but may also report attacks of pruritus, whealing (reddening and swelling of skin lesions), and flushing (erythematous diffuse rash with feeling of warmth) [54, 69]. Diarrhea and abdominal pain are frequent in adults with systemic mastocytosis, and some develop histamine-mediated peptic ulcer disease. Further manifestations and symptoms include osteopenia or osteoporosis, hepatomegaly, ascites, splenomegaly, fatigue, weight loss, mild fever, and night sweats. Palpitations, headache, tachycardia, dizziness, lightheadedness, and syncope may occur. Extensive mast cell mediator release, either spontaneous or triggered, may lead to anaphylaxis [see also chapter on Tryptase Testing in Severe Allergic Reactions to Exclude Mastocytosis]. In adults with SM, the cumulative prevalence of anaphylaxis is much higher than in the normal population, estimated to be between 22% and 49% [56, 70], though not all patients with mastocytosis develop anaphylaxis. Hymenoptera stings are the most common triggers for these reactions. However, anaphylactic reactions triggered by foods, drugs or physical stimuli also occur [56, 71]. Baseline serum tryptase has been reported to trend higher in adults with MPCM/UP who develop anaphylaxis as compared to those who do not [56].

In adult SM, recently and increasingly a substantial fraction of patients with anaphylaxis and later confirmed SM without presence of skin lesions has been reported. In other patients without mastocytosis in the skin, osteoporosis and pathological fractures together with elevated baseline serum tryptase or hematological neoplasms associated with mastocytosis may lead to the diagnosis. Elevated baseline serum tryptase or MPCM/UP have been found in about 5% to 15% of patients with hymenoptera anaphylaxis [71], both indicating a possible underlying mast cell disease, such as SM. The majority of patients with SM without mastocytosis in the skin are diagnosed in the follow-up of anaphylaxis, mostly due to hymenoptera venom allergy, and elevated baseline serum tryptase levels. Specific demographic, clinical, and laboratory features distinguish these patients from those exhibiting typical mastocytosis skin lesions: a male predominance, more cardiovascular as compared to cutaneous symptoms, and more restricted mast cell abnormality/proliferation seen as less bone marrow mast cell aggregates and mast cell lineage-restricted KIT mutations [72, 73]. They also have lower baseline serum

tryptase levels, which may be within the normal range of ≤11.4 ng/mL [74]. In about 6% of ISM patients without skin lesions, baseline serum tryptase levels remain ≤11.4 ng/mL [73]. Thus, in male patients with severe hymenoptera venom anaphylaxis with severe cardiovascular symptoms and without skin symptoms (such as flushing, urticaria or angioedema), a bone marrow biopsy or peripheral blood allele-specific D816V KIT PCR should be considered even in patients with normal baseline serum tryptase levels.

Taken together, monitoring baseline serum tryptase levels has diagnostic and prognostic values in patients with mastocytosis. Tryptase levels >20 ng/mL are a strong indicator of and may initiate a work-up for SM, even though lower levels do not exclude consideration of this disease, e.g., in patients for whom hymenoptera stings triggered systemic anaphylaxis or those with unexplained osteoporosis and pathologic fracture. However, the prognosis is good when tryptase levels are stable over time in cases of cutaneous or indolent SM or poor in cases of rapidly increasing baseline serum tryptase levels.

Tryptase Testing in Mast Cell Activation Syndrome

Mastocytosis is an emerging differential diagnosis in patients with more or less specific symptoms that may be mast cell mediator-related, but without overt mastocytosis in the skin [17]. In some of these patients, typical clinical and laboratory criteria are found and the diagnosis of SM can be established [49]. In other cases, presence of the D816V KIT mutation and/or CD25 positivity of mast cells in the bone marrow aspirate or other sites demonstrate mast cell clonality, but alone do not provide sufficient criteria for the diagnosis SM. The diagnosis in these patients, which suffer particularly from hymenoptera venom or idiopathic anaphylaxis, has been termed monoclonal mast cell activation syndrome (MMCAS) [17, 75, 76]. Other clonal disorders, such as myelodysplastic syndrome associated with a gain-of-function Jak2 mutation [77], or hypereosinophilic syndrome associated with a FIP1L1-PDGFRA gain-of-function mutation [78, 79], result in CD25-positive mast cells, but such mast cells do not form aggregates and do not appear to put such patients at increased risk for either spontaneous or insect venom-triggered anaphylaxis.

This description of a MMAS in patients with anaphylaxis, has led to a search for new unrecognized mast cell activation syndromes. Elevated baseline serum tryptase may be an indicator for a mast cell-driven condition. In a study in Austria, baseline serum tryptase was assessed in 15,298 patients attending an allergy outpatient clinic [24]. In 5.9% of patients, baseline serum tryptase was elevated. Fatigue, abdominal distention, fibromyalgia, bone aches, vertigo, tachycardia, flush, palpitations, diarrhea and edema were associated with increased baseline serum tryptase. Many patients suffer from symptoms, which may point to a possible mast cell disease. In the light of this unmet need, a diagnostic algorithm for patients with suspected mastocytosis was developed and a unifying classification of all mast cell disorders was proposed in order to be able to recognize and describe new entities [17].

