Diploma Thesis

"DERMATOLOGICAL SYMPTOMS IN SYSTEMIC MASTOCYTOSIS"

Disease spectrum in patients with elevated tryptase

Submitted by

Sandra Savić

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Under the supervision of

Ao. Univ. Prof. Dr.med. univ. Elisabeth Aberer

And

Univ. Prof. Dr. med. univ. Werner Aberer

Graz January 2012

Affidavit

I, hereby, declare that the following diploma thesis has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of this thesis other than those indicated in the thesis itself.

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Key words

Mastocytosis

Tryptase

Anaphylaxis

Abstract

Background

Mastocytosis is a rare disease that has systemic and skin manifestations. It is often hard to diagnose, especially in patients with a lack of skin symptoms. One of the characteristics of mastocytosis is an elevated tryptase.

Aims

1. To investigate patients with elevated tryptase for their underlying diseases.
2. To see whether increased tryptase can be used as a diagnostic marker for underlying mastocytosis or whether it is only a sign for a mast cell activation.
3. To see whether or not there is a need for full medical examination for an underlying mastocytosis in patients with a mast cell activation syndrome and elevated tryptase.
4. To define how specific is an elevated tryptase for mastocytosis.

Methods

In this retrospective study conducted at the Department of Dermatology, Division of Environmental Dermatology and Venerology, data of 96 patients with elevated tryptase (>15µg/l) were collected and systematically evaluated. The patients were classified in diagnostic groups, and statistical analysis was performed.

Results

Only 15.6% of our patients had mastocytosis. The rest of the patients was divided as follows: 36.4% had anaphylaxis, 26% urticaria and angioedema, 4.2% increased local reactions after insect stings, 3.1% drug reactions, and 14.6% different diagnoses. Seven patients had systemic mastocytosis (SM) with skin involvement, five SM without skin involvement and three had urticaria pigmentosa (UP). Patients with SM with skin involvement had highest mean tryptase levels (65.9µg/l). followed by SM - patients without skin involvement (53.5µg/l), UP patients (30.9µg/l), patients who had drug reactions (27.4µg/l), urticaria and angioedema patients (25.1µg/l), patients with anaphylaxis (21.7µg/l), patients with increased local reactions (21.3µg/l) and finally patients who had different diagnoses (19.9µg/l).

Conclusion

Elevated tryptase is not only a sign for mastocytosis but also seen in urticaria and angioedema, drug reaction and anaphylactic reactions during an acute event.

Repeatedly elevated tryptase levels should be determined as a specific marker for mastocytosis. In these patients appropriate investigations should be done. Patients with high tryptase and severe anaphylaxis should be inspected for skin lesions of mastocytosis and their baseline serum tryptase should be determined. In the case of elevated baseline tryptase, these patients should get a total diagnostic body work up for SM.

Zusammenfassung

Grundlagen

Die Mastozytose ist eine seltene Erkrankung mit Organ- und Hautbeteiligung. Häufig ist sie schwer zu diagnostizieren, besonders bei Patienten die keine Hautsymptome zeigen. Eine der Besonderheiten der Mastozytose sind erhöhte Tryptasewerte.

Ziele

1. Patienten mit erhöhter Tryptase auf ihre zugrunde liegende Erkrankungen zu untersuchen.
2. Zu ermitteln, ob man erhöhte Tryptase als diagnostischen Marker für Mastozytose verwenden kann oder ob dieser Laborparameter nur ein Zeichen der Mastzellaktivierung ist.
3. Zu erforschen, ob man die Patienten mit Mastzellaktivierung Syndrom und erhöhter Tryptase auf Mastozytose weiter medizinisch untersuchen sollte.
4. Zu ermitteln wie spezifisch eine erhöhte Tryptase für Mastozytose ist.

Methoden

In einer retrospektiven Studie wurden die Krankengeschichten von 96 Patienten der Allergie Ambulanz der Univ.-Klinik für Dermatologie überprüft, bei denen eine erhöhte Tryptase über 15µg/l vorlag. Eine Zuordnung der Krankheitsbilder wurde vorgenommen. Die dabei erhobenen Daten wurden statistisch ausgewertet.

Ergebnisse

Nur 15,6% der Patienten mit erhöhter Tryptase hatten eine Mastozytose. Die übrigen Patienten zeigten in 36,4% eine Anaphylaxie, in 26% Urtikaria und Angioödem, in 4,2% verstärkte Lokalreaktion nach Insektenstichen, in 3,1% eine Arzneimittelreaktion und in 14,6% verschiedene andere Diagnosen. Sieben Patienten hatten eine systemische Mastozytose (SM) mit Hautbeteiligung, fünf eine SM ohne Hautbeteiligung und drei wiesen eine Urticaria pigmentosa (UP) auf. Die höchsten Tryptasemittelwerte fanden sich bei SM mit Hautbeteiligung (65,9µg/l) gefolgt von SM - Patienten ohne Hautbeteiligung (53,5µg/l), UP - Patienten (30,9µg/l), Patienten mit Arzneimittelreaktionen (27,4µg/l), mit Urtikaria und Angioödem (25,1µg/l), Patienten mit Anaphylaxie (21,7µg/l), solche mit

verstärkter Lokalreaktion (21,3µg/l) und schließlich Patienten mit verschiedenen anderen Diagnosen (19,9µg/l).

Konklusion

Erhöhte Tryptase ist nicht nur ein Zeichen für Mastozytose, sondern wird auch bei Urtikaria und Angioödem, Arzneimittel- und anaphylaktischen Reaktionen während eines akuten Ereignisses gefunden. Wiederholt erhöhte basale Tryptasewerte können als spezifischer Marker für Mastozytose verwendet werden. Deshalb ist eine Durchuntersuchung bei diesen Patienten erforderlich. Patienten mit schwerer Anaphylaxie und erhöhter Tryptase sollte man dermatologisch untersuchen und ihre basale Tryptase bestimmen. Falls die basale Tryptase erhöht ist, liegt der Verdacht auf SM vor. Diese Patienten sollten auf eine systemische Mastozytose untersucht werden.

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List of Abbreviations

AML	acute myeloid leukaemia
ASM	aggressive systemic mastocytosis
BM	bone marrow
CM	cutaneous mastocytosis
CML	chronic myeloid leukaemia
CT	computer tomography
D	diameter
e.g.	exempli gratia=for example
FACS	Fluorescence Activated Cell Sorting
GI	gastrointestinal
H1-RA	histamine H1 receptor antagonists
H2-RA	histamine H2 receptor antagonists
i.e.	id est=that is
IFN-alpha	interferon-alpha
IgE	Immunoglobulin E
IgG	Immunoglobulin G
ISM	Indolent systemic mastocytosis
MCL	Mast cell leukemia
MCS	Mast cell sarcoma
MDS	myelodysplastic syndrome
MEDOCS	medical documents system
MPD	myeloproliferative disorder
NSAID	nonsteroidal antiinflammatory drug
n.p.	not performed
n.u.	not usable
SCF	stem cell factor
SM	systemic mastocytosis
SM-AHNMD	SM with an associated clonal hematologic non-mast cell lineage disease
TMEP	telangiectasia macularis eruptiva perstans
TK	tyrosine kinase

UP	urticaria pigmentosa
VIT	venom immunotherapy
WHO	World Health Organisation

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1 Introduction

Mastocytosis is a skin and systemic disease with involvement of multiple organs, that appears both in children and adults. The disease is characterized by elevated serum tryptase and the occurrence of anaphylaxis and other mediator induced symptoms.
Symptoms and clinical course are very heterogeneous due to a variable degree of local or systemic mediator release or organ dysfunction as a result of mast cell infiltrates. Pruritus, wheals, flushing and gastrointestinal symptoms are often described. (1)

In mastocytosis the risk for anaphylactic reactions following an insect sting (and other causes of mast cell activation) is increased significantly. (1)

Diagnostic hallmarks are biopsies from skin and bone marrow using tryptase antibodies for staining as well as serum tryptase levels. The determination of serum tryptase levels (normal value under 11.4µg/l) is in principle a good diagnostic and differential diagnostic parameter, also in evaluation of the disease course. The level correlates with the mast cell load and its activation. (1)

In the dermatological allergy outpatient clinic several patients with allergic reactions are seen, which have elevated serum tryptase. However, it is unknown which of these patients have underlying mastocytosis. Within one year in our allergy outpatient clinic, 96 patients with elevated tryptase were recorded. Aim of our study was to evaluate which underlying diseases these patients have, especially mastocytosis.

2 Review of Literature

2.1 Mastcell

Mast cells are found in all vascularized tissue, especially in the skin and mucous membranes of the respiratory and gastrointestinal tracts and are important effector cells of the immune system. They originate from CD34+ hematopoietic stem cells of the bone marrow and differentiate into mast cell precursors that are distributed to different tissues through blood. (1)

Mast cells have a rather unique position among cells of the immune response. Their progenitors are bone marrow derived, yet under normal conditions appear in the mature state only within vascularized tissues, where they have a long life expectancy. (2) After migrating into tissues, these immature mast cells assume their typical granular morphology. Circulating mast cell precursors express CD34, the tyrosine kinase kit (CD117), and IgG receptors, but not high-affinity IgE receptors. (3)

Mediators of human mast cells include proteoglycans, proteases, and histamine. They are preformed and stored in cytoplasmic granules within the mast cell. These mediators appear together in structured complexes that allow components such as histamine to be quickly disassociated upon contact with the extracellular environment. Tryptases and chymases are the major protein components of mast cell secretory granules and are released upon mast cell degranulation. (2)

Various mast cell proteases have been associated with a number of potential functions, including bronchoconstriction, and the degradation of fibrinogen, extracellular matrix proteins, and endogenous and exogenous peptides. These proteases also hydrolyze chemokines and cytokines and inactivate allergens and neuropeptides. (2)

