Clinical Aspects of Pediatric and Adult Onset Mastocytosis in the Skin

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Klinische aspecten van mastocytose in de huid bij kinderen en volwassenen

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Clinical Aspects of Pediatric and Adult Onset Mastocytosis in the Skin

Klinische aspecten van mastocytose in de huid bij kinderen en volwassenen

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Contents

CHAPTER 1

Introduction and Aims of the study

Preface

The term mastocytosis can be summarized as an accumulation of mast cells without an apparent cause. In the majority of the cases the disease is manifest in the skin and can be diagnosed on clinical suspicion and skin biopsy. Mastocytosis has a wide clinical spectrum and it is not a disease limited to the skin. As cutaneous manifestations of mastocytosis form one of the key features of this rare disease, the dermatological aspects of this disease continue to be relevant.

History of the mast cell and mastocytosis

In 1878 Paul Ehrlich was the first investigator to describe cells that stained reddish—purple with aniline dyes in connective tissues. He called them "mästzellen"[1, 2]. Nettleship and Tay described mastocytosis in the skin in 1869 as a symmetrical maculopapular eruption with urtication of lesions after rubbing[3]. In 1878 Sangster proposed the term 'urticaria pigmentosa' (UP) for such lesions [4] a term now replaced by maculopapular mastocytosis. Mast cells were recognized as the cellular substrate of skin lesions by Unna in 1887[5]. The term mastocytosis was first used by Sézary et al in 1936[6]. Multiple organ involvement in a patient with mastocytosis in the skin was reported by Ellis in an autopsied patient in 1949[7]. Bony changes in patients with UP were observed by Sagher in 1957 and a malignant form of mastocytosis presenting as leukemia was reported in 1957 by Efrati[8,9].

Biology of the mast cell

Mast cells (MCs) originate from CD34+ hemopoietic progenitor cells (stem cells) in the bone marrow (BM) [10,11]. Stem cells are self-regenerating and may differentiate into various types of committed progenitors. Commitment of differentiation is considered to be determined randomly by intrinsic mechanisms. Erythroid, mast cell, eosinophil and neutrophil progenitors can survive, differentiate, and proliferate only in the presence of the appropriate growth factors[12]. Human MCs require a much longer period to develop compared with other cell lineages[12]. Generally, MCs mature in tissues in which they are normally present. Mature MCs are not present in peripheral blood. Mature MCs have the capacity to proliferate[11].

Studies in rodents showed that the life span of MCs varied from weeks to months. Mast cells are normally present in all connective tissues where they may be particularly numerous beneath the epithelial surfaces of the skin, in the respiratory system, in the gastrointestinal and the genitourinary tracts, adjacent to blood or lymphatic vessels, and near or within the peripheral nerves[13].

There are two types of MCs in tissue—the mucosal (MC_T) and the connective tissue (MC_{TC}) MCs. The two types are distinguished on the basis of structural, biochemical, and functional differences and have been well characterized. The connective tissue MCs are the predominant type in the normal skin and in the skin of patients suffering from maculopapular mastocytosis.

Staining of mast cells

Mast cells are identified in tissue sections by their characteristic granules that stain metachromatic after exposure to basic dyes[11,14]. A distinct array of cytoplasmic and cell surface antigens are also expressed in MCs[15]. The surface antigen stem cell factor receptor (KIT/CD117) is the most important growth factor receptor in MCs. It is typically expressed on MCs in various organs independently of the maturation stage of MCs or cell activation[15]. Mast cells differ from each other in the expression of mediators, the response to diverse stimuli and the expression of cell surface antigens depending on the environmental and other factors[16-19].

Staining of tissue sections with hematoxylin & eosin, which is routinely used in pathology laboratories is neither specific nor reliable for establishing the presence of MCs. Other histochemical and immunohistochemical stains, such as toluidine blue, mast cell tryptase and chymase, Leder stain, and CD117 are more specific for MCs. The classic histochemical stain used to demonstrate MCs is the metachromatic stain toluidine blue. The membrane-bound granules in the MC cytoplasm contain biologically active mediators including acidic proteoglycans, which bind basic dyes such as toluidine blue. Since the stained granules typically acquire the color that is different from that of the native dye, they are referred to as metachromatic granules[20].

The most specific method for identifying MCs in tissues is by immunohistochemical staining for mast cell tryptase. During the past 10 years, a number of useful techniques for the enumeration and phenotypic analysis of MCs in the bone marrow have been developed. Most of these techniques are based on the unique expression of the stem cell factor receptor KIT on these cells. In fact, in the bone marrow, KIT is expressed on MCs and CD34+ hemopoietic progenitors, but not on other mature hemopoietic cells [21-27].

Stem cell factor

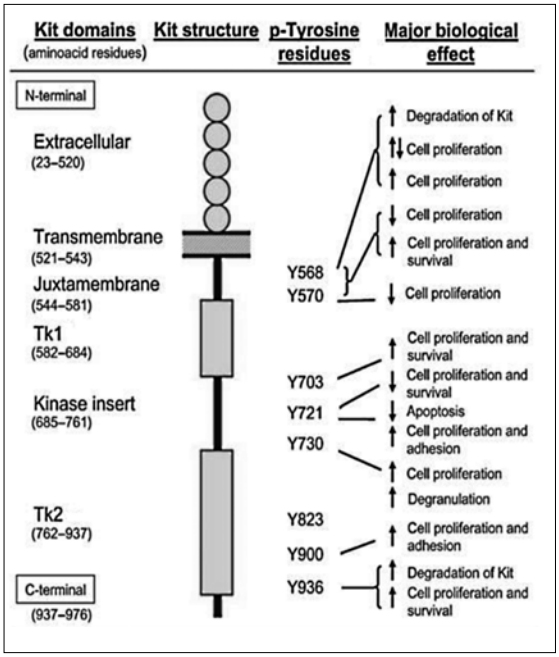
Stem cell factor (SCF) is a major migration, proliferation, maturation and survival factor. It is a cytokine produced by a variety of cells including mesenchymal cells and fibroblasts. It is synthesized as a transmembrane protein and is proteolytically cleaved to generate a soluble form[28]. Soluble SCF molecules exist as homodimers in the plasma and cross-link two KIT receptor molecules when they bind to the cell surface. Cross-linking of KIT results in the activation of the tyrosine kinase enzymatic activity[29].

Stem cell factor is expressed in various tissue microenvironments in which MCs normally develop. Precursors

of MCs require the expression of the proto oncogene c-kit, which gives rise to KIT a transmembrane receptor with intrinsic tyrosine kinase activity, for the normal response to SCF[30-32].

In normal cells, KIT has been shown to play a major role in hematopoiesis (in the differentiation of erythroid, lymphoid, megakaryocytic and myeloid precursors)[33], gametogenesis[34], MC development and function[35], melanogenesis[33] and gastrointestinal function[36]. Activation of the SCF/KIT signaling pathway in MCs is associated with multiple biological effects depending on the activated cell. Among others, these effects include cell proliferation, maturation/differentiation, and suppression of apoptosis, degranulation and changes in the adhesion properties and motility of the activated cells[37]. Expression of the KIT protein has been reported in both normal progenitors, on normal mature MCs and various other cell types as well as neoplastic cells from the gastrointestinal tract, lung, breast and myeloid and lymphatic origin[38]. In humans, the encoding gene for KIT (c-kit) is located on chromosome 4q12. Genomic DNA of human c-kit spans approximately 89 kb and contains 21 exons which are transcribed/translated into a receptor molecule with a molecular mass of 145 kD and 976 amino acids in length[39,40].

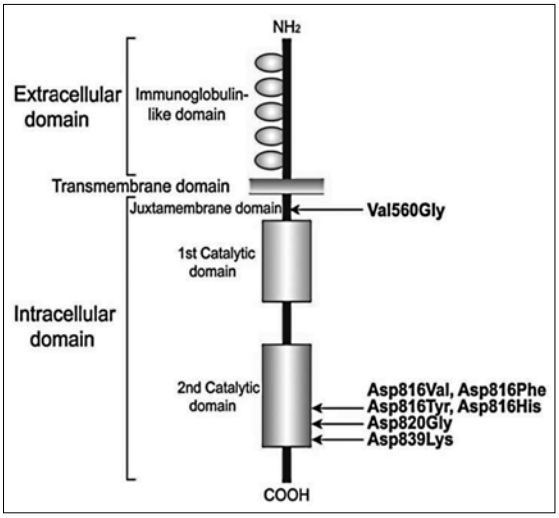
Figure 1. KIT structure and effects.



Adapted from Orfao A, Garcia-Montero AC, Sanchez L, Escribano L; REMA. Recent advances in the understanding of mastocytosis: the role of KIT mutations. Br J Haematol 2007, 138(1):12-30

The following domains are recognized; the extracellular domain: five immunoglobulin-like loops of the extracellular domain of KIT. The first three immunoglobulin (Ig)-like loops of the extracellular domain form the binding site for SCF[41,43]. The fourth and fifth loops play a role in stabilizing the SCF-induced KIT dimer[44,45]. The transmembrane domain, the juxtamembrane domain; this auto inhibitory juxtamembrane domain contains alpha-helical elements whose proper configuration is essential for the down regulation of tyrosine phosporylation[44,46,47]. The TK domain, this domain is the kinase portion of KIT is composed of two domains which are separated by a kinase insert: (1) the TK1 domain contains the ATP binding site, and; (2) the TK2 domain containing the phosphotransferase site and the activation loop (Fig 1)[38]. Based on the localization of the phospho (p)-tyrosine binding sites, three preferential regulatory sites of KIT have been identified with activating and/or inhibitory effects on one or more downstream signaling transduction pathways: (1) the juxtamembrane domain; (2) the tyrosine kinase insert; and (3) the activation loop in the TK2 domain[38].

Figure 2. Sites of most common points of mutation in mastocytosis in KIT.



Adapted from:Yanagihori H, Oyama N, Nakamura K, Kaneko F. c-kit Mutations in patients with childhood-onset mastocytosis and genotype-phenotype correlation. J Mol Diagn 2005, 7(2):252-7

Occurrence of different point mutations and frame deletions/insertions of KIT have been shown to cause alterations of the downstream KIT signaling pathways that convert the KIT proto-oncogene into an active, dysregulated (ligand independent) oncoprotein capable of inducing neoplastic transformation of normal KIT expressing cells[48]. The more frequently observed gain of function

mutations in the KIT sequence, in association to a specific disease or group of diseases include acute myeloid leukemia; gastrointestinal stromal tumor; nasal and nasal-type NK/T-cell lymphoma; mastocytosis; melanoma; myeloproliferative disorder; seminoma/germinoma. Known loss of function mutation in C-kit in human leads to piebaldism which in fact is inherited in an autosomal manner[49]. Figure 2 shows a scheme of the most common sites of mutation in mastocytosis.

C-kit mutations

Multiple KIT mutations have been reported in patients with mastocytosis; many of these mutations are associated with KIT phosphorylation and downstream activation, independent of SCF binding[50]. In order to better understand the impact of KIT mutations Longley et al (2001) proposed that the activating KIT mutations be classified into two major groups based on their topological localization[51].

The '*regulatory type*' mutations that typically affect regulation of the kinase activity of the KIT molecule by disrupting the auto inhibitory a-helix at the juxtamembrane domain of KIT. These mutations affect the binding of signal transducing or regulatory molecules to KIT and/or inducing ligand-independent dimerisation and activation; most frequently these 'regulatory type' mutations occur at the juxtamembrane domain of KIT[52].

The second type are the '*enzymatic pocket type*' mutations which directly affect the enzymatic site at the TK2 activation loop and induce activation of KIT in the absence of dimerisation of the receptor[51].

The large majority (>90%) of adult cases with systemic mastocytosis (SM), mutations in the activation loop of KIT (most frequently D816V) are detected in MC in association with an aberrant CD25+ phenotype[53]. Despite the fact that the D816V KIT mutation is present in >90% of SM, with the exception of rare cases of SM and MCL the exact frequency of this mutation in patients with cutaneous mastocytosis (CM) remains unknown[53]. Accordingly, while a significant proportion of CM cases with a childhood onset do not show the D816V KITmutation[54] KIT mutations at codons 509, 533, 815, 816 and 839 have been reported in adult CM patients [55-58]. Interestingly an analysis of c-kit mutation in skin biopsy specimens from a large group of adults with mastocytosis in the skin showed a significantly different proportion of D816V mutation depending on the age of onset. The patients with disease starting at childhood had D816V in 44% of the cases versus 77% in adult onset mastocytosis[59]. Altogether, the greatest frequency of KIT mutation is found at codon 816 and is consistent with

Table 1. Known KIT mutations in mastocytosis

Domain	Exon	Mutation	Consequense	Frequency of mutation	Comments	Reference
Extracellular	8	delD419	Unknown	<5	Familial SM	Hartmann et al (2005)
	9	K509I	Unknown	<5	Familial SM	Zhang et al (2006)
Transmembrane	100	F522C	Activating	<5	SM	Akin et al (2004)
	10	A533D	Activating	<5	Familial CM	Tang et al (2004)
Juxtamembrane	11	V559I	Activating	<5	ASM	Nakagomi and Hirota (2007)
	11	V560G	Activating	<5	ISM, MCL	Furitsu etal (1993); Buttner etal (1998)
Activation loop	17	R815K	Unknown	<5	Paediatric up	Sotlar et al (2003)
	17	D816V	Activating	>90	Adult SM, Paediatric UP	Garcia-Montero et al (2006)
	17	D816V	Activating	…	Paediatric up, Paediatric UP	Yanagihori et al (2005)
	17	D816Y	Activating	<5	SM	Longley etal (1999)
	17	D816H	Unknown	<5	SM-AML	Pullarkat etal (2003)
	17	D816F	Activating	<5	SM	Longley etal (1999)
	17	I817V	Unknown	<5	WDSM	Garcia-Montero et al (2006)
	17	insV815I 816	Unknown	<5	SM	Garcia-Montero et al (2006)
	17	D820G	Unknown	<5	ASM	Pignon etal (1997)
	17	E839K	Inactivating	<5	UP	Longley etal (1999)

CM, cutaneous mastocytosis; SM, systemic mastocytosis; AML, acute myeloblastic leukemia; ISM, indolent systemic mastocytosis; UP, urticaria pigmentosa (=maculopapular mastocytosis); CML, chronic myeloid leukemia; MCL , mast cell leukemia MF, myelofibrosis; MPD, myeloproliferative disorder; ASM, aggressive systemic mastocytosis; WDSM, well-differentiated systemic mastocytosis

Adapted from Orfao A, Garcia-Montero AC, Sanchez L, Escribano L; REMA. Recent advances in the understanding of mastocytosis: the role of KIT mutations.Br J Haematol. 2007, 138(1):12-30

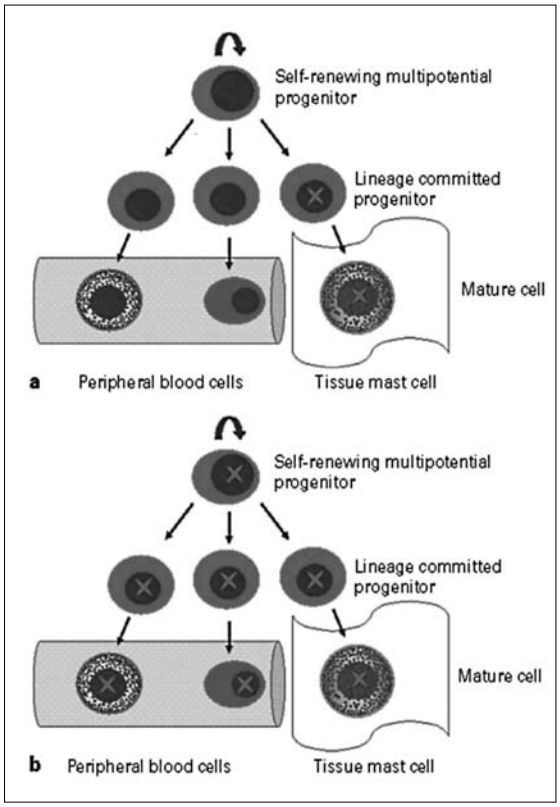
systemic mastocytosis[53,59]. These observations support the notion that genetic examination of the KIT mutational status of MCs from lesional skin, BM or other extracutaneous organs (peripheral blood, spleen, liver, lymph nodes and pleural fluid), is of great help for the differential diagnosis of CM versus SM[60]. Furthermore the CD25 expression on cutaneous mast cells in mastocytosis in the skin appears to be predictive of systemic mastocytosis[61].

Impact of KIT mutation/activation

One of the most frequent and evident clinical manifestations of mastocytosis is the increased proliferation and accumulation of neoplastic MCs in different organs and tissues. This increased proliferation and survival of MCs is because of constitutive activation of KIT. Although, there is an increased numbers of MCs in the skin, BM and other tissues in patients with SM, a large variation in the overall MC burden is encountered in individual patients. KIT mutations have been identified not only in MCs from SM patients, but they may also be found in other BM hemopoietic cell compartments, particularly among CD34+ hemopoietic progenitor and precursor cells (HPC), eosinophil , neutrophil and monocytic precursors[53]. Interestingly, the frequency of patients with involvement of KIT mutation in BM cell compartments other than MCs is significantly lower among patients included within those types of mastocytosis associated with a good prognosis, in comparison with cases of aggressive systemic mastocytosis (ASM), mast cell leukemia (MCL) and systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease (SM-AHNMD). C-Kit mutation may involve the mast cell progenitors at different levels of commitment with varying potentials for expansion. The D816V KIT mutation may affect a committed MC progenitor in patients with limited indolent disease. In contrast, patients with more extensive disease variants, such as smoldering systemic mastocytosis or aggressive mastocytosis, may have an earlier pluripotential progenitor cell affected, resulting in multilineage hemopoietic involvement similar to other myeloproliferative disorders (Fig. 3)[62-64].

Altogether, these results suggest that SM patients showing multilineage involvement of BM hemopoietic cells may represent more advanced stages of the disease. However, the relatively stable course of the disease in most SM patients and the observation that the same KIT mutation (e.g. D816V) may be associated with indolent (good prognosis) and malignant tumors[53] underline the potential role of other genetic and/or epigenetic factors in determining the progression/outcome of the disease[64]. It is recently suggested that KIT D816V alone is sufficient to cause indolent systemic mastocytosis with a favorable course. Additional defects may be required to cause severe types of systemic mastocytosis[65].

Figure 3. Explanation of varying clonal expansion



Hypothetical explanation of varying clonal expansion patterns in mastocytosis. The D816V c-kit mutation (denoted by 'x') may occur in a mast cell lineage-committed progenitor (a) or a multipotential progenitor (b) resulting in single or multilineage involvement, respectively. The mutation would be detectable in peripheral blood in the latter but not in the former scenario. Patients with systemic smoldering mastocytosis or those with an associated myeloproliferative disorder often carry the D816V c-kit mutation in multiple peripheral blood lineages, while patients with limited systemic indolent disease have this mutation detectable only in lesional tissue mast cells.

Taken from Akin C. Clonality and Molecular Pathogenesis of Mastocytosis. Acta Haematol 2005, 114:61-69

Classification of mastocytosis

The rapidly accumulating knowledge on mastocytosis has led to a speedy evolution in the classification of mastocytosis:

1. The Kiel classification (Lennert & Parwaresch, 1979) was followed by others in which mastocytosis was divided into well defined clinico-biological entities[66].
2. The first consensus classification of mastocytosis was proposed by Metcalfe in 1991[67].

The associations between mastocytosis and increased serum Tryptase levels[68] , the presence of the D816V-activating KIT mutation[69-71] and an aberrant CD25+ and CD2+ immunophenotype of BM MCs [21] were most relevant. The identification of these new biological markers has facilitated a better understanding of the molecular mechanisms involved in mastocytosis and has also contributed towards improving the classification and the diagnosis of the disease and promotes the search for effective molecular-targeted therapies[64,72,73].

3. The World Health Organization (WHO) proposed new criteria for the classification and the diagnosis of mastocytosis in 2001[74]. An International Working Conference proposed new standards last year [75]. In addition, this International Working Conference discussed the differential diagnosis of new poorly defined subgroups of patients with increased and/or altered MCs.

Over the years, the descriptive types of classification have been of lost in value but may be applicable in the cutaneous mastocytosis.

The WHO classification published in 2001 defines 7 disease-variants: cutaneous mastocytosis (CM), indolent systemic mastocytosis (ISM), SM with an associated clonal hematological non-MC-lineage disease (SM-AHNMD), aggressive SM (ASM), MC leukemia (MCL, leukemic SM-variant), MC sarcoma (MCS), and extracutaneous mastocytoma (Table 2) [74,76].

Table 2. WHO classification of mastocytosis

1.	Cutaneous mastocytosis Maculopapular CM[1] Diffuse CM Mastocytoma of skin (Mast cell sarcoma of skin)	CM MPCM DCM
2.	Indolent systemic mastocytosis Smouldering SM Isolated bone marrow mastocytosis	ISM SSM BMM
3.	Systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease	SM-AHNMD[2]
4.	Aggressive systemic mastocytosis Lymphadenopathic SM with eosinophilia[3]	ASM
5.	Mast cell leukemia Typical MCL Aleukemic MCL[4]	MCL
6.	(Extracutaneous) mast cell sarcoma	MCS
7.	Extracutaneous mastocytoma	

[1] Also termed urticaria pigmentosa;
[2] The subtype of the 'AHNMD' has to be defined by WHO criteria as well;
[3] In a subgroup of these patients, the FIPL1-PDGFRA fusion gene is detectable;
[4] Circulating mast cells are <10%.

Taken from Horny H-P, Sotlar K, Valent P: Mastocytosis: State of the Art. Pathobiology 2007;74:121-132

Systemic mastocytosis is defined using major and minor SM-criteria in this classification system. The diagnosis is SM if at least one major and one minor or at least three minor SM-criteria are fulfilled. Criteria defining the MC-burden, involvement of non-MC-lineages, and aggressiveness of disease (C-Findings), are to sub classify SM. In addition, a thorough hematological evaluation is undertaken to reveal or exclude an AHNMD[74-76].

Table 3. Diagnostic WHO criteria for systemic mastocytosis: SM criteria

Major	
Multifocal compact infiltrates of MCs in bone marrow or other extracutaneous organ(s) (>15 MCs)	
Minor	
a	MCs in bone marrow or other extracutaneous organ(s) show an abnormal spindle-shaped morphology (>25%)
b	c-kit mutation D816V in extracutaneous organ(s)[1]
c	MCs in the bone marrow express CD2 or/and CD25
d	Serum tryptase >20 ng/ml (does not count in patients who have an associated hemopoietic clonal non-MC lineage disease (= AHNMD)

If at least one major and one minor criterion or three minor criteria are fulfilled, the diagnosis SM can be established. [1]Other activating mutations at codon 816 of c-kit also count as a minor criterion.

Taken from Horny H-P, Sotlar K, Valent P: Mastocytosis: State of the Art. Pathobiology 2007;74:121-132

In the recently published "Standards and standardization in mastocytosis: Consensus Statements on Diagnostics, Treatment Recommendations and Response Criteria" by Valent, Akin, Metcalfe et al. diagnostic algorithms and procedures are highlighted in great details. However, it is beyond the scope of this dissertation to present an in depth description of hematological, pathological and diagnostic issues in systemic mastocytosis[75].

Management of mastocytosis

There is no curative treatment for mastocytosis. However, since the clinical picture and the severity of the disease are widely varied, the management of the disease also varies accordingly. The large majority of the patients require no more than reassurance and advice, while other fatal systemic cases may be candidates for cytoreductive therapies. The treatment in all cases is tailored to alleviate patient discomfort.

Treatment targets include constitutional symptoms, cutaneous symptoms, skeletal complaints, gastrointestinal symptoms and neurological symptoms. Constitutional

symptoms manifest as dizziness, hypotension, flushing, headache and shock. Cutaneous symptoms are pruritus, wheals, and redness, swelling and cosmetic disturbances such as nodules, macules. Skeletal complaints include osteoporosis, osteopenia and bone pain. Gastrointestinal symptoms constist of peptic ulcer, bleeding, cramping, nausea, vomiting and diarrhea. Neurological symptoms include headaches and specific IgE-mediated allergic reactions.

In case of systemic mastocytosis other than indolent forms choice of targeted and cytoreductive therapy depends on the presence of B- or C-findings. B-findings include signs of multilineage-involvement (hypercellular marrow, dysplasia), a massive MC burden (huge MC marrow infiltration, serum Tryptase level > 200 ng mL-1), and organomegaly (spleen, liver, lymph nodes). C-Findings are the result of a clinically relevant impairment or loss of organ function caused by local infiltrates of MCs. The diagnosis is "smoldering SM" if B-Findings are present. The prognosis and the natural course in SSM are varied. Many patients remain in a smoldering state for decades. Response criteria to cytoreductive drugs strictly relate to C-findings. These have been generally agreed upon and should be applied in all patients and thus are only applicable to patients with ASM or MCL[77]. A detailed discussion of cytoreductive therapy in mastocytosis is beyond the scope of this dissertation.

In conclusion, the present insight into the operative mechanisms in mastocytosis has led to the understanding that mastocytosis is a clonal systemic disease in most if not in all of the adult cases as well as a part of the juvenile cases. The dermatological aspects of mastocytosis are refined or even redefined with this notion in mind. Mastocytosis in the skin is considered to be the starting point from which systemic mastocytosis must be excluded. The diagnostic end point is either called systemic mastocytosis, in case mastocytosis is diagnosed elsewhere in the body or cutaneous mastocytosis, if this is not the case. Published data or data that were collected in the period before this algorithm was in practice are included in this thesis. From this point of view, it is now also possible to re-write already published articles using a different classification of mastocytosis in the skin.

In this thesis, a collection of already published articles on dermatologically oriented work dealing with practical issues concerning patients with mastocytosis is presented. Therefore, the main theme of this thesis is the dermatological aspects of mastocytosis with particular attention to the differences between childhood onset mastocytosis and adult onset mastocytosis.

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Aims of the thesis

The aims of the studies described in this thesis were:

1. To provide an overview and an update of the clinical aspects of mastocytosis from a dermatologist's perspective with focus on the differences between pediatric and adult onset of the disease.

2. To describe current diagnostic methods for mastocytosis in dermatological practice.

3. To present a method on the clinical evaluation of the disease severity in cutaneous mastocytosis.

4. To update the already existing diagnostic and therapeutic guidelines in pediatric mastocytosis.

Various approaches described in the various chapters were pursued in order to achieve these objectives.

CHAPTER 2

Recent Advances in Mast Cell-Related Skin Diseases: Particular Focus on Mastocytosis and Urticaria

Expert Rev. Dermatol 3(1), 65-72 (2008)

EXPERT REVIEWS

Recent advances in mast cell-related skin diseases: particular focus on mastocytosis and urticaria

Expert Rev. Dermatol. 3(1), 65 72 (2 008)

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Mast cells have a prominent, although not completely understood, participation in both immunity and disease. Well-known disorders in which mast cells play a prominent and undisputed role are mastocytosis and urticaria. Mastocytosis has been classified more clearly, based on international consensus meetings and reports of the European Network on Mastocytosis (Consensus Mastocytosis 2007) in the last 5 years. Urticaria is elicited by a great diversity of factors and entities. Treatment points at avoidance, elimination or treatment of the eliciting stimulus or cause, inhibition of mast cell mediator release or therapy of target tissues of mast cell mediators (Consensus Urticaria 2006). Chronic urticaria is believed to have an underlying autoimmune pathogenesis in almost 50% of cases. In a disease such as atopic dermatitis, the role of mast cells is probably underexposed, but is beyond the main scope of this review. In this review; we stress the important differences between children and adults with these disorders. Recent advances in mast cell-mediated skin diseases, such as mastocytosis and urticaria, and differences based on age are subject of discussion, with focus on the literature published in the last 5 years.

KEYWORDS: mast cell mastocytosis ur ticaria

Our understanding of the contribution of mast cells (MCs) in skin diseases, particularly in wound healing, angiogenesis and proliferation, is still in its infancy. The exact role of MCs in different tissues is poorly understood with varying characteristics of MCs in different tissues. MCs play a prominent role in several inflammatory and proliferative disorders.

The most important MC disease is mastocytosis, which can be roughly distinguished into cutaneous mastocytosis (CM) and systemic mastocytosis (SM). Mastocytosis is characterized by a deviant MC proliferation. MC proliferation may be limited to the skin, which is called CM. It may also involve one or more extracutaneous organs, such as the bone marrow, when it is called SM, which is considered to be a clonal disease.

The most well-known inflammatory conditions in which MCs play a role are urticaria and atopic dermatitis (AD), although its role in the latter is underexposed and probably underappreciated (FIGURE 1 & BOX 1) [1].

MCs originate from pluripotent hematopoietic bone marrow stem cells. Precursor cells leave the bone marrow and migrate into the blood and invade the tissue, where they proliferate and differentiate into mature MCs.

MCs are most abundantly present in the dermis close to the epidermis where they may stimulate or enhance neoangiogenesis in normal and pathological conditions. These new vessels facilitate increased transport of complement components and antibodies, although complement and antibodies reach all tissues, particularly via normal blood vessels. Both complement components and antibodies play a role against penetrating antigens [1]. MCs are not only increased in number in lesions of AD, but also in nummular eczema. In both conditions, MCs are able to maintain neurogenic inflammation [2]. Urticaria is a common disorder with various pathogenic mechanisms, but the result is cutaneous MC activation with release of histamine and other vasoactive or proinflammatory mediators being a central process [3 5].

This review focuses on recent developments in the last 5 years in MC-mediated skin diseases,

such as mastocytosis and urticaria. Other diseases with a possible or suspected role of MCs will only be mentioned briefly.

Disorders in which MCs play a role

Disorders in which MCs play an established role are listed in Box 1. These disorders can be broadly divided into dermatoses, proliferative processes and others.

Effective measures against MCs or against mediators released by MCs are the cornerstone in the treatment of mastocytosis and urticaria. Other diseases in which the MC may be important are AD (most presumably), hemangioma (speculative), scars (speculative) and keloid (speculative).

Mastocytosis

Mastocytosis consists of a heterogeneous group of diseases characterized by abnormal proliferation and accumulation of MCs in one or more organs, particularly the skin [6]. A cutaneous form of mastocytosis with different variants and several systemic forms of mastocytosis are distinguished. For practical pediatric and general dermatology aspects, we recognize differences between pediatric and adult-onset manifestations. However, in the recent consensus, this difference was not highlighted as such [6]. Pediatric mastocytosis is considered in most cases to be reactive, whereas the adult form is clonal and tends to progress slowly. Pediatric mastocytosis can also be persistent and progressive. The adult variants may more frequently develop into serious conditions, such as malignant MC processes [6]. However, most adult variants may stay indolent for decades. Malignant forms of the disease are generally apparent at the initial diagnosis and rarely evolve from the common indolent variants. By contrast, pediatric mastocytosis is also not always reactive, as reported in some recent studies in which D816V *c-kit* mutation was demonstrated in a significant number of cutaneous lesions in pediatric patients [7]. Clinically, CM expresses as maculopapular mastocytosis (solitary mastocytoma, urticaria pigmentosa, diffuse CM and telangiectasia macularis eruptive perstans). Rubbing and trauma of the affected skin results in a wheal with a flare (Darier s sign). SM can occur with and without skin lesions and symptoms. It is known as indolent SM when there are no symptoms. If there are symptoms, then they are primarily of systemic origin. Based on Tryptase serum values, presence of hematological aberrations and presence of *c-kit* mutations in non-MC lines and other investigations, one can stage the disease severity of mastocytosis (Figure 2).

There is no specific treatment for mastocytosis. For treatment options, roughly three practical categories can be generally recognized: pediatric, adult cutaneous and (adult and pediatric) SM [2]. For a more detailed classification, we recommend the 2003 WHO classification of mastocytosis recognizing more than three categories of mastocytosis, which are further defined by the presence of B and/or C findings, which determine the indicated therapeutic modality in each case. However, this review is limited to skin manifestations only (Figure 3 & Box 2) [1] .

Treatment of mastocytosis

Treatment of mastocytosis in childhood is usually unnecessary. In general, treatment of mastocytosis, both in children and adults, is only indicated when symptoms are present [8 1 0].

Similar to that in other diseases, patient education is an important tool for guiding the patients and affected children and their parents. Affected children and their parents are advised to avoid agents such as aspirin, NSAIDs, codeine, morphine, alcohol, thiamine, quinine, opiates, gallamine, decamethonium, procaine, radiographic dyes, dextran, polymyxin B, scopolamine and D-tubocurarine. All these agents precipitate the release of MC mediators. This list is especially important for adults, because several of the agents are not used in children. Diets are of little value in patients in whom food allergy or intolerance are suspected. However, histamine-liberating foods, such as banana, kiwi and others, should be avoided.

Therapy is aimed at alleviating clinical symptoms of mastocytosis. If mastocytosis presents in children aged younger

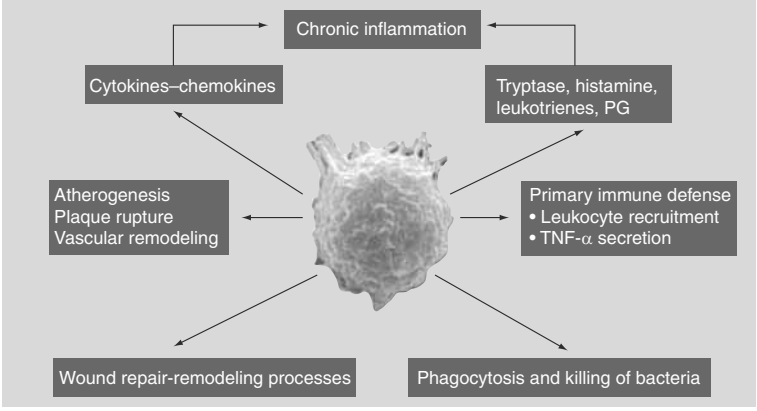

Figure 1. Functions of mast cells in physiological and pathological states.
Mast cells may play crucial roles in various disease states, including vascular disease, fibrotic states, rheumatological disease, certain malignancies and in host defense against infectious pathogens. The probable roles of the mast cell in human diseases are summarized.
PG: Prostaglandin.
Adapted from [28].

Box 1. Cutaneous inflammatory and proliferative disorders in which mast cells play an established or a speculative role.

Allergy, drug eruptions
Dermatitis, exanthemas
Atopic dermatitis
Urticaria
P soriasis
Proliferative disorders
Mastocytosis
Hemangioma?
Malignant skin processes?
Others
S cars?
Keloid?

than 5 years, most cases of maculopapular mastocytosis (urticaria pigmentosa and mastocytoma) follow a benign course. Maculopapular childhood mastocytosis often spares the sunlight-exposed areas, indicating why UV light is a therapeutic option. Therapy is often limited to reassurance of the parents and consists of advice on avoiding factors known to stimulate or induce MC degranulation and to prevent serious events.

Bullae may be present in the first 2 years of life, and can be treated by local general care and preventing infection. This presentation mimics staphylococcal scalded skin syndrome [8]. Bullae formation may occur in active mastocytosis, as well as maculopapular mastocytosis (formerly known as urticaria pigmentosa) and diffuse CM.

The first step of treatment is relief of pruritus, urtication and flushing, which can be achieved by H1 receptor antagonists (e.g., dimentidene 0.05 mg/kg daily in three doses, hydroxyzine 2 mg/kg daily in three doses, cetirizine 0.250 mg/kg daily in two doses) or levocetirizine 0.125 mg/kg daily, which have proved to be very safe. Higher doses than registered are probably required for effectiveness.

H2 receptor antagonists (e.g., ranitidine 4 mg/kg daily) may be added, especially if H1 receptor antagonists have insufficient effect or when gastrointestinal symptoms of hyperacidity or ulceration are present. Patients with diarrhea may also benefit from treatment with an H2 receptor antagonist.

For gastrointestinal symptoms, disodium chromoglycate may be a useful addition, especially for diarrhea. Ketotifen, another MC stabilizer, was reported not to have more advantages when compared with hydroxyzine.

Treatment of children with maculopapular mastocytosis (formerly urticaria pigmentosa) may also include topical steroids (diluted to 25%) under wet dry wraps (double wraps application, rewetted every 2 h) [10,11]. Strict monitoring of cortisol levels, weight and growth are essential. This treatment is contraindicated in puberty for reasons of development of striae. Isolated mastocytomas may be treated with topical steroids with occlusive dressings. This is especially indicated if there are persistent severe symptoms. If this fails, excision may be considered on rare occasions.

Children with a history of anaphylaxis must be equipped with an epinephrine autoinjector and parents and (older) children should be instructed on self medication (for instructions also see [102]). Taking preventive measures in patients with mastocytosis undergoing anesthesia is controversial.

When flushing or pruritus is prominent, antihistamines are used, but the current generation has limited affectivity. In the case of chronic diarrhea, sodium cromoglycate is indicated [12].