The preclinical checkpoint of mast cell activation was defined as a prerequisite for diagnosing a mast cell disorder [17]. Mast cell activation cannot be defined by symptoms alone, as different diseases and conditions may have similar or overlapping symptoms [80]. In severe reactions, mast cell activation can be documented by a substantial increase in the serum tryptase level above baseline. It was proposed that an increase of tryptase of 2 ng/mL + 20% of baseline serum tryptase above baseline serum tryptase is diagnostic for mast cell activation, such as present in anaphylaxis [17]. When symptoms are recurrent, are accompanied by such an increase in tryptase, and are responsive to treatment with mast cell–stabilizing or mediator-targeting drugs, the diagnosis of mast cell activation syndrome (MCAS) is appropriate [80]. Mast cell activation syndromes were subclassified into primary MCAS where clonal mast cells are detected [mastocytosis and MMAS], secondary (e.g. IgE-mediated allergy, but no KIT-mutated cells) and idiopathic forms, where neither an allergy or other underlying disease, nor KIT-mutated mast cells are detectable [17].

Until now, only few patients have been diagnosed as “idiopathic” MCAS according to these criteria in the literature. A group of 18 patients have been described presenting with a combination of abdominal pain, flush and urticarial dermographism [81]. Whereas the number of patients with compatible symptoms and response to therapy was higher in the total patient cohort [personal communication Cem Akin], only these patients also demonstrated at least one increased mast cell mediator at ≥ one time point, including total tryptase (>15 ng/mL), mature tryptase (≥1 ng/mL), urinary histamine (>386 mmol/g creatinine) or urinary prostaglandin D2 (>280 ng/24 h). Another group of 25 patients with symptoms compatible with MCAS has been evaluated at the Mayo Clinic and the frequency of elevated mast cell mediators was analyzed [82]. Urinary PGD2 metabolite 11β-prostaglandin-$F_2\alpha$ (11β-$PGF_2\alpha$) was the most frequently elevated mediator in these patients and serum tryptase level was elevated in 10 patients, whereas the urinary histamine metabolite N-methyl histamine level

was elevated only in 2 patients. The authors recommend measurement of 24-hour urine 11β-$PGF_2\alpha$ and serum tryptase levels of patients with symptoms suggestive of MCAS to avoid misdiagnosis and over interpretation of MCAS symptoms in clinical practice. However, although PGD2 metabolites may be more sensitive than tryptase of such reactions, tryptase is likely to be more specific for mast cell activation due to its preferential storage in mast cell secretory granules and because other cell types can produce PGD2. For example, lipocalin-PGD synthase produces PGD2 from non-mast cells in CNS, male genitalia, adipose tissue and heart, while hematopoietic PGD synthase is expressed in megakaryocytes, dendritic cells and TH2 lymphocytes as well as mast cells.

Tryptase Testing in Allergy and Anaphylaxis

Tryptase determination has been used for more than 20 years for the confirmation of a mast cell degranulation in experimental or clinical allergic reactions. It has been measured in the serum, plasma as well as in different tissue fluids, such as skin blister fluid, tears, gut mucosa, bronchoalveolar, ear and nasal lavage fluids and in tissue culture supernatant [39, 83–88]. Increased levels in comparison to healthy controls are confirmative of an allergic reaction.

Anaphylaxis is a systemic or generalized life-threatening and potentially fatal systemic hypersensitivity reaction [89]. Criteria for anaphylaxis have been defined [90]. Typically, it involves ≥ two out of the four organ systems most involved, including skin [e.g. flush, urticaria, angioedema], gastrointestinal tract (e.g. abdominal pain, nausea, diarrhea), pulmonary system [e.g. wheezing, dyspnea] and cardiovascular system [e.g. hypotension, shock]. Hymenoptera venom, food and drugs account for > 90–95% of identified elicitors for anaphylaxis [89]. In some cases, the culprit elicitor cannot be found and the diagnosis "idiopathic anaphylaxis" is made.

Systemic anaphylaxis is the most severe form of an immediate-type allergic reaction and is typically associated with high amounts of mast cell mediator release [16, 20, 91, 92]. As other mediators are more difficult to determine and less specific, tryptase is the preferred mast cell mediator measured in such a clinical situation, even though it may be less sensitive. The commercially available tryptase test is robust and provides reproducible results [11]. A significant increase of tryptase over the baseline serum tryptase is a strong sign for an allergic or anaphylactic reaction and supports this diagnosis. The chance and magnitude of tryptase elevation depends on the severity of the reaction and on the elicitor of the reaction. An increase of tryptase levels is more often detected in insect venom as well as in drug-triggered anaphylaxis [particularly perioperative anaphylaxis] as compared with food anaphylaxis [92]. A significant correlation between tryptase levels and severity of the reaction was found for insect sting-triggered systemic anaphylaxis [16, 91]. In severe anaphylaxis with circulatory failure and decreased blood pressure, high levels of tryptase are detected, whereas mild urticaria may not be sufficient to increase blood tryptase levels over baseline [93].

