

Mastocytosis: State of the Art

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Abstract

Mastocytosis is a neoplastic disease involving mast cells (MC) and their CD34+ progenitors. Symptoms in mastocytosis are caused by biological mediators released from MC and/or the infiltration of neoplastic MC in various organs, the skin and the bone marrow being predominantly involved. A WHO consensus classification for mastocytosis exists, which is widely accepted and includes three major categories: (1) Cutaneous mastocytosis (CM), a benign disease in which MC infiltration is confined to the skin, is preferentially seen in young children and exhibits a marked tendency to regress spontaneously. (2) Systemic mastocytosis (SM) which is commonly diagnosed in adults and includes four major subtypes: (i) indolent SM (ISM, the most common form involving mainly skin and bone marrow); (ii) a unique subcategory termed SM with an associated non-mast cell clonal hematological disease (SM-AHNMD); (iii) aggressive SM usually presenting without skin lesions, and (iv) MC leukemia, probably representing the rarest variant of human leukemias. (3) The extremely rare localized extracutaneous MC neoplasms, either presenting as malignancy (MC sarcoma) or as benign tumor termed extracutaneous mastocytoma. Diagnostic criteria for mastocytosis are available and are widely accepted. SM criteria include one major criterion (multifocal compact tissue infiltration by MC) and four minor criteria: (1) promi-

nent spindling of MC; (2) atypical immunophenotype of MC with coexpression of CD2 and/or CD25 (antigens which have not been found to be expressed on normal/reactive MC); (3) activating (somatic) point mutations of the c-kit proto-oncogene usually involving exon 17, with the imatinib-resistant type D816V being most frequent, and (4) persistently elevated serum tryptase level (>20 ng/ml). To establish the diagnosis of SM, at least one major and one minor criterion, or at least three minor criteria, have to be fulfilled. The natural clinical course of mastocytosis is variable. Most patients, in particular those with CM and ISM, remain in an indolent stage over many years or even decades, while others, in particular those with aggressive SM, SM-AHNMD, or mast cell leukemia, show a progressive course, usually with a fatal outcome.

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Introduction and Basic Concepts

Mast cells (MC) are medium-sized cells that are found predominantly in perivascular spaces of almost all tissues and are easily recognizable by their metachromatic intracytoplasmic granules when dyes like Giemsa or toluidine blue are used. These specific granules contain proinflammatory and vasoactive mediators, which are released after IgE receptor cross-linking induced by allergens or other stimuli [1]. Of diagnostic importance is α -tryptase (usually referred to as tryptase), which is constitutively secreted from MC. Tryptase thus can be

measured in the serum (baseline levels in healthy individuals 0–15 ng/ml) and thus serves as an indicator of the total MC number [2, 3]. MC are derived from CD34+ multipotent hematopoietic progenitor cells. These cells reside in the bone marrow and in the peripheral blood, and can transmigrate through the endothelium before undergoing differentiation and maturation into MC in tissues. MC especially differentiate under the influence of stem cell factor, which represents the ligand for KIT (CD117), a transmembranous tyrosine kinase receptor constitutively expressed by MC and their progenitors [4–6]. During differentiation, MC acquire distinct morphological and phenotypical properties with four defined stages of maturation: (i) the non-granulated (tryptase-positive) blast cell; (ii) the metachromatic blast cell; (iii) the promastocyte which is also termed atypical MC type II and contains bi- or multilobed monocytoid nuclei, and (iv) the mature MC [7]. Immature or atypical MC can usually be detected in bone marrow smears of patients with aggressive SM (ASM), and also in those with mast cell leukemia (MCL) [7].

Definition

Mastocytosis is a very heterogeneous disease of bone marrow origin and characterized by abnormal growth and/or accumulation of clonal MC in one or more organs. In systemic mastocytosis (SM), at least one extracutaneous organ is involved by definition [8, 9]. The presence of multifocal compact MC infiltrates represents the major diagnostic criterion of SM [8, 9]. It cannot be overemphasized that mastocytosis basically must be diagnosed morphologically, by investigating biopsy specimens of skin (cutaneous mastocytosis (CM)) and/or bone marrow (to reveal or exclude SM). Cytomorphological diagnosis of SM in bone marrow smears is also possible in a minority of cases but is inevitable in all cases of MC leukemia [10].

Diagnostic Criteria (table 1)

Morphological Criteria

The diagnosis of mastocytosis becomes likely when multifocal compact MC infiltrates, consisting of at least 15 cells, are detected in a given tissue [8–10]. Usually a significant proportion of lesional MC in the bone marrow (or in other extracutaneous organs) in SM exhibits a spindle-shaped appearance, fulfilling a minor diagnostic SM criterion, thereby leading to the definitive diagnosis of

Table 1. 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 hematopoietic clonal non-MC lineage disease (= AHNMD))

MCs = Mast cells.