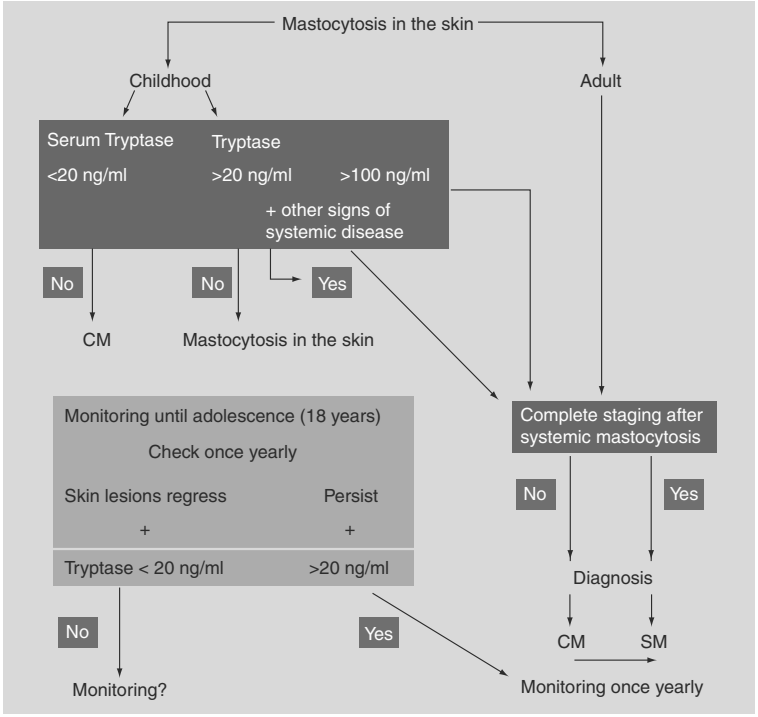

Figure 2. Algorithm of disease staging.
CM: Cutaneous mastocytosis; SM: Systemic mastocytosis.

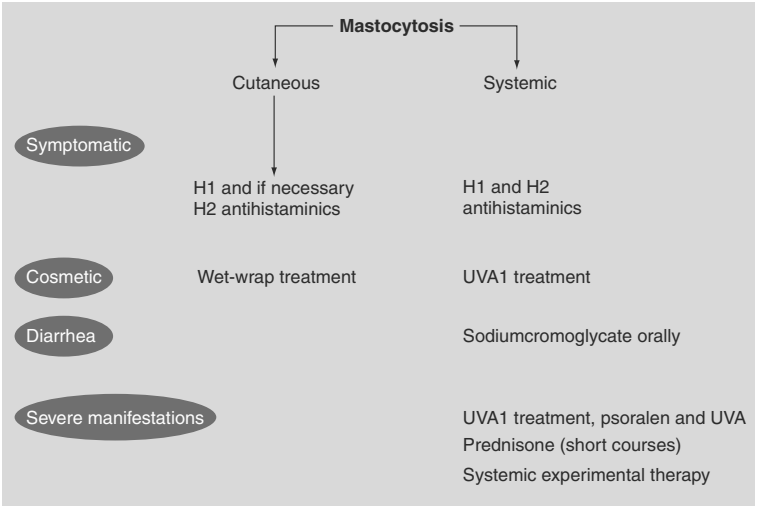

Figure 3. Algorithm of treatment of mastocytosis.

In most adults, the disease is more severe and progressive. The percentage of SM is higher, but less common in dermatology practice. Internal medicine physicians often see more complicated cases. National or international registration would improve knowledge on mastocytosis. However, many cases are, or at least begin as cutaneous forms, in which there is special danger for wasp stings. Infrequently, cases are first encountered after anaphylaxis following a wasp sting [13,14].

Adults with a history of anaphylaxis are treated in the same way as children (see earlier).

Taking preventive measures in patients with mastocytosis undergoing anesthesia is indicated in those with serum Tryptase values of 20 g/ml and more. We recommend the following perioperative measures, but evidence-based data are lacking:

Close monitoring of all patients, the avoidance of known histamine-releasing drugs and a continuous availability of emergency drugs;

Patients are hospitalized 1 day before anesthesia and surgery;

Prednisolone at a stress dosage and antihistamines are started 1 day before and continued 1 day after.

Treatment with drugs, such as prednisone (short courses) or UV therapy with UVA or psoralen and UVA (PUVA), may have a place in adults (they do not have a place in first-line treatment of pediatric mastocytosis) [8].

Systemic mastocytosis

Systemic mastocytosis is only dealt with briefly, because it is not within the scope of this review.

Systemic mastocytosis (diagnosis based on WHO criteria) is a disease characterized by multifocal MC proliferation in the bone marrow or other extracutaneous organs. It is a rare condition that often involves the bone marrow. Many patients with SM exhibit the D816V point mutation in the tyrosine kinase domain of the transmembrane receptor protein *c-kit*. Therefore, if a codon 816 *c-kit* mutation is detected, it counts as a minor diagnostic criterion in the WHO diagnostic criteria for SM. Mutational status of the *c-kit* gene has also pharmacogenomic implications for the best future therapy options [14,15]. Currently, there is no standard cure for SM. Prolonged courses of systemic corticosteroids have been used. Others have tried to influence SM by systemic treatment with IFN-α combined with systemic corticosteroids [16]. Recently, cladribine was effectively used in

Box 2. Diagnostic WHO criteria for systemic mastocytosis: criteria also adjusted for children.

Major criteria
Multifocal compact infiltrates of MCs in bone marrow or other extracutaneous organ(s) (>15 MCs)
Minor criteria
MCs in bone marrow or other extracutaneous organ(s) show an abnormal spindle-shaped morphology (>25%)
Mutation D816V in *C-kit* in extracutaneous organ(s)*
MCs in the bone marrow express CD2 and/or CD25
Serum Tryptase > 20 ng/ml (does not count in two categories: children aged < 15 years and in patients who have an associated hematopoietic clonal non-MC lineage Disease
Diagnosis systemic mastocytosis, based on two possibilities
At least one major and one minor criterion
Three minor criteria

*Other activating mutations at codon 816 of *c-kit* also count as a minor criterion.

MC: Mast cell.

Derived from [31].

patients with SM [17]. In a pilot study, Droogendijk demonstrated that imatinib mesylate was effective in patients with SM, including those with and without the D816V mutation. They studied 14 patients, of whom ten had D816V mutation. Most responded to therapy [18]. SM is often complicated by early osteoporosis; in one study, osteoporosis in mastocytosis was estimated to be present in up to 30% of cases. Mastocytosis is associated with skeletal problems, which include a decrease in bone density and pathological fractures [19].

Urticaria

Urticaria is characterized by the rapid appearance of wheals that may be accompanied by angioedema (Figure 4). A wheal consists of a central swelling of variable size, almost invariably surrounded by an erythema, often with associated itching or sometimes a burning sensation. The individual lesions have a fleeting nature, with a duration of usually 1 24 h [20 22]. In small children, urticaria can have a blue hue, which often leads to misinterpretation as urticarial vasculitis or erythema multiforme (Figure 5).

Urticaria results from a localized capillary vasodilation and transudation of protein-rich fluid in the superficial dermis. Besides histamine, other mediators, including prostaglandins, leukotrienes, cytokines and chemokines produced at different times following MC activation also contribute to the changing character of the urticaria. The mechanism by which MC activation is induced can be either immunological (IgE mediated, complement activation components, antiFcεRI autoantibodies and anti-IgE autoantibodies) or nonimmunological (direct histamine releasers).

Increasing understanding of the pathomechanisms involved in urticaria has highlighted the heterogeneity of different subtypes. Recent consensus guidelines distinguish spontaneous urticaria, physical urticaria, different diseases related to urticaria for historical reasons and syndromes including urticaria/angioedema.

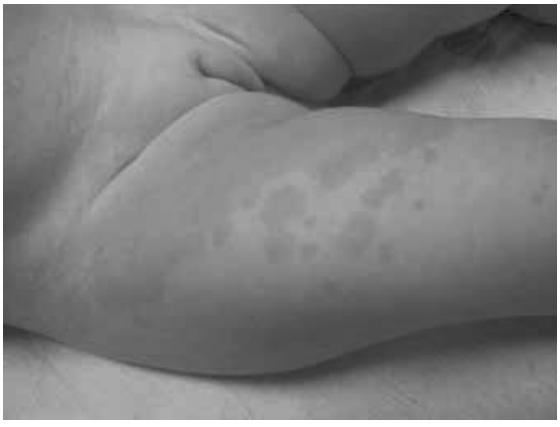

Figure 4. Urticaria with normal appearance in a child.

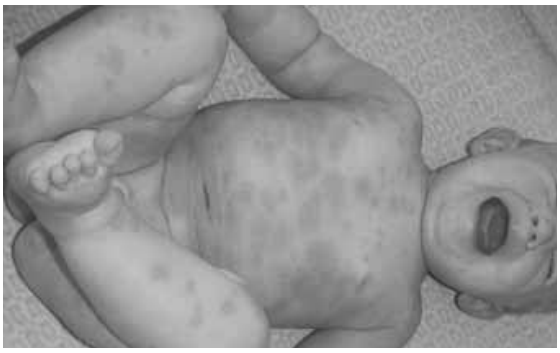

Figure 5. Urticaria with a blue hue often misdiagnosed in young children.

In acute spontaneous urticaria, attacks last less than 6 weeks. Infection, drugs and food allergy are the main known causes; however, most cases of acute urticaria remain idiopathic. If attacks last longer, it is referred to as chronic spontaneous urticaria. Attacks may last several months to years. One distinguishes immunological and nonimmunological urticaria. Immunological urticaria is a hypersensitivity reaction mediated by antibodies and/or T cells, which results in MC activation. IgE-mediated type I hypersensitivity is a major immunological pathway associated with MC activation, especially in acute urticaria. However, it is not the only mechanism. In particular, chronic urticaria may result from the binding of IgG autoantibodies to IgE and/or to the receptor for IgE molecules on MCs, thus corresponding to a type II hypersensitivity reaction. Approximately 50% of patients with chronic urticaria have histamine-releasing autoantibodies in their blood. The term autoimmune urticaria is increasingly being accepted [23]. In children, autoimmune urticaria has also been described in chronic cases. The next most frequent form of long-lasting urticaria is physical urticaria.

Treatment of urticaria can be divided into three basic approaches based on the recent review by Zuberbier *et al.* [21,24]. Acute attacks of urticaria can be managed by short courses of systemic corticosteroids, but preferably as crisis intervention.

Avoidance, elimination or treatment of the eliciting stimulus or cause

This approach is most desirable but, in adults and more rarely in children, only applicable in a minority of cases. It is performed in IgE-mediated urticaria, such as food allergy and physical urticaria. In children, most urticaria are caused by infections and food allergy that are acute in origin and can be treated more easily [22].

Another category that can be treated similarly is physical urticaria in adults and children. Factors can be eliminated as much as possible.

It is often impossible to treat chronic urticaria by avoidance, elimination or by treatment of the eliciting stimulus or cause. Chronic urticaria in childhood is rare. Possible pediatric causes are parasitosis, infectious diseases, drugs and physical factors.

In adults with chronic urticaria, treatment of associated infections, *Helicobacter pylori* gastritis, parasitic disease, cancer or drug allergy can be curative or helpful [21].

A carefully taken history will eliminate most of the causes in the majority of the cases and one should avoid extensive investigations or provocations in such patients. The cause of chronic urticaria often remains undetected.

Inhibition of MC mediator release

Medicines in this category include steroids and ciclosporine. PUVA therapy is an alternative. These approaches are not recommended in children.

The most severe patients may require protracted treatment with low-dose alternate-day steroid or ciclosporine [21]. Ciclosporine 0.5 5 mg/kg /day has a confirmed benefit in more than half of the patients with chronic recalcitrant urticaria. It has also been shown to be effective even in autoantibody-negative patients [23]. Intravenous immunoglobulin, azathioprine and methotrexate are alternatives if ciclosporine is not effective [25].

Therapy of target tissues of MC mediators

The mainstay of treatment for urticaria is orally administered H1 antihistamines as they reduce itch, wheal duration and frequency of attacks. Management is achieved more effectively by taking antihistamines daily, not only when the patient is symptomatic. The sedating H1 antihistamines are as effective as or more effective than classical H1 antihistamines. Additional therapy aimed at pruritogenic mediators other than histamine would be expected to improve urticarial pruritus. It is not uncommon to exceed the licensed dose in severely affected adult and pediatric patients. As a general rule, antihistamines are safe, have few significant adverse effects and interactions with other drugs are rare. If no symptom control is achieved with H1 antihistamines, co-administration of an H2 antihistamine can be considered. In the next step, a leukotriene receptor antagonist may be used as add-on therapy.

If possible, it is best to avoid all antihistamines in pregnancy, although none has proven to be teratogenic. If one must be used, the consensus is that loratadine and chlorphenamine are among the safest [24].

Hydroxizine and chlorphenamine are the only drugs licensed for children under the age of 2 years. Other very extensively studied drugs in children are levocetirizine and cetirizine. Levocetirizine is L-cetirizine and is the active component of cetirizine. In children aged 1 5 y ears, the oral clearance of levocetirizine is rapid and will increase with bodyweight and age. Therefore, levocetirizine dose should be based on bodyweight and age in children [26].

Rupatadine is a nonsedating, selective and long-acting new anti H1-drug with an additional potent antagonist activity towards platelet-activating factor receptors [27]. The use of rupatadine is currently indicated in adult and adolescent patients (>12 years of age). It is more effective than the current drugs, especially in chronic urticaria.

Prognosis of urticaria

The prognosis of acute urticaria is usually excellent and the symptoms will completely disappear over a short period of time. However, the prognosis is less favorable in chronic urticaria, in which the cause is often not found.

Other diseases in which MCs may play a role

Many forms of mucocutaneous drug and other allergic manifestations are mediated by MCs. MCs are involved in allergic and anaphylactic reactions and are also involved in many inflammatory diseases affecting different organs, including the heart, joints, lungs and skin. However, their exact role is poorly understood. In the inflammatory processes involving different organs, MCs appear to be activated by triggers other than aggregation of their IgE receptors, such as anaphylatoxins, immunoglobulin-free light chains, superantigens, heat-shock proteins, neuropeptides and cytokines, leading to selective release of mediators without degranulation.

Expert commentary

More therapeutic options directed against MC antigens and mediators with continued research into MC biology and pathophysiology will become available. This in turn will facilitate the development of more specific therapeutic agents. Oral imatinib and related drugs are promising new therapeutic agents warranting further clinical evaluations. Long-term monitoring of possible side effects of these new agents will help to develop safer therapeutic options for diseases in which MCs play a regulatory role.

Five-year view

Mast cell research has provided new insights into a range of diseases. MCs are recognized as important players, and not just bystanders, in many diseases. As such, targeting MCs will play a prominent role, which may be relevant in the development of new therapeutic options in several skin diseases. In mastocytosis, diagnostic and therapeutic implications of *c-kit* mutations as well as other less common molecular abnormalities may be considered as possible targets for more specific new therapies. New surface antigens in normal and neoplastic MCs have been discovered, which may be potential targets for specific molecular biological therapy.

The use of biologicals is currently under critical review and is expected to lead to more specific treatment modalities for mastocytosis and urticaria in the near future.

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Key issues

The contribution of mast cells (MCs) in inflammatory diseases, angiogenesis and other conditions is only partially understood.

MCs play a prominent role in several inflammatory and proliferative disorders, particularly of the skin. Their role is undisputed in mastocytosis and urticaria.

At present, there is no causative treatment and no cure for mastocytosis.

Pediatric mastocytosis in most cases is not treated, particularly because it is asymptomatic and limited to the skin. Treatment is only indicated for alleviating severe symptoms.

In the majority of cases, chronic urticaria occurs only in adults. Chronic urticaria is believed to have an underlying autoimmune pathogenesis in almost 50% of cases. The role of MCs in autoimmune diseases has not yet been completely elucidated.

MCs play a role in several inflammatory and proliferative disorders. The presence of MCs is important for treatment in the following diseases:

Mastocytosis:

For treatment options, three categories must be recognized: pediatric, adult cutaneous and systemic mastocytosis.

Urticaria: acute or chronic.

Atopic dermatitis in which the MCs play a role; although recently an important role of the skin barrier function has also become clear.

The role of MCs in hemangioma is speculative, but may be interesting as a target for future therapeutic options.

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CHAPTER 3

Mastocytosis in Childhood

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Mastocytosis in Childhood

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Mastocytosis is a primary, abnormal accumulation of mast cells in the absence of an apparent cause. Mast cell infiltrates may be present anywhere in the body. The skin is the most frequent site of involvement (1,2). Mastocytosis is associated with a broad range of local and systemic symptoms primarily caused by the release of mast cell mediators. Adult-onset mastocytosis and childhood onset mastocytosis vary in both clinical symptoms and course. They also differ in association with genetic mutations of growth factor receptor c-kit. In this review, attention has been focused on pediatric mastocytosis.

PATHOGENESIS

The pathogenetic mechanisms leading to mast cell proliferation in cutaneous mastocytosis or systemic mastocytosis are still not completely understood. However, molecular biology techniques have made it possible to obtain new insights into growth regulation of mast cells and to allow a better understanding of the variations in the clinical course of mastocytosis (3). At present, attention is focused on mutations in proto-oncogene receptor c-kit (4–7). Systemic mastocytosis and adult-onset mastocytosis are often linked to Asp-816-Val mutation of c-kit, while in children, different mutations have been found. Typical childhood mastocytosis is linked to Gln-839-Lys c-kit mutation. Mutations such as Asp-816-Tyr and Asp-816-Phe are associated with atypical childhood mastocytosis. The Asp-816-His mutation is associated with mastocytosis and acute myeloid leukemia (8). Consequently the classification schemes of mastocytosis have been revised to incorporate both molecular-genetic and clinical data (8). C-kit mutation analyses are extremely important for predicting sensitivity to therapy and prognosis (8).

CLASSIFICATION

The present classification of mastocytosis is based on the location of the mast cell infiltrates, the accompanying symptoms, and the course of the disease. In 1991 Metcalfe defined a basic classification scheme involving four prognostic categories: an indolent variant, a variant associated with hematologic disorders, an aggressive variant with rapid development of lymphadenopathy and eosinophilia, and mast cell leukemia (3,9). A descriptive classification based on clinical signs is preferable for practical application and is shown in Table 1 (9).

CLINICAL MANIFESTATION

Childhood onset mastocytosis is defined as histologically proven mastocytosis presenting before the age of 15 years. Most of the cases of childhood onset mastocytosis (60–80%) present during the first year of life, whereas congenital mastocytosis was reported in 18–31% of the cases (2,10–13). The sex ratio is equal and data on racial differences are limited. Mastocytosis is not considered a hereditary disease, although familial cases have been described. Children with mastocytosis always have skin lesions, but only 60% experience mast cell mediator-related symptoms (11,13).

The most common type of childhood onset mastocytosis is urticaria pigmentosa (UP) (Fig. 1), which represents about 65% of all cases (12,13). The typical efflorescences consist of red-brown-yellowish macules, papules, or nodules that vary in size from several

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TABLE 1. *Clinical Signs of Mastocytosis*

Disease	Age predominance	Characteristics
Mastocytoma	0–6 months	1–5 red/brown hyperpigmented or skin colored nodules(s)
Urticaria pigmentosa (UP)	3–9 months; most frequent presentation	Multiple red-brown hyperpigmented macules and papules
Diffuse cutaneous mastocytosis	Rare; often at birth	Thickened, lichenified skin with papules, rarely without skin abnormalities; bullae after minimal trauma
Telangiectasia macularis eruptiva perstans (TMEP)	Adults	Numerous hyperpigmented telangiectatic macules
Systemic mastocytosis	Adults; rare in children	Mast cell infiltrates in skin and internal organs
Mast cell leukemia	Adults; rare in children	Anemia; mast cells in peripheral blood

millimeters to centimeters in diameter. Erythema, swelling, and blister formation as well as itching of the lesions may occur spontaneously or after stroking or rubbing. The blistering heals without permanent scarring. The lesions are generalized and randomly disseminated. The palms, soles, scalp, and sunlight-exposed areas of the body are often less affected, especially in older children. Dermatographism and pruritus may be present, but these features per se have no diagnostic value.

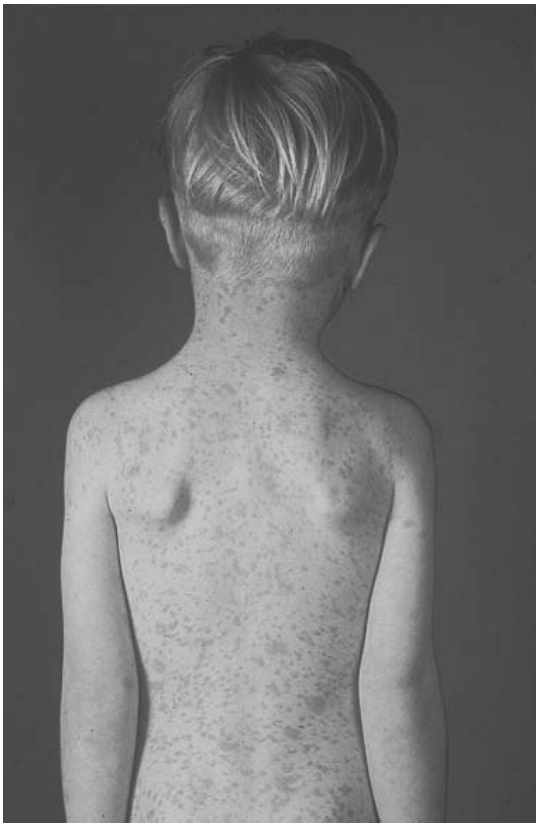

Figure 1. Urticaria pigmentosa.

Complete resolution of the lesions may occur in 10% of patients and significant improvement may be expected in up to 70% of patients by the age of 10 years (9,11,13). Data on the number of persistent cases of childhood-onset mastocytosis are limited and range from 25% to 57% (13,14).

The second most common cutaneous presentation of childhood-onset mastocytosis is solitary mastocytoma (Fig. 2A) defined by the presence of one to several lesions (commonly five separate lesions or less) that have characteristics similar to those of urticaria pigmentosa. It is encountered in 10–35% of the cases of childhood-onset

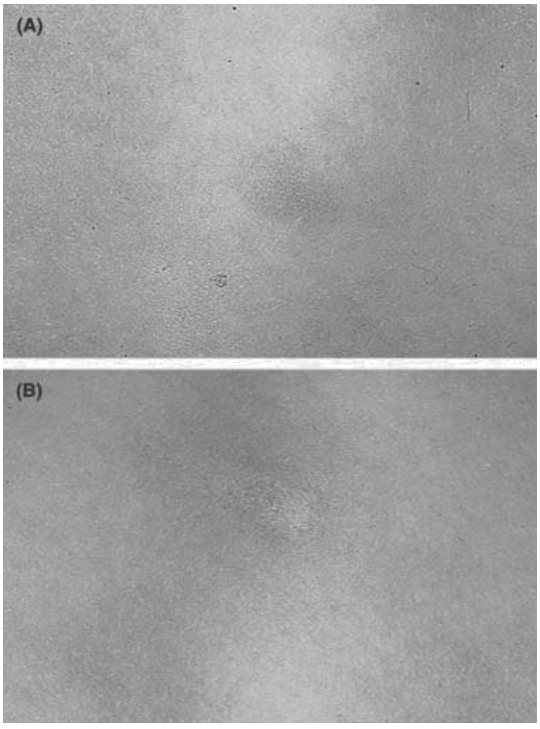

Figure 2. (A) Solitary mastocytoma. (B) Solitary mastocytoma, Darier sign positive.

mastocytosis (11,13,15). However, this figure may be an underestimate, as an asymptomatic solitary lesion may be overlooked or mistaken for a mole or juvenile xanthogranuloma. Of all clinical subtypes of childhood onset mastocytosis, solitary mastocytoma invariably follows a mild course with complete resolution, generally before adulthood.

Diffuse cutaneous mastocytosis (Fig. 3) is rare and most patients have been described in single case reports. By definition, the whole skin is involved, although the central region and the scalp are primarily affected. The clinical features become prominent at birth or in early infancy (1,15,16). These consist of widespread spontaneous blistering with erosions and crusts, various degrees of erythroderma, strong dermatographism, and itching. The diagnosis of mastocytosis must be ruled out in newborns with blistering and bullae. The blisters may be hemorrhagic in diffuse cutaneous mastocytosis. The skin may be leathery and thickened, especially in the flexural regions. In toddlers the skin becomes less reactive, but diffuse hyperpigmentation and positive dermatographism may persist into adulthood.

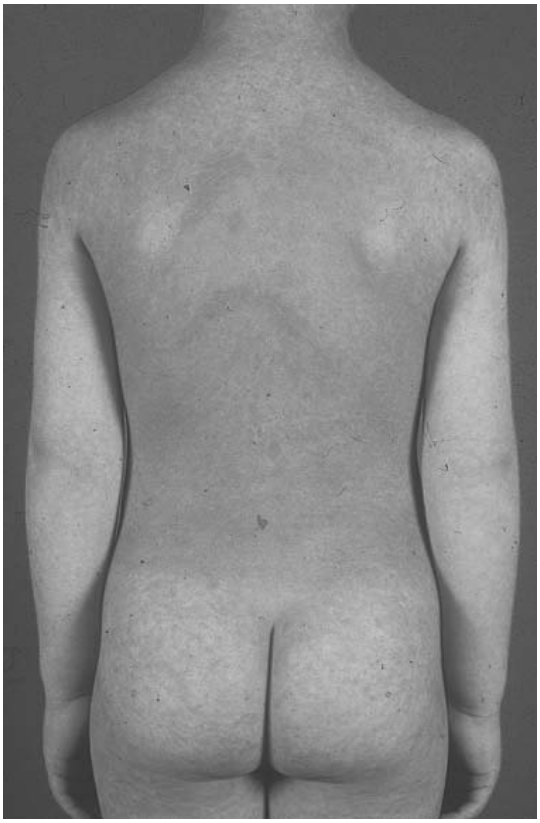

Figure 3. Diffuse cutaneous mastocytosis.

Telangiectasia macularis eruptiva perstans (TMEP) is the least common cutaneous manifestation of mastocytosis. The lesions consist of persistent red telangiectatic areas without any signs of macules or papules. The lesions are poorly demarcated and sometimes difficult to recognize. Darier sign (swelling and redness induced by rubbing of the lesion) is generally positive (1). As an entity, it is said to occur only in adults. The clinical features overlap with those of UP. TMEP should be distinguished from telangiectatic UP in which guttate telangiectatic macules are observed. Underestimation of its incidence is plausible, especially in children.

SYMPTOMS

Essentially all types of childhood-onset mastocytosis may be associated with localized and systemic symptoms caused by mast cell mediators (Table 2) (1,17). The most common symptoms are pruritus, redness, and swelling. These may occur spontaneously or secondarily after thermal, mechanical, or chemical stimuli. In infants, blistering is common. On rare occasions these blisters may have hemorrhagic content, as skin mast cells have been shown to regulate hemostasis (18).

Gastrointestinal complaints are common and consist primarily of diarrhea and abdominal pains in up to 40% of children with mastocytosis (10,11,13,19). Hyperacidity and subsequent peptic ulcers secondary to high histamine levels in pediatric mastocytosis have been

TABLE 2. *Mast Cell Mediators*

Skin specimens	Histamine Leukotriene B_4 (LTB_4) 5-hydroxyeicosatetraenoic acid (5-HETE) Tryptase Chymase Heparin
Cutaneous blister fluid	Histamine Prostaglandin D_2 (PGD_2) Platelet-activating factor (PAF)
Plasma	Histamine α-Tryptase
Urine	Histamine Histamine metabolites N_T-methylhistamine N_T-methylimidazole acetic acid PGD_2 metabolites 9-α-hydroxy-11, 15-dioxo-2,3,4, 5-tetra-norprostane-1,20-dioic acid 9-α, 11 β-dihidroxy-15-oxo-2, 3,18,19-tetranorprost-5-ene-1, 20-dioic acid (PGD-M) Chondroitin sulfate B Hyaluronic acid Arylsulfatases A and B

reported (12,19). However, peptic ulcer disease is extremely rare in children with mastocytosis. Constitutional symptoms ranging from flushing spells in 20–65% to states of hypotension and shock may occur (10,11,13,19). Flushing spells are extremely uncommon in solitary mastocytoma. Reported bone pain, headache, and mild cognitive changes are easily dismissed as common complaints (13,19). Peptic ulcer disease, intestinal bleeding, lymph node swelling, hepatic obstruction, and anaphylactic reactions to wasp sting venom characterize childhood-onset mastocytosis with a potentially severe course. Fatal cases of childhood-onset mastocytosis, although very rare, have been reported (20–22).

SYSTEMIC MASTOCYTOSIS

Systemic mastocytosis is referred to as mast cell accumulation in one or more organs other than the skin. Its diagnosis is based on the demonstration of typical mast cell infiltrates, especially in the bone marrow. It is very rare in children. Children with urticaria pigmentosa do not show the typical pattern of bone marrow lesions as described in adult urticaria pigmentosa and systemic mastocytosis. Nonspecific mast cell accumulation in the bone marrow is observed in 17–35% of adult patients (10,11). Data on intestinal, hepatic, splenic, and lymph node involvement are sporadic. Nonetheless, hepatosplenomegaly has been noted in uncomplicated childhood-onset mastocytosis (11).

The presence of symptoms that are not limited to the skin is not predictive for mast cell accumulation elsewhere in the body. It is oversimplification to accept the presence of systemic symptoms as systemic mastocytosis. Since childhood-onset mastocytosis has a good prognosis in almost all patients, the demonstration of mast cell infiltrates in organs other than the skin is of limited value in patient management.

DIAGNOSIS

Diagnosis of childhood-onset mastocytosis is based on clinical suspicion of mast cell accumulation and confirmed by histologic demonstration of a significant increase in mast cell numbers. Clinical suspicion may be aroused in cases of persistent pigmented lesions with swelling, redness, blistering, and itching provoked by thermal, mechanical, or other stimuli. The patient should always be tested for Darier sign. The test is very informative if conducted correctly. The procedure consists of rubbing the lesion with a blunt object for approximately 10 seconds. The lesion is then observed for swelling and redness for 5 minutes. It is advisable to repeat the procedure on an adjacent nonlesional area of the skin. By definition, Darier sign is positive if it can be provoked only in the lesion but not elsewhere (Fig. 2B). Dermatographism, tested by drawing a "train track crossroad," is not informative as an indicator of mastocytosis. It does, however, provide a clinical impression of the irritability of mast cells in the skin.

In diffuse cutaneous mastocytosis (Fig. 3), it is not possible to provoke Darier sign on a lesion because large areas of the skin are affected. The history of spontaneously occurring profuse redness, swelling, itching, and blistering in very young children may point to diffuse cutaneous mastocytosis. Dermatographism in these cases is very impressive.

The demonstration of elevated levels of mast cell mediator histamine or its metabolites in blood or urine is useful for staging and follow-up of patients with proved childhood-onset mastocytosis. The levels of mast cell mediators may be elevated in various conditions associated with increased mast cell activation (23–25). Therefore elevated levels of any mast cell mediator may support, but not establish, the diagnosis of childhood-onset mastocytosis.

Examination recommendations include complete blood count with differential, serum chemistry, liver enzymes, and the levels of mast cell mediator N-methyl histamine or tryptase at regular intervals. Analysis of c-kit mutations in skin biopsy specimens by polymerase chain reaction (PCR) may differentiate patients likely to have chronic disease (c-kit mutation positive) from those likely to have a transient form of mastocytosis.

Many authors agree that invasive diagnostic procedures should be reserved for patients with hematologic aberrations, persistent, localized bone pain, and persistent, severe, gastrointestinal symptoms or biochemical evidence of hepatic insufficiency (10,11,26).

IMMUNOHISTOCHEMISTRY

The diagnosis of mastocytosis is generally based on the presence of typical dense or minimal mast cell infiltrates in the skin and/or bone marrow (27), which are generally toluidine blue and chloroacetate esterase positive (28). The mast cell enzyme tryptase is increasingly used as a serum (29) and as a sensitive and reliable immunohistologic marker for detecting extremely small mast cell infiltrates (30), which may be missed after toluidine blue or chloroacetate esterase staining.

The expression of cell surface membrane phenotype markers such as the stem cell growth factor (SCF) receptor c-kit (CD117) (31), CD68 (32), and possibly CD2 (LFA-2) can be used for confirming the diagnosis of mastocytosis. Normal tissue mast cells express

considerable amounts of c-kit (CD117) on their surface. It is also expressed on immature normal or neoplastic mast cells, as well as on multilineage hematopoietic ($CD34^+$) progenitors, and is useful in distinguishing mast cells from basophils (31). In recent years c-kit has been used as a diagnostic marker in patients with suspected mast cell disease (33,34). Bone marrow mast cells (BMMCs) in normal subjects and patients with mast cell proliferative disorder can be identified and quantified using specific antibodies against c-kit (CD117) and multiparameter flow cytometry (33). Clear immunophenotypic differences were noted between BMMC in normal or reactive bone marrow and BMMC ($c\text{-}kit^+/CD34^-$) in patients with mastocytosis.

A noteworthy finding was that BMMC in patients with adult-onset indolent or aggressive systemic mastocytosis expressed CD2 (LFA-2) and CD25 (33). The expression of CD2 (LFA-2) is particularly interesting because its expression is restricted to T and NK cells. It is not expressed on normal mast cells. Abnormal expression of CD2 on neoplastic mast cells may be of pathophysiologic importance because mast cells also express a natural ligand LFA-3 (CD58) for CD2 (CD2/CD58-mediated accumulation of neoplastic mast cells). The absence of granulocyte antigens (e.g., CD15) also confirms the presence of a mast cell lineage disease (7). The clinical significance of new histologic markers for mastocytosis is currently under investigation and preliminary results indicate that they may be valuable in defining definite criteria in various forms of masto cytosis.

CURRENT AND EXPERIMENTAL THERAPY

General Approach

In general, treatment of mastocytosis is only indicated when symptoms are present (Fig. 4) (9,35,36). If mastocytosis presents in children younger than 5 years of age, most cases of urticaria pigmentosa and mastocytoma follow a benign course. Therapy is often limited to reassuring the parents and providing advice on avoiding factors known to stimulate or induce mast cell degranulation and prevention of serious events. Bullae present in the first 2 years of life, sometimes mimicking staphylococcal scalded skin syndrome, can be treated by local general care and prevention of infection (16,37). When the presence of symptoms dictates more active intervention, the following five-step procedure should be undertaken.

Step 1

The first step in treatment is relieving pruritus, urtication, and flushing. This may be achieved by H_1-receptor antagonists, such as hydroxyzine and cetirizine (38,39).

Step 2

H_2-receptor antagonists such as cimetidine and ranitidine may be added, especially if H_1-receptor antagonists have insufficient effect or when gastrointestinal symptoms of hyperacidity or ulceration are present (40). Patients with diarrhea may also benefit from treatment with an H_2-receptor antagonist (41).

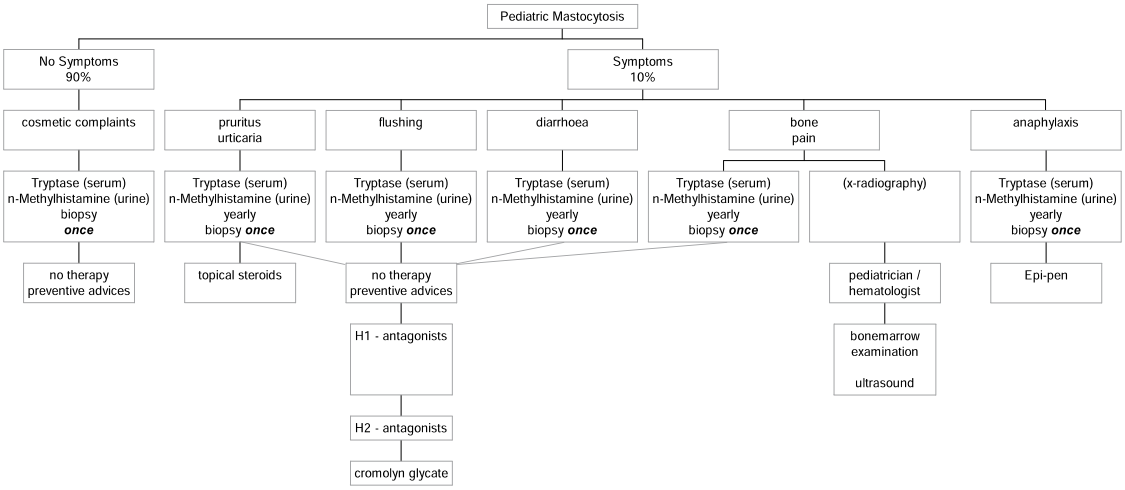

Figure 4. Flow chart of pediatric mastocytosis.

Step 3

For gastrointestinal symptoms, disodium chromoglycate may be a useful addition, especially for diarrhea. This is also effective for flushing and pruritus (42,43), although conflicting results have been reported (44). Ketotifen is another mast cell stabilizer, but showed no advantage as compared with hydroxyzine (10).