In order to support the diagnosis "anaphylaxis", the increase of acute tryptase 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 ≥11.4 ng/mL per se are not indicative of mast cell activation and a normal baseline serum tryptase level does not exclude anaphylaxis [80]. In primary MC disease [mastocytosis and MMCAS], baseline serum tryptase levels are usually elevated [15]. 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 a mast cell activation, should be at least 20% plus 2 ng/mL over the baseline serum tryptase level [17]. For example, if a patient has a baseline serum tryptase level of 10 ng/mL, the acute peak tryptase level should be ≥14 ng/mL (10 + 0.2*10 + 2 ng/mL). A prospective and retrospective clinical investigation to validate this mast cell activation criterion in various clinical situations is under way.

An important prerequisite for using tryptase as an indicator for mast cell activation is the blood collection timing. Peak tryptase levels after anaphylaxis onset have to be compared to baseline serum tryptase levels. If blood is drawn after inadequate time intervals, the increase in tryptase levels may not be detected. At least two blood samples are mandatory, one after the acute reaction and one for baseline serum tryptase. As mature tetrameric tryptase has to diffuse from the tissue to the blood, elevation of serum tryptase may not be detectable during the first 15 to 30 minutes [12]. In the blood, mature tryptase or the rise in total tryptase returns to baseline with a half-life of about two hours [12]. The recommended time frame for drawing blood after suspected anaphylaxis is between 30 minutes and two hours. In an individual patient, the chance is higher to detect an increase, if several acute samples are taken, e.g., after 30 minutes and 1 or 2 hours. In most patients with anaphylaxis, no baseline serum tryptase has been determined before the reaction. With a half-life of two hours for tryptase, blood for baseline serum tryptase should be drawn at least 24 hours after the reaction has completely subsided. If baseline serum tryptase remains elevated

after 24 or more hours, then an investigation for SM and MMCAS should be initiated. Systemic mastocytosis is a well-known underlying disease in patients with hymenoptera venom and idiopathic anaphylaxis and may also be present in food and drug anaphylaxis. Many patients with anaphylaxis and SM do not have additional symptoms and would not have been diagnosed without the anaphylactic episode [94]. In addition to patients with SM, there are patients with anaphylaxis that carry clonal mast cells expressing the D816V KIT mutation, i.e., MMCAS [17].

Tryptase Testing in Severe Allergic Reactions to Exclude Mastocytosis

Anaphylaxis may be the first manifestation of a primary mast cell disorder [56]. The severity of anaphylaxis in adults with ISM as compared to nonmastocytosis patients appears to be increased. In one study, 48% of reactions were classified as severe and 38% resulted in unconsciousness [56]; another study reported unconsciousness occurring in 72% of patients with mastocytosis [95]. In about 5% to 15% of patients with hymenoptera anaphylaxis, elevated baseline serum tryptase levels or urticaria pigmentosa have been found, both indicating a possible underlying clonal mast cell disease, i.e., SM or MMCAS [71, 96]. Mastocytosis is a risk factor for severe anaphylaxis in the group of patients with anaphylaxis to hymenoptera venom [22, 96]. Thus, in all patients with hymenoptera venom anaphylaxis, an underlying clonal mast cell disease should be excluded by baseline serum tryptase determination, D816V cKit allele-specific PCR test in peripheral blood (if available) [97], skin examination and in patients with suspicion of SM (e.g. male with severe reaction, increased baseline serum tryptase or MPCM /UP) by bone marrow examination [98]. In a multicenter study of patients with honey bee or vespid venom allergy, the strongest risk factor for severe anaphylaxis was the elevation of baseline serum tryptase values irrespective of mastocytosis [99]. In this study, there was a continuous log-linear relationship between baseline serum tryptase and the risk for severe anaphylaxis particularly for vespid allergy and even levels within the normal range <11.4 ng/mL had higher adjusted odds ratios. However, elevated tryptase levels may have been a surrogate marker for mast cell clonality, which was not directly examined in this study. Also the risk of allergic adverse reactions during the induction phase of hymenoptera venom immunotherapy was associated with baseline serum tryptase levels, again, perhaps reflecting underlying mast cell clonality [100]. For vespula immunotherapy, the odds ratio reached 2, 5 and 10 for baseline serum tryptase levels of 7.5, 10 and 20 ng/mL, respectively. Thus, a higher risk for severe reactions may be more closely linked to the total body mast cell load or to mast cell clonality as reflected by baseline serum tryptase levels.