The mast cell was, until recently, foremost viewed as an effector cell in allergic diseases. New findings have shown, however, that mast cells also play a crucial part in defense against pathogens and have an important immunoregulatory role in many processes such as wound healing, tumor control, and transplant tolerance. (4) Mast cells and IgE have long been associated with the pathogenesis of the

acute manifestations of the immediate hypersensitivity reaction, the pathophysiologic hallmark of allergic rhinitis, allergic asthma, and anaphylaxis. Their central role in these disorders is widely accepted. (2)
Growth, differentiation, proliferation, survival and in part mediator activation of mast cells are controlled by (among other factors) the tyrosine kinase receptor c-kit (cognate receptor, CD117) and its ligand stem cell factor (SCF), also of key importance in the development of mastocytosis. (1)

2.2 Mastocytosis

2.2.1 Definition

Mastocytosis is an uncommon disorder of the hematopoietic stem cell that has quite heterogeneous clinical manifestations. A detectable clonal expansion of mast cells is present in adults, often with inadequate mediator release. The causal mechanisms are less well-understood in children. Most frequently affected are the skin and bone marrow, whereas a mast cell infiltration of other organs, i. e. spleen lymph nodes, gastrointestinal tract or liver, is less frequently observed. The location and degree of infiltration and cell activation determine the highly variable clinical and morphological characteristics. (1)

2.2.2 Epidemiology

Mastocytosis is a very rare disease, exact numbers with regard to frequency of mastocytosis are lacking. In various studies an incidence of 5 to 10 new cases per one million population per year was calculated. (1) Mastocytosis can present at the time of birth or develop any time thereafter into late adulthood. Childhood mastocytosis is defined as that with an onset before puberty. Approximately 55% of mastocytosis patients develop their disease by 2 years of age, and another 10% experience disease onset between the ages of 2 and 14 years. This disorder has no gender preference, and it has been reported in all races. While most patients with mastocytosis have no family history of the disorder, to date there have been approximately 70 familial cases of mastocytosis reported, including at least 15 sets

of monozygotic twins (although several monozygotic twin pairs discordant for mastocytosis have also been described). Familial mastocytosis has been documented in three generations of a single kindred. (3)

2.2.3 Pathogenesis

Kit receptor of the mast cell is the protein product of the proto-oncogene c-kit located on chromosome 4q12, and it belongs to the type III receptor thyrosine kinase subfamily. (3) It is expressed on a variety of cell types, including mast cells, hematopoietic progenitor cells, melanocytes, germ cells, and gastrointestinal pacemaker cells. Kit is down-regulated from the surface of progenitor cells as they differentiate into their respective mature forms. Mast cells are one exception to this phenomenon, as they retain surface kit expression at high levels as mature cells. (2)

The ligand for kit is stem cell factor (SCF), which is an important growth factor for mast cells. SCF is produced by bone marrow stromal cells, fibroblasts, keratinocytes, endothelial cells, and reproductive Sertoli and granulosa cells. (3)

The activation of the kit receptor (via SCF) leads to increased proliferation, prolonged survival and increased mediator release in both normal as well as clonal-neoplastic mast cells. (1)

In over 90 % of adults with mastocytosis the somatic activating kit point mutation on exon 17 at codon 816 (substitution of aspartate by valine in the tyrosine kinase domain of the kit receptor or Asp-816-Val or kitD816V) can be detected. This mutation leads to autophosphorylation of the receptor resulting in endogenous-autonomous mast cell proliferation. The detection of kitD816V does not allow for a prognosis on the course of mastocytosis. Other rare forms of activating point mutations, not only on exon 17, have been reported. In children mutagenesis is much less homogeneous as in adults. In a recent systematic study on cutaneous biopsies of 50 children with mastocytosis the kit mutation at codon 816 on exon 17 was detected in 42 % of cases, in one-half of cases mutations were observed in the extracellular domain of kit on exon 8 and 9. All mutations were somatic and led to kit activation. (1)

2.2.4 Clinical Findings

2.2.4.1 Signs and Symptoms

The symptoms of mastocytosis vary widely. (4) Many children and adults have few, if any, symptoms. When symptoms do occur, they are due to the diverse physiologic effects of secreted mast cell mediators, such as histamine, eicosanoids and cytokines. These complaints and findings may range from pruritus, dizziness and syncope. Of interest is the relative absence of pulmonary symptoms in mastocytosis. Complaints of fever, night sweats, malaise, weight loss, bone pain, epigastric distress, and problems with mentation (cognitive disorganization) often signal the presence of extracutaneous disease. Deaths associated with extensive mast cell mediator release also are rare, but have been reported in both children and adults. Symptoms of mastocytosis can be exacerbated by exercise, heat or local trauma to skin lesions. In addition, alcohol, narcotics, salicylates and other non-steroidal anti-inflammatory drugs (NSAIDs), polymyxin B, and anticholinergic medications have been implicated in precipitating symptoms of mastocytosis. Some systemic anesthetic agents may precipitate anaphylaxis. (3)

Pregnancy can also intensify the symptoms of SM. SM is very rare in children; here signs and symptoms consist of attacks of flushing and diarrhea. (1)

Anaphylactic reactions have been reported in all forms of mastocytosis; the cumulative incidence is between 22 to over 50 %, much lower in children. Wasp or bee stings are the most common triggers of acute reactions. (1)

A mixed organic brain syndrome with a constellation of symptoms - including irritability, fatigue, headache, poor attention span and motivation, limited short-term memory, inability to work effectively, and difficulty in interacting with other people - have been described in patients with mastocytosis. (3)

2.2.4.2 Cutaneous Lesions

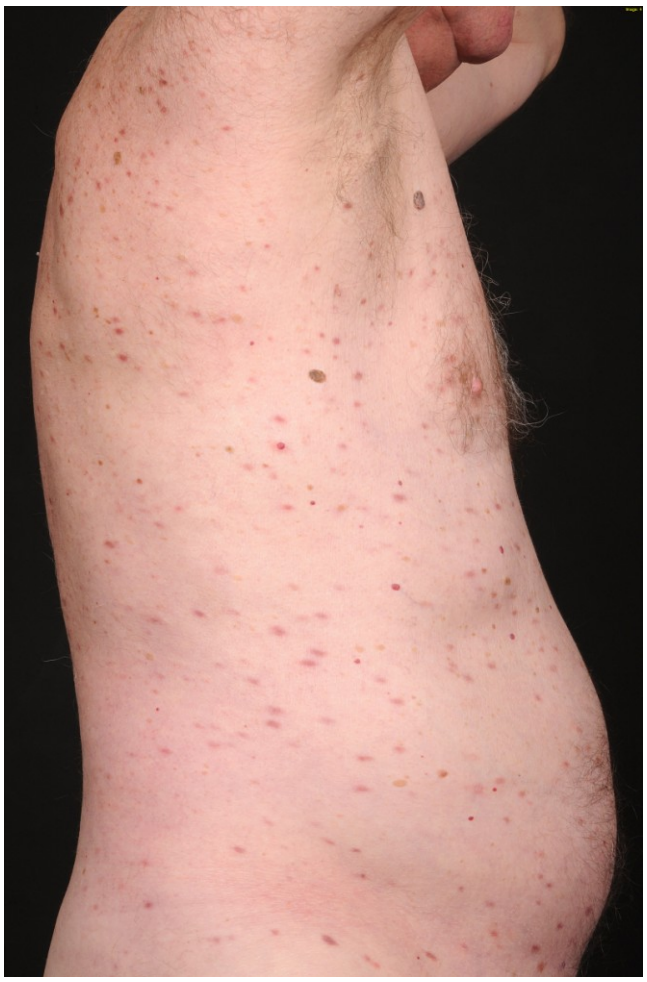

Figure 1 Typical dark-red maculopapular skin lesions (urticaria pigmentosa) in adults

Around 80% of patients develop characteristic brownish-red skin lesions. In adults, these maculopapular lesions are generally less than 0.5 cm in diameter and initially occur particularly on the thighs and trunk (Figure 1). In children, the efflorescences are usually larger (0.5 to 3 cm) and often affect the entire integument (Figure 2), typically including the head and the lateral face. Both forms are referred to as maculopapular cutaneous mastocytosis (previously: urticaria pigmentosa). Solitary mastocytoma of the skin is rare (Figure 3). Mastocytomas are brown or brownish-red, usually well defined and raised. After mechanical irritation with a wooden spatula, the various mastocytic skin lesions show reddening and urticarial swelling (Darier's sign). After the reddening has worn off it may be followed by a white anemic discoloration of a few minutes in duration. (4) An unusual cutaneous subtype is diffuse erythrodermic mastocytosis, characterized by uniform yellowish-red coloration of the entire integument (Figure 4). Mechanical irritation of the mastocytosis foci leads to release of mast cell mediators and thus to reddening, urticarial swelling, and itching (Darier's sign). The small maculopapular skin lesions can occur not only in the purely cutaneous but also in the systemic mastocytoses. Patients with skin lesions frequently suffer from itching and hives, particularly when exposed to heat or cold. In young children the mastocytosis lesions may be associated with vesicles (bullous cutaneous mastocytosis). (4)