Step 4

Treatment of children with urticaria pigmentosa may also include topical steroids. We have had positive experience with diluted steroids under wet wraps (double-wrap applications which are rewetted every 2 hours) (45,46). Strict checks of cortisol levels, weight, and growth are indicated. This treatment is contraindicated in puberty because of the increased risk of development of striae (47,48).

Solitary mastocytomas may be treated with topical steroids with occlusive dressings. This is especially indicated if there are persistent, severe symptoms. If this fails, on rare occasions, excision in toto may be considered (47).

Step 5

Only patients with a history of anaphylaxis must be equipped with injectable adrenaline in the form of Epi-Pen, and parents and (older) children should be instructed and trained in self-medication (49). Because the occurrence of anaphylaxis secondary to wasp sting or other stimuli is unpredictable, all patients and parents should be informed about this. However, we do not think it is mandatory to equip patients with a negative history of anaphylactic reactions with an Epi-Pen.

Treatment with drugs such as prednisone and aspirin, or therapy with ultraviolet A (UVA) or psoralen plus UVA (PUVA) have no place in the management of childhood-onset mastocytosis (9). Preventive measures taken in patients with mastocytosis undergoing anesthesia are controversial (50). We take the following perioperative measures: close monitoring of all patients, avoidance of known histamine-releasing drugs, and the continuous availability of emergency drugs. Patients are hospitalized 1 day before anesthesia and surgery. Prednisolone at a stress dosage and antihistamines are started 1 day before and continued 1 day after anesthesia.

FUTURE ASPECTS

Due to advances in molecular biology, information is becoming available on c-kit mutation-related growth enhancement of mast cells. As a result, the origin of some clinical manifestations of mastocytosis may well be elucidated in the near future. One of the ways to explain the diversity in the course of mastocytosis may be by means of the moment of c-kit alteration in the mast cell lineage.

Cells in early stages in the myeloid (mast cell committed) cell lineage still have a huge potential to multiply compared to those cells in late stages of development. If c-kit mutation arises in these later stages of development, cells have a limited potential for clonal expansion. Consequently, if c-kit mutations cause increased growth of mast cells, the duration of this process of increased growth will still be limited. However, if there is a large potential to multiply, a prolonged, chronic course is likely.

In the future c-kit research will provide knowledge to improve the treatment of adult-onset mastocytosis. The treatment of childhood-onset mastocytosis will probably benefit less from these developments, and symptomatic treatment is likely to remain the method of choice.

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CHAPTER 4

Pediatric Mastocytosis: Current State of Affairs 2002-2007

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Mastocytosis in Children

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Definition

The term mastocytosis denotes a heterogeneous group of disorders, characterized by local or diffuse increased non-malignant proliferation and accumulation of mast cells (MC) in the skin and/or in internal organs. The clinical signs and symptoms are caused by the functional effects of mast cell mediators that are produced and released at various anatomical sites. The skin is most frequently involved, but other organs may also be involved. Mastocytosis can be classified according to prognosis, or according to organ systems and symptoms. [1]

Classification

A basic classification scheme dividing mastocytosis into four prognostic categories was developed by Metcalfe as follows:[2]

1 An indolent variant with either skin disease or specific organ involvement and an excellent prognosis.

2 A variant, associated with hematological disorders.

3 A rare group of patients with an aggressive form of mastocytosis with rapid development of prominent lymphadenopathy and eosinophilia; the survival time of these patients after diagnosis without chemotherapy is 2 4 years.

4 A very rare and fatal form: mast cell leukemia.

Recently the classification was modified several times by the European Network on Mastocytosis.[3] In that concept, the diagnosis of cutaneous mastocytosis (CM) is based on clinical and histological findings in the skin in the

absence of criteria that would allow the diagnosis of systemic mastocytosis. Systemic mastocytosis is a clonal disorder of the mast cell and its progenitor. The symptoms of systemic mastocytosis are due to the pathologic accumulation and activation of mast cells in various tissues such as bone marrow, skin, gastrointestinal tract, liver, and spleen.[4]

Systemic mastocytosis (SM) criteria are divided into major criteria and minor criteria. Major criteria relate to major histological and immunohistochemical (Tryptase staining of tissue sections) findings. The most important aspect here is the dense focal infiltrate of MC that consists of a considerable number of MC (> 15) and is detectable at more than one site in the tissue(s) (multifocal pattern). Minor criteria relate to typical cytomorphological aspects of MC (in tissue sections and/or bone marrow smears) as well as to novel biochemical markers that indicate some degree of specificity for SM. It is proposed that the diagnosis is systemic mastocytosis if one major and one minor or three minor criteria for SM are fulfilled (Table1). Likewise, if dense multifocal infiltrates in the bone marrow consist of > 15 MC that appear to be spindle-shaped (> 25% MC are spindle-shaped), the diagnosis of SM can be established on histology without further investigation (and irrespective of the stain used). If MC are round, one should ask for additional criteria of SM (morphology of MC in bone marrow smears; CD2/CD25-expression on MC; serum tryptase, c-kit mutation) to establish the diagnosis of SM, because focal accumulations of round MC have also been observed in reactive MC hyperplasia.

Four major variants of SM have been defined by the working group: indolent systemic mastocytosis (ISM), systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease (SM-AHNMD), aggressive systemic mastocytosis (ASM), and mast cell leukemia (MCL). This classification is more useful in adult mastocytosis.[55]

Epidemiology

The exact prevalence of mastocytosis in the general population is unknown. Children without skin lesions or with indolent cutaneous mastocytosis may remain undiagnosed or unreported. The incidence figures vary widely from 1 in every 1000 to 1 in 8000 new patients at various dermatological units. Mastocytosis may occur at any age, but in approximately half of the cases, the onset is between birth and the age of 2 years; the disease is congenital in 15%, a further 30% of patients develop mastocytosis before the age of 6 months, another 10% by the age of 2 years, and about 10% between 2 and 15 years of age. There is no clear-cut sex predominance, and children of all races are affected. Although some 50 cases of familial mastocytosis have been reported the overall occurrence is sporadic. It has not been possible to discover the pattern of inheritance in the few cases in which more than one generation was involved.

Pathogenesis

The Stem cell factor (SCF) receptor is encoded by the protooncogene c-kit

Table 1. Proposed criteria to diagnose mastocytosis (adapted from Valent et al, 2001)

Cutaneous mastocytosis:

Typical **skin lesions** (urticaria pigmentosa, diffuse cutaneous mastocytosis, mastocytoma) and
positive **histology** with typical infiltrates of MC (diagnostic infiltrate-pattern: multi/focal or diffuse)

Systemic mast cell disease:

'Systemic Mastocytosis (SM) criteria'

Major : Multifocal dense infiltrates of MC (> 15 MC aggregating) detected in sections of bone marrow and/or of other extracutaneous organ(s) by tryptase-inununohistochemistry or other stains

Minor: a. In MC infiltrates detected in sections of bone marrow or other extracutaneous organs, >25% of MC are spindle-shaped or: in bone marrow smears, atypical MC (type I plus type II) comprise >25% of all MC

b. Detection of a c-kit point mutation at codon 816 in bone marrow or blood or other extracutaneous organ(s)

c. Kit+ mast cells in bone marrow or blood or other extracutaneous organ(s) co-express CD2 or/and CD25

d. Serum total tryptase concentration persistently > 20 ng/ml (in case of an associated clonal hematologic non-mast cell lineage disease (AHNMO), d. is not valid) [a]

If one major and one minor, or three minor criteria are fulfilled
then the diagnosis is systemic mastocytosis (SM)

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[a] In acute myeloid leukemia or myelodysplastic syndrome or myeloproliferative syndrome, elevated serum tryptase levels have been detected without increase in mast cell numbers or signs of mastocytosis.

and belongs to the type III transmembrane receptor tyrosine kinase subfamily. Apart from MC and their progenitors, c-kit is also expressed on other hematopoietic and nonhematopoietic (progenitor) cells. Binding of SCF by c-kit induces receptor dimerization followed by transphosphorylation of tyrosine residues becoming docking sites for the recruitment and activation of various cellular substrates.[1] The activated substrates then induce multiple intracellular signaling pathways responsible for MC differentiation, proliferation, survival and activation. Somatic c-kit mutations leading to ligand-independent (constitutive) activation of the receptor have been detected in several MC-related and non-MC neoplasms. In typical pediatric cases of CM, most studies failed to report recurrent abnormalities in the sequence of c-kit. Nevertheless, a point mutation of codon 839 with substitution of lysine for glutamic acid (c-kitE839K) was found in a case of CM. In the rare familial cases, no

classical c-kit mutations were found. Yanagihori et al. (2005) compared adults with mastocytosis with childhood-onset and adult-onset. They confirmed a high incidence of two distinct c-kit mutations, Asp-816-Val and Asp-816-Phe, in patients with childhood-onset cutaneous mastocytosis. These results underlined the importance of c-kit mutation analysis in children with mastocytosis for prognostic reasons.[6] The heterogeneity of c-kit mutations contribute to difficulties in characterizing genotype-phenotype correlation in the disease.

Clinical features

The clinical expressions of cutaneous mastocytosis (CM) are solitary mastocytoma, urticaria pigmentosa, diffuse cutaneous mastocytosis and Telangiectasia macularis eruptive perstans (TMEP).

The most frequent clinical form of mastocytosis was urticaria pigmentosa followed by mastocytoma and diffuse cutaneous mastocytosis. Darier s sign was present in 94% of cases. A negative Darier s sign does not rule out mastocytosis.[7]

In CM the visible cutaneous abnormalities are frequently of major concern to the patients and their family. However, in some patients the signs and symptoms produced by the functional effects of mast cell mediators may dominate the clinical picture, pruritus being the most frequent symptom. Pruritus tends to be paroxysmal, is most frequently mild to moderate, being only rarely severe, and frequently remains confined to the sites of mast cell infil-

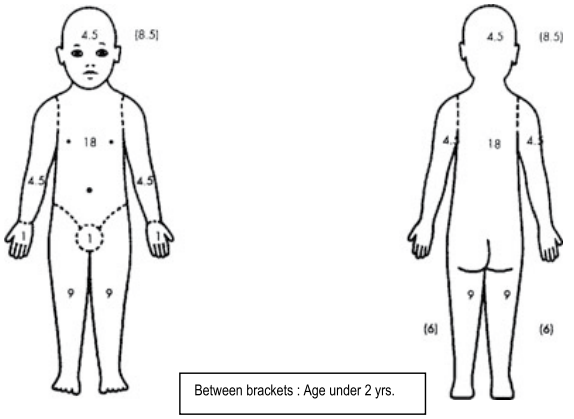
Table 2. Diagnostic work-up scheme

Inspection of cutaneous lesions, clinical examination
Darier's sign (eliciting urtication by rubbing the lesion)
Skin biopsy (pathology diagnosis and C-kit mutation)
Measurement of mast cell mediators and/or their metabolites in serum and/or urine, preferably serum tryptase.
Further investigations (if systemic mastocytosis is suspected): full blood count and peripheral blood smear; bone marrow aspirate and biopsy; skeletal survey, bone scan and bone densitometry; gastrointestinal investigations (contrast studies or endoscopy, abdominal ultrasound scan)
Immunophenotyping of blood cells and bone marrow aspirate C-kit mutation analysis (if available)

Note: In children often a blood sample and skin biopsy for routine histology are enough.

SCORMA INDEX

Institution	:	Name of patient	:
Physician	:	Date of birth	:
Date of visit	:	Patient number	:



Between brackets : Age under 2 yrs.

A: Extent please indicate the area involved [ ]

B: Intensity average representative area [ ]

Criteria	Intensity	Intensity items
1. Pigmentation / erythema	[ ]	0 = absent
2. Vesiculation	[ ]	1 = mild
3. Elevation	[ ]	2 = moderate
4. Positive Darier's sign	[ ]	3 = severe

C: Subjective Symptoms [ ]

	Visual Analog Scale (by parents if child < 5 years)
1. Provoking Factor(s)	0 ---------- 10
2. Flushing	0 ---------- 10
3. Diarrhea	0 ---------- 10
4. Pruritus	0 ---------- 10
5. Localized Bone Pain	0 ---------- 10

Scorma index: A/5 + 5B + 2C/5 [ ]

Figure 1. Mastocytosis SCORMA index.

tration. Systemic mastocytosis may occur with or without skin lesions. Symptoms appear to be derived primarily from the systemic and local effects of mast cell mediators and only secondarily from the space-occupying nature of the mast cell infiltrate.

In about the half of the children the manifestations disappear before, during or in late puberty. [5] Most cases of pediatric mastocytosis are sporadic and appear during the first 2 years of

life, especially on the trunk. Urticaria pigmentosa is the most frequent variant. The prognosis of pediatric mastocytosis, is generally good.[8]

Severity of mastocytosis can be scored by using the by us described SCORMA. (Figure 1)

A diagnostic work scheme is documented in Table 2.

Treatment of mastocytosis in childhood is usually unnecessary. Only when there are complications, treatment will be indicated to complications. When flushing or pruritus is prominent, antihistamines are used. In case of chronic diarrhea sodium cromoglycate is indicated.

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CHAPTER 5

Comparison of Mastocytosis with Onset in Children and Adults

J Eur Acad Dermatol Venereol, 16(2): 115-120

Comparison of mastocytosis with onset in children and adults

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ABSTRACT

Objective To compare the incidence, symptomatology and course of mastocytosis with onset in childhood and in adults.

Design Retrospective study of 101 patients with mastocytosis who were referred from 1980 to 1998.

Patients Medical records of 65 cases of mastocytosis with onset in childhood and 36 in adulthood were analysed. The clinical course was assessed in a subgroup consisting of 33 subjects with childhood onset who were followed up until at least adolescence and 12 subjects with adult onset who were followed up for at least 10 years.

Results The onset of the disease occurred before the age of 2 years in 50% and between the ages of 2 and 15 years in 14% of cases (childhood onset). In 36% of patients onset occurred at the age of 16 years and older (adult onset). An incidence peak of 60% was noted in the first year of life. Mast cell-mediated symptoms were not experienced by 21 of 36 adult onset mastocytosis patients nor by 27 of 65 childhood onset mastocytosis patients. Complete resolution was observed in five of 33 children. The majority of childhood onset cases (21 of 33) showed some improvement. Complete resolution was achieved in three of 12 adults. The majority of the remaining adults (eight of 12) showed no improvement.

Conclusions We confirm the incidence of onset of mastocytosis previously reported in the literature. We conclude that childhood onset mastocytosis is much less transitory than generally is assumed, although improvement occurs in the majority of cases. Symptomatology and clinical course of adult onset mastocytosis is less severe than suggested in the literature.

Key words: adults, children, follow-up studies, mastocytosis

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Introduction

Mastocytosis is a heterogeneous disease that is characterized by an increased number of tissue mast cells.[1] Mast cell proliferation is located mainly in the skin, but can also be found in internal organs, such as the liver, spleen, lymph nodes, bone marrow, lungs and gastrointestinal system. The clinical symptoms are caused by the release of mast cell mediators. Mastocytosis can be classified according to prognosis as reported by Travis *et al.*[2] and modified by Metcalfe.[3] In daily practice we prefer to use the dermatological classification that divides the disease into four cutaneous forms.[4] Urticaria pigmentosa (UP) is the most common type and represents about 65% of all paediatric cases.[5] The typical lesions consist of red–brown to yellowish macules, papules or nodules that may vary in size from several millimetres to centimetres in diameter. Erythema, swelling and blister formation as well as itching of the lesions may occur spontaneously or after stroking or rubbing. The blistering heals without permanent scarring. The lesions are generalized and randomly disseminated. The palms, soles, scalp and sunlight exposed areas of the body are often less affected.

Solitary mastocytoma (SM) is the second most common cutaneous presentation of childhood onset mastocytosis. SM is defined by the presence of one to several lesions (commonly five separate lesions or less) that have characteristics similar to those of UP. It is encountered in 10–35% of cases of childhood onset mastocytosis.[6,7] However, this figure may be an underestimate

as an asymptomatic solitary lesion may be overlooked or mistaken for a mole or juvenile xanthogranuloma. Of all clinical subtypes of childhood onset mastocytosis, SM invariably follows a mild course with complete resolution generally before adulthood.

Diffuse cutaneous mastocytosis (DCM) is rare and most patients have been described in case reports. By definition, the entire skin is involved, although the central region and the scalp are primarily affected. The clinical features become prominent at birth or in early infancy.[7–9] These consist of widespread spontaneous blistering with erosions and crusts, various degrees of erythroderma, strong dermographism and itching. The diagnosis of mastocytosis must be ruled out in new-borns with blistering and bullae. The blisters may be haemorrhagic in diffuse cutaneous mastocytosis. The aspect of the skin may be leather-like and thickened, especially in the flexural regions. In toddlers, the skin becomes less reactive, but diffuse hyperpigmentation and positive dermographism may persist into adulthood.

Telangiectasia macularis eruptiva perstans (TMEP) is the least common cutaneous manifestation of mastocytosis. The lesions consist of persistent red or light brown macules with visible telangiectases. The lesions are poorly demarcated and sometimes difficult to recognize. Darier's sign (swelling and redness induced by rubbing of the lesion) is generally positive.[8] As an entity, it is said to occur only in adults. The clinical features overlap with those of UP. Thus, underestimation of the incidence of this form is plausible, especially in children.

Based on the clinical picture and course, mastocytosis may also be divided into childhood onset mastocytosis (≤ 15 years) and adult onset mastocytosis (> 16 years). The age of the subject at onset is one of the main prognostic factors for the course of the disease.[10,11] Approximately 50–80% of children with UP is expected to undergo resolution of lesions and symptoms by adolescence, whereas the remainder exhibits a marked reduction of cutaneous lesions.[12] Children with SM typically exhibit complete involution of their lesions during childhood.[10] Childhood onset DCM is also known to resolve spontaneously. Adult forms of mastocytosis tend to be chronically progressive.[13]

Lately there has been much discussion on the differences in the aetiology of mastocytosis in children and adults. Evidence-based studies indicate that a somatic mutation in c-Kit receptor, of which stem cell factor is the ligand, may be responsible for a chronic progressive course in adults and sporadically in children.[14,15] However, no satisfactory explanation has been found for the majority of childhood onset cases. In the current study we analysed the medical records of 101 patients with mastocytosis examined in our dermatology department during the period 1980–98. The aim of the study was to investigate whether clinical types of mastocytosis clearly separate the children from the adults, as is generally accepted in the literature.

Table 1 Distribution of the cutaneous forms of mastocytosis in the study population (n = 101) for childhood onset mastocytosis and adult onset mastocytosis

Cutaneous form	Childhood onset	Adult onset	Total
UP	44 (44%)	27 (27%)	71 (70%)
SM	18 (18%)	2 (2%)	20 (20%)
DCM	2 (2%)	3 (3%)	5 (5%)
TMEP	1 (1%)	4 (4%)	5 (5%)
Total	65 (64%)	36 (36%)	101 (100%)

UP, urticaria pigmentosa; SM, solitary mastocytomas; DCM, diffuse cutaneous mastocytosis; TMEP, telangiectasia macularis eruptiva perstans.

Patients and methods

Patients

Our series comprised 65 cases of childhood onset mastocytosis and 36 cases of adult onset mastocytosis who had undergone dermatological evaluation between the period 1980–98 at the outpatient clinic of the Department of Dermato-Venereology at the University Hospital Rotterdam, The Netherlands. Male to female ratio was 1.7 : 1 (63 : 38). Skin lesions were the first manifestation of the disease in all cases. In 44 cases (44%) the diagnosis was based only on clinical examination, including a positive Darier's sign. In 57 cases (56%) the diagnosis was confirmed by histopathological examination. Skin biopsy was omitted in uncomplicated cases with evident UP or mastocytoma. The distribution of the cutaneous forms is shown in Table 1. The follow-up period for the whole group, defined as the period between initial symptoms and the last day of consultation, ranged from 0 years up to 61 years (mean: 8 years). Thus, for cases of childhood onset mastocytosis the maximum period reviewed was 61 years (mean: 9 years) and for cases of adult onset mastocytosis up to 21 years (mean: 6 years).

In 45 of the 101 cases the medical records were sufficient for long-term assessment of the clinical course. All subjects underwent a standard routine work up on a yearly basis, which included physical examination, blood counts, blood chemistry and urine analysis; additional investigations were decided on an individual basis. From 1994 onward measurement of urine *N*-methyl histamine was added to the work up. In well established uncomplicated cases of mastocytosis, diagnosed in the skin, bone marrow biopsy was excluded from routine investigations, because it is irrelevant in the management of the disease.

This subgroup of 45 patients consisted of 33 cases with childhood onset and 12 cases with adult onset mastocytosis, all chosen based on one of the following three criteria:

1 Childhood onset patients followed until at least the age of 15 years.
2 Adult onset patients followed for at least 10 years.
3 Childhood and/or adult onset patients reporting marked improvement of lesions and/or mast cell-mediated symptoms.

Table 2 An overview of symptoms per cutaneous form in childhood and adult onset mastocytosis

Symptoms	UP		SM		DCM		TMEP		Total		
	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	P-value
No symptoms	18	14	7	2	–	–	–	2	25	18	0.145
Symptoms (at least once)	26	13	11	–	2	3	1	2	38	15	
Pruritus	22	10	5	–	2	3	1	2	30	15	
Flushing	10	–	8	–	0	2	1	1	19	3	
Abdominal pain	8	1	6	–	2	–	1	–	17	1	
Diarrhoea	7	–	3	–	1	1	1	–	12	1	
Headache	3	1	2	–	2	1	–	–	7	2	

Ch, childhood onset mastocytosis; Ad, adult onset mastocytosis; UP, urticaria pigmentosa; SM, solitary mastocytomas; DCM, diffuse cutaneous mastocytosis; TMEP, telangiectasia macularis eruptiva perstans.

Methods

All medical records were analysed retrospectively. We developed a standardized form on which several items were recorded, including: the cutaneous form, mast cell-mediated symptoms, Darier's sign and factors provoking or aggravating the symptoms. Neither additional investigation used for diagnosis or follow-up purposes nor given therapy was recorded due to incomplete or sporadic mention in the medical records. Care was taken to record the items in chronological order to obtain insight into the course of the disease.

Statistical analysis

The items were recorded on a spreadsheet and were translated to the statistical package SPSS for Windows to enable statistical analysis. The first part of the evaluation consisted of analysing the items listed, whereas the second part consisted of statistical calculations of quantified items. Differences in prevalence rates between two groups were compared using Fisher's exact test. Two-sided $P < 0.05$ was considered to be statistically significant.

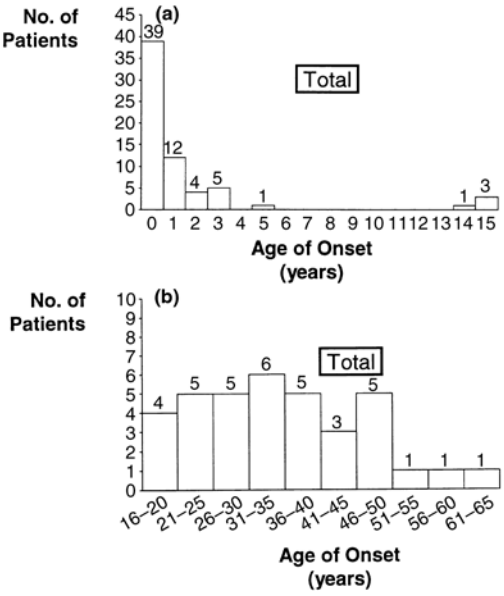

fig. 1 (a) Number of patients with childhood onset mastocytosis per age of onset; (b) Number of patients with adult onset mastocytosis per age of onset.

Results

Age of onset

An overview of the age of onset is shown in fig. 1(a,b). In the majority of patients with childhood onset mastocytosis the disease started before the age of 5 years, with an incidence peak in the first year of life. The incidence of adult onset mastocytosis was stable over the years. In this series only three new cases were diagnosed in subjects after the fourth decade of life.

Familial involvement

One female with childhood onset UP also had a twin sister with childhood onset UP, although the disease was less severe in the sister. In contrast, we also encountered a female with adult onset UP whose twin sister had no evident mastocytosis. One male with childhood onset DCM reported that several male relatives in different generations on his paternal side also had similar complaints.

Symptomatology

The distribution of the different cutaneous forms in our population is listed in Table 1. The most frequently cited mast cell-mediated symptoms in childhood and adult onset mastocytosis are listed in Table 2. Half of the patients with adult onset mastocytosis had never experienced any mast cell-mediated symptoms, but 25 of the 65 childhood onset mastocytosis

Table 3 Course of lesions and/or mast cell-related symptoms in childhood and adult onset mastocytosis

	UP		SM		DCM		TMEP		Total		
	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	Ch	Ad	P–value
Change	18	3	8	1	–	0	0	0	26	4	
Complete resolution	2 (10)	2 (37)	3 (18)	1 (31)	–	0	0	0	5	3	
Improvement	16 (10)	1 (28)	5 (6)	0	–	0	0	0	21	1	
No change	6 (28)	5 (44)	0	0	–	1 (44)	1 (52)	2 (68)	7	8	0.010
Total	24	8	8	1	–	1	1	2	33	12	

Numbers in brackets represent the mean age at which changes took place or the mean age at the end of the review period. ; Ch, childhood onset mastocytosis; Ad, adult onset mastocytosis; UP, urticaria pigmentosa; SM, solitary mastocytomas; DCM, diffuse cutaneous mastocytosis; TMEP, telangiectasia macularis eruptiva perstans.

patients had. However, the difference was not statistically significant ($P = 0.145$). Mast cell-mediated symptoms, such as those involving the skin (pruritus and urticaria) or the gastrointestinal system (abdominal pain, nausea, vomiting and diarrhoea), and constitutional symptoms (including flushing, headaches, dizziness, malaise and collapse) were reported. Subjects with childhood onset mastocytosis had statistically significant ($P = 0.004$) more gastrointestinal symptoms than those with adult onset mastocytosis.

Provoking factors

A total 64 subjects reported provocation or worsening of the symptoms under certain circumstances. Heat or cold was by far the most frequently cited provoking factor, followed by friction, psychological stress, physical effort, alcohol, food, certain medications, flu, fever and insect bites. The number of provoking factors mentioned per patient ranged from 0 to 5. The majority of subjects reported only one provoking factor.

Clinical course

The clinical course could only be assessed in a subgroup consisting of 33 childhood onset and 12 adult onset mastocytosis patients as shown in Table 3. The clinical course was divided into improvement vs. no improvement. Improvement was defined as complete or partial resolution of skin lesions and/or symptoms. Compared with adult onset patients, patients with childhood onset mastocytosis experienced more often statistically significant improvement in the clinical picture (Fisher's Exact Test; $P = 0.010$). Progression of the disease resulting in haematological disease was not found in the study group.

Discussion

The aim of this retrospective study was to establish whether there are differences between the clinical symptoms and the course in childhood onset and adult onset mastocytosis. As it is postulated that childhood and adult onset mastocytosis might have two different aetiological mechanisms, an insight into their course may increase the understanding of the aetiology of the disease, in the hope that such subjects will be followed up and treated more effectively. In this analysis of 101 medical records we focused exclusively on the clinical forms of mastocytosis and the course followed by the disease. It is evident that the results reflect those cases of mastocytosis in which skin symptoms are the main manifestation of the disease.

In our population, 64% of the cases of mastocytosis started in childhood. One-half (50%) of the subjects developed the first lesions before age 2 and 14% between the ages of 2 and 15 years. The remaining 36% developed the first lesions after the age of 15 years (adult onset mastocytosis). These incidence figures closely resemble and confirm those reported in the literature.[11] Although we confirmed that there is a clear peak of incidence (60%) in the first year of life (fig. 1a), we did not observe a second peak of incidence in adult onset mastocytosis (fig. 1b).[10] The incidence from the age of 16 years onward was fairly regular over the years with a decrease in incidence as the age increased.

The distribution of the cutaneous forms within our population is consistent with that reported in the literature[16] and is shown in Table 1. We noticed that lesions of adulthood onset UP more often tended to be small in diameter, whereas those in childhood onset UP were more often large and widespread. We also observed some exceptional cases in our population. For DCM, a form considered mainly to be of childhood onset, the number of adult onset DCM patients was one more (three) than the number (two) of patients with childhood onset DCM. There were five patients with TMEP, one with childhood onset, which is noteworthy considering the rarity of this form and the fact that TMEP is very rarely observed in childhood.[8] These findings point out that all the cutaneous forms of mastocytosis may occur at any age.

Familial cases of mastocytosis are rare.[17–19] Two of our patients reported familial involvement. The first patient was a female with childhood onset UP, whose twin sister also had UP. However, this sister had a less severe form, as the skin lesions were less pigmented and lower in number and she had no mast cell-mediated symptoms. In contrast, another patient in our

population, a female with adult onset UP, had a twin sister who did not have mastocytosis. An autosomal dominant inheritance with incomplete penetrance has been suggested as an explanation for the differences in the occurrence of mastocytosis in twins.[20] We cannot draw any conclusions about the mode of inheritance from such small numbers. The second patient reporting familial involvement was a male with childhood onset DCM, who had several male relatives in different generations on his paternal side with similar complaints.

The typical symptomatology of mastocytosis is caused by the release of mast cell mediators. The five most frequent symptoms in our population (Table 2), can all be attributed to the action of mast cell mediators, especially histamine.[1] The number of patients reporting gastrointestinal symptoms was significantly higher in the childhood onset than in the adult onset mastocytosis group. However, the possibility of bias cannot be ruled out, as children are known to be more susceptible to gastrointestinal afflictions. Although headache, the fifth most frequent symptom, is known to be a symptom of mastocytosis,[16] its cause was difficult to establish.

It is worth of note that 43 (43%) of the 101 patients had not experienced any mast cell-mediated symptoms. In the adult onset group this was the case for 18 of the 36 subjects. This observation is important because of the assumed chronic progressive nature of cutaneous mastocytosis in adults.

The course of mastocytosis in the adult onset group, for which there was adequate follow-up, was more favourable than expected; this was in contrast to the childhood onset group, also for which there was adequate follow-up. For the childhood onset group adequate follow-up was defined as follow-up until adolescence (age 15 years), as this is the age at which the disease is expected to resolve.[11] For the adult onset group this follow-up had to be at least 10 years. Subjects with either childhood or adult onset mastocytosis reporting improvement of lesions and/or symptoms automatically fell into this subgroup. Interestingly, only two of 24 childhood onset UP patients showed complete resolution of lesions and/or symptoms, compared with the prognosis of 50–80% experiencing complete resolution reported by Caplan.[12] The majority of patients with childhood onset mastocytosis, namely 21 of the 33 showed only partial improvement in their lesions and/or symptoms, but the remaining seven subjects showed no changes in the clinical picture at all. The same trend was observed in the SM group. Considering the fact that SM is the form of mastocytosis that has the best prognosis,[20] only three of the eight children were completely cured. Again, the majority of childhood onset SM patients, namely five of the eight patients had partial improvement of lesions and/or mast cell-mediated symptoms during the follow-up period. These findings provide a different view on the prognosis of childhood onset and adult onset mastocytosis. Childhood onset mastocytosis is not as transitory as suggested in the literature, where a higher number of complete resolutions is reported.[12]

Although five of the eight adult onset UP subjects did not show any changes in the clinical picture, as expected, two subjects achieved complete resolution and one partial resolution. The only adult with SM also experienced complete resolution of the lesion. No changes in the disease were noted in adults with DCM and TMEP. The favourable course of adult onset mastocytosis in our group indicated that this type of mastocytosis located in the skin had a better prognosis than expected based on the literature.

While analysing the patient records, we noted the absence of an objective standardized method of evaluating mastocytosis. The clinical findings were always classified subjectively in the physician's own words. This introduced numerous interpersonal and intrapersonal variations. Variations in the description as well as in the assessment of severity and the course of the disease were common. A uniform and reproducible standardized questionnaire for scoring mastocytosis would eliminate this problem. It would also facilitate an objective comparison of cases and reduce confusion in communication between physicians. Therefore, we are developing such a questionnaire for scoring mastocytosis and will not discuss the problem further here.

From the results of this retrospective study, we can conclude that the incidence for the four cutaneous forms of mastocytosis generally corroborate those reported in the literature.[11] We could not confirm that a second peak of incidence occurs in adults, as the incidence was evenly distributed over the years. It was noteworthy that we observed marked differences in comparison of findings reported in the literature,[12] with respect to the clinical course for cutaneous mastocytosis, both in the childhood onset and adult onset group. A considerable number of both groups had never experienced any mast cell-mediated symptoms. In contrast to what is reported in the literature, in our series childhood onset mastocytosis was not a transitory disease as it rarely showed complete resolution, although there was some improvement in the majority of cases. In our study, the prognosis of adult onset mastocytosis with respect to symptomatology and course of the disease was noted to be much better than that reported in the literature.

These observations shed new light on the discussion that childhood onset and adult onset mastocytosis are two different clinical entities. Molecular biological research focused on identifying changes within the mast cell itself, especially on growth regulation, may further clarify the observations reported here.

Acknowledgments

We thank Jan van der Sluis, PhD, for his helpful advice.

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CHAPTER 6

Clinical Aspects of Diffuse Cutaneous Mastocytosis in Children

Submitted: Arch Dermatol

Clinical Aspects Of Diffuse Cutaneous Mastocytosis in Children

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Submitted for publication

Objective: This paper describes the different forms of diffuse cutaneous mastocytosis (DCM), based on the largest series published to date.

Design: We undertook a case controlled analysis of eight children with DCM. Results of laboratory testing including mast cell mediator levels and clinical symptoms on presentation and during follow up were analyzed.

Setting: Department of Dermatology and Venereology (Pediatric Dermatology), Erasmus MC, University Medical Center – Sophia Children's Hospital , Rotterdam. The Netherlands Department of Pediatrics, Onze Lieve Vrouweziekenhuis, Aalst, Belgium. Department of Dermatology, Radboud University Nijmegen Medical Centre, Nijmegen. The Netherlands. Department of Dermatology and Allergology, University Medical Center Utrecht. The Netherlands. 1st Pediatric Department, Athens University, Aghia Sophia Children's Hospital, Athens. Greece

Results: The levels of urinary N-methylhistamine and serum Tryptase were initially high in all cases, but declined sharply later on. Two of seven cases in which mast cell mediator decline could be calculated showed a reduction of 20%, while the remaining five showed a reduction of 80%. Clinical improvement showed the same pattern.

Conclusions: Diffuse cutaneous mastocytosis (DCM) is a rare variant of cutaneous childhood onset mastocytosis. Various forms show the same or overlapping features at various times. It appears to follow a course similar to that in other types of childhood onset mastocytosis taking into account the decreased symptoms and the levels of mast cell mediators during the follow up. The markedly elevated levels of N-methylhistamine and serum Tryptase are not the only factors that should be considered in deciding whether to obtain a bone marrow biopsy for diagnosis according to the recent consensus on mastocytosis.

Author affiliations are listed at the end of this article

Classification of mastocytosis is structured since the publication of WHO guidelines. The WHO classification divides cutaneous mastocytosis (CM) from systemic mastocytosis, the latter being subdivided into four categories of increasing severity[1,2]. Thorough and broad based further recommendations in the standardization and work up of the recognized subtypes of mastocytosis have been published by Valent et al [3]. To date systemic mastocytosis is regarded as a clonal myeloproliferative disease based on the presence of a somatic mutation predominantly in codon D816V encountered in many patients with systemic mastocytosis. Over 50 other c-kit mutations of varying clinical relevance are known[4]. However, it is debated whether pediatric mastocytosis should be regarded as clonal[5]. In contrast to adult onset mastocytosis this clinical variant of mastocytosis may regress in time. Of all cases of mastocytosis, the majority are pediatric cases with cutaneous abnormalities and lack sufficient criteria for classification as systemic cases. The clinical expressions of CM in adults and children recognized by the WHO are maculopapular; diffuse cutaneous mastocytosis and solitary mastocytoma. From a dermatologist's perspective the WHO sub-classification may be an over-simplification of cutaneous mastocytosis. This issue was raised by Hartmann and Henz[6]. They argued based on course and prognosis that maculopapular mastocytosis in fact consists of different entities of cutaneous mastocytosis; maculopapular cutaneous mastocytosis with small lesions that occurs in children and in adults rarely resolves spontaneously and will often eventually be categorized as indolent systemic mastocytosis. In contrast to this they point out that the plaque type

cutaneous mastocytosis with lesions of several centimeters in diameter and solitary mastocytomas do not evolve but tend to disappear[6]. Pediatric mastocytosis is predominantly encountered in patients during the first 2 years of life. Maculopapular mastocytosis is the commonest variant, but this may depend on the setting of the institution. The prognosis of pediatric mastocytosis is mostly good [5,7,8]. The visible cutaneous abnormalities in CM are frequently of major concern to the patients and their family. In about the half of the children the manifestations disappear before, during or in late puberty. As a subtype of pediatric mastocytosis diffuse cutaneous mastocytosis (DCM) remains a rarity[9,10]. The clinical picture may be impressive and treatment options for severe cases include oral steroids and PUVA, mild cases may benefit form anti-histamine agents. Eight patients with DCM are reported in this communication.