In anaphylaxis to drugs and food, baseline serum tryptase levels ≥11.4 ng/mL are less frequently found and the diagnosis of SM is less often made [101, 102]. Nevertheless, food allergy in children has been related to higher levels of baseline serum tryptase [103]. In this study, median serum baseline tryptase levels among 167 children with food allergy with a history of anaphylaxis (4.0 ng/mL]) or no such history (3.6 ng/mL) were compared to a control group (3.3 ng/nL), showing a significantly increased level in the anaphylaxis group. Cutoff baseline tryptase values of 5.7 and 14.5 ng/mL were associated with 50% and 90% predicted probabilities for moderate to severe anaphylaxis, respectively. Children with tree nuts or peanut allergies had significantly higher levels of serum baseline tryptase than children with milk and egg allergy. Only a minority of cases with drug-induced anaphylaxis are associated with mastocytosis [102]. In patients with NSAID-hypersensitivity, no association to elevated basal serum tryptase levels was found, which is in contrast to patients with hymenoptera venom allergy [104]. Nevertheless, also in patients with severe anaphylaxis to food or drugs, determination of baseline serum tryptase and skin examination is advised and cases with newly diagnosed SM have been described [94]. As idiopathic anaphylaxis is occasionally associated with SM, the same holds true for these patients [76].

Tryptase Testing Postmortem

The postmortem diagnosis of anaphylaxis can be difficult without specific biomarkers. Tryptase may be used as a biomarker for mast cell activation and anaphylaxis to support these diagnoses. Tryptase is significantly elevated in the femoral blood of anaphylactic deaths as compared with controls [105]. Very high levels of serum tryptase have been reported after fatal anaphylaxis [20, 105]. However, the diagnosis should not be based on this test alone, as moderately elevated tryptase values are also found in deaths that apparently were not IgE-mediated and a considerable overlap exists between values in the anaphylaxis and control groups [106–108]. Asphyxia was associated with higher postmortem femoral tryptase levels [109]. It has been recommended to do postmortem measurements from femoral blood, because cardiac blood may give higher tryptase values also in nonanphylactic deaths and be more nonspecific [109]. Also, as no baseline sample of serum is typically available, a measurement of mature trytase along with total tryptase might be useful.

Summary

Measuring a total tryptase level under acute and/or baseline conditions can facilitate detection of mast cell involvement related to elevated mast cell numbers as found in systemic mastocytosis, mast cell activation as in systemic anaphylaxis or tryptase overproduction as in alpha-tryptasemia. Also, a normal level reduces the likelihood of such conditions. In systemic mastocytosis, particularly advanced forms, cytoreductive therapy can be monitored, in part, by following the tryptase level, reflecting the mast cell burden. Such information provides more precise information than available from clinical features alone. As cutaneous manifestations can be present in all of these mast cell disorders, dermatologists should be familiar with tryptase testing.

Conflict of interest statement

The authors have written this paper upon a request and with remuneration from Thermo Fisher Scientific and have granted Thermo Fisher Scientific a right to use the paper in its marketing.