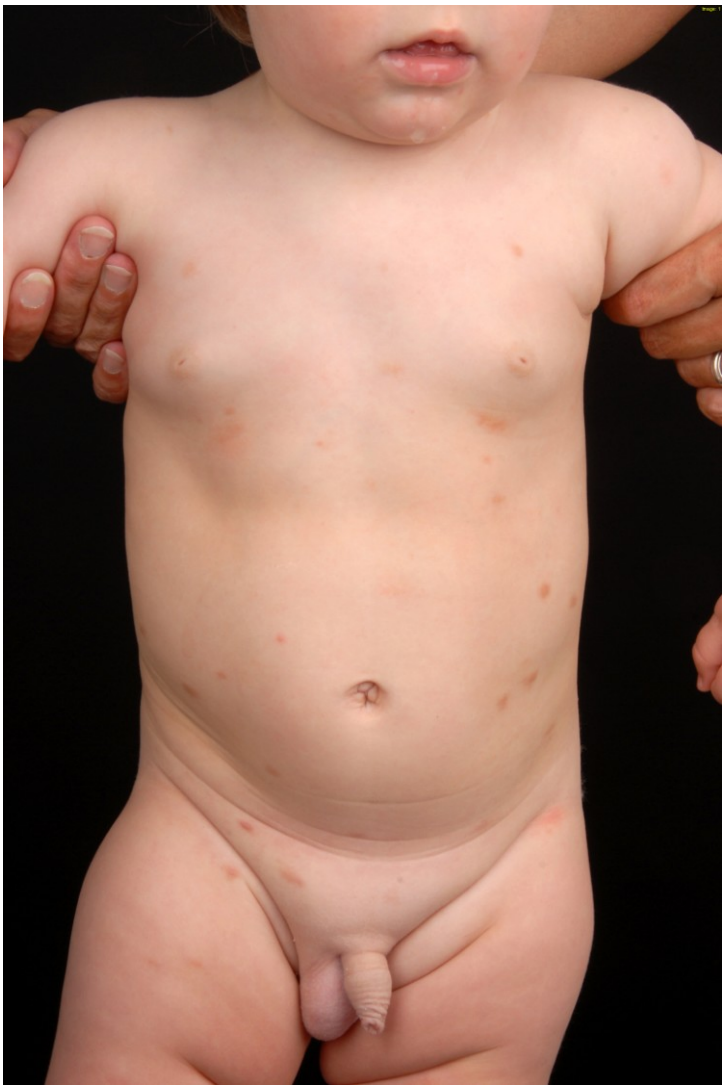

Figure 2 Urticaria pigmentosa in a child with brownish maculo-papular lesions

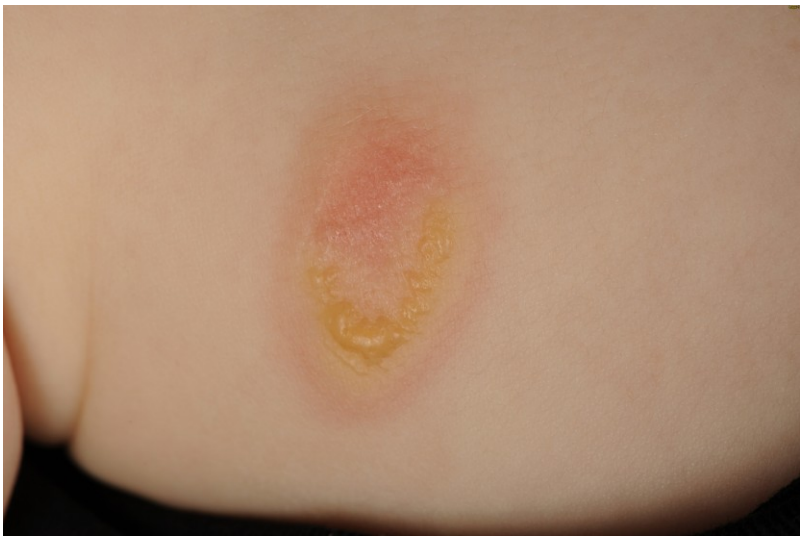

Figure 3 Solitary mastocytoma of the upper right leg with bullous reaction

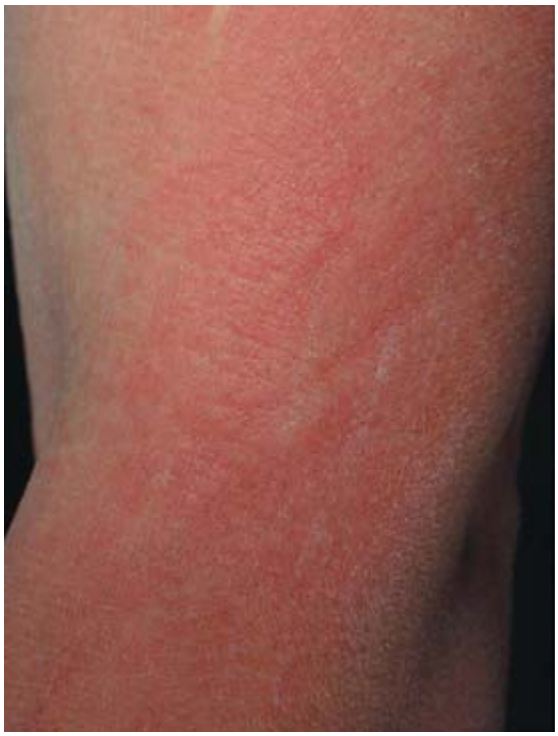

Figure 4 In diffuse cutaneous mastocytosis the skin is uniformly yellowish-red in color and thickened; histological examination shows marked mast cell proliferation in the dermis (4)

2.2.4.3 Systemic Manifestations

Skeletal lesions commonly occur in adult patients with mastocytosis, but they are rarely seen in children. The scull, spine and pelvis are most commonly involved. In one large study of 58 adult systemic mastocytosis patients, 57% had diffuse bone involvement, wheras only 2% had focal lesions. Demineralisation was the most common change in patients with diffuse skeletal disease, followed by osteosclerosis and osteoporosis. (3)

Splenomegaly, detected either clinically or by CT scan, has been reported in 50% to 60% of adult mastocytosis patients. Increased numbers of mast cells and eosinophils are frequently observed in the spleen, as are various degrees of fibrosis and hematopoiesis. Lymph node enlargement is uncommon in most mastocytosis patients, but occurs in patients with more advanced systemic disease. (3)

Hepatomegaly has also been documented in some systemic mastocytosis patients. (3)

2.2.5 Histopathological Findings

The diagnosis of mastocytosis is established by demonstrating characteristic mast cells in one or more organs. For patients with cutaneous lesions, mast cell infiltrates can be demonstrated in a biopsy of lesional skin. Special stains, such as toluidine blue, Giemsa and Leder, or monoclonal antibodies that recognise tryptase or CD117 (kit) are helpful for identifying tissue mast cells. Biopsy specimens of normal-appearing skin from patients with mastocytosis have normal concentrations of mast cells, and thus are not helpful in establishing the diagnosis. A biopsy of the bone marrow or GI tract may be indicated for patients in whom the

diagnosis of mastocytosis is possible, but who lack skin lesions. Increased mast cell numbers in association with variable numbers of eosinophils are observed in these tissue specimens. In addition, the combination of monoclonal antibodies against CD117 (kit) and tryptase may be particularly useful for identifying atypical mast cells in various tissues. (3)

The bone marrow, spleen, liver, and lymph nodes have been recognized as the most common sites of pathologic mast cell infiltrates in systemic mastocytosis. The bone marrow is currently thus recognized as the most useful biopsy site, and is required for establishing the pathologic diagnosis and for staging, and should include inspection of the aspirate. Examination of the bone marrow both reveals diagnostic infiltrates and allows study of the hematopoietic marrow, which provides important prognostic information. Immunohistochemical staining of the bone marrow biopsy with antitryptase used to characterize mast cells into subsets is now the method of choice to visualize mast cells in paraffin-embedded decalcified specimens. (2) (Figure 5)

The morphologic appearance of mastocytosis-related bone marrow infiltrates in trephine core biopsy sections is distinctive. The majority of infiltrates are focal, although the lesions may be diffuse. Focal mast cell lesions are most commonly situated paratrabecularly, followed by perivascular and parafollicular distributions.

Focal aggregates of spindle-shaped mast cells are often accompanied by lymphocytes and eosinophils. In the case of patients with tryptase-positive round cell infiltrates where the infiltrates comprise greater than 95% round cells and less than 5% spindle-shaped cells, application of additional immunohistochemistry markers to confirm the diagnosis of mastocytosis should be applied, since basophils and sometimes blast cells also express tryptase. Mastocytosis infiltrates in the bone marrow may be associated with osteosclerotic or osteolytic changes in the bone trabeculae. (2)

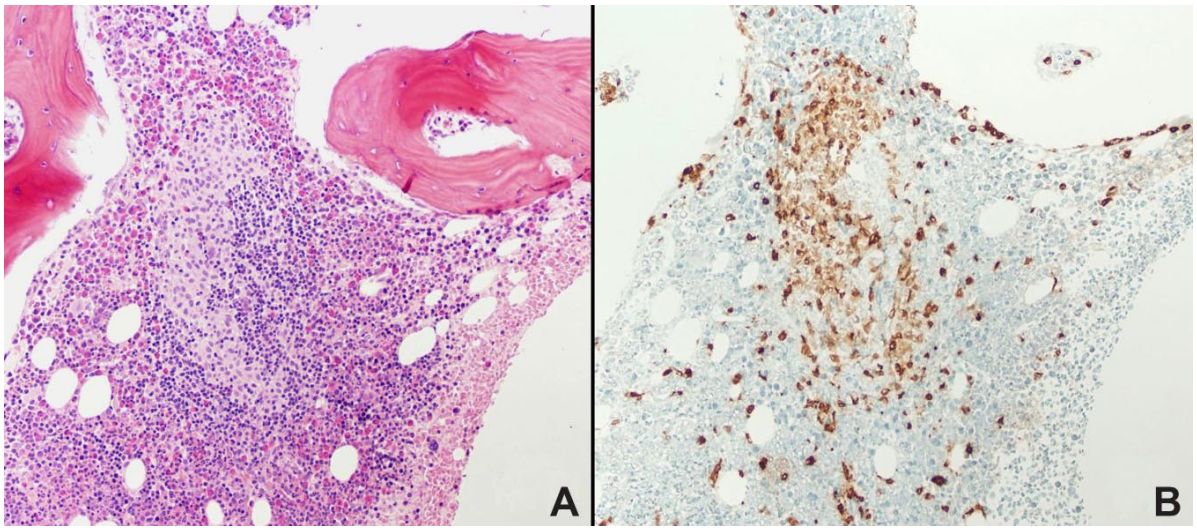

Figure 5 Bone marrow biopsy from a patient with systemic mastocytosis. Hematoxylin and eosin staining is shown in panel A, tryptase staining in panel B. Note the focal collections of mast cells, major criteria for the diagnosis of systemic mastocytosis. (2)

2.2.6 Classification of Mastocytosis

The first attempt to classify the mastocytoses was made in 1979 by the pathologists Lennert and Parwaresch of Kiel, Germany. After various modifications, in 2000 an international group of experts agreed on the categorization that was then adopted by the WHO in 2001 and remains largely unchanged in the revised 2008 WHO publication on the classification of hematological neoplasias. (4)