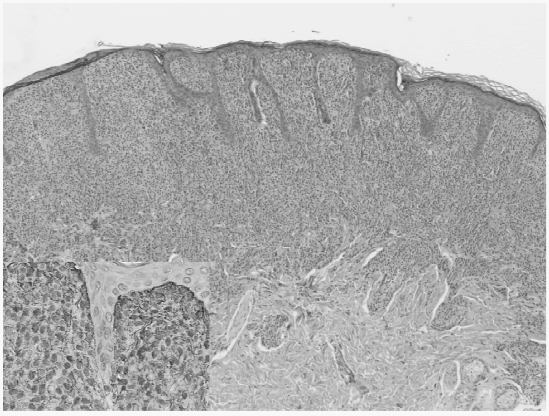
PATIENTS AND METHODS

The combined group of patients with predominantly cutaneous mastocytosis presently under surveillance of the authors consists of 8 patients with DCM. Consent for publication of data and clinical photographs were obtained either from the patients or their parents and approval of the medical ethical committee was obtained.
Four patients were seen at the Pediatric Dermatology Unit of the department of dermatology Erasmus Medical Center, Rotterdam. The remaining 4 patients were seen at the dermatology outpatient clinic of University Medical Center, Utrecht, at the outpatient clinic of Radboud University Medical Center, Nijmegen, at the department of pediatrics, Onze Lieve Vrouweziekenhuis in Aalst, Belgium, and at the 1st Pediatric Department, Athens University, Aghia Sophia Children's Hospital in Athens. Greece. The follow up ranged from 2 to 19 years, with a mean follow up of 9 years. The characteristics of all 8 patients are shown in **Tables 1a and 1b.**
The diagnostics included history, physical examination, skin biopsy, serum Tryptase and urinary N-methylhistamine levels and abdominal ultrasound[11,12]. In the cases with a follow up of over 5 years, initial mast cell mediator analysis included urinary N-methylhistamine which was later replaced with serum Tryptase as the preferred indicator of mast cell activity. The average decline in the level of serum Tryptase was 65% over a period of 4.5 years in seven patients. One patient had serum Tryptase level of higher than 200 ng/l which exceeded the range of laboratory test. In this case no changes in the level of serum Tryptase were observed.
Two cases (Patients no.1 and 8) are described in details. Patient no. 1 represents a typical case starting in the first months of life. Bone marrow biopsy was not obtained from this patient. Patient no. 8 represents an impressive case of the same disease manifestation illustrating the diversity of DCM. In this case neither bone marrow analysis nor c-kit mutation analysis showed significant changes.

HISTOPATHOLOGY

Histopathological examination of the skin biopsy in all cases showed the same pattern of a diffuse infiltration of mast cells as a broad band under the epidermis, which was accompanied by either a large or a small blister formation. The clinically involved skin was somewhat more infiltrated than the not involved skin, but the general histopathological picture was the same.

Figure 1a and 1b



Histopathology of Diffuse Cutaneous Mastocytosis
(1a H&E staining 100X, 1b Tryptase staining – inset- 400X)

CASE REPORT PATIENT NO. 1

A boy aged 2-3 months with progressive blistering and red skin, but without fever or pruritus was examined at the Pediatric Dermatology Unit of the department of dermatology Erasmus Medical Center, Rotterdam. There was no history of skeletal pain, diarrhea, vomiting or other complaints. The initial diagnosis was impetigo bullosa, but treatment was not successful. Dermatological examination showed vesiculo-bullous areas on the left shoulder and on the trunk. Some lesions appeared to be infiltrated. Some were more yellow. Dermography and Darier's sign (lesional dermographism) were present. Diagnosis of DCM was based on clinical examination, histological examination of lesional and apparently non-lesional skin, indicating that the entire skin was involved. Histopathological examination of a skin biopsy showed dense infiltrates of Tryptase positive mast cells.

Treatment was started with oral anti-histamines (ketotifen) and topical sulphur 5% in Zinc linament. As the effect was minimal, the oral medication was changed into dimetindene (and later into oxatomide), both with minimal or no effect. The symptoms became less prominent during the follow up of 8 years.

CASE REPORT PATIENT NO. 8.

A boy aged 33 months with slowly progressive diffusely distributed firm infiltrative subcutaneous nodules and severe accumulation of nodules in the head and neck area was under treatment at the 1th Paediatric Department, Athens University, Aghia Sophia Children's Hospital in Athens Greece since the age of 3 months. The Medical history revealed diffuse erythema and blistering at birth, evolving into a maculopapular – nodular appearance at 3 months, and further evolving into the nodular infiltrative lesions at the present age. The blistering diminished in time, but flushing and pruritus persisted. Diarrhoea from the third month onwards caused retardation of growth. Disodium chromoglycate treatment form the fifth month onwards stopped the diarrhoea.

The diagnosis of diffuse cutaneous mastocytosis was based on clinical examination, histological examination of lesional skin. Serum Tryptase levels were measured at the age of 3, 12, 18 and 21 months and showed values exceeding 200μg/l. The level of serum Tryptase had decreased to 160μg/l at the age of 32 months. Other blood chemistry and blood cell count and white blood cell count were normal. The bone marrow analysis showed diffuse infiltration of mast cells. C-kit analysis did not show any mutations. Currently the patient is treated orally with disodium chromoglycate, cetirizine, ranitidine and L- Thyroxine. Additional therapy with topical corticosteroids the last two months showed improvement of the nodular lesions and the pruritus.

SERIES OF PATIENTS

The initial symptoms were noticed in all patients during the first months of life, before the age of 6 months. The disease began with blistering in all the cases. Two variations in the initial presentation were recognized – starting with red skin and extensive blistering (cases 1, 2, 4-6) and directly starting with yellow-orange infiltrates with only limited blistering (cases 3,4,8) Blistering disappeared before the age of 2 years, but was most prominent in the first year of life.

Table 1a

Patient number	blistering	redness	Dariers sign	dermo-graphy	infiltrates	diffuse infiltrates	Yellow Skin	flushing	urticaria	liver enlarged	prutitus	fever
1*	yes	yes	yes	yes								
2**	yes	yes	yes	yes								
3	yes				yes		yes	yes		yes		
4	yes				yes		yes	yes	yes			yes
5		yes	yes	yes							yes	
6	yes					yes	yes					
7						yes	yes			yes		
8	yes	yes			yes							

Presenting clinical characteristics of eight DCM cases

Table 1b

Patient number	follow up (yrs)	mediator levels[#]	Period (yrs)	c-Kit mutation analysis
1	9	Nmh -93%	3	n.a.
2	19	Nmh -28%	5	asp816val
3	13	Nmh -72%	6	n.a.
4	4	Tryptase -58%	3	n.a.
5	6	Tryptase -22%	1	n.a.
6	3	Tryptase -83%	3	n.a.
7	15	Nmh/Tryptase -85%	10	n.a.
8	2	Tryptase change not esthablished[##]	2	negative

n.a. = information is not available

Clinical characteristics of eight DCM cases

Published cases

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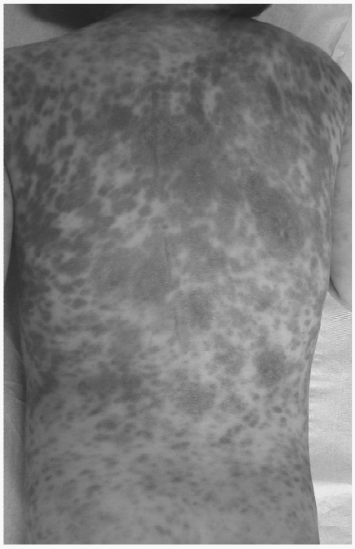
 # Note:

The mast cell mediators urinary N-methylhistamine and serum Tryptase are both valid parameters of mast cell activity in mastocytosis. As the markers were not available in all patients at all times, therefore the changes in time of the measured parameters are mentioned.

Note:

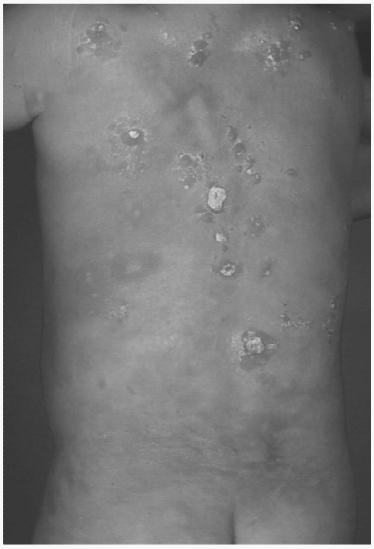
The laboratory report did not specify values in excess of 200 µl/l. The initial Tryptase level present in this patient exceeded of 200 µl/l.

Figure 2



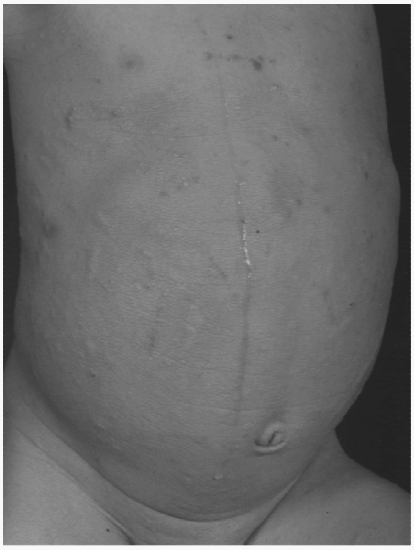
Red large blister type (in patient no 5.)

Figure 3



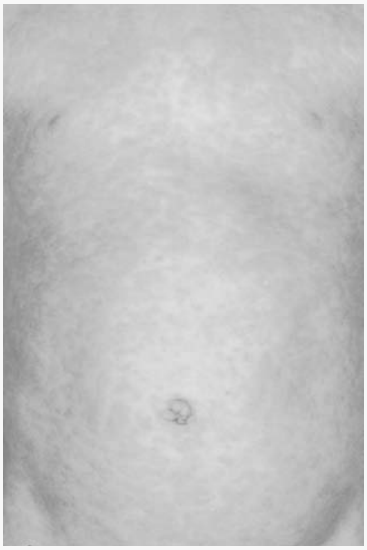
Red large blister type (patient no 1.)

Figure 4



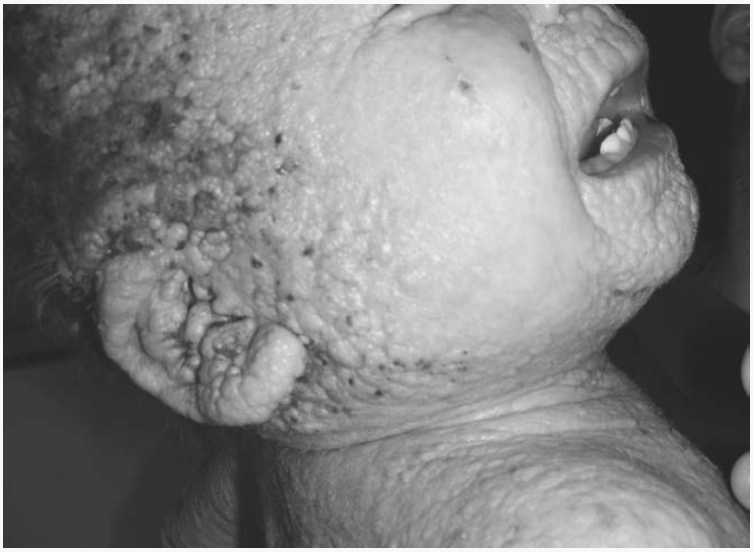
Yellow infiltrated small blister type
(patient no 7.)

Figure 5



Yellow infiltrated small blister type
(patient no 4.)

Figure 6



Yellow infiltrated small blister type extreme presentation (patient no 8.)

The levels of urinary N-methylhistamine and serum Tryptase were initially high, but decreased slowly during the course of time, except in case 4, where there was a temporary and unexplained increase in the serum Tryptase level lasting for 3 months. Bone marrow biopsy was refused by the parents of this child. The child continued to improve and the serum Tryptase levels did decrease later on.

In **Table 1b** it can be seen that there was a decrease of approximately 20% in the levels of mast cell mediators in two cases (cases 2, 5), whereas there was a decrease of 80% in the levels of mast cell mediators in five cases (cases 1, 3, 4, 6, 7).
At the time of writing of this paper, one of the cases with a limited decline in the levels of mast cell mediators has been diagnosed with indolent systemic mastocytosis, with a positive asp816val mutation. The patient is doing fine and receives no specific treatment. The other patient is now six years old and a bone marrow biopsy is likely to be obtained if elevated levels of serum Tryptase continue to persist.

DISCUSSION

Diffuse cutaneous mastocytosis (DCM) is very rare as can be concluded from the small number of studies on pediatric patients that have been reported. Inamadar and Palit reported 6 cases of cutaneous mastocytosis of which 3 patients suffered from diffuse cutaneous mastocytosis [13]. Kiszewski described 71 children with mastocytosis of whom 6 (8%) had diffuse cutaneous mastocytosis [14]. In most series of patients with pediatric mastocytosis, the number of cases with DCM was low. Ben-Amitai et al, described 180 children with cutaneous mastocytosis but none of the patients had DCM [9]. Hannaford reported 3 cases with DCM among 173 pediatric patients with mastocytosis[10]. We reported 2 cases of DCM among 65 cases of childhood onset mastocytosis in 2002 [7]. To our knowledge this case study is the largest series of DCM reported.

On the one hand, clinical presentation begins with a variable degree of generalized blistering early in life. The blistering may be extensive and even life-threatening. The bullous type of DCM has widespread bullae and redness as the main cutaneous feature. These patients often show widespread erythema. It mimics other diseases such as Staphylococcal Scalded Skin Syndrome (SSSS) and bullous erythema multiforme[15-18]. On the other hand, the disease shows yellow-orange infiltrations (xanthogranuloma like abnormalities) and slight blistering. This presentation has been reported in a very limited number of cases [19,20]. Walker reported a rare case of diffuse cutaneous bullous mastocytosis with pachydermia and unusually extensive skin folding in a 3-week-old girl who fits in the subgroup of the yellow-orange variant[21]. Whether the two variants are indeed different or are manifestations of the same disease presenting with different clinical features during the course has no influence on the management of the patients. There is limited information on the prognosis of DCM, but it seems to be similar to that in other forms of CM in childhood. None of the patients reported here experienced severe life-threatening events and treatment was restricted to anti-histamine agents and disodium cromoglycate. Oral steroid treatment and photo-chemo therapy with UV-A light are also other suitable strategies in severe persistent cases [24, 25] The authors are not in favor of using the latter two therapies as first line treatment in infancy.

Bone marrow biopsies were not taken as a routine from any of our patients because of reluctance of the parents due to clinical improvement, decrease in the levels of serum Tryptase or urinary N-methylhistamine. Arguably, bone marrow analysis is important for the choice of therapy, especially in the case of persistent rising serum Tryptase levels, c-Kit abnormalities, and suspicion of myeloproliferative disease. Diffuse cutaneous mastocytosis was shown to be a part of systemic cases of mastocytosis [22, 23]. Furthermore, in SM there is substantial risk of osteoporosis, a condition which must be treated.
The clinical features of red large blistering and orange-yellow small blistering in DCM raises the question of whether there is a single clinical description that fits all presentations. The very low incidence of DCM makes the initial diagnosis difficult as demonstrated in our series in 4 out of the 8 cases where a faulty initial diagnosis was made. Diffuse cutaneous mastocytosis in all the cases presented as a cutaneous disease with a benign course. The levels of serum Tryptase or urinary N-methylhistamine were highly elevated similar to that in other forms of mastocytosis classified as systemic. However, in DCM the number of the cutaneous mast cells alone may be sufficient to cause the elevated levels of the mediators. This is supported by the observed simultaneous decrease in the cutaneous symptoms and the levels of the mediators. If one would apply the consensus standard algorithm as published by Valent et al in 2007, then almost all the patients would be candidates for a bone marrow biopsy, especially the youngest patients. In this series, one patient showed a temporary increase in serum Tryptase level instead of the expected decrease. The significance of this still remains unknown. At the present the patient is doing fine and has no sign of hematological disease, but would have qualified for bone marrow analysis. Two of seven cases in whom the percentage of decline in

the level of mediators was calculated showed a reduction of approximately 20%. This is a fourfold difference compared with five other cases in whom there was a reduction of 80%. The less drastic improvement could be explained in one patient who is now twenty years old and in whom indolent systemic mastocytosis (asp816val positive) has been diagnosed. The other patient is now six years old and in whom bone marrow investigations are awaited.

Given the rarity of DCM, one is unable to clearly recommend when to pursue bone marrow analysis in infants suspected of this disease.- One must realize that the severity of the disease dictates whether one should do this, considering the effects of such an intervention for the patients and their parents. Determining the levels of mast cell mediators alone may not be the best tool to decide the most appropriate therapeutical approach because both serum Tryptase and urinary N-methylhistamine levels tend to be very high, but decrease in time. Whether newly reported plasma IL-6 levels may be of use in such cases is uncertain since there are correlations between plasma IL-6 levels and total serum Tryptase levels, severity of bone marrow pathology, organomegaly, and the extent of skin involvement[26]. Therapeutic recommendations in cases of DCM are tailored and dosage schemes of H1 and H2 antagonists and mast cell stabilizing drug may exceed the recommended dosage. Special care is needed when anesthesia and radiography with contrast medium is undertaken. A protocol on the treatment of mastocytosis in childhood by our group has recently been published [27].

The cases of DCM (in infancy) reported in the literature and from our own experience tend to improve in time. Therefore, we recommend to include the clinical presentation of symptoms, progress of disease and elevated levels of serum Tryptase before deciding whether to obtain bone marrow biopsies and choosing the most effective therapy. An algorithm for the work up of patients suspected of DCM is presented in the appendix.

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Appendix.

An algorithm of work up for patients suspected of suffering from diffuse cutaneous mastocytosis (DCM)

1) A newborn or an infant with an extensive or a generalized urticating, blistering or maculopapular erythematous eruption without any apparent cause and cases with anaphylactic episodes.
2) Consider the diagnosis of DCM
3) Establish any provoking factors
4) Provoke Darier's sign or dermatography
5) Determine serum Tryptase levels (persistently raised serum Tryptase levels makes the diagnosis of mastocytosis likely but does not confirm it)
6) Obtain skin biopsies from lesional and non-lesional skin and also stain for CD25 and undertake Kit mutation analysis
7) Repeat measurements every 3 – 6 months in case of inconclusive results
8) Team up with a pediatrician in case of positive findings :
9) Abdominal ultrasound and hematological work up is recommended
10) Treatment and follow up is tailored according to the laboratory findings, imaging and severity of symptoms
11) Lack of improvement of symptoms over a 12-month period in established DCM, hematological aberrations, established mutation in Kit are indications for bone marrow investigations

CHAPTER 7

Mast Cell Distribution in Normal Adult Skin

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Mast cell distribution in normal adult skin

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Aims: To investigate mast cell distribution in normal adult skin to provide a reference range for comparison with mastocytosis.

Methods: Mast cells (MCs) were counted in uninvolved skin adjacent to basal cell carcinomas and other dermatological disorders in adults.

Results: There was an uneven distribution of MCs in different body sites using the anti-tryptase monoclonal antibody technique. Numbers of MCs on the trunk, upper arm, and upper leg were similar, but were significantly different from those found on the lower leg and forearm. Two distinct groups were formed—proximal and distal. There were 77.0 MCs/mm^2 at proximal body sites and 108.2 MCs/mm^2 at distal sites. Adjusted for the adjacent diagnosis and age, this difference was consistent. The numbers of MCs in uninvolved skin adjacent to basal cell carcinomas and other dermatological disorders were not different from those in the control group. Differences in the numbers of MCs between the distal and the proximal body sites must be considered when MCs are counted for a reliable diagnosis of mastocytosis. A pilot study in patients with mastocytosis underlined the variation in the numbers of MCs in mastocytosis and normal skin, but showed a considerable overlap. The observed numbers of MCs in adults cannot be extrapolated to children.

Conclusions: MC numbers varied significantly between proximal and distal body sites and these differences must be considered when MCs are counted for a reliable diagnosis of mastocytosis. There was a considerable overlap between the numbers of MCs in mastocytosis and normal skin.

Mast cells (MCs) are multifunctional cells that play an important role in inflammatory and allergic reactions. They attract other key players of the immune system by releasing cytokines. Skin MCs are easily recognised by their metachromatic granules, which release their contents after activation by surface antigen or cytokine dependent events. The number of skin MCs can increase under certain conditions. In mastocytosis, the increase in the number of MCs is considered a primary event with an unknown pathogenesis.[1] Cutaneous mastocytosis presents with mildly to severely pruritic macules and papules that host an increased number of MCs.[2] Systemic mastocytosis affects several internal organs and presents with a wide range of symptoms, such as hypotension, seizures, skeletal pain, abdominal pain, and changes in defaecation.[2 3] These symptoms may occur in various diseases and in the absence of dermatological signs, making the diagnosis of mastocytosis very difficult, so that it may even be missed by the clinician. Several criteria should be met for the diagnosis of mastocytosis. An increased serum tryptase concentration (> 13.5 ng/ml), dense infiltrates of mast cells in the cutaneous lesions or in the bone marrow, the expression of CD2 and CD25 on bone marrow mast cells, or the presence of a c-kit mutation may assist in the diagnosis.[2 4] When systemic mastocytosis is suspected, a skin biopsy from lesional skin, or even from non-lesional skin, may be helpful in determining the number of MCs in the skin. In some cases, a slight increase in the number of MCs is seen, and in the absence of reliable reference values for the numbers of MCs in healthy skin, it remains unclear whether there is a pathological increase in the number of MCs or whether the values are within the normal range. Therefore, a reference value for the normal numbers of MCs in healthy skin may provide a valuable tool for improving the accuracy of diagnosis.

''Tryptase is considered to be an immunohistochemical marker for mast cells''

In previous studies, different numbers of MCs in normal skin were reported, but they could not serve as reference values because the methods that were used for counting MCs in skin biopsies were not uniform.[5–10] Furthermore, researchers used different staining techniques and often the studied groups were very small.[11] Staining with anti-tryptase monoclonal antibody (ATA) is now considered to be the gold standard for identifying MCs.[12] The enzyme tryptase is also present in basophilic granulocytes, but its concentration is so low that they are stained very weakly with ATA. Therefore, tryptase is considered to be an immunohistochemical marker for MCs.[12] The aim of our present study was to determine a reliable reference value for the numbers of MCs in healthy skin that could be used to establish the diagnosis of mastocytosis with certainty.

MATERIALS AND METHODS

Biopsies

Paraffin wax embedded blocks of skin biopsies, collected between 1996 and 2001 at the department of pathology of the Erasmus MC, Rotterdam, the Netherlands, were used. The biopsy blocks were divided into three groups. The first group consisted of perilesional skin from 25 basal cell carcinoma (BCC) biopsies. The mean age of the patients in this group was 66.8 years (range, 47–81). The second group consisted of perilesional skin from 95 biopsies taken from patients with various dermatological disorders (compound naevi, Spitz naevi, scars, dermatofibromas, and others). All biopsies were from the trunk, upper arm, forearm, upper leg, or lower leg. Exclusion criteria were inflammatory changes in the skin, massive degranulation of MCs, or other abnormalities. The mean age of the patients in this group was 49.7 years (range, 15–87). The third group consisted of skin biopsies of 21 healthy women (mean age, 37.7 years; range, 18–63) who

Abbreviations: ATA, anti-tryptase monoclonal antibody; BCC, basal cell carcinoma; MC, mast cell; PBS, phosphate buffered saline

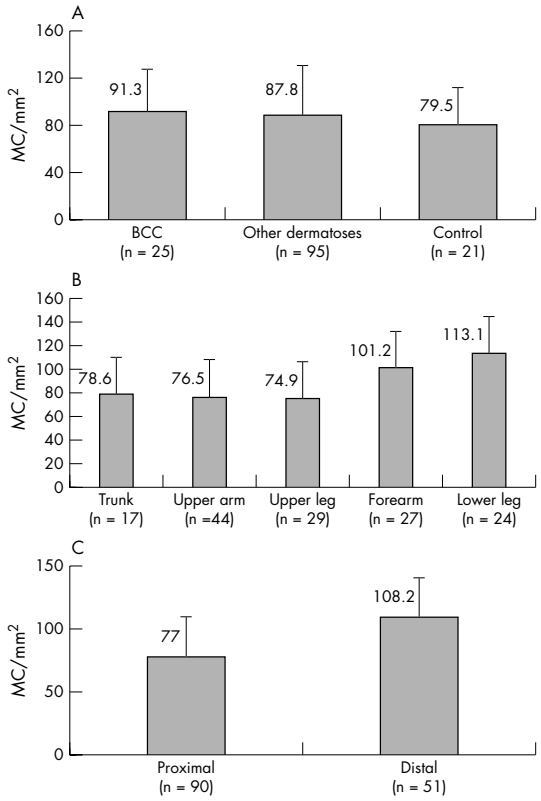

Figure 1 (A) The numbers of mast cells (MCs) were determined in three different biopsy groups, using anti-tryptase antibody staining and expressed as MC/mm^2. No differences were found between perilesional skin biopsies around basal cell carcinomas (BCCs), perilesional skin around various dermatological disorders, and control skin ($p = 0.560$). (B) From these three biopsy groups, the numbers of MCs were determined in five different body sites; p values for differences in MC numbers in the trunk, upper arm, and upper leg were not significant (0.929), and neither were p values for differences between the forearm and lower leg ($p = 0.240$). (C) Body sites with similar MC numbers were placed into two groups. The ''proximal'' group was formed by the trunk, upper arm, and upper leg, whereas the ''distal'' group was formed by the forearm and lower leg (unpaired Student's *t* test, $p < 0.001$).

underwent elective mamma reduction or abdominoplasty at the department of plastic and reconstructive surgery. None of these women had dermatological disorders and there was no report of systemic use of immunosuppressives or glucocorticoids. These biopsies were collected directly after the surgical intervention and were obtained and included after informed consent. This group served as the control group.

Staining procedure

Formalin fixed, paraffin wax embedded sections (4 μm) were used. Staining was performed using mouse ATA clone AA1 (Dako, Glostrup, Denmark) as the primary antibody, as described previously.[12] Briefly, 141 skin slides were dewaxed in xylene three times for five minutes, washed in ethanol three times for five minutes, and then washed in phosphate buffered saline (PBS; pH 7.4) three times for five minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. To block non-specific antibody binding, the slides were preincubated with protein blocking reagent (Immunologic/Labvision, Klinipath, Duiven, the Netherlands) for five minutes. The slides were incubated with primary antibody AA1, diluted 1/ 200 in PBS/5% bovine serum albumin for 30 minutes, rinsed in PBS/0.1% Tween twice for five minutes, and incubated with polyvalent biotinylated antibody (Immunologic/Labvision) for 10 minutes. After washing with PBS three times for five minutes, the slides were incubated with streptavidin–peroxidase (Immunologic/Labvision) for 10 minutes. The reaction was visualised using 3,3′ diaminobenzidine (Fluka Sigma-Aldrich, Zoetermeer, the Netherlands) and 30% hydrogen peroxide (Merck, Darmstadt, Germany) in PBS for seven minutes, then rinsed with tap water for two minutes. All sections were counterstained with Mayer's haematoxylin (Fluka AG, Buchs, Germany) for 10 seconds and rinsed in running tap water for 10 minutes. Slides were immersed three times in ethanol for three minutes after incubation in PBS for five minutes. The sections were mounted in Pertex (Histolab, Goteborg, Sweden). The negative controls consisted of omitting the staining with the primary antibody.

MC quantification

The numbers of mast cells were determined at a magnification of ×400 using a Zeiss Axioplan microscope (Carl Zeiss, Weesp, the Netherlands). Each section was photographed using a Sony colour video camera 3 CCD connected to the microscope. For accuracy of counting, a graticule with 100 fields measuring 0.22 × 0.22 mm (0.0484 mm^2) under ×400 magnification was projected over each photograph. This counting method was based on the morphometric point counting technique, and the size of overlaying fields was fitted to the size of one mast cell.[13 14] Ten photographs in different dermal layers were analysed for each section. To provide an overview of the dermal MCs, four photographs were taken from the stratum papillaris, three from the mid stratum reticularis, and three from the bottom stratum reticularis. Every square overlaying an MC scored one point and the sum of MCs in 10 photographs was calculated for each section. Capillaries, skin appendages, and the whole epidermis were scored in the same way. In addition to true MCs with a nucleus, clustered positively stained granules were counted as MCs and scored one point for each cluster. The numbers of MCs in each section were expressed as MCs/mm^2.

MCs in all the three different biopsy groups were counted. The numbers of MCs in perilesional skin around BCCs and other skin lesions were counted and compared with those in the skin from healthy women (control group).

MC numbers, sample characteristics, and patient assessments were recorded in SPSS for Windows. Both paired and unpaired Student's *t* tests were used to compare the means. A p value of < 0.05 was considered significant.

Table 1 Mean mast cell (MC) numbers in the 3 biopsy groups

Group	Mean (SD) MC numbers	p Value*
Group 1: BCC (n = 25)	91.3 (36.0)	0.560
Group 2: other dermatological disorders (n = 95)	87.8 (41.8)	0.560
Group 3: controls (n = 21)	79.5 (31.8)	0.560

Group 1, mean number of MCs/mm^2 in biopsies from perilesional skin around basal cell carcinomas (BCCs); group 2, mean number of MCs/mm^2 in biopsies from perilesional skin around other dermatological disorders; group 3, mean number of MCs/mm^2 in skin biopsies from healthy women.
*p Value for differences in MCs/mm^2 between the three groups; Student's *t* test.

Table 2 Mast cell (MC) numbers according to the different body sites

Body area	N	Mean (SD) MC/mm^2
Trunk	17	78.6* (31.5)
Upper arm	44	76.5* (32.7)
Upper leg	29	74.9* (38.8)
Proximal	90	77.0** (33.6)
Forearm	27	101.2*** (32.6)
Lower leg	24	113.1*** (46.7)
Distal	51	108.2** (41.4)

Perilesional skin around basal cell carcinomas, various dermatological disorders, and the control group.
*Difference in MCs/mm^2 between trunk, upper arm, and upper leg: $p=0.929$; **difference of MCs/mm^2 between proximal and distal body areas: $p=0.000$; ***difference of MCs/mm^2 between forearm and lower leg: $p=0.240$ (all Student's *t* test).
Proximal: trunk, upper arm, and upper leg; distal: forearm and lower leg.

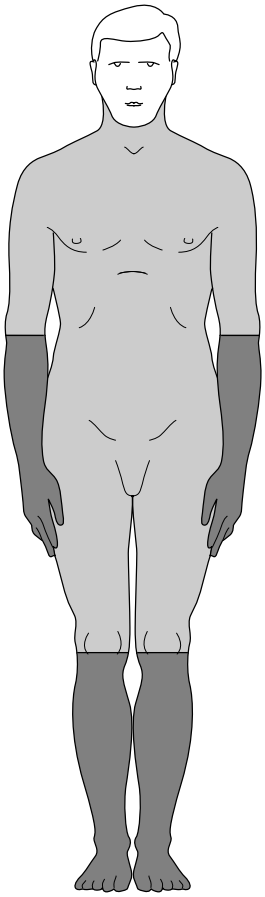

Figure 2 Mast cell distribution in human adult skin. Darker areas, highest numbers of mast cells in human skin (distal areas); lighter areas with the lowest numbers of mast cells in human skin (proximal areas). Mast cells in the facial area were not determined so that this area is left white.

RESULTS

Numbers of MCs in healthy non-lesional and perilesional skin

In sections stained with ATA, the numbers of MCs in the sections of skin from healthy women who underwent mamma reduction or abdominoplasty (control group) were compared with those in the other two biopsy groups using an unpaired parametric Student's *t* test (table 1; fig 1A). The numbers of MCs in perilesional skin in BCCs ($n = 25$), perilesional skin from various dermatological disorders ($n = 95$), and skin from the healthy group ($n = 21$) were not significantly different from each other. The 25 BCC biopsies and 95 other dermatological disorder biopsies were obtained from five different body sites and not exclusively from the trunk. To determine the possible influence of the body site and of the adjacent diagnosis of perilesional skin on the numbers of MCs, a multiple linear regression analysis was performed. When adjusted for the body site from which the biopsies were obtained, the numbers of MCs did not depend on the adjacent original diagnosis ($p = 0.992$).

MCs in various body sites

Perilesional skin samples stained with ATA were used for counting MCs in biopsies from different body sites. Adjusted for the diagnosis, the numbers of MCs were significantly dependent on the body site from which the skin samples were obtained ($p = 0.001$) (figs 2, 3A, B).

Table 2 and fig 1B and C show the numbers of MCs at different body sites. The highest numbers of tryptase positive MCs were found in the lower leg and the forearm. These were significantly higher ($p < 0.001$) than was seen on the trunk, upper leg, and upper arm. As shown in table 2 and fig 2, two body site groups were formed according to this finding. The proximal group comprised biopsies obtained from the trunk, upper leg, and upper arm. The mean number of MCs in this group was 77.0 MCs/mm^2 (SD, 33.6). The distal group comprised biopsies obtained from the lower leg and forearm. The mean number of MCs in this group was 108.2 MCs/mm^2 (SD, 41.4). Adjusted for the diagnosis around which the MCs were counted (BCC or other skin lesions), a similar difference was found in the numbers of MCs at proximal and distal body sites.

The number of capillaries in a biopsy does not affect MC counts

The numbers of MCs were calculated by dividing the total number of MCs by the total area of the biopsy. When the numbers of capillaries and skin appendages were subtracted from the total area of one skin biopsy, the numbers of MCs/mm^2 were computed. It appeared that the numbers of capillaries and skin appendages subtracted from the total surface area had no significant influence on the accuracy of the numbers of MCs/mm^2. However, the number and surface area of capillaries appeared to correlate ($p < 0.001$) with the number of MCs (B coefficient, 1.198). When adjusted for age, there was a significant effect of the number of capillaries on the number of MCs in proximal body sites (B coefficient, 1.5; $p < 0.001$). The number of capillaries had no effect on the number of MCs at distal body sites (B coefficient, -0.4; $p = 0.639$).

Pilot study in patients with mastocytosis (comparison)

Numerous MCs are present in mastocytosis and occur as oval to spindle shaped cells with a centrally located round to oval nucleus. They are concentrated in the upper dermis and around the blood vessels (fig 3C, D).

The numbers of MCs in a pilot study group of 14 patients with mastocytosis ranged from 78 to 2409 MC/mm^2 (mean, 821; SD, 582). The ages of these patients ranged from 4 to 49 years, with six patients younger than 16 years. The numbers of MCs in mastocytosis were increased nearly 10 fold compared with those seen in normal skin. Figure 3E and F shows immunohistochemical staining for CD117, and an example of c-kit analysis, which is only possible in digested tissue, is shown in fig 4.

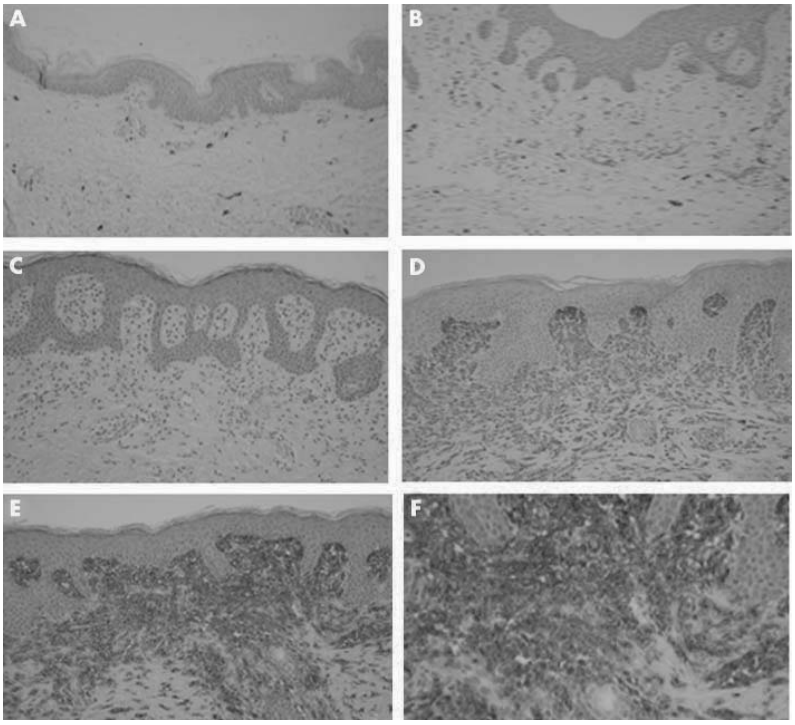

Figure 3 (A) Normal skin near basal cell carcinoma (original magnification, ×100). Mast cells occur in the normal dermis in small numbers as oval to spindle shaped cells. They are concentrated around the blood vessels. (B) Normal skin of mamma reduction tissue (original magnification, ×100). Mast cells occur in the normal dermis in small numbers as oval to spindle shaped cells. They are concentrated around the blood vessels. (C) Haematoxylin and eosin staining of mastocytosis (urticaria pigmentosa) (original magnification, ×100). Mast cells in the normal skin are indistinguishable from other perivascular cells. A small amount of granular cytoplasm is seen. (D) Urticaria pigmentosa, dense infiltrate of mast cells in the upper dermis located directly under the basal membrane. The infiltrate is also more concentrated around the blood vessels (original magnification, ×300). (E) Urticaria pigmentosa: positive staining of the mast cell infiltrate with CD117 (original magnification, ×300). (F) Urticaria pigmentosa: positive staining of the mast cell infiltrate with CD117 (original magnification, ×700).