References

1. Schwartz LB. Tryptase, a mediator of human mast cells. J Allergy Clin Immunol. 1990;86:594-8.
2. Schwartz LB, Irani AM, Roller K, Castells MC, Schechter NM. Quantitation of histamine, tryptase, and chymase in dispersed human T and TC mast cells. J Immunol. 1987;138(8):2611-5.
3. Foster B, Schwartz LB, Devouassoux G, Metcalfe DD, Prussin C. Characterization of mast-cell tryptase-expressing peripheral blood cells as basophils. J Allergy Clin Immunol. 2002;109(2):287-93.
4. Jogie-Brahim S, Min HK, Fukuoka Y, Xia HZ, Schwartz LB. Expression of alpha-tryptase and beta-tryptase by human basophils. J Allergy Clin Immunol. 2004;113(6):1086-92.
5. Samorapoompichit P, Kiener HP, Schernthaner GH, et al. Detection of tryptase in cytoplasmic granules of basophils in patients with chronic myeloid leukemia and other myeloid neoplasms. Blood. 2001;98(8):2580-3.
6. Vitte J. Human mast cell tryptase in biology and medicine. Mol Immunol. 2015;63(1):18-24.
7. Sperr WR, Valent P. The tryptase test: clinical use in hematology Thermo Fisher Scientific; 2015.
8. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. Immunol Allergy Clin North Am. 2006;26(3):451-63.
9. Miller JS, Moxley G, Schwartz LB. Cloning and characterization of a second complementary DNA for human tryptase. J Clin Invest. 1990;86(3):864-70.
10. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. N Engl J Med. 1987;316(26):1622-6.
11. Schwartz LB, Bradford TR, Rouse C, et al. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. J Clin Immunol. 1994;14(3):190-204.
12. Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. J Clin Invest. 1989;83(5):1551-5.
13. Sargur R, Cowley D, Murng S, et al. Raised tryptase without anaphylaxis or mastocytosis: heterophilic antibody interference in the serum tryptase assay. Clin Exp Immunol. 2011;163(3):339-45.
14. van Toorenenbergen AW, van Daele PL, Boonstra JG. False-elevated serum tryptase assay result caused by heterophilic antibodies. J Allergy Clin Immunol. 2005;116(5):1159-60.
15. Schwartz LB, Sakai K, Bradford TR, et al. The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. J Clin Invest. 1995;96(6):2702-10.
16. Lin RY, Schwartz LB, Curry A, et al. Histamine and tryptase levels in patients with acute allergic reactions: An emergency department-based study. J Allergy Clin Immunol. 2000;106:65-71.
17. Valent P, Akin C, Arock M, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. Int Arch Allergy Immunol. 2012;157(3):215-25.
18. Brockow K, Abeck D, Hermann K, Ring J. Tryptase concentration in skin blister fluid from patients with bullous skin conditions. Arch Dermatol Res. 1996;288(12):771-3.
19. Deleuran B, Kristensen M, Larsen CG, et al. Increased tryptase levels in suction-blister fluid from patients with urticaria. Br J Dermatol. 1991;125(1):14-7.
20. Brockow K, Vieluf D, Puschel K, Grosch J, Ring J. Increased postmortem serum mast cell tryptase in a fatal anaphylactoid reaction to nonionic radiocontrast medium. J Allergy Clin Immunol. 1999;104(1):237-8.
21. Gonzalez-Quintela A, Vizcaino L, Gude F, et al. Factors influencing serum total tryptase concentrations in a general adult population. Clin Chem Lab Med. 2010;48(5):701-6.
22. Guenova E, Volz T, Eichner M, et al. Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly. Allergy. 2010;65(7):919-23.
23. Min HK, Moxley G, Neale MC, Schwartz LB. Effect of sex and haplotype on plasma tryptase levels in healthy adults. J Allergy Clin Immunol. 2004;114(1):48-51.
24. Fellinger C, Hemmer W, Wohrl S, Sesztak-Greinecker G, Jarisch R, Wantke F. Clinical characteristics and risk profile of patients with elevated baseline serum tryptase. Allergol Immunopathol (Madr). 2014;42(6):544-52.
25. Belhocine W, Ibrahim Z, Grandne V, et al. Total serum tryptase levels are higher in young infants. Pediatr Allergy Immunol. 2011;22(6):600-7.
26. Komarow HD, Hu Z, Brittain E, Uzzaman A, Gaskins D, Metcalfe DD. Serum tryptase levels in atopic and nonatopic children. J Allergy Clin Immunol. 2009;124(4):845-8.
27. Sahiner UM, Yavuz ST, Buyuktiryaki B, et al. Serum basal tryptase levels in healthy children: correlation between age and gender. Allergy Asthma Proc. 2014;35(5):404-8.
28. Sverrild A, van der Sluis S, Kyvik KO, et al. Genetic factors account for most of the variation in serum tryptase--a twin study. Ann Allergy Asthma Immunol. 2013;111(4):286-9.
29. Schliemann S, Seyfarth F, Hipler UC, Elsner P. Impact of age and heterophilic interference on the basal serum tryptase, a risk indication for anaphylaxis, in 1,092 dermatology patients. Acta Derm Venereol. 2012;92(5):484-9.
30. Sperr WR, El-Samahi A, Kundi M, et al. Elevated tryptase levels selectively cluster in myeloid neoplasms: a novel diagnostic approach and screen marker in clinical haematology. Eur J Clin Invest. 2009;39(10):914-23.
31. Furuta T, Murao LA, Lan NT, et al. Association of mast cell-derived VEGF and proteases in Dengue shock syndrome. PLoS Negl Trop Dis. 2012;6(2):e1505.
32. Dugas-Breit S, Schopf P, Dugas M, Schiffl H, Rueff F, Przybilla B. Baseline serum levels of mast cell tryptase are raised in hemodialysis patients and associated with severity of pruritus. J Dtsch Dermatol Ges. 2005;3(5):343-7.
33. Sirvent AE, Gonzalez C, Enriquez R, et al. Serum tryptase levels and markers of renal dysfunction in a population with chronic kidney disease. J Nephrol. 2010;23(3):282-90.
34. Velasquez CV, Roman AD, Lan NT, et al. Alpha tryptase allele of Tryptase 1 (TPSAB1) gene associated with Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) in Vietnam and Philippines. Hum Immunol. 2015;76(5):318-23.

35. Ombrello MJ, Remmers EF, Sun G, et al. Cold urticaria, immunodeficiency, and autoimmunity related to PLCG2 deletions. N Engl J Med. 2012;366(4):330-8.

36. Hoffman HM, Rosengren S, Boyle DL, et al. Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. Lancet. 2004;364(9447):1779-85.

37. Lyons JJ, Sun G, Stone KD, et al. Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities. J Allergy Clin Immunol. 2014;133(5):1471-4.