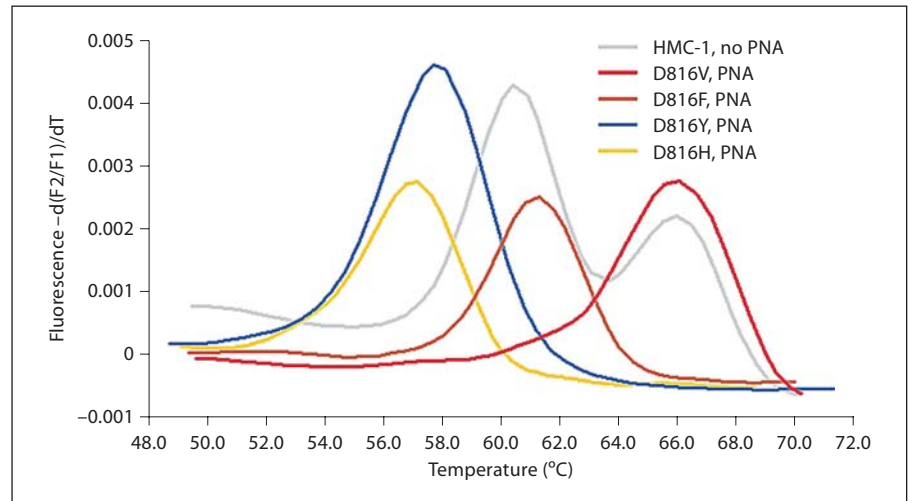
If at least one major and one minor criterion or three minor criteria are fulfilled, the diagnosis SM can be established.

¹ Other activating mutations at codon 816 of c-kit also count as a minor criterion.

SM [11]. These neoplastic MC often show a reduced content of metachromatic granules. This hypogranulation can be regarded as a sign of cytomorphological atypia. Usually the compact MC infiltrates also contain intermingled eosinophils and lymphocytes. Compact lymphocytic infiltrates in the immediate vicinity of MC aggregates are commonly found in indolent SM (ISM) and have been shown to be reactive in nature in almost all cases [12]. When compact infiltrates consist of round mature-appearing MC exclusively, other minor SM criteria must be fulfilled to achieve the definitive diagnosis of mastocytosis [13]. In very rare instances there is a focal and/or diffuse collagen fibrosis of the bone marrow containing an abundance of loosely scattered spindle-shaped MC which lack both expression of CD25 and an activating point mutation of c-kit. This condition therefore must be regarded as an important mimicker of mastocytosis and might be tentatively termed ‘fibromastocytic lesion’.

In all cases of suspected mastocytosis a limited panel of antibodies against at least three antigens should be applied: (i) Anti-tryptase which is highly specific and sensitive (with the exception of some neoplastic tryptase+ myeloblasts or basophils), and therefore allows screening for both the number of loosely scattered MC and immediate detection of even small compact (diagnostic) MC infiltrates [14]. However, especially in extramedullary tissues (e.g. mucosa of the gastrointestinal tract), non-specific background staining may easily lead to overestimation of MC numbers and misinterpretation of the presence of mastocytosis. (ii) An antibody against KIT (CD117) should

Fig. 1. *c-kit* mutations in cutaneous and systemic mastocytoses, detected with PNA-mediated PCR clamping. Various mutations showing a specific peak after melting-point analysis of the amplification products with LightCycler® hybridization probes: WT, 60°C; D816V, 66°C; D816F, 61°C; D816Y, 58°C; D816H, 57°C.



therefore also be applied to confirm the presence of MC in such cases [15]. Although anti-KIT antibodies are non-specific (KIT is also expressed by hemopoietic stem cell, melanocytes, germ cells, and Cajal cells), they have been found to be of superior sensitivity allowing verification of tryptase+ cells as MC without significant background staining. (iii) An antibody against CD25 which is also a non-specific antigen (expressed preferentially by activated T cells but also by certain B-cell malignancies like hairy cell leukemia) should also be applied in all cases of suspected SM. In fact, neoplastic MC in SM typically express CD25, whereas normal MC as well as MC in reactive tissues usually are CD25-negative cells. Thus, CD25 is a minor diagnostic criterion of SM and allows for the confirmation of an atypical phenotype of tryptase+/KIT+ MC in these patients [16]. Note that CD25 immunohistochemistry is of particular diagnostic value in the bone marrow where CD25+ lymphatic cells are found only in very small number (the reactivity of megakaryocytes is used as internal control). However, CD25 immunohistochemistry is much more difficult to interpret in extramedullary tissues containing significant numbers of activated T cells (e.g. spleen, lymph nodes and gastrointestinal mucosa). The diagnostic value of anti-CD2 antibodies is limited because of the marked difficulties in the interpretation of such stains and the presence of CD2+ T cells in almost all tissue infiltrates of mastocytosis [17].

Molecular Criteria (fig. 1, 2)

In contrast to most other neoplastic processes, there are marked problems for analyzing clonal MC of patients with mastocytosis at a molecular level: (i) In most cases,