The WHO classification differentiates 7 categories: cutaneous mastocytosis, indolent SM, SM-AHNMD, ASM, mast cell leukemia, mast cell sarcoma and extracutaneous (benign) mastocytoma. The most important forms of cutaneous mastocytosis are also cited here. (1) (Table 1)

Disease	Signs and symptoms	Prognosis *quoad vitam*
Cutaneous mastocytosis		
Maculopapular cutaneous mastocytosis (urticaria pigmentosa, UP)	Brown or red-brown maculae and papules; in adults < 0,5 cm D, usually larger in children; positive Darier test	Good
- Special form: plaque form	(red-)brown plaques; positive Darier test	Good
- Special form: nodular form	(red-)brown nodules; positive Darier test	Good
-Special form: telangiectasia macularis eruptiva perstans (TMEP)	Brown maculae and erythema with telangiectases on trunk and limbs; positive Darier test	Good
Diffuse cutaneous mastocytosis	Yellow-red erythroderma-like, diffuse doughy-firm swelling of the entire integument (peau chagrine); positive Darier test	Good
Solitary mastocytoma	Isolated (red-)brown node (1–10 cm D); positive Darier test	Good
Indolent systemic mastocytosis (ISM)	Urticaria pigmentosa; often coincidental finding (e. g. due to osteoporosis/ pathologic fractures, anaphylaxis)	Good
-Special form: smouldering SM	Unspecific; few symptoms despite high tryptase levels and high mast cell numbers in bone marrow	Usually good
-Special form: isolated mastocytosis of the bone marrow without cutaneous involvement	Unspecific; often noticed due to severe anaphylactic reactions after insect sting, mast cell infiltration exclusively in bone marrow	Usually good
SM with an associated clonal hematologic non-mast cell lineage disease (SM-AHNMD)	Unspecific; myelodysplastic or –proliferative symptoms of the concomitant disease, e. g. AML, chronic eosinophilic leukemia, very rarely lymphomas; detection of the KitD816V mutation in the AHNMD compartment	Depends on concomitant hematologic disorder
Aggressive SM (ASM)	Unspecific; organ dysfunction depending on site and degree of mast cell infiltration: bone marrow insufficiency with cytopenia, organo-/ splenomegaly, pathologic fractures, malabsorption, cachexia, liver failure, ascites	Heterogeneous, usually poor
Mast cell leukemia (MCL)	> 20 % mast cells in bone marrow aspirate in contrast to ASM; > 10 % mast cells in the peripheral blood smear with atypical	Poor

	morphology	
Mast cell sarcoma (MCS)	Unspecific; histologically high-degree abnormal mast cell morphology in a destructive tumor, in the course generalization and leukemia	Poor
Extracutaneous mastocytomas	Unspecific; benign tumor composed of mature mast cells	Good

Table 1 Classification of mastocytosis (1)

More than 90 % of patients with ISM display typical maculopapular skin lesions of cutaneous mastocytosis (urticaria pigmentosa). In the other forms of systemic mastocytosis cutaneous lesions are only present sometimes. (1)

The current WHO classification also differentiates purely cutaneous from systemic forms of mastocytosis.

Cutaneous mastocytosis is defined as an accumulation of mast cells limited to the skin. Clinically, macroscopically various subtypes are differentiated:

Maculopapular cutaneous mastocytosis or “typical” urticaria pigmentosa (UP) is characterized in adults by disseminated small plaques (Figure 1).

Diffuse cutaneous mastocytosis can involve extensive areas of the skin.

Solitary mastocytoma, in part with blistering, manifests predominantly in childhood and in most cases heals spontaneously (Figure 3).

Much rarer are the bullous variant (often in the first weeks of life), the plaque form, the nodular form as well as telangiectasia macularis eruptiva perstans (TMEP) with multiple hyperpigmented maculae as well as erythema with telangiectases. These variants do not constitute an independent form in the WHO classification.

Systemic mastocytoses (SM) are highly heterogeneous diseases with by definition involvement of at least one extracutaneous tissue (skin involvement is frequent but not obligatory). (1)

In indolent systemic mastocytosis (ISM) mast cell infiltrates are usually found in skin and bone marrow, but also (even if only rarely morphologically proven) in other extracutaneous organs. ISM is the most common form of SM. It differs from prognostically much more unfavorable forms of SM clinically by predominant skin involvement and by the lack of associated hematologic disorders or organ dysfunction. Usually ISM manifests in adulthood. (1)

The rare smoldering SM is differentiated from the more common indolent variety by massive mast cell proliferation and tryptase levels over 200µg/l. Even in smoldering SM no signs of bone marrow insufficiency should be detectable (important differentiation from aggressive SM). A further rare variety of indolent systemic mastocytosis is isolated bone marrow mastocytosis, where mast cell infiltration is only found in bone marrow, but not in the skin. (1)

In a subgroup of patients with SM an associated clonal hematologic disorder not of the mast cell series is detected: SM-AHNMD (systemic mastocytosis associated with a clonal hematologic non-mast cell lineage disease). SM and AHNMD are usually diagnosed simultaneously in iliac crest biopsy. All subtypes of hematologic neoplasia, even malignant lymphomas and plasmocytomas, have been reported within the context of SM-AHNMD. Myeloid neoplasias are especially frequent, particularly chronic myelomonocytic leukemia. The prognosis of SM-AHNMD usually depends on the AHNMD and not on SM. (1)

In aggressive systemic mastocytosis (ASM) the extensive infiltration of tissue depending on the involved organ leads to highly different symptoms as a result of dysfunction with signs of insufficiency (for bone marrow cytopenia and for the liver ascites) being considered as C findings (grading criteria that describe the aggressiveness of the mast cell infiltrate). The serum tryptase level is usually over 200µg/l. The prognosis of ASM is poor. (1,5)

Mast cell leukemia (probably the rarest form of human leukemia) also is a form of SM and is characterized in typical cases by a large number of circulatory tissue mast cells and over 20 % immature mast cells in the bone marrow. The prognosis is poor. (1)

Mast cell sarcoma must be clearly differentiated from SM subtypes. Not more than five sufficiently documented case reports exist and it is also a tumor with a very poor prognosis. (1)

On the other hand, the extremely rare extracutaneous mastocytomas are independently cited as benign tumors of mature mast cells in the WHO classification. (1)

2.2.7 Diagnostic Criteria

The diagnosis of cutaneous mastocytosis and a mastocytoma can usually be made based on history and clinical criteria with careful skin inspection, with cutaneous findings being extremely variable. The Darier sign with urticarial swelling after mechanical rubbing of macules or papules is almost always positive. Inspection of the skin is supplemental by palpation of peripheral lymph node stations and abdominal organs. (1)

For the diagnosis of systemic mastocytosis according to the WHO the following criteria should be fulfilled: (1)

Major criterion

Multifocal compact mast cell infiltrate (with mast cell aggregates of 15 or more cells) in histological sections.

Minor criteria

1. More than 25 % of mast cells have a spindle shape.
2. Activating kit point mutations (most often kitD816V).
3. Atypical mast cell immunophenotype with expression of CD25 (and/or CD2) on mast cells in tissue sections or in FACS analysis.
4. Serum tryptase levels permanently > 20µg/l (with the exception of associated clonal myeloid disorder; criterion not valid in this case).

The diagnosis SM is established in patients in whom at least one major and one minor or at least three minor SM criteria are detected. (5)

On suspicion of mastocytosis is following procedure advisable: (4)

1. Determination of serum tryptase
2. Dermatological examination to confirm or exclude cutaneous mastocytosis
3. Histological/immunohistochemical investigation of the bone marrow (iliac crest trephination) or another tissue (e.g., gastrointestinal mucosa)
4. Molecular investigation of the tissue to confirm or exclude an activating kit mutation in exon 17 (possibly also investigation of microdissected pooled mast cells and, in the case of SM-AHNMD, cells from the associated neoplasia).

Whether a biopsy should be performed in children with a clear clinical diagnosis of mastocytoma depends on the individual case. Due to differential diagnostic discrepancies it is probably wise to advise it even when parents are hesitant. (1)

2.2.8 Differential Diagnosis

Because of the frequently nonspecific symptoms, the differential diagnosis of mastocytosis embraces an unusually broad spectrum of clinical syndromes, e.g. immunological processes such as autoimmune diseases, inflammatory bowel diseases, and carcinoid flush. Many patients have morphologically mild tissue infiltration (as a rule those with indolent systemic mastocytosis) yet suffer greatly from their mediator symptoms, e.g., diarrhea. The morphological differential diagnosis of mastocytosis includes very rare hematological neoplasias such as chronic basophilic leukemia, myelomastocytic leukemia, and tryptase-positive acute myeloic leukemia. The differential diagnosis of the variants with unfavorable prognosis, such as ASM and mast cell leukemia, includes malignant lymphomas as well as myelodysplastic and myeloproliferative diseases. Mastocytosis often presents a special diagnostic challenge for the experienced clinician and the hematopathologist. (4)

2.2.9 Treatment of Mastocytosis

Curative treatment– with the exception of excision of isolated mastocytomas – is not yet available for mastocytosis. In over 95 % of patients with cutaneous mastocytosis or ISM, life expectancy is not reduced. In far more than 50 % of cutaneous mastocytosis in childhood, spontaneous healing takes place before adolescence. In mastocytosis in adulthood the course is usually chronic. In the overwhelming majority of patients SM takes an indolent, benign course. Rare aggressive or leukemic courses of SM almost always, just as mast cell sarcoma, end fatally. (1)

Explanation and counseling with regard to the increased tendency towards anaphylactic reactions is especially important for all patients with mastocytosis. Such a reaction can be triggered by, for example, insect stings, intense physical exertion, intense mechanical friction, extreme temperature changes at the skin

surface (leap into cold water). Alcohol, hotly spiced foods, nonsteroidal anti-inflammatory drugs, muscle relaxants and ß-blockers should be avoided. (1, 4)

Group	Examples
Physical stimuli	Acute temperature change, cold, heat, (intense) rubbing/ friction, pressure, strong sweating/(intense) physical exertion, intense UV-exposition
Psycho-vegetative stimuli	Stress, fear
Food stuffs	Alcohol, particularly with a high content of histamine (especially red wine), very hotly spiced food, large amounts of citrus fruits, strawberries, tomatoes, seafood, cheese, smoked sausages, canned fish, fermented vegetables, soy products
Analgesics	Acetylsalicylic acid , NSAID, codeine, morphine
Antibiotics	Vancomycin, polymyxin B, amphotericin B
Drugs used in general anesthesia	Enfluran, etomidate, isoflurane, thiopental, atracurium, doxacurium, d-tubocurarine, metocurine, mivacurium, rocuronium, succinylcholine
Circulatory drugs	Clonidine, metoprolol
Local anesthetics	Lidocaine
Plasma expanders	Dextran
Psychopharmacological agents	Clomethiazole, midazolam
Radiologic contrast media	Ionic radiologic contrast media
Venoms	Insect and snake venom

Table 2 Selection of possible mast cell activating factors (1)

Carrying emergency medication (oral glucocorticosteroid solution, in small children as suppository or oral soluble histamine H1 receptor antagonist; epinephrine autoinjector or diluted epinephrine solution in small children) and information on use are obligatory. A medical alert tag („mastocytosis pass”) is also recommendable.