38. Lyons JJ, Yu X, Hughes JD, et al. Elevated basal serum tryptase identifies a multisystem disorder associated with increased TPSAB1 copy number. Nat Genet. 2016;48(12):1564-1569.

39. Shalit M, Schwartz LB, von Allmen C, Atkins PC, Lavker RM, Zweiman B. Release of histamine and tryptase during continuous and interrupted cutaneous challenge with allergen in humans. J Allergy Clin Immunol. 1990;86(1):117-25.

40. Aberer E, Savic S, Bretterklieber A, Reiter H, Berghold A, Aberer W. Disease spectrum in patients with elevated serum tryptase levels. Australas J Dermatol. 2015;56(1):7-13.

41. Zuberbier T, Asero R, Bindslev-Jensen C, et al. EAACI/GA(2)LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. Allergy. 2009;64(10):1417-26.

42. Siles R, Xu M, Hsieh FH. The utility of serum tryptase as a marker in chronic spontaneous urticaria. Acta Derm Venereol. 2013;93(3):354-5.

43. Ferrer M, Nunez-Cordoba JM, Luquin E, et al. Serum total tryptase levels are increased in patients with active chronic urticaria. Clin Exp Allergy. 2010;40(12):1760-6.

44. Gerdes S, Kurrat W, Mrowietz U. Serum mast cell tryptase is not a useful marker for disease severity in psoriasis or atopic dermatitis. Br J Dermatol. 2009;160(4):736-40.

45. Paolino G, Moliterni E, Lopez T, et al. Serum tryptase levels in melanoma patients: first results of clinicopathological features. Melanoma Res. 2016;26(2):207-8.

46. Lipitsa T, Naukkarinen A, Harvima IT. Mast cell tryptase and chymase in the progress of cutaneous vasculitis. Arch Dermatol Res. 2015;307(10):917-24.

47. Brockow K, Akin C, Huber M, Scott LM, Schwartz LB, Metcalfe DD. Levels of mast-cell growth factors in plasma and in suction skin blister fluid in adults with mastocytosis: correlation with dermal mast-cell numbers and mast-cell tryptase. J Allergy Clin Immunol. 2002;109(1):82-8.

48. D'Auria L, Pietravalle M, Cordiali-Fei P, Ameglio F. Increased tryptase and myeloperoxidase levels in blister fluids of patients with bullous pemphigoid: correlations with cytokines, adhesion molecules and anti-basement membrane zone antibodies. Exp Dermatol. 2000;9(2):131-7.

49. Valent P, Akin C, Escribano L, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. Eur J Clin Invest. 2007;37(6):435-53.

50. Valent P, Akin C, Hartmann K, et al. Advances in the Classification and Treatment of Mastocytosis: Current Status and Outlook toward the Future. Cancer Res. 2017;77(6):1261-1270.

51. Bodemer C, Hermine O, Palmerini F, et al. Pediatric mastocytosis is a clonal disease associated with D816V and other activating c-KIT mutations. J Invest Dermatol. 2010;130(3):804-15.

52. Escribano L, Alvarez-Twose I, Sanchez-Munoz L, et al. Prognosis in adult indolent systemic mastocytosis: a long-term study of the Spanish Network on Mastocytosis in a series of 145 patients. J Allergy Clin Immunol. 2009;124(3):514-21.

53. Arock M, Sotlar K, Akin C, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. Leukemia. 2015;29(6):1223-32.

54. Brockow K. Urticaria pigmentosa. Immunol Allergy Clin North Am. 2004;24(2):287-316, vii.

55. Hartmann K, Escribano L, Grattan C, et al. Cutaneous manifestations in patients with mastocytosis: Consensus report of the European Competence Network on Mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. J Allergy Clin Immunol. 2016;137(1):35-45.

56. Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. Allergy. 2008;63(2):226-32.

57. Alvarez-Twose I, Vano-Galvan S, Sanchez-Munoz L, et al. Increased serum baseline tryptase levels and extensive skin involvement are predictors for the severity of mast cell activation episodes in children with mastocytosis. Allergy. 2012;67(6):813-21.

58. Brockow K, Ring J, Alvarez-Twose I, Orfao A, Escribano L. Extensive blistering is a predictor for severe complications in children with mastocytosis. Allergy. 2012;67(10):1323-4.

59. Carter MC, Clayton ST, Komarow HD, et al. Assessment of clinical findings, tryptase levels, and bone marrow histopathology in the management of pediatric mastocytosis. J Allergy Clin Immunol. 2015;136(6):1673-79 e1-3.

60. Williams KW, Metcalfe DD, Prussin C, Carter MC, Komarow HD. Telangiectasia macularis eruptiva perstans or highly vascularized urticaria pigmentosa? J Allergy Clin Immunol Pract. 2014;2(6):813-5.

61. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. Blood. 2017;129(11):1420-1427.