DISCUSSION

In our present study, the numbers of MCs in adults were determined in biopsies obtained from five different body sites. Higher numbers of MCs were found in the forearm and lower leg (distal extremities) compared with those in the trunk, upper leg, and upper arm (centre and proximal extremities). Mast cells on the face were not counted in our study because diagnostic biopsies for mastocytosis are preferentially not taken from the face. From a pilot study in 14 patients with mastocytosis, we concluded that the lower limit for the number of MCs in mastocytosis may be as low as 78 MCs/mm^2, which is also lower than the mean number of MCs in healthy skin (87.8 MCs/mm^2). Thus, there was an overlap in the range of numbers of MCs in mastocytosis and normal skin.

The first reports on the measurement of MC numbers in different body sites date to 1950.[15] In those early studies,[5 6 10] different counting and staining techniques were used and no differences in the numbers of MCs in relation to the site of origin of the biopsy were noted. The numbers of MCs were found to be between 44 and 50 MCs/mm^2. The uneven distribution in the numbers of MCs in different body sites may be the result of differences in their functional, environmental, and haemodynamic properties. Although still controversial, the numbers of MCs do not appear to be related to variations in the exposure of different body sites to ultraviolet light.[16–20]

We confirm the results obtained by Weber *et al*, who reported increased numbers of MCs on the face compared with other body sites and variations in numbers of MCs at different body sites similar to those reported here.[21] The main difference between their study and ours is that they used the toluidine blue staining technique. It may be the case that more granules were stained in our study than that of *Weber et al* because the ATA technique is more sensitive than the toluidine blue stain used in their study. We also used a high magnification (×400) for analysing stained MCs from a picture on the computer screen. This may have magnified groups of granules that would have otherwise remained undetected.

> ''There was an overlap in the range of numbers of mast cells in mastocytosis and normal skin''

Collecting a large number of skin samples, using perilesional skin biopsies from BCCs or various dermatological disorders was useful, making biopsies from healthy individuals (controls) unnecessary. Cohen and Rogers emphasised the increased numbers of MCs around BCCs.[22] They reported significant differences between the numbers of MCs in skin directly adjacent to a BCC compared with the surrounding skin, independent of the overall inflammatory cell response in the area. An increase in the numbers of MCs was described above and around multiple and single dermatofibromas and other benign epithelial tumours.[23 24] In contrast, in other

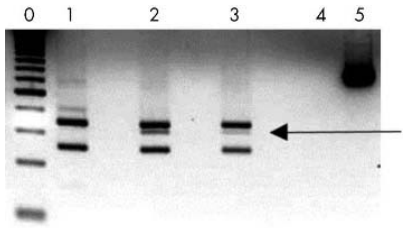

Figure 4 Digestion fragments from polymerase chain reaction (PCR) products obtained from genomic DNA of one patient, analysed on a 4% agarose gel. Lane 0, 50 bp DNA ladder; lane 1, Asp816 (negative control); lane 2, Asp816/Val816 (positive control); lane 3, patient 43002; lane 4, blank; lane 5, PCR product. The 157 bp fragment, indicating the presence of the mutation encoding the Asp-816-Val change, is present in the tested patient (lane 3). Courtesy of R van Schdik.

studies there was no difference in the numbers of perilesional MCs between benign and malignant skin lesions.[11] By using perilesional skin biopsies without obvious signs of inflammation and determining the numbers of MCs as far away from the original lesion as possible, we avoided possible influences of inflammation on MC numbers.

Although MCs are mostly seen around dermal capillaries,[6] in our study we found that adjusting for the number of capillaries does not contribute to a more precise quantification of MCs/mm^2. In other studies on MC quantitation, an attempt was made to define the numbers of MCs in different dermal layers.[5 7] In our study, the dermis was not divided into different dermal layers because the border between the papillary dermis and reticular dermis was not always clearly visible, which would make counting in relation to different dermal layers less accurate.

Based on our results, it is impossible to draw a strict distinguishing line between the upper limit of MC numbers in normal skin and the lower limit of MC numbers in mastocytosis. We suggest that a figure of up to 75 MCs/mm^2 should be considered as normal and more than 250 MCs/mm^2 as abnormal. Between 75 and 250 MCs/mm^2 is the borderline area in which a diagnosis of mastocytosis should certainly be considered. These figures are crude and an individual approach still remains essential. Further studies in a large number of patients with mastocytosis are necessary to clarify the lower limit of the numbers of MCs in these patients.

Take home messages

- There was a significant difference between the numbers of mast cells (MCs) on the trunk, upper arm, and upper leg (distal location) and those found on the lower leg and forearm (proximal location)
- These differences between distal and proximal locations must be considered when MCs are counted for a reliable diagnosis of mastocytosis
- A pilot study in patients with mastocytosis underlined the variation in the numbers of MCs in mastocytosis and normal skin, but showed a considerable overlap, and further studies are needed to clarify the lower limit of the numbers of MCs in patients with mastocytosis

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CHAPTER 8

Urinary N-Methylhistamine as an Indicator of Bone Marrow Involvement in Mastocytosis

Clin Exp Dermatol 2002, 27(6): 502-506

Urinary N-methylhistamine as an indicator of bone marrow involvement in mastocytosis

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Summary

Thirty-seven patients with mastocytosis and unexplained elevated levels of urinary N-methylhistamine who were undergoing bone marrow biopsy were studied with respect to the diagnosis of mastocytosis and the manifestations of the disease. These patients were from a group of 66 patients from whom a bone marrow biopsy was obtained and urinary N-methylhistamine levels were measured in the period 1990–1998. In seven (19%) of the 37 patients, mastocytosis was limited to the skin. Five (14%) of the 37 patients showed accumulation of mast cells in the bone marrow without characteristic skin lesions, whereas seven (19%) of the 37 patients showed increased numbers of mast cells both in the skin and the bone marrow. Eighteen (49%) of the 37 patients with elevated N-methylhistamine did not have mast cell accumulation in either the skin or the bone marrow biopsy. The median level of N-methylhistamine in the urine of patients with mastocytosis limited to the skin was 245 μmol/mol creatinine. The average level of N-methylhistamine was 509 μmol/mol creatinine in patients with mast cell accumulation in the bone marrow and cutaneous mastocytosis. There was a significant difference in the levels of N-methylhistamine in patients with mast cell accumulation in the bone marrow biopsy compared with those without. The likelihood of mastocytosis with mast cell accumulation in the bone marrow biopsy at a given level of N-methylhistamine was calculated. It was established that an N-methylhistamine level of 297 μmol/mol creatinine or higher may be considered as a threshold indicator for obtaining a bone marrow biopsy in patients suspected of mastocytosis with mast cell accumulation in the bone marrow. For practical purposes, we propose to consider the cut-off level of $\approx$ 300 μmol/mol N-methylhistamine creatinine for this assay.

Introduction

The diagnosis of mastocytosis is based on the clinical symptoms and the pathology.[1–3] Extracutaneous mastocytosis is difficult to diagnose. Measuring the levels of mast cell mediator metabolites may be helpful in obtaining clues for this disease. However, biopsy of the bone marrow or other organs is necessary for establishing the diagnosis. Mastocytosis may be classified as indolent, associated with haematological disorders, aggressive or malignant.[3] Information on prognostic factors for the disease is limited.

Mastocytosis in children is more often transient than in adults in whom there is a stable or progressive disease.[4–6] Thorough monitoring of the patients is necessary, given the likelihood of mast cell accumulation in the bone marrow in patients with persistent childhood and adult onset disease.[7–10] Various types of skeletal and bone marrow lesions have been described in mastocytosis of the bone marrow, the most common

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type being nodular or granuloma-like mast cell accumulation. The risk of osteoporosis in patients with mast cell accumulation in the bone marrow is considerable and estimated at up to 85%.[11,12] The risk of early skeletal decalcification appears to be proportional to the amount and the activity of the mast cells in the bone marrow. Heparin and prostaglandins are believed to have a role in the induction of osteoporosis, whereas sclerotic lesions are induced by histamine.[13] A subgroup of the patients with mast cells in the bone marrow develops complications such as haematological disorders or progression to aggressive or malignant mastocytosis. Later onset of symptoms, absence of cutaneous mastocytosis, thrombocytopenia, elevated lactatic dehydrogenase level, anaemia, bone marrow hypercellularity, qualitative peripheral blood smear abnormalities, elevated alkaline phosphatase level and hepatosplenomegaly are all associated with decreased survival.[14] The identification of somatic mutations in the C-kit coding genome in mast cells of adults may prove useful as a predictor of bone marrow involvement and possibly of myelodysplastic or other complications.[15–17] The treatment of mastocytosis is largely symptomatic. However, some potential complications of mastocytosis may be detected before clinical symptoms occur. Osteoporosis especially, may be successfully treated with bisphosphonates and interferon alpha.[18–20] Myelodysplastic and other haematological complications require co-operation with haematologists. Bone marrow biopsy is the most reliable method to identify the patients at risk for these complications. Elevated levels of mast cell mediator metabolites in the urine have been associated with the presence of extracutaneous mast cell accumulation.[21–27] The aim of our study was to assess the diagnostic value of N-methylhistamine levels in identifying patients with the (tentative) diagnosis of mastocytosis who are likely to have mast cell accumulation in the bone marrow.

Materials and methods

Routine measurements of N-methylhistamine in urine samples performed during 1990–1998 were reviewed with respect to the levels, concurrent bone marrow biopsy and the diagnosis of mastocytosis.

N-methylhistamine in the urine samples was determined in duplicate using a competitive radioimmunoassay according to the manufacturer's instructions (Pharmacia & Upjohn, Woerden, the Netherlands), as described previously.[28] The levels of N-methylhistamine in all samples were expressed as micromoles N-methylhistamine per mole creatinine. Reference values of urinary N-methylhistamine for adults using this method ranged between 50 and 155 μmol/mol creatinine. An elevated level of N-methylhistamine was defined as 156 μmol/mol creatinine or higher.

N-methylhistamine levels were measured and bone marrow biopsy was obtained from 66 patients for various reasons. Levels of the N-methylhistamine, outcome of the bone marrow biopsy and clinical observations were analysed statistically using SPSS. The significance of differences in N-methylhistamine levels between a group of patients with accumulated mast cells in the bone marrow biopsy and a group of patients without was determined using the Mann–Whitney test. If there is a significant difference, the relevance of N-methylhistamine as a diagnostic test for predicting bone marrow involvement may be investigated further. The Mann–Whitney test is particularly relevant here, because the value of the test statistic is essentially equal to the area under the ROC-curve.[27] A ROC-curve is the relationship between sensitivity and 100 − specificity obtained from varying the cut-off level of the diagnostic test parameter (here N-methylhistamine). A significant Mann–Whitney test coincides with an area under the ROC-curve that is significantly larger than 50% and hence with sums of sensitivity and specificity that are larger than 100%. A measure for the validity of a diagnostic test is the sum of its sensitivity and specificity. The criterion used in this paper for choosing a cut-off level for N-methylhistamine is the maximum of the sum of sensitivity and specificity.

The likelihood of mastocytosis with mast cell accumulation in the bone marrow at a given N-methylhistamine cut-off level was calculated using a 2×2 table. The validity of the test is expressed here as the sum of sensitivity and specificity. Sensitivity is defined as the chance that the test result is positive in a patient with the disease. In the 37 patients with elevated levels of N-methylhistamine an optimal cut-off value for detecting mast cell accumulation in bone marrow biopsy was 297 μmol/mol creatinine. At this cut-off level the sum of specificity and sensitivity was maximum: the specificity was 84% and the sensitivity was 67% as shown in Table 1.

Results

Thirty-seven (56%) of the 66 patients from whom a bone marrow biopsy had been obtained also had elevated levels of N-methylhistamine. Nineteen (51%) of these 37 patients had biopsy proven mastocytosis. Of 29 patients without elevated N-methylhistamine levels and from whom a bone marrow biopsy had been

Table 1 Sensitivity and specificity at an N-methylhistamine cut-off level of 300 μmol/mol creatinine in relation to mast cell numbers in bone marrow (borderline significance range, 250–350 μmol/mol creatinine).

Urine N-methyl histamine level > 250–350 μmol/mol creatinine	Mast cell number in bone marrow not increased ($n = 25$)	Mast cell number in bone marrow increased ($n = 12$)	Total number of patients ($n = 37$)	
Test result negative	14 (56%)	3	(25%)	17 (47%)
Test result doubtful*	7 (28%)	1 (8%)	8 (21%)	
Test result positive	4 (16%)	8 (67%)	12 (32%)	

*In cases where the test result is doubtful we advise to repeat the N-methylhistamine test.

obtained, only three had mastocytosis, which was limited to the skin.

Of the 19 patients with biopsy-proven mastocytosis and elevated urinary N-methylhistamine levels seven (37%) had mastocytosis of the skin. Five patients (26%) showed bone marrow mast cell accumulation without characteristic skin lesions and seven (37%) showed increased numbers of mast cells in both skin and bone marrow. The other 18 (49%) of the 37 patients with elevated levels of N-methylhistamine did not have mast cell accumulation in the skin or bone marrow.

In patients with mast cell accumulation in the bone marrow, but without the typical skin lesions, the average level of N-methylhistamine was 795.8 μmol/-mol creatinine. The average level of N-methylhistamine in patients with mastocytosis in the skin and mast cell accumulation in the bone marrow, was 781.7 μmol/-mol creatinine.

There was a significant difference in the levels of N-methylhistamine in patients with accumulated mast cells in the bone marrow biopsy compared with those without as shown in Fig. 1. In the 37 patients with elevated levels of N-methylhistamine, the optimal predictive value for detecting mast cell accumulation in bone marrow biopsy was observed at an N-methylhistamine level of 297 μmol/mol creatinine at which the specificity was 84% and the sensitivity was 67% (Table 1 and Figure 2).

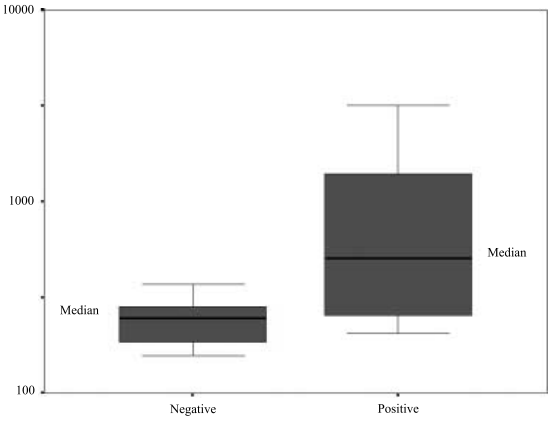

Figure 1 Urinary N-methylhistamine levels in patients with mastocytosis with (positive) and without (negative) involvement of the bone marrow. Data are median and SD. y-axis: values in μmol/mol creatinine.

Discussion

The aetiology of mastocytosis has not yet been elucidated. A minority of the patients with mastocytosis develops complications that require extensive monitoring and treatment. These complications may be subdivided into those related primarily to the large number of mast cells, excessive amounts of mast cell mediators and complications related to changes in the myeloid cell lineage. Both types of complications are

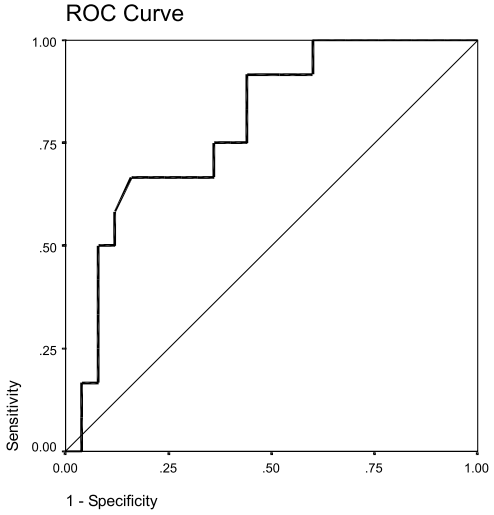

Figure 2 ROC-curve of the sensitivity in relation to 1 − specificity for various cut-off levels of urinary N-methylhistamine. (area under the ROC-curve equals 0.79 with 95% CI of 0.64–0.94).

likely to occur in patients with mastocytosis who develop significant mast cell accumulation in the bone marrow. Bone marrow biopsy is the method of choice to identify patients who may be at risk of such complications. Bone marrow biopsy is an invasive procedure with potential morbidity. Care must be taken to identify patients who may benefit from bone marrow biopsy.

The current method of staging mastocytosis depends on repeated evaluations of the clinical condition of the patient, blood- and urine chemistry, haematological screening as well as bone marrow biopsy and bone density measurement. N-methylhistamine in the urine indicates mast cell activity and mast cell density. In the reported group of patients, N-methylhistamine levels were determined in order to stage mastocytosis in patients suspected of having mastocytosis without skin involvement and those with established mastocytosis. Levels of N-methylhistamine >156 μmol/mol creatinine always indicated that a bone marrow biopsy was essential.

The likelihood of mast cell accumulation in the bone marrow could be predicted on the basis of elevated N-methylhistamine levels. In the given group of patients, an N-methylhistamine level of 297 μmol/mol creatinine was the cut-off point of choice for obtaining a bone marrow biopsy. We propose to handle in practise as follows: Below 250 μmol/mol no bone marrow examination indicated; from 250 to 350μmol/mol creatinine doubtful, repeat test; and ≥ 350μmol/mol creatinine bone marrow examination indicated.

The level of N-methylhistamine as a diagnostic aid for levels of mastocytosis has limited value. There are several conditions that lead to elevated levels of N-methylhistamine. The most important of these are allergic reactions,[29] histamine-rich diets,[30] and interstitial cystitis.[31] Persistent levels of N-methylhistamine exceeding 297 μmol/mol creatinine may indicate mast cell accumulation in the bone marrow.

Based on the results of this study it is recommended to restrict bone marrow biopsy to patients with established or suspected mastocytosis and unexplained osteoporosis, osteosclerosis or haematological aberrations and in cases in which the diagnosis cannot be confirmed by a skin biopsy.

Long-standing mastocytosis with elevated levels of N-methylhistamine, but without signs of osteoporosis or osteosclerosis on densitometry are not primary candidates for bone marrow biopsy unless there are haematological disturbances. The process of diagnosing mastocytosis may be difficult in some patients, especially in patients without the classical skin lesions. In patients suspected of having mastocytosis, persistent levels of N-methylhistamine >300 μmol/mol creatinine may indicate accumulation of mast cells in the bone marrow. In patients suspected of having mastocytosis, but without elevated levels of N-methylhistamine a 'wait and see' approach is justified. Where there is doubt about whether or not to obtain a bone marrow biopsy it may be helpful to determine the level of N-methylhistamine.

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CHAPTER 9

C-Kit Asp-816-Val Mutation Analysis in Patients with Mastocytosis

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C-kit Asp-816-Val Mutation Analysis in Patients with Mastocytosis

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Key Words
Mastocytosis · C-kit Asp-816-Val mutation · Mast cells

Abstract
Background: Mastocytosis is a heterogeneous group of disorders characterized by abnormal accumulation of mast cells. ***Objective:*** Skin biopsies from 24 patients (23 with proven mastocytosis) were screened for the presence of the c-kit Asp-816-Val mutation. ***Methods:*** In frozen biopsies, RNA was isolated, cDNA synthesis and PCR, the expected PCR product of 346 bp was obtained from 23 patients. ***Results:*** In patients with urticaria pigmentosa, the mutation was detected in 38% of the adults and 25% of the children. With regard to the clinical presentation of the disease, no difference was found between adult patients with and without the mutation, as detected with our assay. One out of the 2 children with the mutation had an atypical presentation of the disease. ***Conclusion:*** the mutation could not be detected in all the patients, probably due to lack of sensitivity of the methods.

Introduction

Mastocytosis is a heterogeneous group of disorders characterized by proliferation and accumulation of mast cells in one or more organ systems [1, 2]. In mastocytosis, the disorder becomes clinically manifest at sites where mast cells are normally present. The organ most frequently affected is the skin, but the gastrointestinal tract, bone marrow, bones, liver, spleen and lymph nodes can also be involved [1–3].

Mastocytosis can be divided into a classification system based on consensus [1]. There are two main variants, cutaneous and systemic forms [3, 4]. Based on the clinical presentation, six subtypes can be distinguished: mastocytoma, urticaria pigmentosa, diffuse cutaneous mastocytosis, telangiectasia macularis eruptiva perstans (TMEP), systemic mastocytosis and mast cell leukemia (table 1). This classification has been preferred in children [3, 4]. Mastocytosis in adults frequently is a persisting or progressive disorder, whereas in many children the disorder resolves with age [4, 5]. Although generally mastocytosis is a rare disorder, families have been described in which more than one member is affected [6]. In patients with mastocytosis, symptoms are primarily caused by the release of mast cell mediators, although they may also be due to local accumulation of mast cells. Recent studies have shown that mutations in c-kit may cause some forms of mastocytosis [7]. C-kit is a proto-oncogene encoding for a tyrosine kinase receptor (fig. 1). Apart from the presence of the receptor on mast cells, c-kit is also expressed on various other cells, including hematopoietic stem cells and melanocytes. Mutations in c-kit may lead to SCF-independent activation of the receptor, preventing proliferation and apoptosis of mast cells [7, 8]. In familial forms of mastocytosis, the Asp-816-Val mutation has never been demonstrated [6, 7].

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Table 1. Subtypes of mastocytosis with common average age of onset and clinical characteristics [4]

Subtype	Age	Characteristics
Mastocytoma	0–6 months	1–5 reddish brown hyperpigmented/skin colored noduli/nodi
Urticaria pigmentosa	3–9 months; if onset after the age of 10 years, average age at onset 26.5 years	Multiple reddish brown hyperpigmented maculae and papules
Diffuse cutaneous mastocytosis	In general before the age of 3 years, frequently at birth	Thickened lichenified skin with papules, rarely without skin abnormalities, formation of bullae following minor trauma
TMEP	Almost exclusively at adult age	Hyperpigmented telangiectatic maculae
Systemic mastocytosis	Adult age, rarely childhood	Mast cell infiltrates in skin and internal organs
Mast cell leukemia (rare)	Adult age	Mast cells in peripheral blood

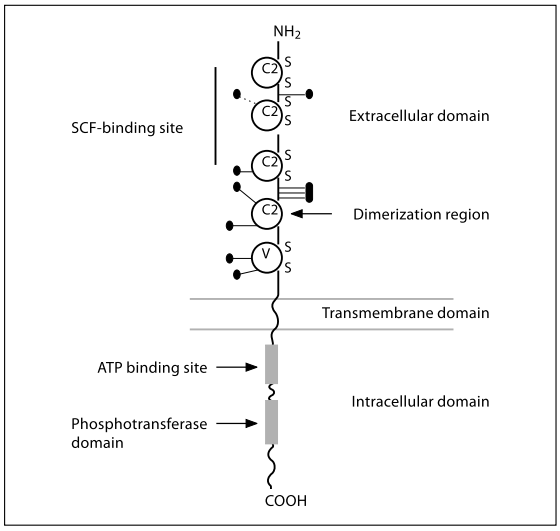

Fig. 1. Schematic representation of the structure of the c-kit receptor. Adapted from reference [3].

Table 2. Mutations in c-kit in patients with mastocytosis

C-kit mutation	Demonstrated in
Asp-816-Val	Systemic mastocytosis
	Mastocytosis with associated hematologic disorder
	Adults with cutaneous mastocytosis
	Children with atypical mastocytosis
Asp-816-Tyr	Children with atypical mastocytosis
Asp-816-Phe	Children with atypical mastocytosis
Asp-816-His	Mastocytosis associated with acute myeloid leukemia
Asp-820-Gly	Aggressive mastocytosis
Val-560-Gly	Adults with indolent cutaneous mastocytosis
Gln-839-Lys	Children with typical mastocytosis

From references [2, 7, 9, 11].

Besides the Asp-816-Val mutation, a number of other mutations in c-kit have been found in patients with mastocytosis (table 2) [2, 9, 10]. Because the presence of certain mutations may be relevant to the prognosis (mastocytosis in children with the Asp-816-Val mutation may be chronic) and the sensitivity to therapy, it is important to demonstrate such mutations [11, 12]. Because of the possible clinical implications, skin biopsies from 24 patients with mastocytosis were screened for the presence of the Asp-816-Val mutation, and in this patient group the prevalence of the mutation was determined. A possible relation between the presence of the mutation and the clinical presentation of the patients was also investigated.

Material and Methods

Patients and Biopsies

At the time the study was performed, 72 patients with mastocytosis of the skin were recorded at the Dermatology outpatient clinic of the Academic Hospital Rotterdam. Frozen biopsies from 24 patients (n = 24), 12 adult and 12 children (table 3) were used. Among these patients, 9 adults suffered from sporadic urticaria pigmentosa (patients 1–5 and 7–10), and in 3 of those 9 patients multiorgan involvement was demonstrated (patients 3, 5 and 10), 1 adult had sporadic TMEP (patient 6), a father and son were diagnosed with familial urticaria pigmentosa (patients 12 and 13), and 8 children with sporadic urticaria pigmentosa (patients 14–16

Table 3. Patient characteristics

Patient	Gender	Type	Age at onset	Age at biopsy	Clinical diagnosis
1	m	sporadic	34 years	38 years	UP
2	f	sporadic	70 years	71 years	UP
3	f	sporadic	29 years	47 years	UP+Sys[a]
4	f	sporadic	24 years	24 years	UP
5	m	sporadic	31 years	40 years	UP+Sys[a]
6	f	sporadic	49 years	50 years	TMEP
7	f	sporadic	29 years	41 years	UP
8	f	sporadic	30 years	40 years	UP
9	m	sporadic	38 years	49 years	UP
10	f	sporadic	20 years	28 years	UP+Sys[b]
11	f	–	–	29 years	–
12	m	familial	childhood	49 years	UP
13	m	familial	3 months	14 years	UP
14	m	sporadic	6 months	4 years	UP
15	m	sporadic	3 months	8 years	UP
16	m	sporadic	birth	7 months	UP/Juv Xanth
17	m	sporadic	birth	7 years	DCM
18	m	sporadic	early infancy	9 months	DCM
19	f	sporadic	2 years	7 years	UP
20	f	sporadic	4 months	6 years	UP
21	f	sporadic	6 months	5 years	UP
22	m	sporadic	at birth	2 years	UP
23	m	sporadic	10 days	12 years	UP
24	m	sporadic	neonatal	11 years	DCM

UP = Urticaria pigmentosa; DCM = diffuse cutaneous mastocytosis; Sys = systemic extension demonstrated; Juv Xanth = juvenile xanthogranuloma.

[a] Infiltration of mast cells demonstrated in bone marrow; in other patients systemic extension not tested.

[b] Infiltration of mast cells demonstrated in intestine; in other patients systemic extension not tested.

and 19–23), of which 1 also showed juvenile xanthogranuloma (patient 16), and 3 children suffered from sporadic diffuse cutaneous mastocytosis (patients 17, 18 and 24). Moreover, a skin biopsy of the twin sister of patient 10 was screened. In this adult, mastocytosis could not be diagnosed, despite the fact that some complaints were possibly consistent with mastocytosis (patient 11).

From the skin biopsies from patients 2–24, 3–5 sections of about 20 μm were cut. Due to the small tissue biopsy taken from patient 1, no additional sections could be made for use in further analysis.

Mutation Analysis

RNA Extraction

Cells were lysed in Trizol (Life Technologies) and total RNA was extracted according to the manufacturer's protocol (method based upon the acid guanidinium/phenol/chloroform extraction [13]). RNA was dissolved in 25 μl RNAse-free water.

Synthesis of cDNA and Polymerase Chain Reaction

From 6 μl of isolated total RNA, cDNA was synthesized using 2 μl AMV reverse transcriptase (9 U/μl, Promega), 2 μl random hexamers (500 μg/ml, Roche), 5 μl dNTPs (4 mM, Roche), 4 μl RT buffer (Promega) and 1 μl RNAsin (40 u/μl, Promega). This cDNA was used as a template in a polymerase chain reaction (PCR) (45 cycles: 1 min 94°C, 1 min 55°C, 1 min 72°C) using the primers 5′-ACATAGAAAGAGATGTGACTCCCG-3′ (P187, nucleotide 2261-2284) and 5′-AGCTCCCAAAGAAAAATCCCATAGG-3′ (P188, nucleotide 2606-2582) (GenBank No. 1:X06182), resulting in a PCR product of 346 bp.

Digestion of the PCR Product

Two digests were performed on the PCR product: 5 μl PCR product was cleaved with restriction enzyme *Hin*f I and 5 μl PCR product was cleaved with restriction enzymes *Hin*f I and *Hae* III, the latter for a better resolution on a gel [13]. If an A→T substitution occurs at nucleotide 2468, an extra cleavage site for *Hin*f I will be formed, and the expected fragments will be 188, 127, 17 and 14 bp (*Hin*f I) and 157, 127, 31, 17, 14 bp (*Hin*f I and *Hae* III). If the mutation is not present, the fragments will have a length of 202, 127 and 17 bp (*Hin*f I) and 171, 127, 31 and 17 bp (*Hin*f I and *Hae* III).

In the *Hin*f I digest, the presence of a 188-bp product is indicative of the presence of the Asp-816-Val mutation; in the *Hin*f I and *Hae* III digest, the presence of a 157-bp product is indicative of the mutation.

Table 4. Results of the c-kit Asp-816-Val mutation analysis

	Clinical diagnosis	Patients	Patients without Asp-816-Val mutation	Patients with Asp-816-Val mutation
Adults (n = 11)		total 10	7/10 (70%)	3/10 (30%)
	Sp UP/Sp UP+Sys	8	5/8 (63%)	3/8 (38%)
	Fam UP	1	1/1 (100%)	0/1 (0%)
	Sp TMEP	1	1/1 (100%)	0/1 (0%)
	No mastocytosis	1	1/1 (100%)	0/1 (0%)
Children (n = 12)		total 12	10/12 (83%)	2/12 (17%)
	Sp UP	8	6/8 (75%)	2/8 (25%)
	Fam UP	1	1/1 (100%)	0/1 (0%)
	Sp DCM	3	3/3 (100%)	0/3 (0%)

Sp UP = Sporadic urticaria pigmentosa; Sp TMEP = sporadic TMEP; Sys = systemic extension; Sp DCM = sporadic diffuse cutaneous mastocytosis; Fam UP = familial urticaria pigmentosa.

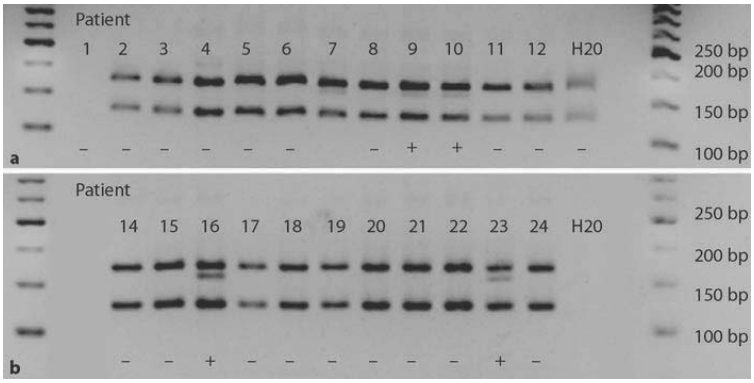

Fig. 2. *Hinf* I/*Hae* III digestion fragments from PCR products obtained from genomic DNA of 24 patients, analyzed on a 4% agarose gel. Left and right: 50-bp DNA ladder. The 171-bp and 127-bp digestion products are visible in all samples except patient 1. The 157-bp fragment, indicating the presence of the mutation encoding the Asp-816-Val change, is present in patients 7, 9, 10, 16 and 23. **a** Patients 1–12. **b** Patients 13–24; in patient 13 the PCR was negative.

Gel Electrophoresis

The cleaved PCR products were mixed with loading buffer, and separated on a 2% agarose gel (product cleaved with *Hinf* I) and 4% agarose gel (product cleaved with *Hinf* I and *Hae* III), and stained with ethidium bromide.

Results

Following RNA isolation, cDNA synthesis and PCR, the expected PCR product of 346 bp was obtained from patients 2–24. No PCR product from patient 1 was found; this patient was subsequently excluded from the study. Following cleavage of the PCR product with *Hinf* I and analysis on a 2% agarose gel, fragments of 202 and 127 bp were found for all patients, but no fragments of 188 bp.

For better separation of fragments, the PCR product was cleaved with *Hinf* I and *Hae* III and applied to a 4% agarose gel. In 5 patients (patients 7, 9, 10, 16 and 23), another extra fragment of 157 bp was found in addition to fragments of 171 and 127 bp. This is in accordance with the c-kit Asp-816-Val mutation (fig. 2).

Therefore, the c-kit Asp-816-Val mutation could be demonstrated in 3 out of 10 adult patients (30%) and in 2 out of 12 children with mastocytosis (17%; table 4).

In the adult patients, the mutation was found in 3 patients with sporadic urticaria pigmentosa (patients 7, 9 and 10). In one of the patients with sporadic urticaria

pigmentosa with the mutation, involvement of the intestine had been established (patient 10). Adult patients with the mutation do not differ clinically from patients with sporadic urticaria pigmentosa that do not carry the mutation. The mutation was demonstrated neither in the patient with familial urticaria pigmentosa (patient 6), nor in the patient with TMEP (patient 12). In patient 11 (no mastocytosis diagnosed) no Asp-816-Val mutation was found, in contrast to her twin sister with mastocytosis (patient 10).

With regard to the children, the mutation was demonstrated in 2 patients with sporadic urticaria pigmentosa (patients 16 and 23). Besides urticaria pigmentosa, patient 16 also showed juvenile xanthogranuloma, consistent with an abnormal clinical presentation. Patient 23 showed no abnormal clinical presentation. The mutation was not found in the child with familial urticaria pigmentosa (patient 13) and in the children with sporadic diffuse cutaneous mastocytosis (patients 17, 18 and 24).

Discussion and Conclusion

Because of the potential prognostic significance of the Asp-816-Val mutation and the possible relationship between certain mutations and the sensitivity to therapy, a PCR was carried out for c-kit Asp-816-Val mutation analysis in skin biopsy sections [11, 12]. In 23 out of 24 patients a PCR product was found. The absence of PCR product for one patient might be due to the fact that in this patient the whole biopsy was used instead of sections: because of the thickness of the material, this may have warmed up before the buffer was able to inhibit RNAse activity, resulting in insufficient amounts of intact RNA. The best analysis results were obtained using the double digest *Hinf* I and *Hae* III, followed by electrophoresis on a 4% agarose gel.

The prevalence of the c-kit Asp-816-Val mutation in the total patient group examined by us was 30 and 17% for adults and children, respectively. Within the groups with sporadic urticaria pigmentosa the prevalence was 38% (adults) and 25% (children). In contrast to earlier publications by Longley et al. [7] and Büttner et al. [9], the mutation was not demonstrated in all adults with sporadic cutaneous mastocytosis. No distinction was made between the patient groups with or without demonstrated systemic extension, because recent investigations have shown that on bone marrow testing in patients with cutaneous mastocytosis, in virtually all patients bone marrow involvement, and therefore systemic extension, will be found [14]. The Asp-816-Val mutation could not be demonstrated in familial urticaria pigmentosa. This is consistent with earlier publications, although we only studied 2 cases [6, 7]. The suggested absence of Asp-816-Val mutation in familial cases of mastocytosis must be further evaluated in such populations.

Mastocytosis is very heterogeneous and the manifestations differ from one to other cases in several ways. Tebbe et al. [15] illustrated it in an observational study in 14 adults with cutaneous mastocytosis. Bone marrow involvement was observed in only 7 of 13 patients investigated [15].

The prevalence of the Asp-816-Val mutation was found in 2 children with mastocytosis (17%). One child with the mutation showed an atypical clinical presentation of urticaria pigmentosa and juvenile xanthogranuloma. According to Longley et al. [7], children in whom the Asp-816-Val mutation is found will be characterized by an atypical presentation. Up to now, the second child in which the mutation was demonstrated did not show an atypical clinical presentation. In patient 11 (no diagnosed mastocytosis), the Asp-816-Val mutation was not found. Detection of the mutation in her (monozygotic) twin sister confirms that this mutation did not occur in early embryonic development (germ cell mutation). In this case, it is an acquired somatic mutation associated with the disease. An illustration that atypical manifestations not always are Asp-816-Val positive was demonstrated by Walker et al. [16], who described a child with neonatal mastocytosis with pachydermic bullous skin without the Asp-816-Val mutation.