Due to the increased perioperative risk of mastocytosis patients, the patient, anesthesist and surgeon should discuss this comprehensively before the

procedure, particularly before intubation narcosis to lower the risk of anaphylaxis. As premedication should be selected antihistamines and glucocorticosteroids in a sufficiently high dosage. Perioperatively should be administered only selected drugs that are proven not to be relevant histamine liberators and provoke mast cell activation. (1)

Most of the patients with mastocytosis have no subjective symptoms, and need no medication. However, at those patients who have complaints, is the treatment based on the improvement of symptoms with pharmacologic agents that inhibit the actions of mediators identified as mast cell derived, especially histamine and the leukotrienes. Treatment of gastric hypersecretion with H2-antihistamines and proton pump inhibitors, treatment of osteoporosis with calcium, vitamin D, and bisphosphonates, treatment of associated inflammation with glucocorticoids, treatment of systemic anaphylaxis-like reactions with epinephrine, and with strategies to treat an associated hematologic disorder or decrease the mast cell compartment. (2) (Table 3)

Symptom	**Therapy**	**Note**
Pruritus, wheals, dermographism, flushing, burning sensation of skin	Non-sedating H1-RA of the 2nd and 3rd generation	• Perhaps high-dose therapy with non-sedating H1-RA • Perhaps combination therapy with H2-RA • Oral PUVA therapy is also effective for pruritus due to urticaria pigmentosa • Acetyl salicylic acid (up to 5 g daily) is effective in some cases of H1-RA-resistent flushing, but can rarely also cause anaphylaxis or lead to exacerbation of symptoms; perhaps gradual dose increase • Perhaps in combination with leukotriene receptor antagonists • In anaphylactoid reactions guidelines on emergency treatment apply • Perhaps omalizumab in therapy-refractory anaphylactic reactions or as an add-on in insect venom hyposensitization
Nausea, vomiting, abdominal pain, colics, diarrhea, gastritis, peptic ulcers, bleeding, malabsorption	Combination of H1- and H2-RA, cromoglycic acid, antacids or proton pump inhibitors, anticholinergic agents, oral steroids	Gradually increase cromoglycinic acid dose

Headaches and other neurological symptoms	Combination of H1- and H2-RA	Perhaps paracetamol (acetaminophen), no NSAID
Bone/ skeletal pain, osteoporosis, pathologic fractures	Calcum, vitamin D, bisphosphonates	• In advanced stages perhaps IFN-alpha • In intense bone pain additional electron beam radiation
Tachycardia, palpitations, hypotension, recurrent syncopes	Combination of H1- and H2-RA, perhaps anticholinergic agents	
Rhinitis, asthma, dyspnea	Combinatin of H1- and H2-RA	• Perhaps additional leukotriene receptor antagonists • Perhaps additional topical antihistamines nasally • Perhaps additional topical corticosteroids • Further therapy according to the guidelines for asthma therapy
Bone marrow insufficiency with cytopenia, organo-/ splenomegaly, pathologic fractures, malabsorption, cachexia, liver failure, ascites	IFN-alpha, cladribine, within clinical studies TK inhibitors (e. g. midostaurine, dasatinib imatinib, among others), hydroxyurea, polychemotherapy	• No fixed dosage schedules exist • IFN-alpha and cladribine are in part combined with systemic corticosteroids • The TK inhibitor imatinib is ineffective in the presence of a KitD816V mutation

Table 3 Recommendations for systemic pharmacotherapy in mastocytosis (1)

2.3 Tryptase

Tryptase is a neutral protease, a mediator produced and secreted by mast cells, and stored in its mature form in the secretory granules of mast cells and basophils. Tryptase is released after crosslinkage of immunoglobulin E receptors by allergens or other degranulating agents.This degranulation of mast cells can be mediated either immunologically or nonimmunologically. Two forms (alpha and beta) of mast cell tryptase have been identified. Both alpha- and pro-beta tryptase are secreted continuously, mature beta-tryptase is stored in mast cell granules and released following mast cell activation, mostly during IgE-mediated allergic reactions,

together with histamine, prostaglandins, leukotrienes and other mediators. The commercially available fluorescence immunoassay measures both alpha- and beta- tryptase. (3, 6, 7)

Detection of circulating mast cell mediators and/or their metabolites can offer indirect evidence of mastocytosis. Alpha-tryptase is elevated in patients with systemic mastocytosis, regardless of whether or not they are experiencing acute symptoms, and therefore may be useful in assessing total body mast cell burden. Beta-tryptase, however, is often detected both in mastocytosis patients and in patients without mastocytosis who are experiencing anaphylactic symptoms. Total (alpha- and beta-) serum tryptase levels have been correlated with the extent of mast cell disease. (3)

The baseline serum level in the absence of an allergic or anaphylactic reaction is considered as an indicator of the whole body mast cell load and, if elevated, may indicate the presence of systemic mastocytosis. When the serum tryptase level increases significantly within 1–5 h after the first symptoms of a suspected systemic hypersensitivity reaction it confirms its mostly allergic, IgE-mediated origin. (6)

2.3.1 Serum Tryptase

The median serum tryptase level in healthy adults is approximately 5µg/l. It may be slightly lower in children. Tryptase levels are not affected by pregnancy, food-intake, or physical exercise. Severely impaired renal function is associated with a slight increase in tryptase. In severe allergic reactions, tryptase may become markedly elevated. Here, the recommended standard is to wait at least two days following clinical resolution before measuring basal tryptase levels. If tryptase remains elevated, SM should be considered. (5)

2.3.2 Tryptase and Mastocytosis

Tryptase remains the recommended serum test to employ in the diagnostic work-up for suspected SM. Almost all patients with SM have serum total tryptase levels exceeding 20µg/l, which is a minor SM-criterion. (5)

The differential diagnosis of an elevated tryptase also includes myeloid non-MC-lineage neoplasms such as acute (AML) or chronic myeloid leukaemia (CML),

myeloproliferative disorders (MPD), and MDS. In these patients, serum tryptase levels may be elevated in the absence of a coexisting SM. Therefore, a tryptase level > 20µg/l counts as minor SM-criterion only in the absence of an AHNMD. (5)
A normal tryptase level does not rule out clonal mast cell disease. However, the likelihood of diagnosing mast cell disease by identifying characteristic multifocal bone marrow aggregates diminishes significantly in those with tryptase levels of less than 20µg/l. (8)

2.3.3 Tryptase Levels in Subtypes of Mastocytosis

Tryptase levels tend to be higher in patients with a high mast cell burden. In most patients with CM, tryptase levels are < 20µg/l. In typical ISM and SM-AHNMD, tryptase levels range from normal to markedly elevated. In ISM, tryptase levels usually remain reasonably constant over years, whereas in ASM or MCL, tryptase levels often increase, especially as the disease progresses. (5)

2.3.4 Changes in Serum Levels of Tryptases in Systemic Anaphylaxis

The concentration of total serum tryptase increases after severe anaphylaxis and anaphylactoid reactions. Tryptase levels are more likely to increase in severe than in mild reactions. For example, in insect sting challenges an increase in serum tryptase levels correlates with hypotension. Therefore a normal tryptase level does not rule out anaphylaxis, but it does make severe anaphylaxis less likely if the sample is drawn within a few hours of the onset of symptoms. Increases of serum tryptase levels in postmortem and forensic cases can support anaphylaxis as a cause of sudden infant death syndrome and other cases of unexplained death. However, serum levels of tryptase can increase nonspecifically shortly before death in trauma and other cases of severe illness, thereby increasing tryptase concentrations in samples obtained after death. Thus a high serum tryptase level in a postmortem specimen by itself might be inadequate to establish a diagnosis of anaphylaxis without other supporting clinical evidence. (9)

2.3.5 Involvement of Tryptases in the Pathogenesis of Anaphylaxis

Although several of lines of evidence in animals and human subjects suggest a role for tryptases in the pathogenesis of asthma and allergic inflammation, there is no direct evidence of a role for tryptases in shock and other clinical manifestations of anaphylaxis. Speculatively, tryptases contribute to the pathology of anaphylaxis by spreading the degranulation signal from mast cell to mast cell. (9)

Tryptases can increase the egress of plasma from blood vessels by inactivating procoagulant proteins and promoting fibrin clot lysis and can promote bronchoconstriction, which could give them a role in rash, tissue swelling, and bronchospasm in anaphylaxis. With specific regard to anaphylactic shock, the time course is such that the increase in tryptase level in blood occurs later than the onset of shock and rash and well after the serum histamine peak. Therefore the appearance of tryptases in blood most likely reflects mast cell activation in a variety of tissue locations but is not itself a cause of anaphylactic shock. (9)

2.4 Anaphylaxis and Mastocytosis

Anaphylaxis is a severe, potentially fatal, systemic allergic reaction that occurs abruptly after contact with an allergy causing substance, it is rapid in onset and may cause death. The most frequent causes of anaphylaxis are foods, insect stings, medications, allergen immunotherapy injections, and idiopathic anaphylaxis. (10)