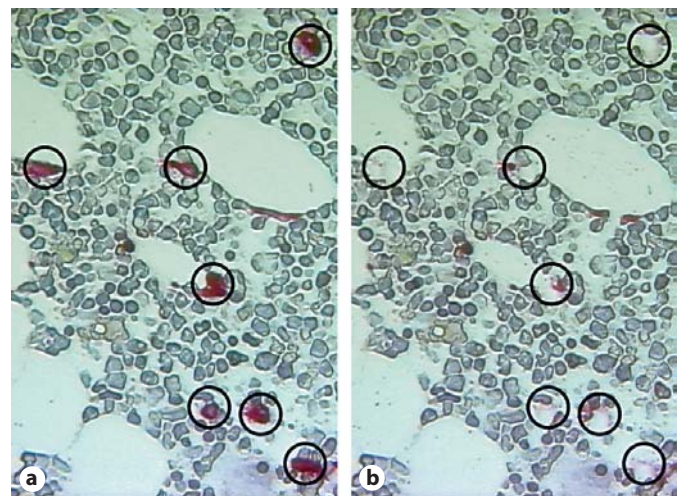


Fig. 2. **a** Bone marrow with loosely scattered partly spindle-shaped tryptase-positive MC. **b** Microdissection of single MC which are then pooled to a total of 50 cells per reaction tube, proteinase-K digested and amplified by nested PCR. In positive samples, further genotyping is performed by melting-point analysis.

the numbers of MC are low and often outnumbered by the surrounding normal/reactive cells; (ii) mRNA cannot be analyzed in paraffin-embedded routinely processed tissues (available in mastocytosis); (iii) the heterozygous point mutations detected in mastocytosis are somatic mutations, and (iv) the sensitivity of several techniques (e.g. direct sequencing) are relatively low. Therefore, more sensitive methods have to be developed to investigate routinely processed, formalin-fixed and paraffin-embedded

tissues, such as PNA-mediated PCR clamping, which leads to a predominant suppression of the amplification of wild-type alleles during PCR [18]. Even more sensitive but very laborious is the analysis of microdissected pooled single tryptase-positive cells [19]. In SM, by far the most frequently detected activating point mutation is D816V in exon 17, resulting from substitution of asparagine by valine [20–22]. D816V is found in more than 90% of all patients with SM. Other activating point mutations of exon 17 include D816Y, D816H, and D816F. Each of these mutations is detected only in a small proportion of cases [23]. The presence of such point mutations (at codon 816) can be used as a minor diagnostic criterion, especially in cases without compact infiltrates. Here, demonstration of a significant number of spindle-shaped MC with aberrant expression of CD25 and the presence of D816V are sufficient to establish a diagnosis of SM on the basis of three minor criteria. It is of importance to note that activating point mutations in exon 17 may interfere with imatinib therapy and therefore are not only of diagnostic relevance but have also crucial therapeutic implications [24, 25]. It is also noteworthy to realize that D816V is not specific for mastocytosis but is also found in patients with testicular seminoma, especially in patients being at increased risk of bilateral disease [26]. Moreover, the occurrence of D816V has also been reported in cases of AML although it is not clear whether these cases had coexisting SM, as SM was usually not excluded by histological/immunohistochemical investigation of the bone marrow in these patients. In fact, many of these cases may have occult SM, detectable only after chemotherapy and thus significant reduction of blast cells [27–29].

The presence of KIT D816V must not be regarded as a major diagnostic criterion. Otherwise the demonstration of spindle-shaped MC in the immediate vicinity of a testicular seminoma carrying this mutation could be misinterpreted as mastocytosis. A single case of SM with the mutation F522P within the transmembranous domain of c-kit has been published. This patient showed some morphological peculiarities in that MC were exclusively round and showed an abundance of granules leading to the descriptive term of ‘well-differentiated’ mastocytosis (this peculiar type of mutation was not associated with resistance to imatinib) [30].

Serological Criterion

In almost all patients with SM the serum tryptase level is >20 ng/ml, whereas in pure CM, most patients have a normal tryptase level. Serum tryptase can be used for monitoring patients with CM and SM [3]. A significant

Table 2. WHO classification of mastocytosis

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

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-PDGFRα fusion gene is detectable.

⁴ Circulating mast cells are <10%.

increase of the serum tryptase in a patient with hitherto CM can be regarded as indication of an increase in the MC burden, and thus will lead to a histological re-evaluation of the bone marrow to assess or exclude (indolent) SM. An increasing serum tryptase in ISM should lead to the suspicion of disease progression and thus will lead to re-examination of the patient for the presence of B and C findings. However, it should also be kept in mind that a variety of myeloid neoplasms can produce significantly elevated tryptase levels, in particular tryptase+ AML, myelomastocytic leukemia (which does, by definition, not belong to the spectrum of mastocytosis), or AML-M4eo. In patients with SM with an associated non-mast cell clonal hematological disease (SM-AHNMD), an elevated serum tryptase level should not be used as minor diagnostic criterion [31].

WHO Classification for Mastocytosis (table 2)

Based on significant advances in mastocytosis research, an updated consensus classification for mastocytosis was proposed in 2001 [8]. This new classification

system was fully adopted by the WHO and has now become widely accepted because it provides criteria to discriminate not only between SM and MC hyperplasia but also between SM and CM, and even allows clear-cut separation of SM from related myeloid disorders with signs of MC differentiation. Three major subgroups of the disease were defined: (i) CM; (ii) SM including ISM, ASM, SM-AHNMD and MCL, and (iii) extracutaneous mastocytoma.