The discrepancy between our c-kit mutation findings and those from earlier publications may be due to insufficient sensitivity of our assay. As for sensitivity, however, the method used by us (PCR + restriction analysis) is at least comparable to the methods used by Longley et al. [7] (sequencing) and Büttner et al. [9] (sequencing and PCR + restriction analysis). A discrepancy introduced by sampling is very unlikely because the material was obtained in an identical way. A possibility that cannot be ruled out is that the patient populations from studies mentioned before are different from the population examined by us.

Summarizing, we conclude that *Hinf* I/*Hae* III double digests of c-kit PCR fragments separated on a 4% agarose gel can be used for detection of the c-kit Asp-816-Val mutation in skin biopsies. In contrast to the literature, we find the Asp-816-Val mutation only in 38% of the adults with sporadic urticaria pigmentosa. Careful follow-up of

patients will give a decisive answer on the question whether patients with the mutation are more severely affected and/or whether the disease runs a more progressive course in patients with the mutation.

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CHAPTER 10

Clinical Scoring of Cutaneous Mastocytosis

Mastocytosis Study Group Rotterdam

Acta Derm Venereol 2001, 81:273-276

Clinical Scoring of Cutaneous Mastocytosis

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There are still many controversies in defining and evaluating mastocytosis. One of the aspects that is missing is a system for clinical evaluation of mastocytosis of the skin. A calculation based on a semi-quantitative analysis of three aspects of mastocytosis was designed. The method is called the scoring index of mastocytosis (SCORMA). The clinical use of SCORMA is advocated. ***Key words: mast cell disease; skin; SCORMA.***

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Mastocytosis is a rare and heterogeneous disease, which is characterized by an abnormal accumulation of non-malignant mast cells (1–3). Usually symptomatic skin involvement is present and is either localized or diffuse (2). Dermatologists may encounter new cases of mastocytosis at a ratio of one in every 1,000–8,000 new patients (3). Bone marrow involvement can be observed in most of the adult patients (4, 5). Despite rapidly growing knowledge, there are still many controversies in defining and evaluating mastocytosis (6–11). Because there is no system for clinical evaluation of mastocytosis of the skin, we designed a method for quantifying and scoring mastocytosis. The method is called the scoring index of mastocytosis (SCORMA). Details of SCORMA and an evaluation of its application by a panel of dermatologists are presented in this report.

MATERIALS AND METHODS

SCORMA

A semi-quantitative analysis of three aspects of skin mastocytosis was made using the same principles of the "SCORAD" for atopic dermatitis (12–15).

The analysis was divided into 3 parts. In the first part (A) the extent of the skin involvement was assessed. In the second part (B), the activity of the lesions was estimated, and in the third part (C), the subjective symptoms were recorded. Each part was rated separately on a semi-quantitative scale. The combined scores of A, B and C formed the SCORMA index.

Part A. There is a substantial variety in skin lesions in the cutaneous manifestation of mastocytosis. Using a pencil, the extent of the disease is estimated by roughly marking the contours of the skin abnormalities in the picture printed on the SCORMA form (Fig. 1). Thereby, the difference in morphology of the variation in presentations of skin mastocytosis is minimized. The percentage of skin involvement is represented by the marked surface. In the SCORMA setting there are two assumptions. In the case of solitary mastocytomas, each lesion represents 1% skin involvement. In the case of diffuse cutaneous mastocytosis, by definition the skin is completely involved (100%).

Part B. The pattern of the local reactivity of the skin lesions is limited to erythema, swelling and blistering of varying degrees. This may occur spontaneously or be caused intentionally by rubbing the skin lesion (Darier's sign).

In assessing mastocytosis, the activity of the individual mast cell is probably reflected by the activity of the skin lesions. This is exemplified by the observation that, in resolving mastocytosis, reactivity to various stimuli decreases a long time before resolution of the associated hyperpigmentation. Subsequently, it is our opinion that assessing the activity of the lesions provides a close impression of the actual local state of the disease activity. Reduction of mediator-induced symptoms owing to a decrease of degranulation is always established in treatment that is considered successful. The relevance of looking at the lesional activity is therefore evident.

The activity of the disease is measured by one elected lesion. The chosen lesion should be typical in shape, size and colour for the majority of the skin lesions. A lesion on the back of the trunk may be chosen because of the limited exposure to UV radiation from sunlight. Secondly, non-lesional skin will be present close to the lesion. This is used to assess dermographism due to rubbing of unaffected skin.

Four items of the chosen lesion are scored semi-quantitatively: pigmentation/erythema, vesiculation, elevation and Darier's sign (Fig. 1). The score for each item is 0–3. In total, the range of the score is 1 to a maximum of 12.

Part C. In mastocytosis, many patients lack symptoms such as itching or flushing, but they complain about the adverse cosmetic aspects of their disease. Occasionally, patients are restricted in their daily activities because of severe constitutional symptoms caused by mediators released upon mast cell degranulation. Scoring subjective complaints involves a certain degree of inaccuracy caused by the patient's mental state. Subjective complaints were included in the SCORMA index, but have only a limited influence on the final score.

The subjective symptoms prior to consultation were evaluated. A period of 3 weeks was chosen in order to gain an impression of the persistent nature of a given complaint. A shorter period of observation would increase the risk of including non-specific complaints or symptoms of a transient nature, while a longer period of observation would exclude the possibility of evaluation of the effect of therapy on a shorter interval. However, until data are available on the duration of symptoms in mastocytosis, these cannot be included as complaints that are likely to be caused by mastocytosis. Five questions were put to the patient. Question 1 dealt with the daily inconvenience caused by this disease. The remaining questions dealt with specific symptoms caused by mediator release or mast cell accumulation. The severity of the symptoms was scored from 0 to 10. A visual analogue scale was used in children older than 4 years of age. The score ranged from 0 to a maximum of 50.

Formula for calculating SCORMA index

It was our intention to develop a semi-quantitative clinical index to monitor the effect of therapy on skin mastocytosis. We have assumed that the lesional activity was a reflection of the potential degranulating activity of all the mast cells. Consequently, during the course of the disease and treatment, increase or decrease in the lesional activity is probably the first objectively measurable item to change. The SCORMA index was intended to be sensitive for monitoring changes

during the course of the disease. Emphasis was therefore put on lesional activity by multiplying it by a factor of three as compared with extent of complaints and subjective complaints. The multiplication factor 3 for lesional activity was chosen in analogy with the SCORAD (12–15) used in atopic dermatitis.

The resulting scores of each item are incorporated in the SCORMA index formula: $A + 3B + C$.

In order to use the SCORMA index formula: $A + 3B + C$, the ranges of the scores of A, B and C were equalized to 20. This was done by dividing A by 5 (maximum outcome: $100/5 = 20$), multiplying B by 5/3 (maximum outcome: $12 \times 5/3 = 20$) and multiplying C by 2/5 (maximum outcome: $50 \times 2/5 = 20$). Finally, the score of B is multiplied by 3. The SCORMA index formula in practice is $A/5 + 5B + 2C/5$, and ranges from 5.2 to 100.

We assessed whether the SCORMA index also has practical value for dermatologists not involved in basic research programmes on mastocytosis. The reliability of the SCORMA index was determined by measuring the inter-rater agreement on all the items of the SCORMA index. Secondly, the severity of the disease calculated as the SCORMA index was compared with the dermatologists' assessment on severity. The assessment of the severity was documented on a 0–100 scale for each patient separately prior to revealing the SCORMA index calculation.

In 1998, 9 dermatologists were invited to participate in a course to evaluate 9 patients (6 boys and 3 girls) with mastocytosis using the SCORMA index (Fig. 1). The SCORMA index of all the patients is shown in Table I.

All relevant clinical information was provided by a slide presentation of an overview of the skin as well as close-ups of the lesional skin.

None of the dermatologists had information on the SCORMA index formula prior to the course. The data were processed and calculated using SSPS. For all patients and all parts, statistical analysis was made including the inter-investigator variations expressed as inter-rater agreement for each patient (16, 17). The degree of agreement is expressed as the proportion of the possible scope of doing better than by chance. A value between 0.41 and 0.60 is considered moderate, between 0.61 and 0.80 as good and between 0.81 and 1.00 as very good (16, 17).

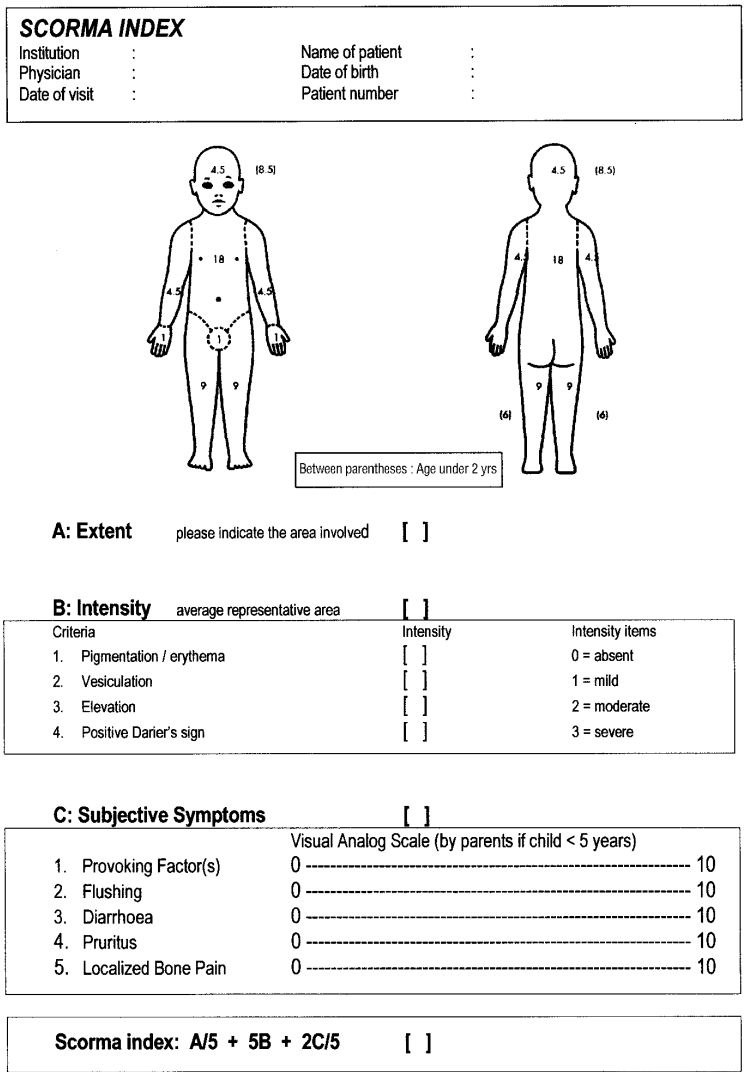

Fig. 1. The scoring index of mastocytosis (SCORMA).

Table I. *Assessment of the severity of mastocytosis by 9 dermatologists in each patient (mean ± SD)*

Patient	Diagnosis	Extent score	Intensity score	SCORMA index	Dermatologist's global evaluation (scale 0–100)*
1	UP	21 ± 14.2	2.4 ± 0.8	19.8 ± 5.4	21.1 ± 12.2
2	SM	1 ± 0	3.6 ± 0.7	20.6 ± 3.5	10.4 ± 7.9
3	UP	17.2 ± 3.8	3.6 ± 0.5	21.4 ± 2.8	19.2 ± 5.9
4	UP	44.1 ± 10.7	2.8 ± 1.0	29.6 ± 6.8	47.8 ± 17.2
5	UP	31.4 ± 7.2	3.4 ± 0.8	30.5 ± 4.7	44.4 ± 17.2
6	UP	52.4 ± 10.9	3.8 ± 0.9	30.7 ± 5.0	51.7 ± 16.4
7	UP	70 ± 9.7	4.6 ± 0.5	37 ± 2.8	69.4 ± 9.2
8	DCM	100 ± 0	3.6 ± 1.2	42 ± 12.3	74.9 ± 12.3
9	DCM	100 ± 0	10.6 ± 1.3	78.6 ± 6.3	92 ± 8.3

UP = Urticaria pigmentosa; SM = solitary mastocytoma; DCM = diffuse cutaneous mastocytosis.
*Impression prior to SCORMA.

RESULTS

The means and standard deviations for the extent of skin involvement for all the 9 patients are listed in Table I. Note that for patients 2, 8 and 9 there is a unanimous assessment. These patients had a defined percentage of skin involvement; 1% for solitary mastocytoma (1 pat.) and 100% for diffuse cutaneous mastocytosis (2 pats.).The mean results show less variety for activity than for extent. This can be explained by the limited width in range for part B.

There was a high degree of inter-rater agreement for all the investigated items, for parts A and B and the outcome of the SCORMA index. The lowest value, 0.43, was noted in subsection B1 (erythema).

The results of the SCORMA index compared with the results of the global evaluation of dermatologists before disclosing the SCORMA formula are also presented in Table I. The two assessments differ in the degree of severity in 7 of the 9 patients. In 4 of those patients the severity was assessed as more than 50% higher by the dermatologists than by the SCORMA index.

DISCUSSION

Mastocytosis is characterized by an indolent or progressive disease. In the majority of cases, mastocytosis is manifested by skin symptoms. In patients with a high suspicion of mastocytosis, histopathological examination of the bone marrow or other organs may be necessary to establish the diagnosis in cases in which histopathological examination of the skin showed no abnormalities. Measurement of mast cell mediator metabolites, such as N-methyl histamine and protryptase, is valuable in staging mastocytosis. However, these measurements have no diagnostic significance, because numerous other conditions may also lead to elevated levels.

No standardized protocol has been published for clinical monitoring of mastocytosis. Likewise, there is no scoring system to monitor symptomatic therapy, either routine or experimental.

We developed a clinical scoring system to monitor the cutaneous symptoms of patients with proven mastocytosis. The method is simple, which makes it reproducible. It imposes no burden to the patient. This method provides standardized information on the extent and the activity of mastocytosis in the skin. It is applicable alone or in combination with other staging investigations. In daily practice, it may prove valuable because it quickly provides a point of reference for the doctor and the patient.

The SCORMA index calculation contains a factor three-weight advantage for lesional activity over extent of the disease and subjective complaints. Consequently, emphasis has been put on the items of mastocytosis of the skin that are likely to be the first features that undergo change in progressive disease as well as in subsiding disease. It serves the goal of monitoring changes in mast cell activity and, to a lesser degree, to monitor changes in subjective aspects of the disease and pigmentation.

At our clinic, many of the mastocytosis patients are enrolled in therapeutic trails for which we use the SCORMA index as well as measurements of several mast cell mediator metabolites. Correlation studies between reduction of the SCORMA index and mediator metabolites are being pursued.

In order to gain insight into the reproducibility of the SCORMA system, it was put to the test by 9 practising dermatologists at regional hospitals. Based on the results of the assessment of 9 patients, a very high degree of agreement was achieved when the SCORMA index was used, even without prior familiarity with the method. Therefore, it also has practical value when used incidentally. The results of the SCORMA index compared with the dermatologists' opinion on the severity of the disease differ with respect to the extent of the disease. In general, patients with a relatively high percentage of involved body surface were considered to be more severely affected than those who had a lower percentage of involvement, the latter, regardless of the activity of skin lesions. This indicates further that standardized observation using the SCORMA index of the skin in mastocytosis could provide a more accurate clinical description of the activity of the disease.

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CHAPTER 11

Serum Tryptase and SCORMA (SCORing MAstocytosis) Index as Desease Severity Parameters in Childhood and Adult Cutaneous Mastocytosis

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Serum tryptase and SCORMA (SCORing MAstocytosis) Index as disease severity parameters in childhood and adult cutaneous mastocytosis

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Summary

Background. Skin lesions are the predominant clinical feature of the commonest form of mastocytosis. Mastocytosis is classified according to World Health Organization criteria. Determination of the levels of mast-cell mediators or their metabolites reflects the mast-cell burden. The extent of cutaneous mastocytosis can be assessed clinically using a scoring system (SCORing MAstocytosis; SCORMA Index) that we have developed.

Objective. Serum tryptase levels were compared with the SCORMA Index in a large group of paediatric and adult patients to investigate whether there was any correlation between the two.

Methods. The SCORMA Index in 64 patients (31 children and 33 adults) was compared with serum tryptase levels. The results of the first visit at which SCORMA and tryptase were evaluated were analysed.

Results. There was a positive correlation between the SCORMA Index and serum tryptase levels, indicating the value of the SCORMA Index in the assessment of mastocytosis with skin involvement.

Conclusion. The results of this study showed that the SCORMA Index is a useful tool for evaluating the severity of cutaneous mastocytosis. The correlation between the SCORMA Index and serum tryptase levels underlines the benefit of the SCORMA Index as a clinical tool. Repeated SCORMA Index measurements can provide a rapid impression of changes in the clinical state of mastocytosis. This is particularly relevant in children, because taking blood samples from this group is much more difficult. The well-established methods for evaluation of disease severity may be expanded by the rapid SCORMA Index method.

Introduction

Mastocytosis is a heterogeneous group of mostly clonal myeloproliferative diseases characterized by aberrant proliferation and accumulation of mast cells in one or more organ systems.[1–3] In the 2001 World Health Organization (WHO) classification, systemic mastocytosis is differentiated from cutaneous mastocytosis (CM) based on clinical, biochemical and pathological criteria, especially bone-marrow smears.[2] Childhood mastocytosis

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accounts for most of the cases of mastocytosis seen.[4] Unlike adult-onset mastocytosis, childhood cases of mastocytosis are primarily cutaneous, and complete remission will develop in a considerable number of patients during puberty.[5,6] The affected skin shows localized or diffuse accumulations of mast cells, resulting in pigmented macules and plaques.[7]

In 2001, we developed a system for scoring the clinical extent of mastocytosis, which was evaluated in nine patients by a group of nine dermatologists.[8] The system is known as the SCORMA (SCORing MAstocytosis) Index. The SCORMA Index consists of three parts and is based on the principles of the SCORAD (SCORing Atopic Dermatitis Index).[9] In the SCORMA Index, the extent of the skin abnormality is evaluated in the first part (A) using the relevant form (Fig. 1). The marked area represents the percentage of exposed skin. By definition, the lesion is 1% of the affected skin in solitary mastocytoma and almost the whole (100%) of the skin in diffuse CM. (R Heide, E Zuidema, AP Oranje, submitted) The intensity of the disorder is dealt with in part B, for which a lesion of a typical form, size and colour representing the majority of the lesions is examined. A lesion that is not affected by sunlight, such as one located on the back, is preferred. This lesion is then judged on its pigmentation/erythema, vesiculation, elevation and Darier's sign. Each item is scored from 0 to 3 (0 = absent, 3 = most severe). Five subjective symptoms (triggering factors, flushing, diarrhoea, itch and local bone pain) that may occur in mastocytosis are dealt with in part C, which is scored by the patient on a 10 point visual analogue scale (VAS), where 0 = absent and 10 = continuously present. The formula: A/5 + 5B + 2C/5 is used to calculate the final SCORMA Index. The value of the SCORMA Index then lies between 5.2 and 100.[8]

In the study reported below, a group of adult and paediatric patients with cutaneous features of mastocytosis were evaluated using the SCORMA. In total, 15 of these patients were classified as having indolent systemic mastocytosis (ISM) and their SCORMA Index was compared with their serum tryptase level to examine whether there was any correlation between the two.

Methods

All patients approved the use of their data for scientific purposes, but written informed consent was omitted, as no additional procedures or investigations took place. The study was approved by the local ethics committee.

Patients

Patients with mastocytosis and evident cutaneous lesions were selected for this study. The medical records of the patients with proven or suspected mastocytosis were drawn from the hospital registry. In total, 67 patients were found in the database up to 5 years before the study, of which 64 (31 children aged 0–18 years and 33 adults aged > 18 years) were evaluated at least once, and these were entered into the final analysis. The inclusion and exclusion criteria for this study are shown in Table 1. In children the peak incidence was between 0 and 5 years.

The diagnoses are listed in Table 2. Most of the patients (n = 40) had maculopapular, plaque-type mastocytosis (urticaria pigmentosa) or telangiectatic CM. A minority of the patients (n = 6) had solitary mastocytoma. Other diagnoses were diffuse CM (n = 3) and ISM (n = 15).

In addition to the SCORMA Index and serum tryptase level, the patients underwent full clinical examination and skin biopsy was obtained (if not obtained before or obtained elsewhere). C-kit mutation analysis was either not performed or unavailable in all cases.

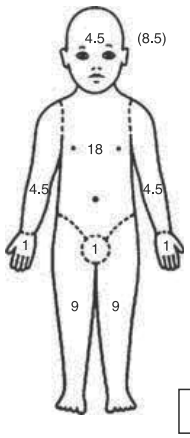
Classification based on clinical signs and conform the WHO guidelines was used to subdivide the patients.[2] Many of the patients reported in this paper were diagnosed and underwent complete investigation before 2001 when the WHO guidelines were published. The diagnosis of SM in this group was made on the basis of the presence of mast-cell accumulation in the skin and in ≥ 1 extracutaneous site. Immunostaining of bone-marrow sections, which is advocated by Working Conference on Mastocytosis, was not performed as a routine staining procedure [2]. The pre-2001 cases were reviewed to confirm their established diagnosis based on the available data during the preparation of this manuscript.

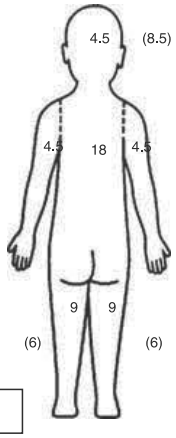
Tryptase levels

Serum tryptase level was determined on the same day as the SCORMA Index during routine checkups for each patient, and not while the patient was experiencing a worsening of the disease. Serum tryptase levels were determined using a commercial fluorescent enzyme immunoassay (UniCAP assay and UniCAP 100 instrument; Phadia, Nieuwegein, the Netherlands) according to the manufacturer's instructions. The 'Directions for Use' of this tryptase assay gives 11.4 μg/L as the 95% upper percentile value for healthy children and adults.

SCORMA INDEX

Institution	:	Name of patient	:
Physician	:	Date of birth	:
Date of visit	:	Patient number	:





Between brackets: Age under 2 yrs.

A: Extent please indicate the area involved [ ]

B: Intensity average representative area [ ]

Criteria	Intensity	Intensity items
1. Pigmentation / erythema	[ ]	0 = absent
2. Vesiculation	[ ]	1 = mild
3. Elevation	[ ]	2 = moderate
4. Positive Darier's sign	[ ]	3 = severe

C: Subjective Symptoms [ ]

	Visual Analog Scale (by parents if child < 5 years)
1. Provoking Factor(s)	0 ---------- 10
2. Flushing	0 ---------- 10
3. Diarrhea	0 ---------- 10
4. Pruritus	0 ---------- 10
5. Localized Bone Pain	0 ---------- 10

Scorma index: A/5 + 5B + 2C/5 [ ]

Figure 1 SCORing MAstocytosis Index.

Table 1 Inclusion and exclusion criteria for the study.

	Inclusion criteria	Exclusion criteria
Description of patient population	Diagnosis of mastocytosis according to WHO criteria	WHO criteria for any type of mastocytosis not met
Comparison SCORMA and tryptase	Diagnosis of mastocytosis SCORMA and tryptase with corroborating data	Uncertain diagnosis, no SCORMA and/or tryptase

SCORMA, SCORing Mastocytosis; WHO, World Health Organization.

SCORMA Index

Clinical evaluation of the patients using the SCORMA system was performed by one or both of two investigators (RH or APO) according to the previously published method. As the interobserver variation in the SCORMA is low, this had no substantial effect on the outcome.[8]

Statistical analysis

The particulars of all patients were entered in a database and analysed using SPSS software (SPSS Inc., Chicago, IL, USA). Pearson's correlation coefficient and the partial correlation coefficient were used for evaluating the relationship between SCORMA Index and serum tryptase. Serum tryptase was logarithmically transformed before analysis as its distribution was strongly skewed to the right. For the partial correlation coefficient, adjustment was made for the age of the patient. Values were also corrected for age because normal values of serum tryptase are influenced by age. $P < 0.05$ was considered significant.

Results

All cases of SM had skin involvement and serum tryptase levels > 20 μg/L. It was noted that patients 15, 27, 39 and 62 with bone-marrow findings showing increased mast-cell numbers had serum tryptase levels < 20 μg/L. Likewise, in patients 10, 12, 17, 32 and 55, who had raised levels of serum tryptase, apparently normal bone-marrow results were found.

The paired serum tryptase levels and SCORMA Index of all 64 patients classified by disease category are shown in Table 3. A scatter plot diagram showing the relationship between the SCORMA Index and serum tryptase level (after natural logarithmic transformation) of the same 64 patients is shown in Fig. 2. The Pearson correlation coefficient was 0.35 ($P = 0.004$). When solitary mastocytoma ($n = 6$) was excluded, making the population $n = 58$, the Pearson correlation coefficient was even higher (0.41, $P = 0.002$). After adjusting for the age of the patient, the partial correlation coefficient was 0.47 ($P < 0.0005$).

Discussion

Mastocytosis is rare, complex and sometimes diagnosed late. However, diagnostic methods are now more readily available and increasingly sensitive.[10] The following clinically distinct variations are recognized in the WHO classification; CM, ISM, SM with an associated clonal haematological non-mast-cell lineage disease, aggressive SM, MC leukaemia (leukaemic SM variant), MC sarcoma and extracutaneous mastocytoma.[2] From a dermatologist's perspective, the WHO subclassification of CM into three categories namely maculopapular CM (also known as urticaria pigmentosa), diffuse CM and solitary mastocytoma of the skin) may be debatable, as reported by Hartmann and Henz.[11] These authors suggested, based on the course and prognosis, that maculopapular mastocytosis in fact consists of different variations of CM. Maculopapular CM has small lesions, occurs in children and in adults, rarely resolves spontaneously and will often eventually be categorized as

Table 2 Types of mastocytosis in the patients in this study.

	Investigated population ($n = 64$)		Children ($n = 31$)		Adults ($n = 33$)	
Diagnosis according to WHO recommendation	%	*n*	%	*n*	%	*n*
Nodular cutaneous mastocytosis	9.4	6	19.4	6	0	0
Maculopapular cutaneous mastocytosis*	62.6	40	64.5	20	60.6	20
Diffuse cutaneous mastocytosis	4.7	3	9.7	3	0	0
Indolent systemic mastocytosis (with cutaneous features as the dominant symptom)	23.4	15	6.5	2	39.4	13

*Includes plaque-type cutaneous mastocytosis, maculopapular cutaneous mastocytosis, telangiectatic cutaneous mastocytosis.

Table 3 Paired serum tryptase levels and SCORMA Index.

Patient no.	Age, years	Tryptase level, ng/L	SCORMA
Nodular cutaneous mastocytosis (n = 6)			
5	0	3.9	36
7	0	3.9	16
38	0	20.3	21
41	0	9.8	21
54	0	5.1	21
64	0	5.8	29
Maculopapular cutaneous mastocytosis (n = 40)			
1	40	9.9	12
3	40	10.3	21
4	1	3.4	24
10	51	24.5	24
11	0	6.4	14
12	39	29.3	37
16	56	25.6	12
17	20	130	40
20	–	7.2	45
22	32	8.4	27
23	20	10.5	12
24	0	5.4	12
25	49	5.2	25
26	1	9.8	15
28	2	6.5	18
29	1	17.2	18
30	65	13.7	22
32	–	25.7	42
33	20	21.3	22
34	0	9.8	30
36	38	13.4	30
43	13	15.1	27
44	0	2.7	27
45	2	8.2	18
46	1	18.2	22
47	23	18.2	40
48	33	4.2	21
52	0	5.1	28
53	1	13.7	26
55	0	69.1	31
56	0	12.3	44
58	14	4.6	34
63	17	7.7	22
60	0	14.7	21
61	0	4.3	31
65	0	3.9	19
14	40	6.8	4
18	49	5.9	11
37	41	10.3	13
59	6	4.3	10
Diffuse cutaneous mastocytosis (n = 3)			
9	0	15.2	23
21	0	32.6	35
40	9	9.5	33
Indolent systemic mastocytosis (n = 15)			
2	53	35.4	23
6	38	28.1	32
8	1	20	47
13	34	182	43
15	30	12.4	22
19	42	28.8	19
27	38	17.5	34
31	20	25.8	42
39	18	14.7	30
42	39	483	30
49	35	22.8	15
50	25	22.5	41
51	57	34.5	11
57	23	221	46
62	2	10.4	27

SCORMA, SCORing MAstocytosis.

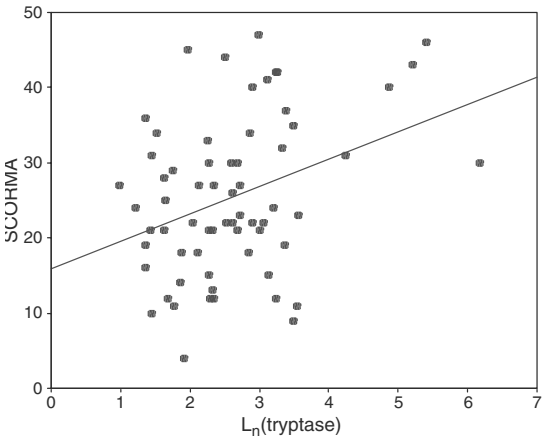


Figure 2 The relationship between the SCORMA index and the natural logarithm (L_n) of serum tryptase levels (μg/mL).

ISM. In contrast, plaque-type CM has lesions of several centimetres in diameter and the solitary mastocytomas do not evolve but tend to disappear. However, in this paper we analysed the results according to the WHO subclassification and included mastocytosis of the skin as a part of an established (indolent) SM. The diagnosis of SM was reconsidered because many of the reported cases were diagnosed before the WHO subclassification, and we were unable to revise the diagnosis on the basis of the available data at the time of collection.

An important aspect in terms of prognosis is to distinguish mastocytosis that has developed in the first 5 years of life from that arising in adulthood.[12,13] Several mediators or their metabolites have been used to evaluate the severity of mastocytosis, which is important for the prognosis. Tryptase and histamine are prominent indicators of mast-cell activation. Urinary

N-methylhistamine (NMH) levels correlate with the extent and the activity of the mastocytosis. In a previous study, a significant correlation between serum tryptase levels and urinary NMH levels was found in 138 patients.[14] Serum tryptase levels are a much better tool than NMH levels to distinguish patients with mastocytosis at an extracutaneous location, thereby making these patients potential candidates for mast-cell targeting therapy.[15] Serum tryptase levels in mastocytosis are normal in most cases of uncomplicated CM and mastocytoma, but are > 20 μg/L in SM.[16] The correlation between exclusive CM and serum tryptase levels may therefore be weak. In adults with SM, however, a positive correlation between density of the skin lesions, duration of the disease and constitutional symptoms, organomegaly and raised serum tryptase levels has been shown, whereas in children such a relationship has not been observed.[16] Our findings show an unexpected high number of cases in which raised serum tryptase levels could not be shown in combination with bone-marrow involvement. Possible explanations for these findings are that bone-marrow lesions in ISM may be difficult to find and require immunostaining instead of Giemsa/Leder or toluidine, which are the commonly used stains. Thus, bone-marrow involvement may be underappreciated in these cases.[3] Normal serum tryptase levels was noted in five of our patients with SM. This has been reported previously, and on its own is not a reason to doubt the diagnosis.[2] In our study we used a previously validated clinical evaluation system for mastocytosis and compared this with serum tryptase levels. Our system for mastocytosis is called SCORMA and may be used to take a clinical snapshot of a patient with mastocytosis. Its clinical applications have been published previously.[8] The SCORMA system is comparable with that used for atopic dermatitis (modified-objective SCORAD, Eczema Area and Severity Index, and Six Area Six Sign Atopic Dermatitis) and psoriasis (Psoriasis and Severity Index). Such a system is particularly beneficial in paediatric and adult patients with maculopapular mastocytosis (urticaria pigmentosa), because the clinical features of the disease may vary during the course of the disease and because of therapeutic interventions.[17]

However, there are several limitations to this observational technique. In patients with an associated haematological disorder such as myelodysplasia, regression of skin lesions may be accompanied by disease progression.[18] In contrast with this, regression of CM in patients with the indolent course parallels with a decrease in disease severity, although abnormal bone-marrow findings are still present.[18] Furthermore, the SCORMA Index requires the input of subjective factors. The degree of discomfort is often difficult to express not only by children but also by adults. In our study, the SCORMA Index in 64 patients (31 children and 33 adults) was compared with serum tryptase levels. The results of the first visit, at which both SCORMA Index and serum tryptase levels were determined were analysed. The SCORMA Index and serum tryptase levels showed a moderate correlation indicating that both are valuable for assessing CM or SM in cases where the skin is involved. The results of this study in children and in adults with mastocytosis show that SCORMA is of practical value for evaluating the severity of the disease. It is plausible that comparing sequential SCORMA values in an individual patient may be a useful tool to monitor disease progression and treatment efficacy.

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CHAPTER 12

Efficacy of 25% Diluted Fluticasone Propionate 0.05% Cream as Wet-Wrap Treatment in Cutaneous Mastocytosis

Dermatology 2007, 214:333-335

Efficacy of 25% Diluted Fluticasone Propionate 0.05% Cream as Wet-Wrap Treatment in Cutaneous Mastocytosis

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Key Words
Mastocytosis · Fluticasone propionate 25% diluted · Wet-wrap

Abstract
Background: Mastocytosis is a disorder that can be subdivided into two forms: cutaneous and systemic. Patients with cutaneous mastocytosis only may suffer from cosmetic problems. Topical steroid application has been shown to be effective in cases of limited skin lesions. ***Methods:*** A case-controlled pilot study was conducted during a 6-weeks treatment using diluted 25% fluticasone propionate 0.05% cream under wet-wrap occlusion in 5 adults and 6 children. Improvement was measured up to the 24th week after treatment using the SCORMA Index. ***Results:*** The results of this pilot study showed a partial but clear cosmetic improvement in 9 of the 11 patients. The mean SCORMA Index decreased after treatment from 38 to 26. ***Conclusion:*** 25% dilution of fluticasone propionate 0.05% cream under wet-wrap occlusion is an alternative treatment modality for alleviating the symptoms of cutaneous mastocytosis, but the improvement may be moderate and fall short of the patient's expectations.

Mastocytosis is an abnormal accumulation of mast cells in the absence of an apparent cause [1, 2]. The skin is the most frequent site of involvement. Mastocytosis is associated with a broad range of local and systemic symptoms primarily caused by the release of mast cell mediators [3]. Rare manifestations of mastocytosis have constantly been reported [4]. Available therapies are symptomatic [5]. These include H1- and H2-receptor antagonists and disodium cromoglycate. PUVA or UVA1 therapy may reduce mast cell numbers and alleviate symptoms for several months [6]. Topical application of highly potent corticosteroids under occlusion has been used successfully in adult patients with urticaria pigmentosa and in solitary mastocytoma in children. UV light therapy or highly potent corticosteroids under occlusion are unsuitable for treating children with urticaria pigmentosa because of potential side effects. Dilutions of 0.5–50% fluticasone propionate 0.05% cream or other topical steroids under wet-wrap occlusion have been safely used in children and adults with atopic eczema covering large surface area [7, 8]. In this open case-controlled pilot study, we investigated the efficacy of topical fluticasone propionate 0.05% cream under wet-wrap occlusion in 5 adults (4 with urticaria pigmentosa, 1 with teleangiectasia macularis eruptiva perstans) and 6 children (4 with urticaria pigmentosa, 2 with diffuse cutaneous mastocytosis).

The extent of lesions in each patient was assessed using a semiquantative scoring system called SCORMA that we

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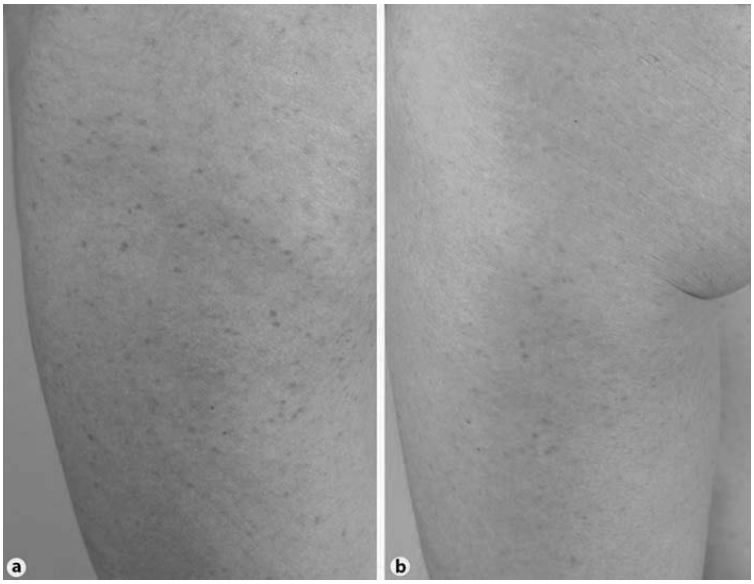

Fig. 1. a Mastocytosis lesions in a patient before treatment with 25% diluted fluticasone propionate 0.05% cream under occlusion. **b** A partial but clear cosmetic improvement in the same patient 24 weeks after treatment.