Many organs are involved in the clinical symptoms of anaphylaxis. These symptoms can be very variable. Intensity grading scale of clinical symptoms of anaphylaxis has proven to be valuable. (Table 4)

Grade	Symptoms			
	Skin	Abdomen	Respiratory tract	Cardiovascular system
I	Pruritus Flush Urticaria Angioedema			
II	Pruritus Flush Urticaria Angioedema (not mandatory)	Nausea Cramping	Rhinorrhea Hoarseness Dyspnea	Tachycardia (>20 beat/min) RR change (>20mmHg systolic) Arrhythmia
III	Pruritus Flush Urticaria Angioedema (not mandatory)	Vomiting Defecation Diarrhea	Laringeal edema Bronchospasm Cyanosis	Shock
IV	Pruritus Flush Urticaria Angioedema (not mandatory)	Vomiting Defecation Diarrhea	Respiratory arrest	Cardiac arrest

Table 4 Grading of anaphylactic reactions according to severity of clinical symptoms (11)

Anaphylaxis begins, most commonly, with skin symptoms like pruritus, flush, urticaria or angioedema. (11) The most severe manifestations of acute anaphylaxis are those involving the respiratory and cardiovascular system. (10)

The risk of an anaphylactic reaction occurring is strongly increased in patients with mastocytosis. (10) Incidents of life-threatening anaphylaxis are a recognized characteristic of a disease. The clinical symptomatology appears to be more severe in allergic patients who also have mastocytosis. These patients may suffer from severe anaphylactic shock after hymenoptera stings or during the course of insect venom hyposensitization. This can even lead to death in some individuals. Anaphylactic episodes may be provoked by certain medications (NSAIDs, codeine, narcotics) in some patients with mastocytosis. Because of the untypical

symptoms, mastocytosis can be masked under the clinical diagnosis of idiopathic anaphylaxis. (12)

According to Brockow et al., the cumulative incidence of anaphylaxis in adult patients with mastocytosis is 49%. They also found that anaphylactic reactions in adults correlated to the absence of urticaria pigmentosa lesions. Adult systemic mastocytosis patients experienced more frequently anaphylaxis (56%) then adult cutaneus mastocytosis patients (9%). Considering the causes of anaphylaxis Brockow et al. found that major observed trigger factors for adults were hymenoptera stings (19%), foods (16%) and medication (9%). However, in 26 % of reactions, only a combination of different triggers preceded anaphylaxis. (12)

3 Aims of the Study

1. To investigate patients with elevated tryptase for their underlying diseases
2. To see if increased tryptase can be used as a diagnostic marker for underlying mastocytosis
3. To see whether or not there is a need for full medical examination of patients with a non mast cell diseases and a mast cell activation symptoms
4. To estimate how specific is an elevated tryptase for mastocytosis

4 Methods

In our study we used data from patients who attended the outpatient clinic for allergic diseases of the Department of Dermatology in Graz. Between 07/2009 and 05/2010 3374 blood samples of patients with different complaints and suspected mast cell activation syndrome were investigated for serum tryptase levels. Out of these 3374 patients, 96 (2.8 %) had an increased serum tryptase (higher than 15µg/l). We conducted a retrospective study that included these 96 Patients. Clinical and laboratory data from these patients were analysed. Clinical history was taken out of the medical documents system (MEDOCS). The patient data were entered into the Excel 2007 data base and descriptive statistical analysis was performed. This project was approved by the local ethics committee.

5 Results

5.1 Clinical Diagnoses in Patients with Elevated Tryptase

Of the total 96 patients with elevated tryptase 35 had anaphylaxis, 25 urticaria and angioedema, 7 systemic mastocytosis with skin involvement, 5 systemic mastocytosis without skin involvement, 4 increased local reactions to insect stings , 3 drug reactions, 3 urticaria pigmentosa and 14 other diagnoses.
Forty-seven patients were males and 49 females (1:1.04). The details about the male/female distribution in each group are shown in Table 5.
The average age of all patients with elevated tryptase was 47 years (max 98, min 18, median 21). The youngest patient was 18 and had urticaria. The oldest patient was 98 years old and had a drug-induced angioedema. Further details in Table 5.

5.2 Tryptase Levels

We examined data from 96 patients with serum tryptase greater than 15µg/l. In 43 patients, serum tryptase was between 15 and 20µg/l and in 53 patients ≥ 20µg/l. Total serum tryptase range was between min 15 and max 195µg/l, mean 27.7, median 21.0.

Number of patients	**Diagnosis**	**Sex (m/f)**	**Serum tryptase**				**Age**		
			mean	**min**	**max**	**median**	**mean**	**min**	**max**
35	Anaphylaxis	17/18	21.7	15.1	38.5	19.2	47	19	79
25	Urticaria and angioedema	15/10	25.1	15.4	111.0	20.0	42	18	98
7	Systemic mastocytosis with skin involvement	2/5	65.9	19.0	195.0	44.3	46	30	73
5	Systemic mastocytosis without skin involvement	1/4	53.5	31.7	77.0	48.6	51	42	70
4	Increased local reactions	2/2	21.3	16.6	33.1	17.8	47	23	65
3	Drug reactions	1/2	27.4	17.9	44.1	20.2	76	51	94
3	Urticaria pigmentosa	2/1	30.9	15.6	46.3	30.9	40	24	51
14	Other diagnoses	7/7	19.9	15.0	26.9	19.2	48	20	79

Table 5 Disease patterns in patients with elevated tryptase

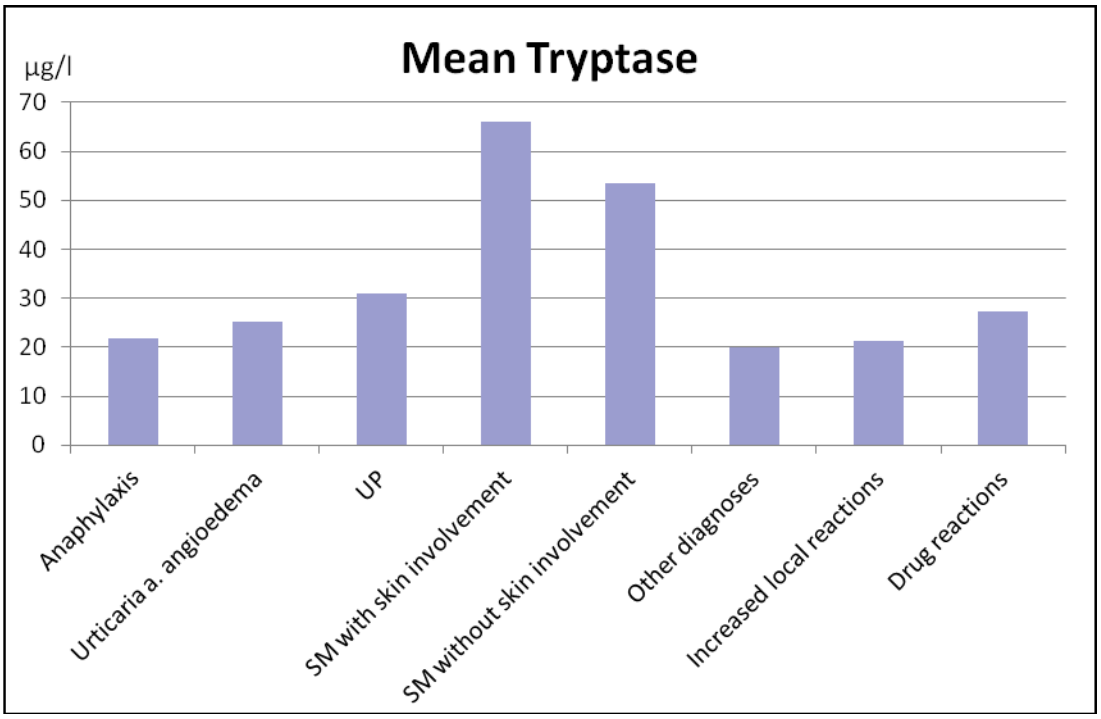

Figure 6 Mean tryptase levels according to diagnoses

5.3 Laboratory Findings

The blood cell count was investigated in 58/96 patients and was in all patients normal.

IgE levels were investigated in 73 patients, of whom 30 patients (41.1%) had IgE levels >100, and 43 (58.9%) IgE levels ≤100. The patients with IgE>100 had anaphylaxis (n=13), urticaria and angioedema (n=8), increased local reactions (n=2), drug reactions (n=2) and in 5 cases other diagnoses.

Specific IgE antibodies could be detected in 11 patients against bee and wasp venom, 9 against wasp, 7 patients against bee, 5 against bee, wasp and hornet, and 2 against wasp and hornet venom.

In 62 patients no specific IgE levels were determined.

5.4 Body Workup and Imaging Analyses for Organ Involvement of Mastocytosis

5.4.1 Bone Marrow Biopsy

In 22 of 96 patients bone marrow biopsies were performed, with the following results. In 12 patients there were positive and bone marrow involvement of systemic mastocytosis could be proven. Seven patients had SM with skin involvement, and 5 SM without skin involvement. Bone marrow biopsies were negative in 8 patients (including one patient who had a small diffuse mast cell infiltration, one patient with a non-specific reactive change and one had a bone marrow biopsy with <10% mast cells) and the diagnosis of SM was not confirmed. One bone marrow biopsy was not usable and one showed myelodysplasia (RAEB-2).

Ten patients were investigated regarding the existence of a kit gene mutation. In 3 patients kit gene mutation was proven. These patients had SM with skin involvement. Six patients had no detectable mutation (2 anaphylaxis, 2 SM without skin involvement, 1 urticaria and 1 UP). In one case the sample was not usable (SM with skin involvement).

5.4.2 Bone Scintigraphy

Bone scintigraphy was performed in 24 patients. Two showed bone marrow involvement in the context of SM (1 SM with and 1 SM without skin involvement). Two scintigraphies were questionable pathologic. This patients had anaphylaxis and increased local reaction. One finding described a central bone marrow activation in a patient with SM with skin involvement. In 19 patients, no changes of the bone marrow was detected.

