

Diagnostic Criteria and Classification of Mastocytosis in 2014

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KEYWORDS

• Mastocytosis • Diagnosis • Classification • World Health Organization • c-kit
• Tryptase

KEY POINTS

- The diagnosis of mastocytosis in skin is established by presence of characteristic maculopapular skin lesions and confirmed by skin biopsy.
- Systemic mastocytosis should be diagnosed based on World Health Organization (WHO) criteria. Presence of unexplained symptoms of mast cell activation should prompt the pathologic investigation; however, the diagnosis cannot be based on symptoms alone.
- According to WHO, mastocytosis is classified into 7 categories, with distinct clinicopathologic and prognostic features that guide the therapy.
- Tryptase level greater than 20 ng/mL is associated with systemic mastocytosis. However, lower tryptase levels can be seen in patients with cutaneous mastocytosis, monoclonal mast cell activation syndrome, and systemic mastocytosis with limited bone marrow involvement.
- More than 90% of adults and 80% of children with mastocytosis are detected to have somatic gain of function mutations in c-kit. In most patients with systemic mastocytosis, the c-kit mutation D816V is detectable.

DEFINITION AND OVERVIEW OF CATEGORIES

Mastocytosis is a disorder characterized by accumulation of pathologic mast cells in tissues, which is accompanied by symptoms of mast cell activation in most patients.^{1–4} Most commonly affected tissues are skin, bone marrow, and gastrointestinal tract, followed by liver, spleen, and lymph nodes. Mastocytosis can affect both children and adults. In children, it commonly presents with skin lesions of urticaria

Conflict of Interest Disclosure: Dr C. Akin has a consultancy agreement with Novartis.

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Immunol Allergy Clin N Am 34 (2014) 207–218

<http://dx.doi.org/10.1016/j.iac.2014.02.003>

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pigmentosa (UP) or mastocytomas within the first year of life.⁵⁻⁷ Mastocytosis diagnosed in infancy has a good prognosis; there is no evidence of pathologic mast cell accumulation in tissues other than skin (cutaneous mastocytosis), and most patients experience resolution or fading of skin lesions by adolescence. In contrast, adult-onset mastocytosis is almost always associated with bone marrow involvement and has a persistent course. When mastocytosis is present in any extracutaneous tissue (proved by biopsy) and shows multifocal or diffuse organ infiltration, it is termed systemic mastocytosis (SM). SM is a heterogeneous group of disorders with variable prognosis.⁸ Most patients have indolent SM (ISM), meaning that bone marrow examination shows abnormal mast cell collections but no other hematologic disease, and there is no evidence of end-organ damage attributable to mast cell infiltration. Patients with ISM have a comparable life expectancy with the general age-matched population.⁹⁻¹¹ Up to 20% of patients with SM may have a second bone marrow disease, usually with myeloproliferative or myelodysplastic features. These patients have SM associated with a hematologic non-mast cell clonal disease (SM-AHNMD). The prognosis in these patients depends on the course of the AHNMD.¹⁰ A few patients (approximately 5%) have evidence of end-organ damage caused by mast cell infiltration (aggressive SM [ASM]), in whom the disease follows an accelerated course resembling a malignancy.^{12,13} Mast cell leukemia (MCL) is a rare subset of SM, which is diagnosed when mast cells in bone marrow aspirate smears are greater than 20%.^{14,15} In several of these cases, circulating mast cells are found. Mast cell sarcoma (MCS) and extracutaneous mastocytosis are extremely rare variants with solid mast cell tumors, bearing malignant and benign pathologic features, respectively.¹⁶

DIAGNOSIS

Mastocytosis commonly comes to clinical attention in one of the following clinical scenarios:

1. The patient (or parents if the patient is a child) notices the hyperpigmented skin lesions of UP (or mastocytoma in a child). Sometimes, these lesions may be noticed in dermatology evaluations for other purposes rather than by patients themselves, because they may resemble freckles earlier in the course.
2. The patient presents with symptoms of mast cell activation such as recurrent flushing, hypotension, near syncope or syncope, abdominal cramps, and diarrhea. Anaphylaxis is suspected, but an allergy evaluation often does not identify a culprit. A variation of this presentation involves patients who experience severe systemic reactions to Hymenoptera stings. Curiously, urticaria and angioedema are not commonly seen in mast cell activation episodes in mastocytosis.
3. The patient is detected to have hematologic abnormalities such as cytopenias or increased white blood or platelet counts, liver or spleen enlargement, fatigue, and weight loss, prompting a hematologic workup.
4. The patient may be detected to have sclerotic or lytic bone lesions in imaging studies, raising concern for metastatic disease.
5. A rare first presentation is osteoporosis and pathologic bone fractures (commonly vertebral compression fractures). This finding should raise suspicion in younger patients (especially males) who do not otherwise have any risk factors for osteoporosis.
6. The patient is referred because of nonspecific gastrointestinal symptoms suggestive of colitis and unexplained splenomegaly, and the hematologic evaluation shows mastocytosis.