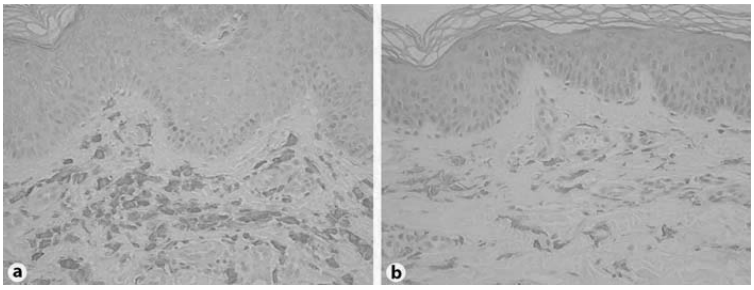

Fig. 2. a A large number of mast cell in a histological section of lesional skin biopsy before treatment. Leder staining. ×160. **b** A decreased number of mast cells in the histological section of lesional skin biopsy after 6 weeks of treatment. Leder staining. ×100.

had previously developed and described in details elsewhere [9]. The SCORMA Index in the 5 adults ranged from 35 to 47 (mean 40), whereas it ranged from 28 to 41 (mean 35) in the 6 children before wet-wrap treatment. The most common subjective symptoms before and after treatment are summarized in table 1.

Prior to this pilot study, wet-wrap treatment with diluted corticosteroids was approved by the Medical Ethical Committee of our institution. After informed consent had been obtained, each patient was treated with 25% dilution of fluticasone propionate cream 0.05% applied daily under a wet-wrap occlusion dressing for 6 weeks. The amount of cream used for the 5 adults ranged from 4 to 12 g (average 8 g) daily and for the 6 children ranged from 3 to 5 g (average 4 g daily) and were performed according to the finger tip method. The therapy compliance was monitored by regularly weighing the amount of cream. The patients were evaluated at 3, 6, 12 and 24 weeks after the start of the treatment. Biochemical parameters such as routine blood and urine examination including the levels of urinary N-methylhistamine and fasting serum cortisol were monitored at each evaluation. Skin infiltrates were stained using the Leder technique [10]. The number of mast cells was also counted in lesional skin biopsies from all patients before and 6 weeks after treatment. Clinical improvement was assessed using SCORMA. The SCORMA Index in the 5 adults ranged from 7 to 41 (mean 27), whereas it ranged from 15 to 46 (mean 26) in the 6 children after wet-wrap treatment.

Table 1. Symptoms before and after treatment

	Cosmetic	Heat intolerance	Pruritus	Flushing	Abdominal pain
Before treatment	11/11	10/11	9/11	6/11	5/11
After treatment	9/11	5/11	5/11	4/11	5/11

Table 2. Clinical improvement 24 weeks after start of the treatment

Clinical assessment	Before treatment		After treatment	
	mean	range	mean	range
Extent	58%	100–17	40%	100–6
Intensity				
Pigmentation/erythema	1.8	3–1	1.3	2–1
Vesiculation	0		0	
Elevation	0.4	1–0	0.1	1–0
Darier's sign	1.8	2–0	0.9	2–0
SCORMA index	38	47–24	26	46–9

The results of this pilot study showed that diluted 25% fluticasone propionate 0.05% cream under wet-wrap occlusion led to a partial but clear cosmetic improvement in 9 of the 11 patients (fig. 1a, b). An overall summary of the clinical results 24 weeks after treatment is shown in table 2.

The mean SCORMA Index decreased from 38 before treatment to 26 after treatment. The number of mast cells counted in skin biopsies from all patients taken before the treatment and after completion at week 6 showed a decrease of between 10–60% (fig. 2a, b). The levels of urinary N-methylhistamine remained unchanged during the course of the treatment in all patients, but serum tryptase levels were not determined. Both of these parameters can be used to establish the intensity of mastocytosis [11]. The treatment was noted to be safe on the basis of fasting serum cortisol levels, which remained unchanged during the course of the treatment in all patients.

From the results, it can be concluded that 25% dilution of fluticasone propionate 0.05% cream under wet-wrap occlusion is an alternative treatment modality for alleviating the symptoms of cutaneous mastocytosis affecting large skin areas. The treatment had no side effects in this series of patients. However, fasting cortisol levels should be monitored. Safety of this therapy in atopic dermatitis has also been reported [12, 13]. Nonetheless, the clinical and cosmetic improvement achieved may fall short of patient's expectation and all patients should be made aware of this aspect of the treatment. Further prospective randomized studies involving a large number of patients are necessary to optimize the effect of this therapy.

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CHAPTER 13

Mastocytosis in Children: a Protocol for Management

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MASTOCYTOSIS IN CHILDREN: A PROTOCOL FOR MANAGEMENT

Abstract: **Mastocytosis is characterized by an increased number of mast cells with an abnormal growth and accumulation in one or more organs. In most children mastocytosis is limited to the skin (cutaneous mastocytosis) and often transient as compared with that in adults in whom mastocytosis is usually progressive and systemic. Generally, we recognize three more common forms of cutaneous mastocytosis: maculopapulous mastocytosis (formerly urticaria pigmentosa), mastocytoma of skin, and diffuse cutaneous mastocytosis. Childhood mastocytosis can further be divided into cutaneous mastocytosis (nonpersisting and persisting) and systemic mastocytosis (extremely rare). An approach to management using a set protocol is described in table form. In most cases of mastocytosis, only yearly checkups are necessary and no treatment is required; preventive recommendations are warranted in those individuals with systemic disease and constitutional symptoms. Symptomatic therapy is advised in only a minority of cases. This article is meant as a guideline for physicians involved in the care of children with mastocytosis and their parents.**

Mastocytosis is defined as a heterogenous group of disorders with an abnormal accumulation of mast cells (MCs) anywhere in the body. All forms of mastocytosis are rare. Mastocytosis has many variants, which share several clinical features (1–3). The course of mastocytosis is varied and depends on the subtype and on the age of onset (4–6). In children, mastocytosis is commonly cutaneous (CM) and often transient when compared with that in adults, in whom the disease usually is progressive and systemic (2,6). However, all variants of mastocytosis may be diagnosed in children. A considerable body of evidence that many forms of mastocytosis are caused by point mutations in the genes coding for growth factor receptor c-kit (2,7–9). Genetic findings also indicate several different pathogenetic forms of mastocytosis. Adult patients and minor subsets of pediatric mastocytosis express activating mutations of the c-kit receptor, whereas in most cases of childhood-onset mastocytosis such mutations were not reported (10). The heterogeneity of c-kit mutations may have contributed to difficulties in characterizing genotype–phenotype correlations of the disease (11).

Traditionally, mastocytosis has been classified on the basis of clinical presentation. In 2001 a consensus classification based on the behavior/course of the disease rather than its clinical description was proposed and approved by the World Health Organization (12). In this consensus, classification mastocytosis is divided into three major groups consisting of CM, systemic mastocytosis (SM), and the extremely rare localized extracutaneous MC neoplasms not described in children (13). In children, mastocytosis is mostly cutaneous and transient. Therefore, for childhood mastocytosis we prefer a classification of five subgroups of increasing severity and morbidity as shown in Table 1. First, CM is distinguished from SM on the basis of criteria shown in Table 2. Second, the severity of the systemic subtype is defined on the basis of investigations of skin lesions, bone marrow, peripheral blood, and serum tryptase levels, as well as the functions of the liver, the spleen, the lymph nodes, and other organs. The skin lesions may be clinically classified as shown in Table 3. In February 2007 additional broad-based thorough recommendations for the standardization of the recognized subtypes and workup of mastocytosis were reported by Valent et al (8). In that article, mastocytosis in adults is considered systemic until proved otherwise. In children this is not the case unless persistently high (or rising) serum tryptase levels and other signs of SM are encountered.

Generally, we recognize three more common forms of childhood CM: maculopapular mastocytosis (urticaria pigmentosa), mastocytoma of skin, and diffuse cutaneous mastocytosis (DCM). Telangiectasia macularis eruptive perstans has not been recognized by the WHO. SM is usually indolent and is extremely rare in children (5).

This protocol forms a practical guideline for the diagnosis, the evaluation, and the treatment of mastocytosis in children. It is primarily intended for dermatologists, allergists, and pediatricians.

RECOMMENDATIONS ON HISTORY, EXAMINATION, DIAGNOSTICS, THERAPY, AND FOLLOW-UP

History

History should include the following topics:

- Duration of the disease. Is there an increase/decrease in the number of lesions?;
- Factors provoking mediator release;
- Increase/decrease in the activity of the lesions in time;
- Itch (attacks/periodic/chronic);
- Mastocytosis in family;
- Heat;
- Exertion;

TABLE 1. *Mastocytosis Classification (12)*

Category	Diagnostics	Characteristic findings
Cutaneous mastocytosis	SM criteria*	Not present
	Skin lesions	Present (mast cell infiltrates in biopsy)
	Bone marrow (not required in most cases)	No mast cell infiltrates
	Peripheral blood profile	Normal
	Serum tryptase	< 20 $\mu g/L$
Indolent systemic mastocytosis	SM criteria*	Fulfilled
	Skin lesions	Generally present
	Bone marrow	Multifocal mast cell infiltrates; $< 20\%$ mast cells in smears
	Peripheral blood profile	Normal or slightly deviating
	Serum tryptase	> 20 $\mu g/L$
	Liver/spleen/lymph nodes	Sometimes enlarged
Systemic mastocytosis with a AHNMD†	SM criteria*	Fulfilled
	Bone marrow and peripheral blood profile	Besides multifocal mast cell infiltrates MDS, MPS, AML of NHL (WHO/FAB criteria)
Aggressive systemic mastocytosis	SM criteria*	Fulfilled
	Skin lesions	Often absent
	Bone marrow	Multifocal mast cell infiltrates; $< 20\%$ mast cells in smear; no AHNMD†
	Peripheral blood profile	Abnormal (leukocyte count $< 1.0 \times 10^9$/L, Hb < 6.2 mmol/L, and/ of thrombocyte count $< 100 \times 10^9$/L)
	Liver/spleen/lymph nodes	Enlarged
	Organ function	Reduced‡
Mast cell leukemia	SM criteria*	Fulfilled
	Skin lesions	Absent
	Bone marrow	Diffuse uncontrolled mast cell growth $\geq 20\%$ mast cells in smear
	Peripheral blood profile	$\geq 10\%$ mast cells (in aleukemic variants) $< 10\%$ mast cells
	Organ function	Reduced

*SM criteria: criteria for establishing the diagnosis of systemic mastocytosis (see Table 2); †AHNMD: associated clonal hematologic illness of an origin other than the mast cell line; ‡reduced organ function: ascites, disturbed liver function and/or portal hypertension (liver), hypersplenism (spleen), malabsorption with hypalbuminemia and loss of weight (gastrointestinal tract); osteolytic centers and/or osteoporosis with fractures (skeleton). MDS, myelodysplastic syndrome; MPS, myeloproliferative syndrome; AML, acute myeloid leukemia; NHL, non-Hodgkin lymphoma; SM, systemic mastocytosis; WHO/FAB, World Health Organization/French American British study group.

TABLE 2. *Criteria for Establishing the Diagnosis of Systemic Mastocytosis (12)*

Major criteria	Multifocal infiltrates of mast cells (> 15 close to each other) observed in bone marrow biopsies and/or mast cells stained for tryptase in biopsies from other extracutaneous organs.
Minor criteria	$> 25\%$ spindle-shaped mast cells in infiltrates in biopsies from bone marrow or other extracutaneous organs or presence of $> 25\%$ atypical mast cells in bone marrow aspirates.
	Demonstration of the c-kit point mutation on codon 816 in bone marrow, blood or other extracutaneous organs.
	CD117 (c-kit receptor) positive cells, which are also positive for CD2 and/or CD25.
	Serum tryptase is > 20 $\mu g/L$.

- Showers;
- Stress; and
- Certain foods.

Physical Examination

Physical examination should include:

- Inspection of whole skin;
- Examine Darier sign in suspected lesions;
- Classify skin profile using; and
- SCORMA (see Appendix "SCORMA").

Diagnostics

Diagnostics are limited to the following:

- Skin biopsy from the lesion; and
- Skin biopsy from nonlesional skin only on indication (high clinical suspicion of DCM).

Interpretation of Skin Biopsies

(1) Histologic findings and MC counts confirm clinical diagnosis of CM; absence of the characteristic findings

and the number of MCs do not exclude the diagnosis in dermatologic patient population.

Bone Marrow

(1) Generally not required; and
(2) Bone marrow investigation should only be performed in symptomatic pediatric cases with suspected hematologic disease or suspected SM. Only serum tryptase levels that are clearly elevated (>20 μg/L) are not enough to undertake investigations of bone marrow.

Therapy

Therapy in isolated CM may be omitted.

Cutaneous mastocytosis with cosmetic complaints only:

- Generally no therapy; and
- Topical therapy in children older than 2 years (15).
  - Confined to about 10% of the body surface area: corticosteroid cream with occlusive dressing and
  - More than 10% of the body surface area: corticosteroid cream (one part vs. three parts) 25% diluted under wet-wrap occlusion (optional) for 3 to 6 weeks.

Cutaneous mastocytosis with complaints of itch, redness, and swelling:

- Avoid foods, which according to the anamnesis provoke the lesions; and
- Systemic therapy, consisting of combination of H1- and H2-blocker (and oral sodium cromoglycate).

Follow-Up

Follow-up in isolated CM is recommended:

Checkup once a year and telephone consultation once every 6 months.

The symptoms in SM may vary considerably and depend on the extent and the site of MC accumulations in an organ. Most of the complaints are caused by the mediators (histamine, leukotrienes, prostaglandins, interleukins, platelet-activating factors, and tryptase) released from MCs. The mediator-related symptoms may be fainting, hypotensive shock, diarrhea with abdominal pain, heartburn, severe bone pain, flushes, and headache. It should be noted that extensive skin involvement alone may also induce these symptoms.

When in doubt on the extent of mastocytosis, the work-up should be broadened.

History should also include the following topics:

- Attacks of flushing;
- Attacks of syncope;
- Attacks of heart palpitations;
- Abdominal cramps or pain (attacks/periodic/chronic);
- Diarrhea (attacks/periodic/chronic);
- Pyrosis (heartburn);
- Reaction to drugs (aspirin, NSAID, codeine, and opiates);
- Reaction to narcosis;
- Reaction to i.v. radiograph contrast fluids or MRI-contrast media;
- Reaction to wasp and bee stings;
- Anaphylactoid/anaphylactic reaction;
- Bone-pain; and
- General symptoms:
  - Weight loss,
  - Nausea,
  - (Severe) headache,
  - Fever/feverish feeling,
  - General depression, and
  - Fatigue.

- Physical examination (preferably in cooperation with a pediatrician) should also include:
  - Length;
  - Weight;
  - Organomegaly; and
  - Other investigations on indication (e.g., constitutional symptoms).

- Indications for screening for SM in the absence of CM are:
  - Unexplainable abdominal pain with diarrhea;
  - Unexplainable bone pain;
  - Unexplainable flushes;
  - Unexplainable itch;
  - Occurrence of an anaphylactic reaction/shock;
  - Organomegaly (liver, spleen, or lymph nodes);
  - Unexplainable (pan)thrombocytopenia; and
  - Combinations of the above.

Diagnostic procedures should include:

(1) Determination of serum tryptase levels; if clearly elevated (>20 μg/L) proceed to step 2;
(2) Skin biopsy (see addendum);
(3) Abdomen ultrasound;
(4) Peripheral blood analysis (thrombocytopenia, leukocytosis, differential cell count); and

TABLE 3. *Descriptive Classification of Skin Lesions in Cutaneous Mastocytosis and Indolent Systemic Mastocytosis Based on Valent et al (12) and Hartmann et al (14)*

Manifestation	Prevalence	Age	Remission partial	Systemic mastocytosis	Remarks
Diffuse cutaneous mastocytosis	Very rare	Infants	In 3rd–5th year of life	Unknown	Often with bleeding, blister formation, and severe initial systemic manifestations
Nodular form (one or several mastocytomas)	Frequent	Before 3rd month	Generally, before puberty	No	
Maculo papular form, subdivided into		Infants, often before 1–2nd month	About 50% before puberty		Formerly urticaria pigmentosa
Plaque variant (large papular lesions)		Children and adults	Little in children, seldom in adults	Frequently in adults	Sometimes hardly visible Formerly urticaria pigmentosa
Medium-size maculopapular (smaller maculae)	Most common form				
Telangiectatic cutaneous mastocytosis	Very rare	Very rare in children		Frequently	Sometimes hardly visible

The term Telangiectasia macularis eruptiva perstans has not been recognized by the WHO (2001).

(5) In cases with organomegaly, lymphadenopathy or abnormalities in the peripheral blood: crista biopsy and bone marrow aspirate (16).

In tissue/smear specimen:

- Morphology of MCs and MC counts;
- Immunologic staining (CD2 and CD25 on CD117 positive MCs);
- Tryptase staining of the MCs; and
- Determination of proto-oncogene c-kit mutation (codon 816).

Preventive measures in systemic and extensive CM in children are strongly recommended:

Prevention of anaphylactic/anaphylactoid reactions in general

- Eliminate allergen(s)/provoking factor(s), if known
- Avoid alcohol, aspirin, NSAIDs, codeine, opiates, polymyxin B, and intravenous radiograph contrast fluids and MRI-contrast media, unless (recently) known tolerance;
- 2x Epinephrine auto injector; for instructions see http://www.anafylaxis.net;
- H1 + H2 blockers at maximum dose (on indication: e.g., high frequency);
- Aspirin or NSAID at high dose (on indication: e.g., high frequency and insufficient effect of H1 + H2 blockade; see remark);
- "Medical alert" card or chain (see Appendix: Example of text "medical alert" and information letter in English);
- Information letter in English for vacations abroad; and
- Hyposensitization for wasp or bee sting allergy is contraindicated.

Remark:

Aspirin and NSAID should be started only at a very low dose, gradually increased under strict monitoring of circulation! [pediatric intensive care (ICP/ICC)].

Prevention of anaphylactoid reactions with anesthetics:

- See Appendix "preventive measures"

Prevention of anaphylactoid reactions to radiograph contrast fluids or MRI-contrast media:

- See Appendix "preventive measures for radiograph contrast fluids or MRI-contrast media"

Therapy in SM in children is symptomatic:

- Anti-mediator drugs (H1 blockers) such as anti-histamines: clemastine, ketotifen, and cromoglycic acid;

- H2 receptor blockers such as ranitidine particularly for heartburn;
- Aspirin for flushes, tachycardia, or fainting; (warning: hypotensive crisis during use of aspirin);
- Adequate intake/formation of vitamin D;
- Adequate calcium intake; and
- Adrenaline auto injector.

Follow-up in SM should include at least:

- Anamnesis (annually);
- Physical examination (annually);
- Laboratory tryptase (annually);
- Abdomen ultrasound (once annually); and
- Additional diagnostics depending on the complaints.

APPENDIX

Skin Biopsy Procedure Indications

- Suspicion of CM; and
- Demonstrated SM in the absence of suspected abnormalities for CM.

Procedure

- Local anesthetics with low histamine liberating potential such as lidocaine or bupivacaine;
- 3 to 4 mm punch biopsy from suspected lesion;
- 3 or 4 mm punch biopsy from inner-side of left lower arm; and
- Send in biopsies on physiologic saline-drenched gauze.

Processing of Biopsy

- Routine Giemsa staining;
- Tryptase staining (else Tolnidine blue staining, if possible);
- Immunophenotyping;
- Mast cell counts; and
- Mutation in proto-oncogene c-kit.

Evaluation

- Counts (17):
  - Healthy controls: max. 58 MCs/mm^2;
  - Nonlesional skin from CM: min. 68 MCs/mm^2;
  - Nonlesional skin from SM: min. 30 MCs/mm^2;
  - Lesional skin from CM: min. 64 MCs/mm^2; and
  - Lesional skin from SM: min. 335 MCs/mm^2.

Remark

In children, 10× higher values are still normal. The number of cells probably depends on the age, site of the biopsy and staining. Therefore, the numbers mentioned above are with reservation.

Preventive measures for children with CM should considered when use of narcotics or anesthesia are planned (18).

Considerations when planning a procedure include:

- Intradermal skin testing with drugs to be used in anesthesia (19);
- Reducing anxiety, preoperative sedation (oral diazepam);
- Minimize the number of pharmacologic agents; and
- Use a relaxing agent with low potential of histamine liberation.

Suggested measures in children with large or unknown disease burden on the day of operation:

- Prednisolone i.v., bolus 2 mg/kg, followed by 1 mg/kg prior to the procedure; and
- Clemastine 3 dd 0.05 mg/kg orally.

Peri-operative period

- Isofluran (or others based on the experience of the attending anesthetist); and
- Keep adrenaline at hand.

Postoperative period

- Paracetamol/acetaminophen.

Aspirin, NSAIDs, codeine, opiates, atropine, polymyxin B, and procaine (warning: mouthwashes, eardrops, and nose ointments) are contraindicated.

No relevant systematic data are available in the literature on the pharmacologic pretreatment of patients with mastocytosis. From a theoretical point of view combined H1- and H2-blockers may be useful because these drugs have been reported to reduce unprovoked attacks (20). Unexpectedly, NSAIDs when given continuously were also shown to reduce attacks in some patients (21). However, NSAIDs also may induce attacks when given without careful up dosing and are thus not considered to be safe as a pretreatment regimen. Anti-IgE (omalizumab) has been shown to reduce attacks in mastocytosis patients (22). Pretreatment with anti-IgE probably will only be effective after several weeks of treatment and only in situations where IgE-mediated processes occur.

Two possible schemes are proposed based on these considerations. Scheme 1 may be used as a default and scheme 2 may be used in patients with a delayed intestinal resorption or preoperatively. If clemastine is not avail-

able, it may be replaced with another injectable drug. Obviously, anti histamines may be continued in mastocytosis patient already on maintenance treatment.

Pretreatment Scheme 1

$T = -13$ hours
Prednisolone 0.5 mg/kg orally;
Cetirizine 10 mg orally; and
Ranitidine 150 mg orally.
$T = -7$ hours
Prednisolone 0.5 mg/kg orally.
$T = -3$ hours
Prednisolone 0.5 mg/kg orally;
Cetirizine 10 mg orally; and
Ranitidine 150 mg orally.
$T = 0$ hour
Radio contrast.

Cetirizine: Children older than 6 years 10 mg orally, children 1 to 5 years 2.5 mg orally.
Ranitidine: Children 2 mg/kg orally.

Pretreatment Scheme 2

$T = -13$ hours
Prednisolone 0.5 mg/kg orally;
Clemastine 2 mg orally; and
Ranitidine 150 mg orally.
$T = -7$ hours
Prednisolone 0.5 mg/kg orally.
$T = -1$ hours
Prednisolone 0.5 mg/kg i.v. and Clemastine 2 mg/kg i.v. (slow i.v. injection)
Ranitidine 50 mg i.v. (in 20 mL, slow i.v. injection)
$T = 0$ hour
Radio contrast.

Clemastine: Children older than 12 years 2 mg orally or i.v., children 6 to 12 years 1 mg orally or i.v.
Ranitidine: Children orally 4 mg/kg; intravenously 1 mg/kg (in 20 mL, slow i.v. injection).

Acute Treatment of an Anaphylactoid/Anaphylactic Reaction in Children

- Eliminate cause (e.g., stop infusion with a provoking substance);
- Clemastine 0.05 mg/kg i.v.;
- Adrenaline (1:1000) 0.01 mg/kg i.m. or 0.1 mL in 10 mL NaCl 0.9% slowly i.v. under monitoring of cardiac rhythm in about 6 minutes; and
- Prednisolone 1 mg/kg i.m. or i.v.

Finally, observation during at least 8 to 10 hours.

SCORMA Index

We need an alternate method for monitoring the severity of childhood mastocytosis because obtaining blood samples from children should be avoided as much as possible (23,24). That was the main reason why we developed a scoring system comparable with already existing methods for monitoring the severity of atopic dermatitis. We also use this system for monitoring the severity of the disease in adults.

The extent of the skin abnormality is evaluated in the first part (A). This is filled in the SCORMA form. The marked area then represents the percentage of exposed skin. In multiple mastocytoma of skin, each lesion is 1% of the affected skin. In DCM, by definition, almost the whole of the skin is affected, thus 100%. The intensity of the disorder is dealt with in part B. Hereby, a lesion of a typical form, size, and color representing the majority of the lesions is examined. A lesion which is not affected by sunlight such as that on the back is preferred. This lesion is then judged on pigmentation/erythema, vesiculation, elevation, and Darier sign. Each item is scored from 0 to 3, whereby 0 is for an absent item and 3 represents the most severity. The 5 subjective symptoms such as provocative factors, flushing, diarrhea, itch, and local bone pain, which may occur in mastocytosis are dealt with in part C. The patient may score these symptoms from 0 to 10 (using the visual analog scale), whereby 0 is for absence and 10 is for continuous presence. The formula: $A/5 + 5B + 2C/5$ is used to calculate the final SCORMA score. The value of the SCORMA score then lies between 5.2 and 100.

Note: this system was validated by a group of dermatologists practicing in greater Rotterdam, The Netherlands (23).

DISCUSSION

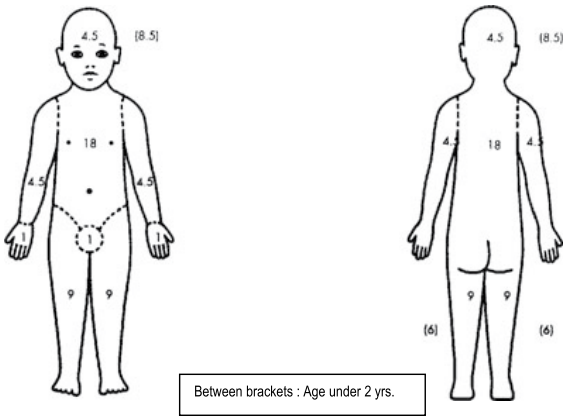
This article is intended as a guideline for physicians who are involved in pediatric mastocytosis care. Difficulties may arise in establishing the diagnosis despite the mild course of the disease in the majority of the children. In cutaneous forms, especially in the macular subtypes the number of MCs may be low after the Giemsa staining, Leder staining, or tryptase staining (18). When the number of MCs is low, the staining for c-kit usually results in a higher number of MCs, whereas the other stains are too insensitive. Anti-c-kit (CD117) staining has a high specificity and a high sensitivity for MCs in paraffin sections. This staining is against c-kit membrane receptor. It stains MCs even after they have degranulated (25).

On the rare occasion when systemic involvement is highly suspected, a multidisciplinary approach in which careful monitoring of mediator-related symptoms, organ function, growth as well as hematologic and bone marrow analysis are performed is imperative.

Preventive measures with respect to wasp stings and anesthesia are only necessary in cases with (subjective) symptoms and clearly elevated serum tryptase levels. We developed the SCORMA index which has proved useful in cases in which obtaining repeated blood samples such

SCORMA INDEX

Institution	:	Name of patient	:
Physician	:	Date of birth	:
Date of visit	:	Patient number	:



Between brackets : Age under 2 yrs.

A: Extent please indicate the area involved [ ]

B: Intensity average representative area [ ]

Criteria	Intensity	Intensity items
1. Pigmentation / erythema	[ ]	0 = absent
2. Vesiculation	[ ]	1 = mild
3. Elevation	[ ]	2 = moderate
4. Positive Darier's sign	[ ]	3 = severe

C: Subjective Symptoms [ ]

	Visual Analog Scale (by parents if child < 5 years)
1. Provoking Factor(s)	0 ---------- 10
2. Flushing	0 ---------- 10
3. Diarrhea	0 ---------- 10
4. Pruritus	0 ---------- 10
5. Localized Bone Pain	0 ---------- 10

Scorma index: A/5 + 5B + 2C/5 [ ]

as that in children with mild symptoms is problematic (23). The SCORMA index scoring system shows a good correlation with serum tryptase levels (submitted) and provides information on eventual disease improvement or aggravation (24).

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CHAPTER 14

Discussion and Summary

in English and Dutch

Discussion

Cutaneous symptoms are the main presenting feature of mastocytosis in almost all children and in the majority of adults. The aim of physicians is to assess the severity of mastocytosis in all cases because at present mastocytosis is considered to be a clonal disease originating in the myeloid precursor cells in the bone marrow. Systemic mastocytosis that is encountered in the majority of adult patients is uncommon in children.

The increasing fundamental knowledge on mastocytosis now comes from the hemato-oncological field, with ongoing refinement in the subdivision of systemic mastocytosis. New insights and developments in this area probably benefit primarily the most severe cases of mastocytosis. The dermatologist's involvement in such severe cases is less relevant, but the involvement in absolute numbers of patients with mastocytosis is not. In mastocytosis a multidisciplinary approach is demanded.

The aim of the investigations presented in this thesis was to provide a global dermatological perspective on mastocytosis with focus on clinical, diagnostic and therapeutic aspects of the disease in children and adults. Current insights into advances in mast cell-related skin diseases and a structured management of pediatric mastocytosis are also presented. For sporadic cases with cosmetic complaints of the disease wet- wrap treatment may be a useful, but well designed clinical studies are necessary to confirm this.

The general conclusions of the studies were that mastocytosis in children remains a different disease category despite the demonstration of clonality in some of those cases. In diffuse cutaneous mastocytosis, a severe subtype of mastocytosis in children, the course of the disease appears to be similar to maculopapular mastocytosis. The dermatologist's perspective on mastocytosis must evolve in the future towards fully supporting their patients in all the aspects of the disease. In this, it is highly imperative that systemic mastocytosis should be ruled out in all cases. Once the diagnosis of mastocytosis has been established, the treatment and the follow-up remain tailored. Progress has been made in developing new tools for defining the extent of the disease, which is supported by mast cell counts in the skin and mast cell mediator measurements in relation to clinical scoring of the disease and bone marrow involvement.

Summary

In chapter 1, various clinical and biological aspects of mastocytosis are presented that are reflected in the studies of this thesis. The aims of the studies described in this thesis are also presented in this chapter. In chapter 2, the current state of affairs on the role of mast cells (MCs) in dermatological diseases including mastocytosis is presented. Key issues include the contribution of MCs in urticaria and mastocytosis and other inflammatory diseases with a brief description on angiogenesis. Mast cells play a prominent role in several inflammatory and proliferative disorders, particularly of the skin. Their role is undisputed in mastocytosis and urticaria. At present, there is no causative treatment and no complete cure for mastocytosis possible. Pediatric mastocytosis in most cases is not treated, particularly because it is asymptomatic and limited to the skin. Treatment is only indicated for alleviating severe symptoms. In the majority of cases, chronic urticaria is encountered in adults. Chronic urticaria is believed to have an underlying autoimmune pathogenesis in almost 50% of the cases. The role of MCs in autoimmune diseases has not yet been completely elucidated. In atopic dermatitis MCs also play a role; although recently an important role of the skin barrier function has also become clear. The role of MCs in hemangioma is speculative, but may be interesting as a target for future therapeutic options.

In 2002, we provided a clinical review on mastocytosis in childhood. In the light of emerging new insights in mastocytosis a place for clinical description of skin lesions was advocated for practical use. This is described in chapter 3. It also became obvious at that time that the classification of mastocytosis was in need for and undergoing a major restructuring. In 2006, an update on the period 2002-2006 dealing with the WHO classification scheme and its relevance in pediatric mastocytosis described in chapter 4 was published.

In 2002, a retrospective analysis of mastocytosis comparing the clinical aspects of 101 children and adults that were diagnosed with mastocytosis in the period of 1980-1998 was conducted. The results are described in chapter 5 and provide a different view on the prognosis of childhood onset and adult onset mastocytosis. Childhood onset mastocytosis is not as transitory as was indicated in the literature, where a higher number of complete resolutions was reported (15% in our study compared with 50-80% reported in the literature). The absence of an objective standardized method of evaluating the disease severity in

mastocytosis was lacking. This made it difficult to evaluate and establish the spontaneous cure rate of pediatric mastocytosis.

A series of eight children suffering from diffuse cutaneous mastocytosis (DCM) is presented in chapter 6. It was the largest series of DCM at the time of this writing. The eight cases presented showed impressive symptoms and sometimes very high serum Tryptase levels, especially in young patients. The disease spectrum varied from red blistering to infiltrative yellowish nodular lesions. Since the disease follows a favorable course, invasive diagnostics such as obtaining bone marrow biopsies should be considered only in case of lack of improvement and other systemic or hematological symptoms.

The aim of the investigations described in chapter 7 was to determine a reliable reference value for the numbers of MCs in healthy skin. The study was inspired by the diagnostic difficulty in cases with suspicion of mastocytosis without clear increase in the number of MCs in the skin. The numbers of MCs were determined in skin biopsies of healthy individuals and compared with those from a group of patients with mastocytosis. There was a significant difference between the numbers of MCs on the trunk, the upper arm, and the upper leg (proximal location) and those found on the lower leg and the forearm (distal location). The differences between the distal and the proximal locations must be considered when the number of MCs is determined to establish a reliable diagnosis of mastocytosis. A pilot study in patients with mastocytosis underlined the variation in the numbers of MCs in mastocytosis and normal skin, but showed a considerable overlap. Further studies are necessary to clarify the lower limit of the numbers of MCs in patients with mastocytosis.

The predictive value of urinary N-methylhistamine as an indicator of bone marrow involvement in mastocytosis was examined in studies described in chapter 8. Elevated levels of N-methylhistamine and bone marrow analysis were matched with respect to the subtype of mastocytosis and clinical manifestation of the disease in 37 adult patients. In the reported group of patients, N-methylhistamine levels were determined in order to stage mastocytosis in patients suspected of suffering from mastocytosis without skin involvement and those with established mastocytosis. Levels of N-methylhistamine > 156 μmol/mol creatinine always indicated that a bone marrow biopsy was essential. The likelihood of mast cell accumulation in the bone marrow could be predicted on the basis of elevated N-methylhistamine levels. In patients suffering from mastocytosis, persistent high levels (> 300 μmol/mol creatinine) of N-methylhistamine may indicate accumulation of MCs in the bone marrow. The level of N-methylhistamine as a diagnostic aid for establishing the severity of mastocytosis was of limited value. There are several conditions that lead to elevated levels of N-methylhistamine. The most important of these are allergic reactions, histamine-rich diets, and interstitial cystitis.

Analysis of C-kit Asp-816-val mutation in skin biopsies of 24 patients is dealt with in chapter 9. A mutation was found in 38% of the adult cases with maculopapular mastocytosis (formerly urticaria pigmentosa) and in 25% of the juvenile cases. The percentage of C-kit Asp-816-val mutation in adults was lower than that reported in literature, but a plausible explanation for this discrepancy could not be found. Both the children positive for C-kit Asp-816-val mutation showed some interesting characteristics. The first patient was one of the homozygous twin; one with mastocytosis the other without. The findings confirmed that the mutation did not occur in early embryonic development (germ cell mutation). Instead it was an acquired somatic mutation associated with the disease. The second child with the mutation showed an atypical clinical presentation of maculopapular mastocytosis (formerly urticaria pigmentosa) and juvenile xanthogranuloma.

In studies described in chapter 10, we developed a clinical scoring system to monitor the cutaneous symptoms of patients with confirmed mastocytosis. We felt it as a shortcoming that there was no satisfactory system available for accurately determining the extent and the severity of the disease, for example, as that in our earlier study (chapter 4). A calculation based on a semi-quantitative analysis of three aspects of mastocytosis was designed. The method is called the scoring index of mastocytosis (SCORMA). In order to gain insight into the reproducibility of the SCORMA system, it was put to the test by 9 dermatologists at regional hospitals. Based on the results of the assessment of 9 patients, a very high degree of agreement was noted when the SCORMA index was used, even without prior familiarity with the method. The clinical use of SCORMA was recommended for establishing the severity of the disease. Such systems were not available at that moment, but a comparable system called Grading of Mastocytosis in the Skin Is available at present from the European Working Conference on Mastocytosis (2007).

A validation study incorporating both serum Tryptase levels and the SCORMA-index conducted in 64 patients (31 children and 33 adults) is described in chapter 11. The results of the first visit on which SCORMA index as well as the serum Tryptase levels were determined were analyzed. The SCORMA index scores and the serum Tryptase levels showed a moderate correlation indicating that both were valuable for assessing cutaneous mastocytosis or systemic mastocytosis whereby the skin was involved. The results of this study in children and in adults with mastocytosis demonstrated that SCORMA index was of practical value for evaluating the severity of the disease. It is plausible that comparing sequential SCORMA index values in one patient may provide a useful tool to monitor disease progression and therapeutic efficacy.