5.4.3 Osteodensitometry

Osteodensitometry was performed in 13 of 96 patients with the following results: 4 had osteoporosis, 4 osteopenia and 5 had a normal bone density. Out of the 4 patients with osteoporosis, 2 had SM with skin involvement, 1 anaphylaxis and 1

urticaria and angioedema. Two of the patients with osteopenia had SM with skin involvement, one had anaphylaxis, and one another diagnosis.

5.4.4 Sonography

In forty four patients an upper abdominal and lymph node sonography was performed. One patient had splenomegaly (SM with skin involvement), one had a mild hepatomegaly (anaphylaxis patient) and one a borderline large spleen (urticaria and angioedema). In the remaining 41 cases the findings were normal.

5.4.5 Endoscopy

Endoscopy (gastroscopy and/or coloscopy) was conducted in 22 of 96 patients whereby no specific mast cell staining was applied. In one patient (with urticaria pigmentosa), a slight increase in mast cells in the ileum and colon was noticed. In 21 cases, no pathological changes were detectable.

	Medical examinations				
Diagnosis (Nr.of patients)	Osteodensitometry	Bone scintigraphy	Bone marrow biopsy	Kit gene mutation	Abdominal sonography
Systemic mastocytosis with skin involvement n=7	-2 osteoporosis -2 osteopenie -3 n.p.	-1BM involvement -1central bone marrow activation -4 normal -1 n.p.	-7 positiv	-3 positiv -3 n.p. -1 n.u.	-1Splenomegaly -6 normal
Systemic mastocytosis without skin involvement n=5	-1 normal -4 n.p.	-1BM involvement -2 normal -2 n.p.	-5 positiv	-2 negativ -3 n.p.	-3 normal -2 n.p.
Urticaria pigmentosa n=3	-3 n.p.	-1 normal -2 n.p.	-1 negativ -2 n.p.	-1 negativ -2 n.p.	-1 normal -2 n.p.

Table 6 Results of body workup in patients with mastocytosis

5.5 Concomitant Symptoms in Patients with Elevated Tryptase

5.5.1 Hypersensitivity Reactions

In 77 patients the following reactions occurred: 26 had urticaria (including 16 acute, 9 chronic and 1 autoimmune form), one of those patients, with chronic urticaria had SM with skin involvement. 44 patients had a grade II-IV anaphylaxis (including 22 grade II, 18 grade III and 4 grade IV), including 3 patients with SM with skin involvement (1 grade II and 2 grade III reaction), and 5 patients with SM without skin involvement (1 grade II, 2 grade III and 2 grade IV anaphylaxis). In 4 patients severe local reactions after insect bite occurred and 3 had drug reactions. In 19 of 96 patients no allergic reactions were noted. (Fig.6)

The causes for anaphylaxis were insect bites in 32 patients, drugs in 6 patients and food in one patient. There was one case of an exercise-induced anaphylaxis and in 2 patients there was a combination of different causes. In 2 patients the causes of anaphylaxis were unknown. (Fig.7)

In the group of urticaria pigmentosa, one patient had anaphylaxis caused by wasp venom. Patients with SM without skin changes had anaphylaxis to wasp venom (n=2), wasp and bee venom (n=2) and wasp venom and food (n=1). SM with skin involvement patients had NSAID caused anaphylaxis (n=2), and wasp venom, bee venom, food and NSAID caused anaphylaxis (n=1).

5.5.2 Other Symptoms in the Contex of Mast Cell Activation

Furthermore the following symptoms occurred: 2 patients had flushing (both with SM without skin involvement), 4 had recurrent diarrhea and colics (1 anaphylaxis, 1 urticaria and angioedema , 1 SM with and 1 SM without skin involvement), 4 had headache (3 anaphylaxis patients and 1 patient with SM with skin involvement), 5 had pruritus (1 urticaria and angioedema, 4 other diagnoses), 5 had depression (2 anaphylaxis, 1 SM with skin involvement, 1 SM without skin involvement, 1 other diagnosis) and 2 had fatigue (both with other diagnoses). None of the patients had polyuria.

5.6 Therapy

5.6.1 Hyposensitization

Specific immunotherapy was carried out in 23 of 96 patients. In12 patients against wasp venom, in 5 against bee venom, in 4 against bee and wasp venom and in 2 against mites.

5.6.2 Treatment

Out of 96 patients, 10 received a treatment for their symptoms. Three patients got PUVA therapy, 2 got H1-blockers, 1got H2-blockers, 1 got H1- and H2 blockers, 1 got H1-, H2-blockers and interferon alpha, 1 got H1-, H2- blockers, PUVA, oral glucocorticoids and imatinib, and 1 cyclosporine A. (Table 6)

Therapy	**Number of patients**	**Diagnosis**
PUVA	3	1 Urticaria pigmentosa, 2 SM with skin involvement
H1-Blocker	2	1 SM without skin involvement, 1 chronic urticaria
H2-Blocker	1	SM with skin involvement
H1-Blocker+H2-Blocker	1	SM with skin involvement
H1-Blocker+H2-Blocker+Interferon alpha	1	SM without skin involvement
H1-Blocker+H2-Blocker+PUVA+oral glucocorticoids+imatinib	1	SM with skin involvement
Cyclosporine A	1	Autoimmune urticaria

Table 7 Therapy in patients with elevated tryptase

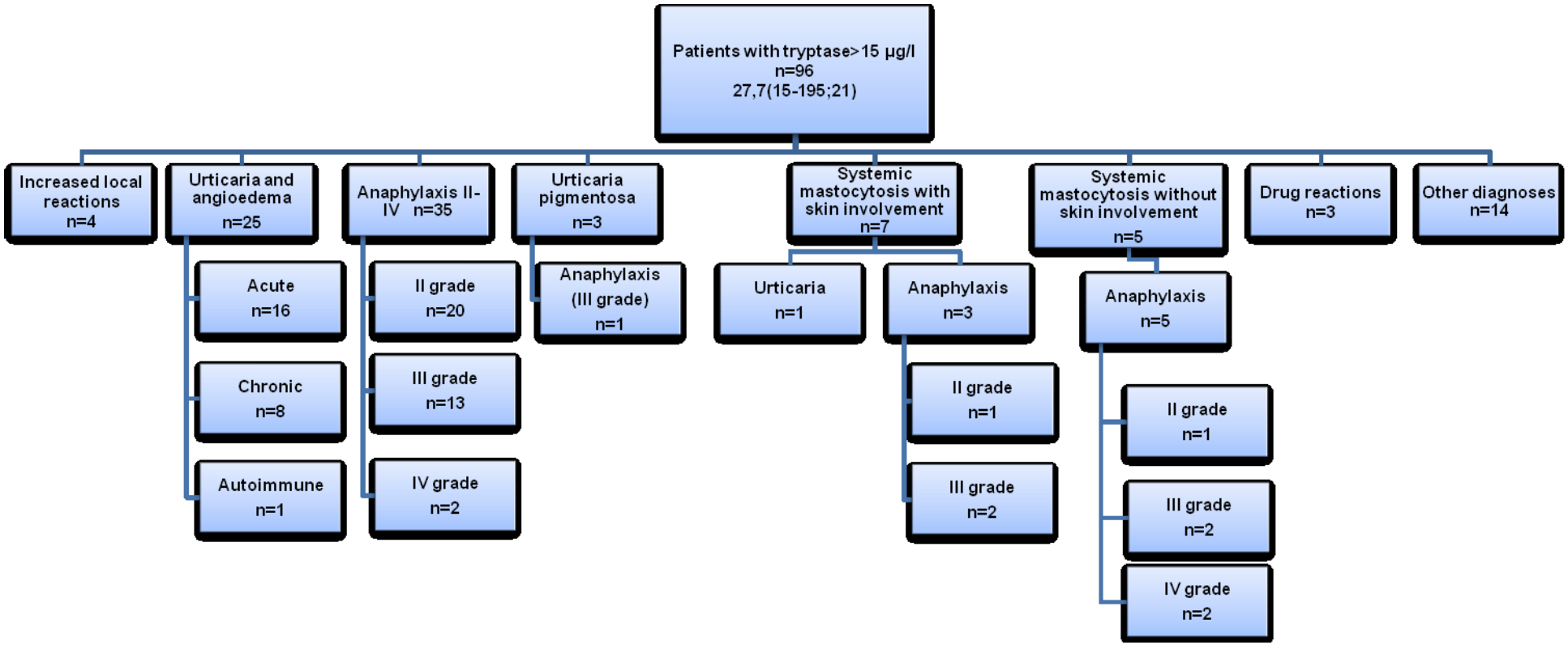

Figure 7 Clinical diagnoses in patients with tryptase>15µg/l mean (min-max;median)