62. Valent P, Horny HP, Escribano L, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res. 2001;25(7):603-25.

63. Schwartz LB. Diagnostic Value of Tryptase in Anaphylaxis and Mastocytosis. Immunol Allergy Clin N Am. 2006;(26):451-463.

64. Brockow K, Akin C, Huber M, Metcalfe DD. IL-6 levels predict disease variant and extent of organ involvement in patients with mastocytosis. Clin Immunol. 2005;115(2):216-23.

65. Akin C, Schwartz LB, Kitoh T, et al. Soluble stem cell factor receptor (CD117) and IL-2 receptor alpha chain (CD25) levels in the plasma of patients with mastocytosis: relationships to disease severity and bone marrow pathology. Blood. 2000;96(4):1267-73.

66. Kristensen T, Broesby-Olsen S, Vestergaard H, Bindslev-Jensen C, Moller MB. Serum tryptase correlates with the KIT D816V mutation burden in adults with indolent systemic mastocytosis. Eur J Haematol. 2013;91(2):106-11.

67. Sperr WR, Jordan JH, Fiegl M, et al. Serum tryptase levels in patients with mastocytosis: correlation with mast cell burden and implication for defining the category of disease. Int Arch Allergy Immunol. 2002;128(2):136-41.

68. Matito A, Morgado JM, Alvarez-Twose I, et al. Serum tryptase monitoring in indolent systemic mastocytosis: association with disease features and patient outcome. PLoS One. 2013;8(10):e76116.

69. Brockow K, Metcalfe DD. Mastocytosis. Chem Immunol Allergy. 2010;95:110-24.

70. Gonzalez de Olano D, de la Hoz Caballer B, Nunez Lopez R, et al. Prevalence of allergy and anaphylactic symptoms in 210 adult and pediatric patients with mastocytosis in Spain: a study of the Spanish network on mastocytosis (REMA). Clin Exp Allergy. 2007;37(10):1547-55.

71. Bonadonna P, Perbellini O, Passalacqua G, et al. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. J Allergy Clin Immunol. 2009;123(3):680-6.

72. Alvarez-Twose I, Gonzalez de Olano D, Sanchez-Munoz L, et al. Clinical, biological, and molecular characteristics of clonal mast cell disorders presenting with systemic mast cell activation symptoms. J Allergy Clin Immunol. 2010;125(6):1269-1278.

73. Alvarez-Twose I, Zanotti R, Gonzalez-de-Olano D, et al. Nonaggressive systemic mastocytosis (SM) without skin lesions associated with insect-induced anaphylaxis shows unique features versus other indolent SM. J Allergy Clin Immunol. 2014;133(2):520-8.

74. Zanotti R, Lombardo C, Passalacqua G, et al. Clonal mast cell disorders in patients with severe Hymenoptera venom allergy and normal serum tryptase levels. J Allergy Clin Immunol. 2015;136(1):135-9.

75. Sonneck K, Florian S, Mullauer L, et al. Diagnostic and subdiagnostic accumulation of mast cells in the bone marrow of patients with anaphylaxis: Monoclonal mast cell activation syndrome. Int Arch Allergy Immunol. 2007;142(2):158-64.

76. Akin C, Scott LM, Kocabas CN, et al. Demonstration of an aberrant mast-cell population with clonal markers in a subset of patients with "idiopathic" anaphylaxis. Blood. 2007;110(7):2331-3.

thermoscientific

77. Valent P, Sperr WR, Sotlar K, et al. The serum tryptase test: an emerging robust biomarker in clinical hematology. Expert Rev Hematol. 2014;7(5):683-90.

78. Klion AD, Noel P, Akin C, et al. Elevated serum tryptase levels identify a subset of patients with a myeloproliferative variant of idiopathic hypereosinophilic syndrome associated with tissue fibrosis, poor prognosis, and imatinib responsiveness. Blood. 2003;101(12):4660-6.

79. Maric I, Robyn J, Metcalfe DD, et al. KIT D816V-associated systemic mastocytosis with eosinophilia and FIP1L1/PDGFRA-associated chronic eosinophilic leukemia are distinct entities. J Allergy Clin Immunol. 2007;120(3):680-7.

80. Valent P. Mast cell activation syndromes: definition and classification. Allergy. 2013;68(4):417-24.

81. Hamilton MJ, Hornick JL, Akin C, Castells MC, Greenberger NJ. Mast cell activation syndrome: a newly recognized disorder with systemic clinical manifestations. J Allergy Clin Immunol. 2011;128(1):147-152.

82. Ravi A, Butterfield J, Weiler CR. Mast cell activation syndrome: improved identification by combined determinations of serum tryptase and 24-hour urine 11beta-prostaglandin2alpha. J Allergy Clin Immunol Pract. 2014;2(6):775-8.

83. Wenzel SE, Fowler AA, 3rd, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. Am Rev Respir Dis. 1988;137(5):1002-8.