CM is an indolent disease and, by definition, can only be diagnosed when SM is excluded by appropriate investigations. Most patients are children, whereas only a few adult patients have pure CM. In adults, a trephine biopsy specimen, including immunohistochemical and molecular analyses as described above, must always be performed. By contrast, in children with a normal serum tryptase level, a bone marrow biopsy is not absolutely required (mastocytosis in the skin). The most common subvariant of CM presents as disseminated macular or maculopapular rash, and is (descriptively) termed urticaria pigmentosa (UP) [32, 33]. Diffuse CM is less frequently diagnosed. The solitary localized mastocytoma (of the skin) is also rare, and has a benign clinical course. Thus, most MC tumors of the skin appear to be benign in most cases. However, more recently, a unique case of a primary cutaneous mast cell sarcoma (with secondary infiltration of the bone marrow) has been documented [pers. unpubl. observation].

ISM is the most common variant of SM comprising about two thirds of all cases. Usually, ISM involves both the skin and bone marrow. The bone marrow infiltration may be difficult to detect in some cases and then can only be diagnosed when appropriate immunohistochemical stains (anti-CD25 antibody) and molecular (D816V) studies are performed. ISM shows a prolonged clinical course in almost all patients with survival times of two decades and more. However, in a small group of patients, transformation into another disease category, such as ASM or SM with an associated hematological malignancy (SM-AHNMD), has been reported [34].

ASM is by far much less common than ISM comprising only about 5% of all SM patients. Clinically, ASM may present with hepatosplenomegaly and/or generalized lymphadenopathy, but usually without typical (UP-like) skin lesions. Although the criteria of ASM relate to clinical findings, ASM is often revealed by histological examination, but not by the clinician [35]. ASM is characterized by progressive infiltration of various organs by often large and confluent MC aggregates with consecutive and clinically significant impairment of organ func-

tion (severe cytopenias, malabsorption, bone fractures, signs of hepatopathy with loss of liver function). Such findings are termed C findings [8]. MC infiltration leading to marked organomegaly should not be regarded as a C finding unless accompanied by signs of impaired organ function since significant organomegaly is also found in patients with an indolent or a smouldering course, and then regarded as a B finding [8]. A rare subvariant of ASM with prominent eosinophilia of blood and tissues and generalized lymphadenopathy (clinically mimicking malignant lymphoma) has been described as lymphadenopathic mastocytosis with eosinophilia [36].

In about one fourth to one third of the patients with SM, an AHNMD is diagnosed making SM-AHNMD the second most frequent subtype of SM [37]. In these patients, WHO criteria for both SM and the AHNMD must be fulfilled. SM-AHNMD is a unique disorder amongst hematological neoplasms in that it combines two completely different histologies and disease categories into one defined entity of SM [38]. The vast majority (about 80–90%) of 'AHNMDs' are myeloid disorders including almost all defined disease entities: MDS, MDS/MPS, MPS, AML, CEL, and CML [39]. Most common are disorders of the MDS/MPS group, usually termed chronic myelomonocytic leukemia. Until now only 1 case of malignant histiocytosis with signs of differentiation towards reticulum cells has been diagnosed in SM [pers. unpubl. observation]. Of special importance is the clear-cut separation of chronic eosinophilic leukemia (CEL) containing loosely scattered CD25+ abnormal MC but not fulfilling the criteria of SM, and SM-CEL with multifocal compact diagnostic mast cell infiltrates and criteria for both SM and CEL fulfilled [40]. In both instances, the FIP1L1/PDGFR α fusion gene product can be detected.

Associated lymphatic malignancies comprise only about 10–20% of all 'AHNMDs' with plasma cell myelomas being most frequent within this group, but cases of ALL, CLL and hairy cell leukemia have also been reported [37]. Since hairy cell leukemia usually contains CD25+ tumor cells, it may be extremely difficult to clearly delineate CD25+ MC. A case of 'triple' SM-AHNMD has also been observed including three diseases: SM, AML, and CLL [pers. unpubl. observation]. Until now, Hodgkin's lymphoma has not been found to occur as AHNMD. The clinical picture and prognosis of SM-AHNMD patients are mainly determined by the 'AHNMD', but cases have been detected that after successful chemotherapy of an AML a hitherto obscured SM was seen ('occult mastocytosis') with persistence or even slight progression of SM even in a stable hematological