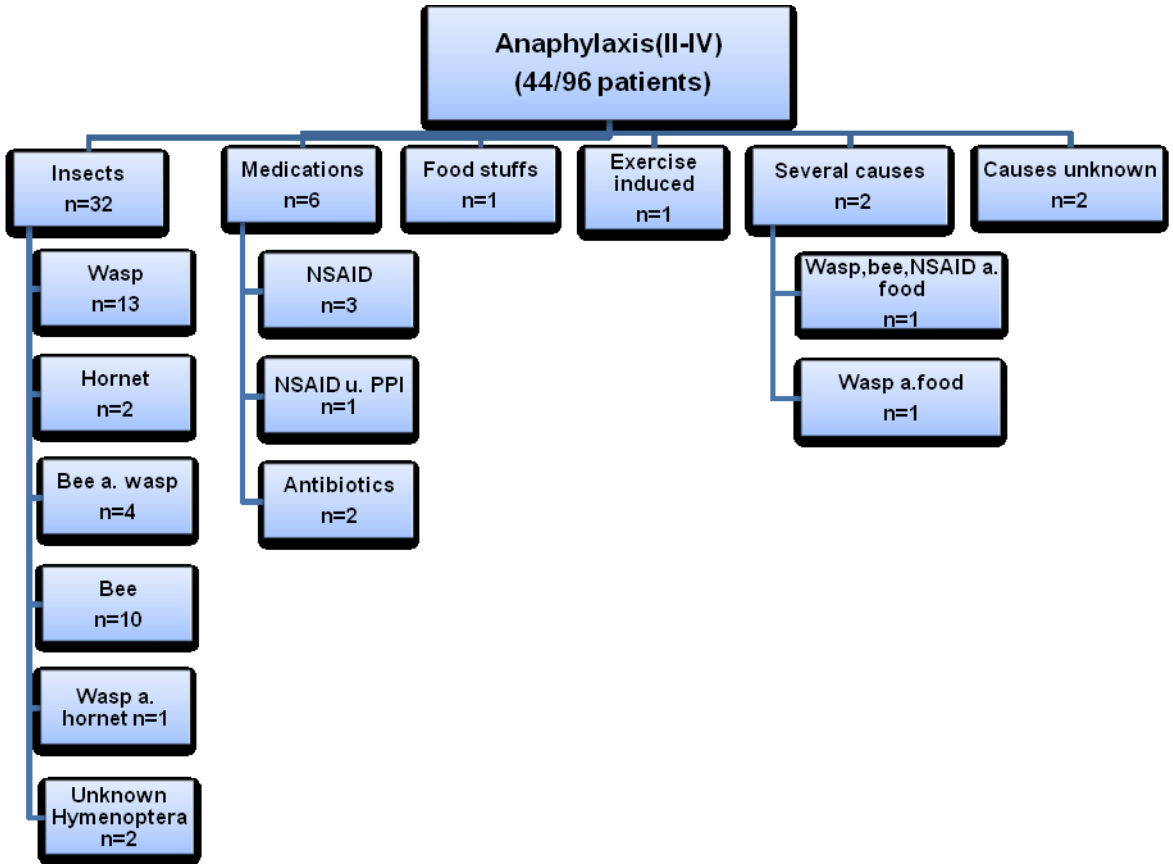

Figure 8 Causes of anaphylaxis(II-IV)

6 Discussion

In our study 96 (2.8 %) of 3374 patients with clinical symptoms of mast cell activation syndrome indicated by pruritus, flushing, urticaria, up to severe anaphylaxis, headache, abdominal pain, diarrhoe and dyspnea showed elevated tryptase levels (higher than 15µg/l).

We have analysed the spectrum of underlying diseases in 96 patients with increased tryptase. Out of 96 patients 36.4% had anaphylaxis, 26% urticaria and angioedema, 7.3% SM with skin involvement, 5.2% SM without skin involvement, 4.2% increased local reactions, 3.1% drug reactions, 3.1% urticaria pigmentosa and 14.6% other diagnoses.

The highest mean tryptase levels were observed in patients with SM with skin involvement, followed by SM without skin involvement and urticaria pigmentosa patients. Furthermore, high mean tryptase was apparent in patients with drug reactions, then in urticaria and angioedema patients, followed by anaphylaxis and increased local reaction patients and finally in patients with other diagnoses. (Table 5, Figure 6)

In the study of Brockow et al. serum tryptase concentrations were higher in those mastocytosis patients with anaphylaxis indicating an association with the severity of systemic involvement. Higher serum tryptase values reflect a higher total systemic mast cell number and pool of effector cells, possibly leading to a higher risk of spontaneous mast cell degranulation. (12)

All together, 45.8% of patients with elevated tryptase had a history of anaphylactic reactions, including 33.3 % of patients with UP, 42.8 % of patients with SM with skin involvement, and even 100% (all 5) of patients with SM without skin involvement . That corresponds to the results of Brockow et al. who found that the risk of anaphylaxis was higher in patients with absence of cutaneous lesions compared with those presenting cutaneous lesions of urticaria pigmentosa. (12)

Elevated IgE (above 100) was found only in patients who had allergic reactions (increased local reaction, drug reaction, urticaria and angioedema, anaphylaxis), and not in mastocytosis patients.

According to Brockow et al. causes for anaphylaxis in mastocytosis do not differ from triggers of anaphylaxis in patients without mastocytosis. These are

hymenoptera stings, foods and medications. (12) In our patients 72.7% of reactions were triggered by hymenoptera stings, and 13.6 % by medications. Other causes were foods, exercise, combined or unknown triggers. Our mastocytosis patients had similar causes of anaphylaxis, that is bee and wasp venom, food and NSAID, as described by Brockow et al.

Rueff et al. found that in patients with hymenoptera venom allergy, baseline serum tryptase are associated with the risk for severe anaphylactic reactions. After a field sting 21.4% of their patients had severe anaphylactic reactions, and the frequency of this event increased significantly with higher tryptase contentrations. (13) That corresponds with our results, in our patients with elevated tryptase, 23% had severe allergic reactions defined as grad III and IV reactions.

Schwartz implied that the individual mast cell burden is an important predictor for the severity of secondary reactions after venom exposure. (14) Therefore, we would expect that our patients who had urticaria and angioedema most likely do not have mastocytosis, because one might expect more severe reaction out of mastocytosis patients.

According to Rueff et al., severe side effects and risks can be expected in patients with mastocytosis during venom immunotherapy (VIT). The frequency of systemic side effects during VIT is about 24%. Rueff et al. found that in patients undergoing VIT, even minor elevations of baseline serum tryptase are associated with more frequent severe systemic reactions during buildup, especially in wasp venom allergic patients, severity of allergic reactions correlates closely with baseline serum tryptase concentration. On the other hand, patients with bee venom allergy need an especially high degree of surveillance during VIT, since a bee venom allergy is an independent predictor for a higher risk during VIT. Rueff et al. concluded that before VIT, measurement of baseline tryptase should be used to identify patients with a high risk for side effects. (15) Specific immunotherapy was carried out in 24% of our patients, but we have no data regarding side effects. We can conclude that a VIT should be performed with particular caution in patients with elevated tryptase, especially in those with mastocytosis.

According to Rueff, it could be useful to include the whole range of tryptase concentrations (currently accepted upper limit 11.4µg/l might be inadequate) in the decision, which patient after a reaction to a field sting, should be offered immunotherapy. (13) It could be shown in this study that the predicted risk for

severe anaphylactic reactions increased markedly above a concentration of approximately 5µg/l. Measurement of baseline serum tryptase concentration in patients with Hymenoptera venom allergy might help identify those with concurrent mastocytosis or a monoclonal mast cell activation syndrome. (13, 16)

Routine screenings of serum baseline tryptase levels in patients with systemic reactions caused by Hymenoptera stings is not a common medical praxis. Usually, only these patients with typical skin lesions of CM get the whole diagnostic work up for SM. However, Olano et al. found that 76% of patients with Hymenoptera venom allergy and SM do not have skin involvement. (16, 17) So it is suggested that patients with elevated baseline tryptase and systemic reactions to Hymenoptera stings should also be investigated for SM. (16) Therefore, in our patients with systemic anaphylactic reactions after Hymenoptera stings, a basal tryptase investigation should be performed in future.

Seidel et al. reported on a 43-year-old patient who died in the consequence of wasp sting with a low tryptase level (few hours after a sting 10.4µg/l, basal level 3.1µg/l). He had an UP and undiagnosed CM with Hymenoptera venom allergy which was not detected before, so the authors suggested that a thorough dermatological examination and detailed medical history should be undertaken in all patients with history of severe allergic reactions. They also recommend lower upper limit for a normal tryptase concentration referring to Rueff et al. (13, 18)

In our group of SM patients, regardless of skin involvement, out of 12 patients, 4 had bone involvement (33%). In fact 2 had osteoporosis (17%) and 2 had osteopenie (17%). One of the patients had no bone pathology and in 7 cases we had no data considering possible bone involvement. According to Barete et al. in their large group of SM patients, half of the patients had bone involvement, and osteoporosis was the most prevalent bone manifestation (31%). (19) It would be advisable to conduct further investigations (skeletal x-rays and bone mineral density (BMD) assessment) in those patients who have not been examined yet.

Considering skin symptoms in mastocytosis, we had 3 patients with CM (UP) and 7 patients with SM with skin involvement. All of these 7 SM patients had urticaria pigmentosa. One of them had additional telangiectasia macularis eruptiva perstans, and in one patient UP regressed after 26 years. Out of 10 patients with skin changes in form of urticaria pigmentosa, in 7 patients (SM) bone marrow

biopsy was positive (70%), in 1(UP) it was negative (10%) and in 2 (UP) it was not performed (20%). This correlates with the results of Topar et al., who found that 60% of patients with urticaria pigmentosa had mast cell infiltrates of the bone marrow. They also found that bone marrow involvement in UP patients does not seem to correlate with clinical symptoms of mast cell activation, and they question whether or not the BM biopsy examination would be useful for the further medical treatment. (20)

In our opinion the knowledge of BM involvement is important for two reasons. Most severe anaphylactic reactions can be expected in these patients which is relevant for immunotherapy and in situations where there is a risk for mast cell degranulation such as after administration of certain drugs. Second, patients with c kit mutation have a risk of disease progression into a more aggressive form. (21)

Escribano et al. found that an indolent systemic mastocytosis in adults has a low disease progression rate, and the great majority of patients have a normal life expectancy. The presence of kit mutations in all hematopoietic lineages is strongly associated with disease progression and indicates the need for periodic follow-up. (21) Three of our patients had a kit gene mutation. These patients have SM with skin involvement, and should consistently get controlled.

The main limitation of our study is that we used one time serum tryptase values and not baseline tryptase for our data evaluation. Sera had been collected in our patients mostly during an acute event, what would explain high tryptase levels.

7 Conclusion

- Only a minority (15.6%) of patients with elevated tryptase had mastocytosis. Most of the patients had anaphylaxis, urticaria and angioedema followed by other diagnoses.
- We found that in our group of patients with elevated tryptase, highest tryptase levels were observed in SM patients, followed by CM patients, so we can conclude that elevated tryptase could be used as diagnostic marker for mastocytosis.
- In those patients who show repeatedly elevated tryptase values, mastocytosis diagnosis should be considered, and adequate investigations should be carried out. Patients with additional anaphylaxis should be inspected for skin lesions of mastocytosis and their baseline serum tryptase should be determined. In the case of elevated baseline tryptase, these patients should get a total diagnostic body work up for SM.
- Only repeatedly elevated baseline tryptase levels are a specific marker for mastocytosis.

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