84. Butrus SI, Ochsner KI, Abelson MB, Schwartz LB. The level of tryptase in human tears. An indicator of activation of conjunctival mast cells. Ophthalmology. 1990;97(12):1678-83.

85. Castells M, Schwartz LB. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. J Allergy Clin Immunol. 1988;82(3 Pt 1):348-55.

86. Hagel AF, deRossi T, Zopf Y, et al. Mast cell tryptase levels in gut mucosa in patients with gastrointestinal symptoms caused by food allergy. Int Arch Allergy Immunol. 2013;160(4):350-5.

87. Jang CH, Jung JK. Expression of mast cell tryptase in pediatric otitis media with effusion. Int J Pediatr Otorhinolaryngol. 2003;67(11):1185-8.

88. Leonardi A. Allergy and allergic mediators in tears. Exp Eye Res. 2013;117:106-17.

89. Simons FE, Ardusso LR, Bilo MB, et al. World Allergy Organization anaphylaxis guidelines: summary. J Allergy Clin Immunol. 2011;127(3):587-93.

90. Sampson HA, Munoz-Furlong A, Campbell RL, et al. Second symposium on the definition and management of anaphylaxis: summary report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol. 2006;117(2):391-7.

91. van der Linden PW, Hack CE, Poortman J, Vivie-Kipp YC, Struyvenberg A, van der Zwan JK. Insect-sting challenge in 138 patients: relation between clinical severity of anaphylaxis and mast cell activation. J Allergy Clin Immunol. 1992;90(1):110-8.

92. Sala-Cunill A, Cardona V, Labrador-Horrillo M, et al. Usefulness and limitations of sequential serum tryptase for the diagnosis of anaphylaxis in 102 patients. Int Arch Allergy Immunol. 2013;160(2):192-9.

93. Vadas P, Perelman B, Liss G. Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis. J Allergy Clin Immunol. 2013;131(1):144-9.

94. Schuch A, Brockow K. Mastocytosis and Anaphylaxis. Immunol Allergy Clin North Am. 2017;37(1):153-164.

95. Gulen T, Hagglund H, Dahlen B, Nilsson G. High prevalence of anaphylaxis in patients with systemic mastocytosis - A single-center experience. Clin Exp Allergy. 2014;44(1):121-9.

96. Ludolph-Hauser D, Rueff F, Fries C, Schopf P, Przybilla B. Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to Hymenoptera stings. Lancet. 2001;357(9253):361-2.

97. Kristensen T, Vestergaard H, Bindslev-Jensen C, Moller MB, Broesby-Olsen S. Sensitive KIT D816V mutation analysis of blood as a diagnostic test in mastocytosis. Am J Hematol. 2014;89(5):493-8.

98. Brockow K. Epidemiology, prognosis, and risk factors in mastocytosis. Immunol Allergy Clin North Am. 2014;34(2):283-95.

99. Rueff F, Przybilla B, Bilo MB, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase-a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol. 2009;124(5):1047-54.

100. Rueff F, Przybilla B, Bilo MB, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: The importance of baseline serum tryptase. J Allergy Clin Immunol. 2010.

101. Bonadonna P, Zanotti R, Pagani M, et al. How much specific is the association between hymenoptera venom allergy and mastocytosis? Allergy. 2009;64(9):1379-82.

102. Brockow K, Bonadonna P. Drug allergy in mast cell disease. Curr Opin Allergy Clin Immunol. 2012;12(4):354-60.

103. Sahiner UM, Yavuz ST, Buyuktiryaki B, et al. Serum basal tryptase may be a good marker for predicting the risk of anaphylaxis in children with food allergy. Allergy. 2014;69(2):265-8.

104. Seitz CS, Brockow K, Hain J, Trautmann A. Non-steroidal anti-inflammatory drug hypersensitivity: association with elevated basal serum tryptase? Allergy Asthma Clin Immunol. 2014;10(1):19.

105. Yunginger JW, Nelson DR, Squillace DL, et al. Laboratory investigation of deaths due to anaphylaxis. J Forensic Sci. 1991;36(3):857-65.

106. McLean-Tooke A, Goulding M, Bundell C, White J, Hollingsworth P. Postmortem serum tryptase levels in anaphylactic and non-anaphylactic deaths. J Clin Pathol. 2014;67(2):134-8.

107. Randall B, Butts J, Halsey JF. Elevated postmortem tryptase in the absence of anaphylaxis. J Forensic Sci. 1995;40(2):208-11.

108. Mayer DE, Krauskopf A, Hemmer W, Moritz K, Jarisch R, Reiter C. Usefulness of post mortem determination of serum tryptase, histamine and diamine oxidase in the diagnosis of fatal anaphylaxis. Forensic Sci Int. 2011;212(1-3):96-101.

109. Edston E, Eriksson O, van Hage M. Mast cell tryptase in postmortem serum-reference values and confounders. Int J Legal Med. 2007;121(4):275-80.

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