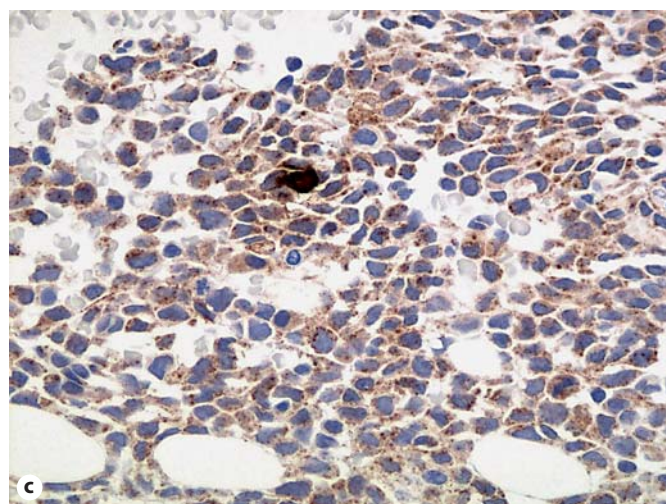
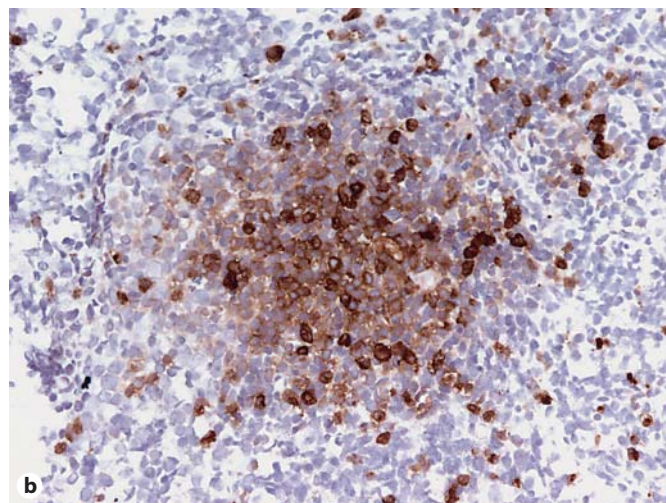
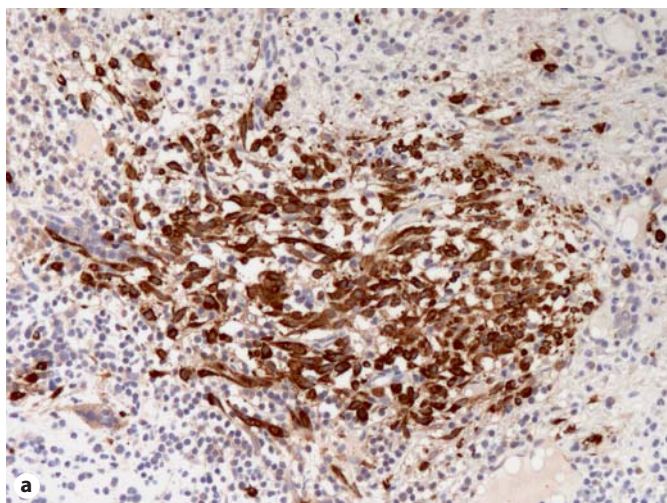


Fig. 3. Tryptase immunohistochemistry. **a** SM-AHNMD with focal infiltration of the bone marrow. Note the abundance of spindle-shaped mast cells. Since the major and one minor criterion are fulfilled, this histological picture meets the defined criteria to establish a diagnosis of SM. The ‘AHNMD’ could be classified as idiopathic myelofibrosis (features not recognizable). **b** Focal tryptase-positive round cell infiltrate of the bone marrow (focal TROCI-bm). Additional stainings applying basophil-related antibodies revealed a secondary chronic basophilic leukemia representing the terminal phase of a Ph1+ chronic myeloid leukemia. **c** Diffuse round cell infiltration of the bone marrow (diffuse TROCI-bm). This case proved to be a tryptase+ acute myeloid leukemia of M1 type according to FAB. Note the single reactive mast cells with strong expression of tryptase. ABC method: AAI (anti-tryptase).

remission of the leukemia [27]. Altogether, SM-AHNMD may present with three histomorphological pictures (fig. 3): (i) Extremely hypercellular marrow with clear-cut infiltrates of SM (usually multifocal) and the ‘AHNMD’ (usually diffuse-compact); (ii) normo- or hypocellular marrow in the few patients with plasma cell myeloma or CLL both exhibiting a multifocal bone marrow infiltration but a widely intact hemopoiesis, and (iii) ‘occult’ mastocytosis which is revealed only after chemotherapy. The uniqueness of SM-AHNMD is further underlined by the finding of a frequent occurrence of activating point mutations of c-kit in both malignancies even within lymphatic tumors [pers. unpubl. observations; 41].

MCL is extraordinarily rare and characterized by leukemic infiltration of various organs by immature neoplastic MC [42]. MCL is the only subvariant of mas-

tocytosis where the cytological diagnosis in smear preparations is needed: accordingly, the bone marrow smear must contain significant numbers of MC which comprise more than 20% (and up to 90%) of all nucleated cells [8]. The cut-off level of 20% for bone marrow MC only refers to the cytological assessment in smears, but not to the percentage of MC in the histological analysis. In most cases with MCL, circulating MC are found. In typical MCL, MC make up more than 10% of blood cells, in the aleukemic variant of MCL, less than 10% of all circulating leukocytes are MC. The prognosis of patients with MCL is grave [8]. The most important differential diagnosis to be considered is myelomastocytic leukemia.

Localized MC proliferations are also extremely rare and include both the extracutaneous mastocytoma (of the lung) and the ‘true’ MC sarcoma of which less than