In chapter 12, a new therapeutic modality for treating mastocytosis in the skin is described. A case controlled pilot study was conducted during a 6-weeks treatment using diluted 25% fluticasone propionate 0.05% cream under wet-wrap occlusion in 5 adults and 6 children. Improvement was measured up to the 24th week after treatment using the SCORMA Index. The results of this pilot study showed a partial, but clear cosmetic improvement in 9 of the 11 patients. The mean SCORMA Index decreased after treatment from 38 to 26. Treatment with 25% dilution of fluticasone propionate 0.05% cream under wet-wrap occlusion is an alternative modality for alleviating the symptoms of cutaneous mastocytosis, but the improvement may be moderate and fall short of the patient's expectations.

The recommendations and a protocol for management of mastocytosis in children are described in Chapter 13. These were compiled and published in co–operation with the Dutch National Mastocytosis Work Group.

Discussie

Bij bijna alle kinderen en bij een meerderheid van de volwassenen die zich met mastocytose presenteren staan cutane symptomen op de voorgrond. Het eerste doel van de behandelende artsen is om vast te stellen wat de ernst van de mastocytose is. Mastocytose wordt op dit moment beschouwd als een clonale aandoening die zijn oorsprong heeft in de myeloide reeks van hematopoetische stamcellen in het beenmerg. Bij bijna alle volwassen patiënten met mastocytose kan systemische mastocytose worden vastgesteld maar dat is ongebruikelijk bij kinderen.
De groeiende basale kennis over mastocytose is afkomstig uit de hoek van de hemato-oncologie. Deze ontwikkeling uit zich in een steeds verder gaande verfijning van de onderverdeling van systemische mastocytose. Van nieuwe inzichten en ontwikkelingen op dit terrein zullen de patiënten met ernstige mastocytose waarschijnlijk het meest profiteren. De bijdrage van dermatologen voor deze beperkte patiëntengroep is minder groot, maar de waarde van de dermatoloog bij de gehele groep van mastocytose patiënten is dat zeker niet. Mastocytose vereist een multidisciplinaire benadering.
Het doel van de onderzoeksprojecten die in dit proefschrift worden gepresenteerd is om een algemeen dermatologisch perspectief te verschaffen op mastocytose. Hierbij ligt de focus op klinische, diagnostische en therapeutische aspecten van deze aandoening bij volwassenen en kinderen. Actuele ontwikkelingen op het gebied van mestcel gerelateerde huidaandoeningen en gestructureerde aanbevelingen voor de aanpak van mastocytose bij kinderen worden gepresenteerd. Voor incidentele gevallen met mastocytose waarbij cosmetische aspecten een rol van betekenis spelen, kan wet wrap behandeling een nuttige therapie zijn, maar verdere klinische studies moeten dit bevestigen.
De algemene conclusie van de onderzoeksprojecten is dat mastocytose bij kinderen een aparte ziekte categorie blijft, ondanks dat bij kinderen in sommige gevallen clonaliteit aantoonbaar blijkt. Bij diffuse cutane mastocytose, een ernstige variant van mastocytose bij kinderen lijkt de prognose overkomstig met maculopapulaire mastocytose. Het perspectief van de dermatoloog op mastocytose moet zich ontwikkelen om de patiënten optimaal te kunnen bedienen in alle aspecten van de aandoening, waarbij de belangrijkste boodschap is dat systemische mastocytose altijd dient te worden overwogen en uitgesloten. Wanneer de diagnose mastocytose is gesteld, blijven keuzes die gemaakt worden ten aanzien van follow up en behandeling maatwerk. Er is vooruitgang geboekt in het definiëren van uitgebreidheid van de ziekte. Dit werd bereikt door het vaststellen van normaalwaarden van mestcelaantallen in de huid, door metingen van mestcelmediatoren in samenhang met klinische scoring van mastocytose en door meting van mediatoren in samenhang met beenmerglokalisatie van de aandoening.

Samenvatting

In hoofdstuk 1 worden diverse klinische en biologische aspecten van mastocytose gepresenteerd. De doelstellingen van dit proefschrift worden ook beschreven in dit hoofdstuk. In hoofdstuk 2 worden de actuele inzichten gepresenteerd met betrekking tot de rol van mestcellen bij dermatologische aandoeningen. Belangrijke punten hierbij zijn de bijdrage van mestcellen aan urticaria, mastocytose en andere inflammatoire ziekten, evenals aan angiogenese. Mestcellen spelen een belangrijke rol in ontstekingsprocessen en proliferatieve ziekten, vooral in de huid. De rol van mestcellen is onomstreden bij mastocytose en urticaria. Op dit moment is er geen oorzakelijke behandeling voor mastocytose. Mastocytose bij kinderen wordt in de meeste gevallen niet behandeld, omdat het beloop in de regel asymptomatisch en beperkt is tot de huid. Behandeling is alleen aangewezen voor het verlichten van ernstige symptomen.
Het merendeel van de gevallen met chronische urticaria treedt op bij volwassenen. Er wordt aangenomen dat in meer dan 50% van de gevallen sprake is van een autoimmuun pathogenese, hoewel dat omstreden is. De rol van mestcellen in autoimmuun ziektes is nog niet volledig bekend. Bij atopisch eczeem spelen mestcellen een rol van betekenis, hoewel recentelijk een belangrijke rol van de huidbarrière functie is komen vast te staan. De rol van mestcellen bij hemangiomen is speculatief, maar dit gegeven zou een interessante target voor therapie kunnen betekenen.
In 2002 hebben wij een klinisch review artikel gepubliceerd over mastocytose bij kinderen. In het licht van de nieuwe inzichten in de pathogenese van mastocytose werd gepropageerd de klinische beschrijving van huidlesies voor de dagelijkse praktijk te behouden. Dit is beschreven in hoofdstuk 3. In deze periode werd het duidelijk, dat de classificatie van mastocytose een grote verandering zou (moeten) ondergaan. Hoofdstuk 4 betreft een update over de periode 2002-2006 gepubliceerd in 2006, waarin de WHO classificatie van mastocytose en de relevantie daar-

van voor mastocytose bij kinderen werden behandeld.
In 2002 werd een retrospectieve analyse uitgevoerd waarbij de klinische aspecten van mastocytose bij 101 volwassenen en kinderen met mastocytose gediagnosticeerd in 1980-1998 onderling werden vergeleken. De resultaten hiervan zijn beschreven in hoofdstuk 5 en geven een andere interpretatie van de prognose van mastocytose ontstaan op kinderleeftijd en ontstaan bij volwassenen. Mastocytose bij kinderen is niet zo transient als aangegeven in de literatuur. Complete genezing werd vastgesteld bij 15%, afgezet tegen 50-80% gesuggereerd in de literatuur. Een systematische methode om de mastocytose te beschrijven ontbrak en klinische scoringssystemen waren niet voorhanden. Dit gegeven maakt het moeilijk om definitieve uitspraken te doen over de spontane genezing van mastocytose bij kinderen.
In hoofdstuk 6 wordt een serie van acht kinderen met diffuse cutane mastocytose (DCM) gepresenteerd. Het is de grootste gepubliceerde serie van DCM op het moment van publicatie en illustreert de variabiliteit in presentatie van de aandoening. De acht gevallen toonden indrukwekkende symptomen en soms zeer hoge serum Tryptase waarden, vooral bij de initiële presentatie. Het spectrum van de aandoening was variabel van rode blaarvorming tot gelige infiltratieve en nodulaire lesies. Aangezien de aandoening een gunstig beloop vertoont moet invasieve diagnostiek, zoals beenmerg biopten, beperkt worden tot gevallen waarin geen verbetering wordt vastgesteld en wanneer er sprake is van andere systemische verschijnselen of hematologische afwijkingen.
Het doel van de studie beschreven in hoofdstuk 7 was het vaststellen van betrouwbare referentie waarden voor mestcelaantallen in de huid. De studie werd geïnspireerd door de diagnostische problemen die kunnen ontstaan bij patiënten verdacht van mastocytose bij wie de toename van het aantal mestcellen in de huid gering lijkt. Het aantal mestcellen in huidbiopten afkomstig van gezonde individuen werd bepaald en vergeleken met het aantal mestcellen in huidbiopten afkomstig van een groep van mastocytose patiënten. Het aantal mestcellen in het romp- , het bovenarm- en bovenbeengebied (proximale locatie) verschilde significant van het aantal gevonden in het onderbeen- en onderarmgebied (distale locatie). Deze verschillen tussen proximale en distale locatie moeten worden overwogen wanneer mestcel aantallen worden bepaald bij het stellen van de diagnose mastocytose. Een pilotstudy bij mastocytosepatiënten bevestigde de variatie in mestcel aantallen zowel in mastocytose als in de normale huid. De studie toonde bovendien een aanzienlijke overlap in mestcelaantallen in de huid tussen mastocytose en de normale huid, zodat verdere studies nodig zijn om de ondergrens vast te stellen voor de diagnose mastocytose in de huid.
De voorspellende waarde van N-methylhistamine in de urine, voor beenmerg infiltratie van mastocytose is onderzocht in de studie beschreven in hoofdstuk 8. Verhoogde waarden van N-methylhistamine in de urine en de gegevens van beenmergonderzoek afkomstig van 37 volwassen patiënten werden vergeleken met betrekking tot het subtype van mastocytose en de klinische manifestatie van de aandoening. In de betrokken patiëntengroep werd de waarde van het N-methylhistamine in de urine bepaald om de mastocytose te stageren bij patiënten verdacht van mastocytose zonder huidlokalisatie en bij patiënten met bewezen mastocytose. Een waarde van N-methylhistamine in de urine > 156 µmol / mol creatinine was altijd aanleiding tot beenmergbiopsie. De kans op ophoping van mestcellen in het beenmerg kon worden voorspeld op basis van een verhoogde waarde van N-methylhistamine in de urine. Een persisterende waarde van > 300 µmol / mol creatinine in de urine bij patiënten verdacht voor of met mastocytose was indicatief voor de ophoping van mestcellen in het beenmerg. De waarde van de bepaling van N-methylhistamine in de urine als graadmeter voor de ernst van de mastocytose is echter beperkt. Er zijn verschillende omstandigheden en aandoeningen die ook leiden tot verhoging van de waarde van N-methylhistamine in de urine. De belangrijkste daarvan zijn allergische reacties, inname van histaminerijk voedsel en interstitiële cystitis.
De analyse van de C-kit Asp-816-val mutatie in huidbiopten van 24 patiënten wordt behandeld in hoofdstuk 9. Deze mutatie werd gevonden in 38% van de volwassen patiënten met maculopapulaire mastocytose (voorheen urticaria pigmentosa genoemd) en in 25% van de kinderen met deze diagnose. Het percentage van C-kit Asp-816-val mutaties bij volwassenen was lager dan gerapporteerd wordt in de literatuur, maar een aannemelijke verklaring voor deze discrepantie kon niet worden gevonden. De twee kinderen met C-kit Asp-816-val mutatie in huidbiopten hadden beide opvallende eigenschappen. De eerste patiënt was onderdeel van een homozygote tweeling; één met en één zonder mastocytose. Deze bevindingen bevestigen dat de mutatie niet is opgetreden in de vroege embryonale ontwikkeling, maar een somatische mutatie betreft. Het tweede kind had een atypische presentatie van de maculopapulaire mastocytose met juveniele xanthogranulomen.
Bij de studie beschreven in hoofdstuk 10 hebben wij een klinisch scoringssysteem ontwikkeld voor het monitoren van cutane symptomen van patiënten met bewezen mastocytose. Aanleiding hiervoor was het ontbreken van een methode voor systematische evaluatie bij onze studie over mastocytose bij kinderen versus volwassen. Het systeem betreft een calculatie gebaseerd op een semikwantitatieve analyse van drie aspecten van mastocytose. De methode heet de scoringsindex van mastocytose (SCORMA). Om inzicht te krijgen in de reproduceerbaarheid van het SCORMA systeem werd het getest met 9 dermatologen

uit ziekenhuizen in de regio Rotterdam. Gebaseerd op de beoordeling van 9 patiënten, werd vastgesteld dat er een hoge mate van overeenkomst is bij het gebruik van deze methode zelfs zonder eerdere bekendheid met deze methode. Het gebruik van de methode voor het vaststellen van de ernst van de mastocytose in een klinische setting werd aanbevolen. Dergelijke systemen waren op dat moment niet beschikbaar, maar in 2007 is er een vergelijkbaar systeem gepubliceerd met de naam "Grading of Mastocytosis In the Skin" afkomst van de European Working Conference on Mastocytosis (2007).

Een validatiestudie is verricht waarbij de waarde van het serum tryptase werd vergeleken met de SCORMA index waardes bij 64 patiënten (31 kinderen en 33 volwassenen). Deze is beschreven in hoofdstuk 11. De resultaten van het eerste bezoek aan de polikliniek, waarbij de SCORMA index werd bepaald en de waarde van het serum tryptase werd gemeten zijn geanalyseerd. Hierbij werd vastgesteld dat beide metingen een matige correlatie vertonen. Op grond hiervan werd geconcludeerd dat beide metingen nuttig zijn voor het in kaart brengen van cutane mastocytose en bij systemische mastocytose waarbij de huid betrokken is. De resultaten van de studie bij zowel kinderen als volwassenen met mastocytose toonde aan dat de SCORMA index van praktisch nut is voor het evalueren van de ernst van de aandoening. Het is aannemelijk dat het verrichten van achtereenvolgende metingen van de SCORMA index in één patiënt nuttig kan zijn om progressie van de aandoening en het effect van therapie te monitoren.

In hoofdstuk 12 wordt een nieuwe therapie voor mastocytose in de huid beschreven. Een case controlled studie werd uitgevoerd gedurende een periode van 6 weken. De behandeling bestond uit 25% verdunde fluticason propionaat 0,05% creme onder wet wrap occlusie bij 5 volwassenen en 6 kinderen. Met behulp van de SCORMA index werd verbetering vastgesteld tot 24 weken na de behandeling. De resultaten toonden partiële, maar duidelijke cosmetische verbetering in 9 van de 11 patiënten. De gemiddelde SCORMA index daalde na behandeling van 38 tot 26. Geconcludeerd werd dat 25% verdunde fluticason propionaat 0,05% creme onder wet wrap occlusie een alternatief kan betekenen voor bestaande therapie voor het verlichten van symptomen van cutane mastocytose maar dat de verbetering beperkt kan zijn en minder dan de patiënt verwacht.

De aanbevelingen en een protocol voor de behandeling van mastocytose bij kinderen worden beschreven in hoofdstuk 13. De publicatie is geschreven in samenwerking met de Nederlandse Mastocytose Werkgroep.

CHAPTER 15

Curriculum Vitae in English and Dutch
Bibliography
Dankwoord
List of abbreviations

Curriculum vitae

The author was born in 1968 in Rotterdam. He obtained his high school diploma (gymnasium) in 1986 and commenced medical studies at the Faculty of Medicine, Erasmus University Rotterdam in the same year. In 1994 he received his medical degree (MD) with honors.
From 1994 to 1995, he worked as a resident not in training (ANIOS) at the department of General Surgery, University Medical Center Utrecht. His duties included pre- and post surgery care of admitted patients as well as assisting in the operating theater. From 1995 to 1996, he worked as a resident not in training (ANIOS) at the department of Dermatology and Phlebology of the Alkmaar Medical Center. His duties included all aspects of dermatological patient care including advanced dermato-surgical procedures. From 1996 to 2001, he was a resident in training (AIOS) at the department of Dermatology and Venereology of the Erasmus MC, University Medical Center in Rotterdam and he was registered as a Dermato-Venereologist in 2001. Investigations into various clinical aspects of children and adults with mastocytosis were initiated at the department of Dermatology and Venereology during his residency and continued later cumulating in the investigations described in this thesis. From 2001 to present, he is a staff member in the department of Dermatology and Phlebology of the Alkmaar Medical Center. He was the head of this department from 2002 to 2006 during which he developed and implemented new strategies to increase the level of dermatological care, which resulted in quality enhancement and an increase of more than 30% in the production figures of the department.

Curriculum vitae

De auteur is in 1968 geboren te Rotterdam. Hij behaalde zijn gymnasium diploma in 1986 en startte in hetzelfde jaar met zijn geneeskunde studie aan de Erasmus Universiteit in Rotterdam. In 1994 behaalde hij zijn artsexamen cum laude.
Van 1994-1995 werkte hij als arts niet-in-opleiding (ANIOS) op de afdeling Algemene Heelkunde van het Universitair Medisch Centrum Utrecht. Zijn taken betroffen pre- en postoperatieve zorg voor opgenomen patiënten en assisteren bij operaties. Van 1995-1996 werkte hij als arts niet-in-opleiding (ANIOS) op de afdeling Dermatologie en Flebologie van het Medisch Centrum Alkmaar, waar hij verantwoordelijk was voor dermatologische zorg in breedste zin, inclusief uitgebreide dermatochirurgische therapie. Van 1996-2001 was hij werkzaam als arts in opleiding (AIOS) op de afdeling Dermatologie and Venereologie van het Erasmus Medisch Centrum Rotterdam. In 2001 behaalde hij zijn registratie als Dermato-Venereoloog. Gedurende zijn opleiding heeft hij diverse onderzoeksprojecten naar diverse klinische aspecten van mastocytose bij kinderen en volwassenen geïnitieerd en uitgevoerd. Een deel van de resultaten daarvan zijn beschreven in dit proefschrift. Van 2001 tot heden is hij werkzaam als staflid van de discipline Dermatologie en Flebologie van het Medisch Centrum Alkmaar. Van 2002-2006 is hij voorzitter van de discipline geweest en heeft hij nieuwe strategieën ontwikkeld en geïmplementeerd om het kwaliteitsniveau van de medisch zorg te verhogen. Tegelijkertijd is onder zijn leiding een productiestijging van de discipline gerealiseerd van meer dan 30%.

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Dankwoord

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Morris (2006) jij bent een charmeur en boefje, veel vaker dan ik wil lukt het jou om toch je zin te krijgen. Voor deze papa is er weinig dat leuker is dan door zijn zoon bij de neus te worden genomen.

Juna (2003) jij bent mijn trots en soms moet ik goed kijken om te zien dat je pas vijf bent, want vaak lijk je veel groter. Je bent voor mij het allermooiste meisje van de wereld en Ik vind het heerlijk om gek met je doen …. Je bent mijn MEIDER.

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Rogier

List of abbreviations

AML	Acute Myeloblastic Leukemia
Asp	D, Aspartic acid, (aspartate)
ASM	Aggressive Systemic Mastocytosis
BM	Bone Marrow
CD34	Cluster of Differentiation no 34 (cell surface glycoprotein and functions as a cell-cell adhesion factor)
CD25	Cluster of Differentiation no 25 (interleukin-2 receptor)
C-kit	encoding gene for KIT
CM	Cutaneous Mastocytosis; mastocytosis only located in the skin
CML	Chronic Myeloid Leukemia
D816V	susbstitution of aspartate to valine in codon 816 (Asp816Val)
Growth Factor	protein capable of stimulating cellular proliferation and cellular differentiation
Ig	Immunoglobulin
ISM	Indolent Systemic Mastocytosis
KIT	CD117, c-kit receptor; cytokine receptor (receptor tyrosine kinase), product of C-kit oncogene
kb	kilo base pairs
kD	kiloDalton
MCs	Mast Cells
MC_{TC}	Mast cells connective tissue type
MC_{T}	Mast cell mucosal type
MCL	Mast Cell Leukemia
MF	Myelofibrosis
MPD	Myeloproliferative Disorder
SCF	Stem Cell Factor; (KIT ligand or Steel factor) is a cytokine which binds CD117 (KIT)
SM	Systemic Mastocytosis; mastocytosis in one or more extra cutaneous organ
TK	Tyrosine Kinase
Tyr	Y, Tyrosine
UP	Urticaria Pigmentosa (=maculopapular mastocytosis)
Val	V, Valine
WDSM	Well-Differentiated Systemic Mastocytosis

Appendix - Color figures

Chapter 2

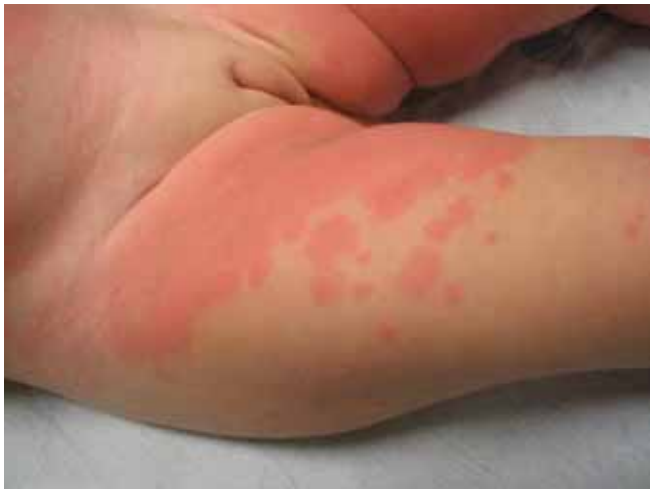

Figure 4. Urticaria with normal appearance in a child.

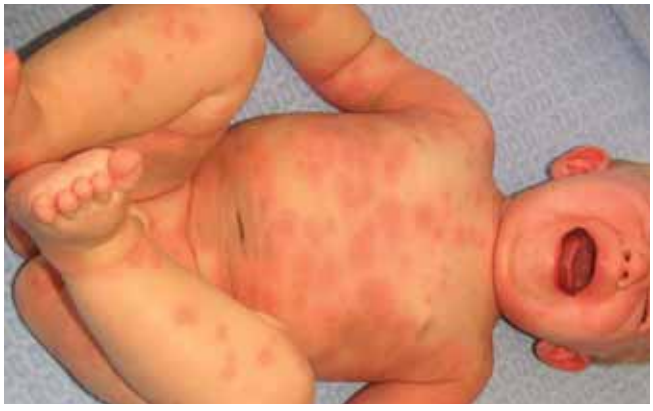

Figure 5. Urticaria with a blue hue often misdiagnosed in young children.

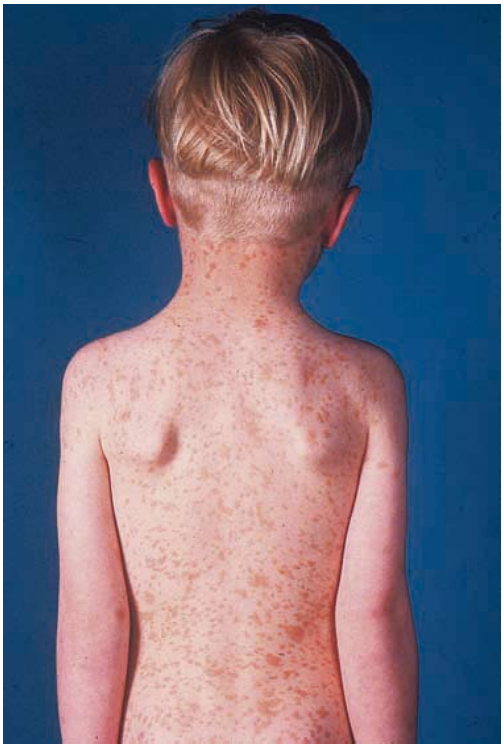

Figure 1. Urticaria pigmentosa.

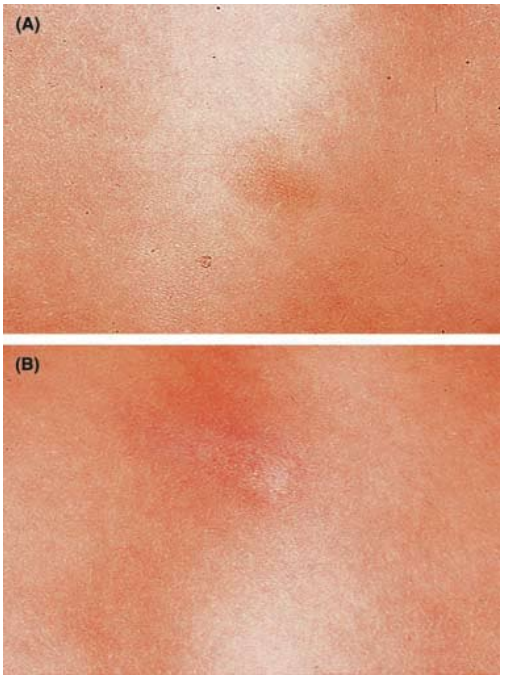

Figure 2. (A) Solitary mastocytoma.(B) Solitary mastocytoma, Darier sign positive.

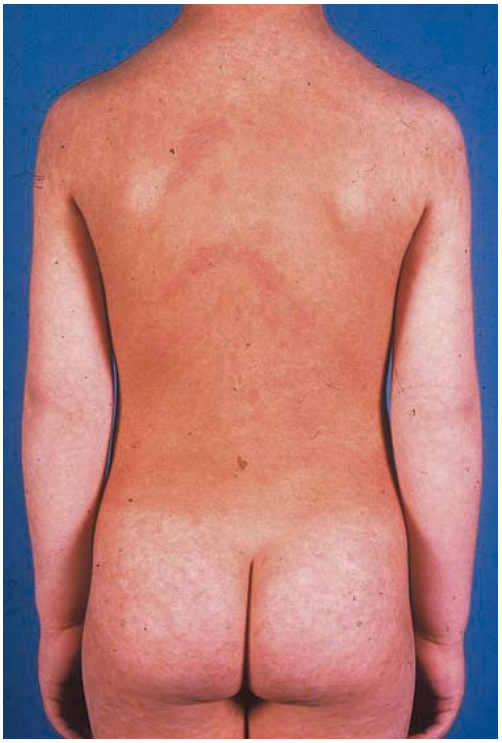

Figure 3. Diffuse cutaneous mastocytosis.

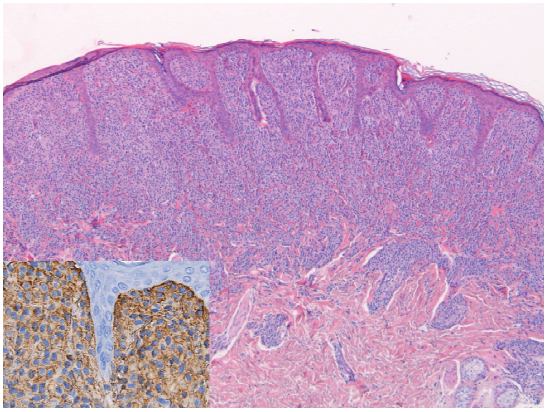

Figure 1a and 1b. Histopathology of Diffuse Cutaneous Mastocytosis (1a H&E staining, 1b Tryptase staining - inset 400x).

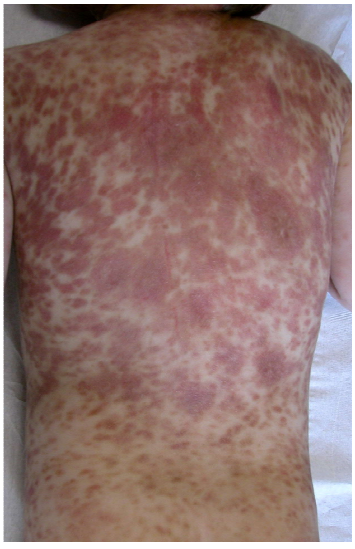

Figure 2. Red large blister type (patient no 5).

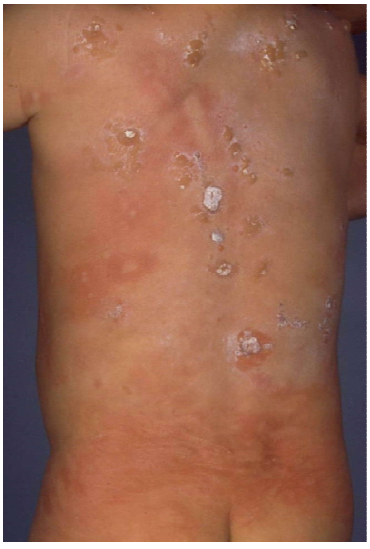

Figure 3. Red large blister type (patient no 1).

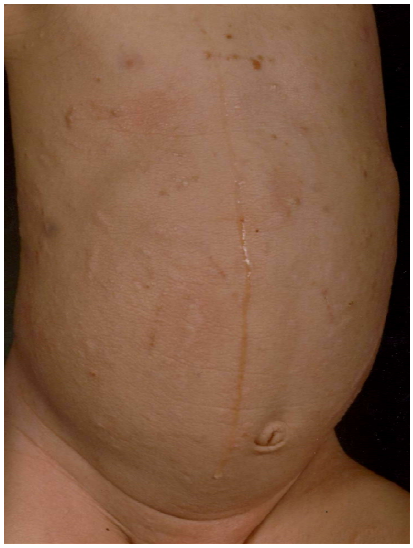

Figure 4. Yellow infiltrated small blister type (patient no 7).

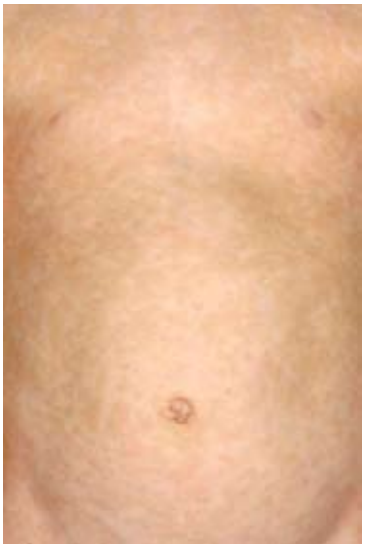

Figure 5. Yellow infiltrated small blister type (patient no 4).

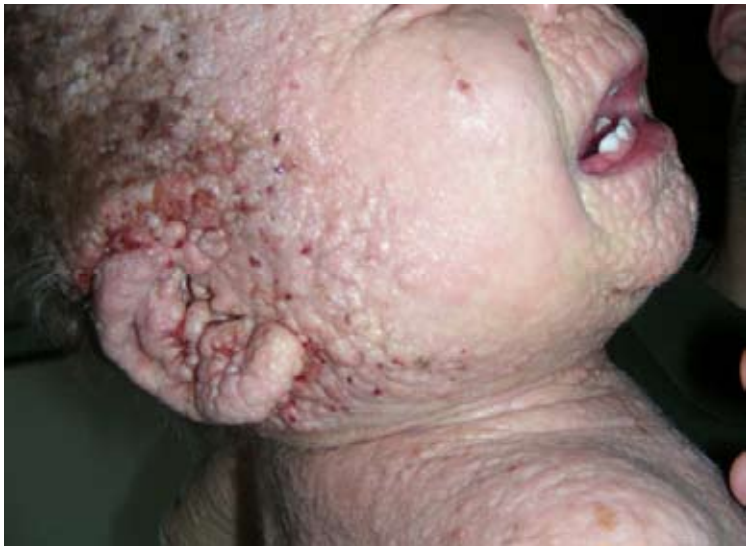

Figure 6. Yellow infiltrated small blister type extreme presentation (patient no 8).

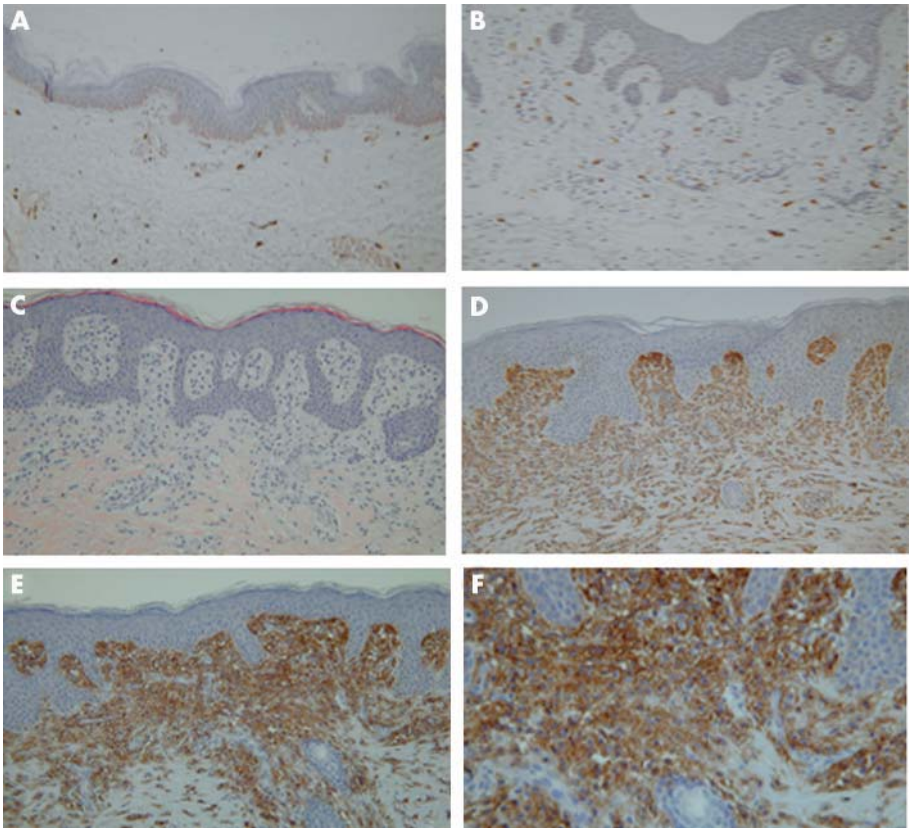

Figure 3.

(A) Normal skin near basal cell carcinoma (original magnification, ×100. Mast cells occur in the normal dermis in small numbers as oval to spindle shaped cells. They are concentrated around the blood vessels.

(B) Normal skin of mamma reduction tissue (original magnification, ×100). Mast cells occur in the normal dermis in small numbers as oval to spindle shaped cells. They are concentrated around the blood vessels.

(C) Haematoxylin and eosin staining of mastocytosis (urticaria pigmentosa) (original magnification, ×100). Mast cells in the normal skin are indistinguishable from other perivascular cells. A small amount of granular cytoplasm is seen.

(D) Urticaria pigmentosa, dense infiltrate of mast cells in the upper dermis located directly under the basal membrane. The infiltrate is also more concentrated around the blood vessels (original magnification, ×300).

(E) Urticaria pigmentosa: positive staining of the mast cell infiltrate with CD117 (original magnification, ×300).

(F) Urticaria pigmentosa: positive staining of the mast cell infiltrate with CD117 (original magnification, ×700).

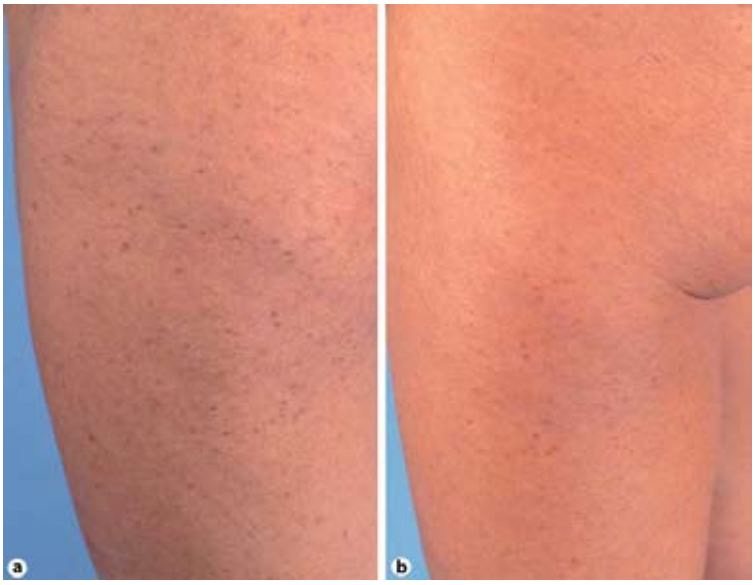

Figure 1.
a. Mastocytosis lesions in a patient before treatment with 25% diluted fluticasone propionate 0.05% cream under occlusion.
b. A partial but clear cosmetic improvement in the same patient 24 weeks after treatment.

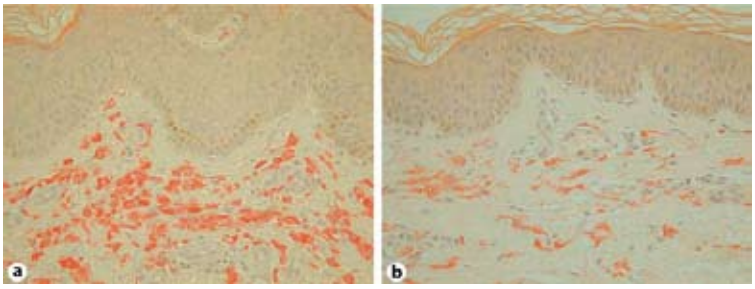

Figure 2.
a. A large number of mast cells in a histological section of lesional skin biopsy before treatment. Leder staining. ×160.
b. A decreased number of mast cells in the histological section of lesional skin biopsy after 6 weeks of treatment. Leder staining. ×100.