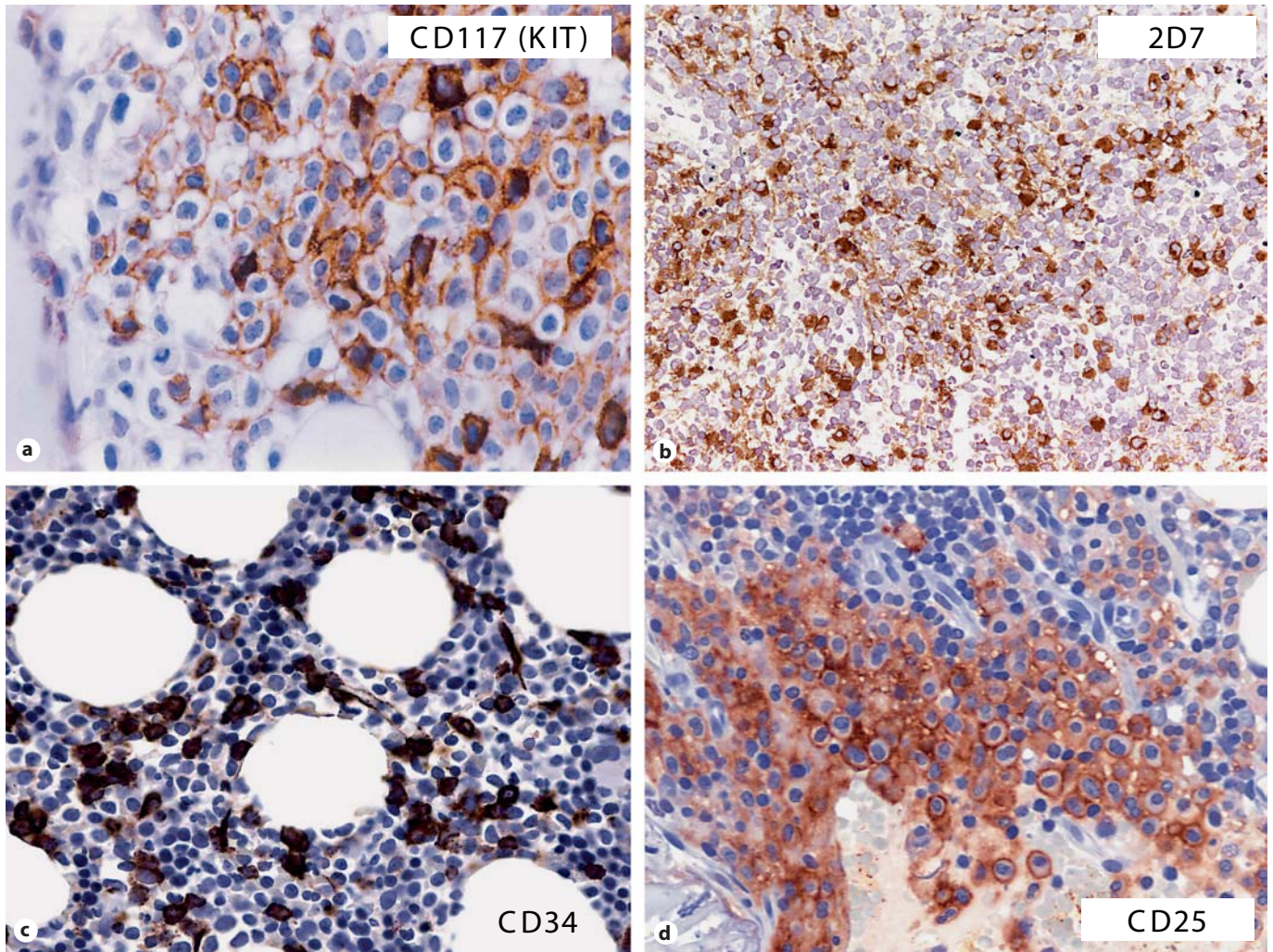


Fig. 4. Immunohistochemistry in mastocytosis and related disorders. **a** Mast cell leukemia with diffuse infiltration of the bone marrow and strong expression of KIT (CD117). Note the specific annular reactivity of the neoplastic cells (in contrast to the granular cytoplasmic staining when anti-tryptase antibodies are applied). **b** Basophilic leukemia (secondary type) with diffuse infiltration of the bone marrow. The neoplastic basophils are high-

lighted by the antibody 2D7 which produces a strong cytoplasmic staining. **c** Myelomastocytic leukemia with marked increase in CD34+ blast cells which clearly separates this condition from 'true' mastocytosis. **d** Systemic mastocytosis with focal infiltration of the bone marrow and strong expression of CD25 which indicates the neoplastic phenotype of the cells. ABC method: anti-CD117; 2D7; QBend 10; anti-CD25.

five published cases exist [43–45]. Since cytomorphological atypia of MC sarcoma is high (according to a grade 3 sarcoma), it is impossible to achieve the correct diagnosis without appropriate immunohistochemical stainings. It is noteworthy that the MC sarcomas reported occurred in tissues not commonly involved by SM (larynx, colon, meningeal site). All cases showed rapid progression and generalization with a terminal phase resembling ('secondary') MC leukemia.

Rare Subvariants of Mastocytosis and Differential Diagnoses (fig. 4)

The following subcategories are either rare subvariants of mastocytosis not yet included in the WHO classification system (1–4) or are disorders closely related and often confused with 'true' mastocytosis (5–7):

(1) *Smouldering mastocytosis (SSM)*: SSM is a well-recognized subcategory of ISM that clinically assumes an intermediate position between ISM and ASM, with a high

degree of tissue infiltration including the bone marrow, a high serum tryptase level (>200 ng/ml), and organomegaly, e.g. lymphadenopathy or splenomegaly (B findings). By contrast, no C findings are detected in SSM, unless the disease progresses into ASM [46, 47].

(2) *Well-differentiated systemic mastocytosis (WDSM)*: WDSM is another subcategory of ISM. Compact tissue infiltrates of mastocytosis consisting exclusively of round mature-appearing MC belong to the spectrum of the so-called tryptase-positive round cell infiltrate (of the bone marrow), preliminary termed TROCI-bm [30]. TROCI-bm may present with either localized or diffuse infiltration patterns, and WDSM belongs to the differential diagnostic spectrum of localized TROCI. Morphologically, WDSM can be separated from 'common' SM by the absence of both CD25 expression on MC and the typical exon 17 point mutations. The only one published case reported on a unique point mutation of c-kit within the transmembranous domain (F522P) which does not lead to imatinib resistance seen in patients carrying the typical D816V mutation [30]. In addition, a unique case of WDSM was recognized within the spectrum of SM-AHNMD presenting with an associated monoblastic leukemia [pers. unpubl. observation].

(3) *Monoclonal mast cell activation syndrome*: This disorder comprises a group of patients clinically presenting with recurrent episodes of anaphylaxis, no skin lesions, and one or two minor diagnostic criteria for SM but lacking the major criterion. In this subdiagnostic condition, MC may display cytomorphological atypia, aberrant expression of CD25 or the presence of D816V but all three features (sufficient for the diagnosis of SM) are not detectable [48]. A follow-up of such patients similar to ISM seems to be required and may reveal SM, whereas progression into high-grade SM seems unlikely (not described so far).

(4) *Occult mastocytosis*: Occult mastocytosis presents in two different variants. On the one hand, it can be a rare occurrence within the spectrum of SM-AHNMD and is detected after eradication of the 'AHNMD' by adequate chemotherapy and then, retrospectively, is also found in the initial biopsy specimens (where it was obscured by the widespread neoplastic non-MC clone) after appropriate immunohistochemical and molecular analysis. Despite complete hematological remission, infiltrates of SM usually persist or even progress signaling that still one part of the neoplastic process is still present [27]. On the other hand, it was possible to analyze tissues which had been removed years before the diagnosis of SM was established. Although there was no morphological evidence of a tissue infiltrate of SM, molecular analysis yielded the

presence of an activating c-kit mutation up to 10 years before morphological manifestation of SM [49].

(5) *Myelomastocytic leukemia (MML)*: MML represents a rare advanced myeloid neoplasm (usually a myelodysplastic syndrome of RAEB type or even AML by WHO criteria) exhibiting more than 10% metachromatic immature cells (often metachromatic blasts) in a bone marrow or blood smear but not fulfilling criteria for diagnosis of SM. In particular, there are no compact MC infiltrates, there is no aberrant phenotype of MC with coexpression of CD25 and there is no activating point mutation of c-kit. Usually, a significant increase in CD34+ progenitor/blast cells is detected [50]. Tentatively, MML is best categorized as a subgroup within the MDS/MPS overlap syndromes.

(6) *Tryptase+ acute myeloid leukemia*: Tryptase+ AML is also a rare finding and characterized by strong expression of tryptase and, less frequently, also of KIT (CD117), by myeloblasts in an otherwise morphologically unremarkable AML (often subtypes FAB M1, M2, or M4eo). Tryptase+ AML lacks criteria for diagnosis of SM [50]. The separation of tryptase+ AML from MML is possible by counting metachromatic cells in blood and/or bone marrow smears: the presence of more than 10% metachromatic cells that can be proven to be MC or MC progenitors argues for the diagnosis of MML [51].

(7) *Basophilic leukemia*: Basophilic leukemia is an extremely rare subvariant of myeloid leukemias and until now could not be diagnosed histologically in bone marrow trephine biopsy specimens. BAL also belongs to the spectrum of TROCI (diffuse type) since it could be shown that neoplastic basophils do express detectable amounts of tryptase (but not normal/reactive basophils). Definitive diagnosis of BAL is only possible when basophil-related antibodies like 2D7 and/or BB1 are used for immunohistochemical analysis of a trephine biopsy specimen [52]. In contrast to MC granules, metachromatic granules of basophils are water-soluble and therefore cannot be detected in routinely processed (formalin-fixed) tissues. In most published cases of BAL, the underlying disease turned out to be a Ph+ CML. Recently, a unique case of secondary basophilic leukemia in a patient with Ph+ CML with associated SM was diagnosed retrospectively in an analysis of almost 200 cases of CML [pers. unpubl. observation].

Therapy of Mastocytosis – Current Status

Since curative therapies are not available, mastocytosis should be treated according to the symptoms recorded in each individual patient [53]. In patients with predom-

Table 3. Practical guide for diagnostic workup in patients with suspected mastocytosis

Initial sign/symptom	Recommended diagnostic procedures
UP-like skin lesions in pediatric cases	<ol style="list-style-type: none"> 1. Skin biopsy (with analysis of c-kit D816V) and serum tryptase (monitoring)¹ 2. Bone marrow investigation in cases with suspected hematologic disease/SM
UP-like skin lesions in adult patients	<ol style="list-style-type: none"> 1. Bone marrow examinations, skin biopsy and serum tryptase (>20 ng/ml in most cases) 2. In case of SM → complete staging: gastrointestinal tract, osteodensitometry, x-ray of bones, ultrasound of abdomen, complete blood count, serum chemistry, coagulation parameters, c-kit mutations
Reported mediator symptoms but no skin lesions (UP) ²	<ol style="list-style-type: none"> 1. Serum tryptase, if >20 ng/ml → 2. 2. Bone marrow examination, if SM → 3. 3. SM – staging²
Severe unexplained allergic reaction/anaphylaxis at presentation	<ol style="list-style-type: none"> 1. Serum tryptase, if >20 ng/ml → 2. 2. Repeat serum tryptase a few weeks later: if then, serum tryptase is >20 ng/ml → 3. 3. Bone marrow examination, if SM → 4. 4. SM – staging²

¹ In young infants, a serum tryptase level slightly exceeding 20 ng/ml is not regarded as safe indicator for systemic mastocytosis. Therefore, it is recommended to wait and to monitor the serum tryptase level over time in these patients (but do not perform a bone marrow puncture) unless other signs for a systemic hematologic disease are found (organomegaly, osteolyses, severe cytopenias, others).

² Especially in patients with aggressive mast cell disorders, skin lesions are absent. Therefore it is of pivotal importance to know the subtype of SM in these patients as soon as possible. In aggressive SM the serum tryptase level is usually higher than in patients with isolated bone marrow mastocytosis (often <20 ng/ml), a benign mast cell disease in which skin lesions are also absent.

inant mediator-related symptoms, ‘mediator-targeting’ drugs must be prescribed [52]. In addition, these patients should avoid any triggering stimuli that may cause mediator secretion from (neoplastic) tissue MC [54]. In case of repeated life-threatening anaphylactoid episodes, the self-administration of epinephrine on demand (through an EpiPen®) has been recommended as an appropriate approach [53]. In addition, some authors have recommended the use of cytoreductive or ‘growth-inhibitory’ drugs for these patients [55]. However, such therapeutic maneuvers and their potential beneficial effects have to be balanced against the long-term risk and side effects of these therapies (often immunosuppressive or/and mutagenic). In patients with SM-AHNMD, separate treatment plans for the SM and for the AHNMD should be established. The general recommendation for these patients is to treat the SM as if no AHNMD had been diagnosed and to treat the AHNMD component as if no SM was diagnosed [53]. In patients with high-grade variants of mas-

tocytosis and a progressive clinical course, cytoreductive drugs are recommended and are prescribed together with anti-mediator-type drugs. The best studied drug is interferon- α (with or without additional glucocorticoids) that is considered to affect growth of early myeloid progenitor cells. IFN- α has been reported to produce major clinical responses in about 15–20% of all ASM/MCL patients [56–58]. Another promising drug is cladribine (2CdA). 2CdA has been found to reduce the MC burden in a subgroup of patients with (slowly progressing) ASM [59–61]. However, not all patients with ASM may respond to 2CdA, especially when the disease progresses rapidly. As those with MCL, these ASM patients are candidates for intensive chemotherapy. More recently, the use of targeted drugs in the treatment of patients with ASM or MCL has been proposed. When considering the use of such drugs (e.g., imatinib = STI571), it is of importance to define the exact molecular defects that underlie mastocytosis and the potential targets expressed in neoplastic cells

[59]. Thus, whereas some of the molecular targets and altered genes (e.g. wild-type c-kit, c-kit with Phe522Cys, or the FIPL1-PDGFR α fusion gene) respond to imatinib, other mutations, in particular D816V, lead to an at least partial resistance against imatinib [61]. The major problem here is that most patients with SM exhibit KIT D816V. Another recently developed drug, namely PKC412, has been reported to exert remarkable effects on neoplastic MC carrying KIT D816V in vitro. In addition, PKC412 (midostaurin) has been described to induce remission in a patient with MCL carrying the activating c-kit mutation of D816V type [62].

Conclusion

Mastocytosis is a term collectively used for a heterogeneous group of disorders presenting with an accumulation of MC in various organs with or without skin lesions or mediator-related symptoms. A widely accepted classification system for mastocytosis with solid disease-specific criteria is available, which is now generally accepted and adopted by the WHO. Using such criteria, it is possible to discriminate between mastocytosis and all other related and unrelated conditions including myelomasto-

cytic leukemia. In addition, much has been learned in recent years about disease-related genetic aberrations occurring in mastocytosis. Some of the altered genes and molecules have recently also been identified as potential targets of therapy. This hopefully will lead to a crucial improvement of therapy of mast cell proliferative disorders in the near future. A practical guide for approaching patients with suspected mastocytosis is shown in table 3. Based on the above-mentioned criteria, such an approach usually starts with screening tests (such as serum tryptase) and a biopsy of lesional tissue. A bone marrow trephine biopsy specimen including immunohistochemical analysis using anti-tryptase, anti-KIT and anti-CD25 antibodies as well as application of further diagnostic criteria must be performed in all cases with suspected SM. Further staging investigations will then reveal or exclude the presence of B and/or C findings, or an AHNMD, and thus will lead to the final diagnosis, i.e. subvariant of SM. A molecular analysis is also crucial in suspected SM, especially when the histology did not yield a major diagnostic criterion. When the screening-PCR for c-kit mutations shows a negative result, one should keep in mind that more sensitive methods like PNA-mediated clamping or investigation of microdissected MC can be applied.

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