7. The patient has been diagnosed with a myelodysplastic syndrome, acute myeloid leukemia (AML), or chronic myelomonocytic leukemia (CMML), and the molecular workup showed a c-kit mutation. In these cases, no histology was available or the pathologist had overlooked SM.

The first step in all patients who are suspected to have mastocytosis is a thorough skin examination to detect typical skin lesions (UP type). These lesions range in size from a few millimeters to a few centimeters and have a brownish hyperpigmentation along with a hyperemic component. The lesions are fixed and do not appear and disappear at different locations like classic urticarial wheals. They are usually nonpruritic at baseline but may be triggered to urticate with friction, temperature changes, fever, emotional or physical stress, exercise, alcohol, and spicy foods. Stroking of the lesion results in a wheal localized to the lesion (Darier sign); however, some patients may lack this sign if they are on H₁ antihistamines. Skin lesions in young children may not be hyperpigmented and may show blistering in the first 3 years of life. Although most experts can diagnose UP-like skin lesions on visual inspection, skin biopsy may confirm the diagnosis of mastocytosis (in the skin) in suspected cases. In these patients, skin biopsy shows a perivascular mast cell infiltrate in the upper dermis. Some scattered eosinophils may be mixed with the infiltrate, but other inflammatory cells are typically absent. Increased melanin deposition is noted in epidermis. Mastocytomas are typically solitary and larger lesions with histologic evidence of a solid mast cell tumor. Physical irritation of mastocytomas may cause systemic symptoms, such as hives. Teleangiectasia macularis eruptive perstans is a rare manifestation of cutaneous mastocytosis, in which the skin has flat teleangiectatic macules in a generalized distribution. Skin lesions in adults generally spare sun-exposed areas, such as face and hands, and tend to aggregate in areas prone to irritation, such as upper thighs and axillae. However, it is not uncommon to have lesions on scalp, face, and neck in young children.

After visualization of skin lesions, the patient is said to have mastocytosis in the skin, and a decision has to be made whether a bone marrow biopsy is necessary to look for evidence (criteria) of systemic mastocytosis or to classify the patient as having cutaneous mastocytosis if bone marrow histopathology is normal or criteria for SM are not fulfilled. A simple guide for this decision is the patient's age. Children in whom the skin lesions start in the first 2 years of life rarely have bone marrow involvement, and these children are presumed to have cutaneous mastocytosis. A bone marrow biopsy in children is not routinely recommended unless the child has a persistently high serum tryptase level (or shows an increasing trend in serial measurements) or has abnormal complete blood count or liver or spleen enlargement. In contrast, most patients with adult-onset disease have evidence of bone marrow involvement when skin lesions are detected, and therefore, a bone marrow biopsy is recommended to establish the diagnosis of SM, to assess bone marrow mast cell burden, and to rule out presence of other hematologic disease.

Therefore, a bone marrow biopsy is recommended in adult patients presenting with skin lesions of UP type as well as those presenting with clinical scenarios 2 to 5, outlined earlier. Biopsy of tissues other than bone marrow is not recommended to establish a diagnosis, because bone marrow is always involved, and the histopathologic aspects of mast cell disease are not well studied in other tissues. Gastrointestinal biopsies are sometimes needed to establish the cause of symptoms such as diarrhea and malabsorption, whether they are caused by mast cell infiltration or simply caused by systemic effects of mast cell mediator release, and a subset of patients were first diagnosed on gastrointestinal biopsies by astute pathologists.¹⁷

Diagnosis of SM is made according to the World Health Organization (WHO) criteria (Box 1). These criteria were established by an international group of experts in a consensus meeting in Vienna in 2000.^{18–20} Until that time, the diagnosis was mainly made by visualization of large aggregates of mast cells in bone marrow and subjective assessment of increased mast cell numbers in tissues. The Vienna conference retained the mast cell aggregates as the major diagnostic criterion but also introduced the concept of minor criteria. Thus, the modern diagnostic criteria include 1 major and 4 minor criteria, as outlined in the following sections. The patient needs to have either the major and at least 1 minor, or 3 minor criteria, to diagnose SM.

Major Criterion

Multifocal dense aggregates of mast cells with more than 15 cells per aggregate in an extracutaneous tissue. The tissue of choice in diagnosis is bone marrow. Both a biopsy and an aspirate should be obtained. Biopsy should be of good quality without significant crush or bleeding artifact. Formalin-fixed, paraffin-embedded bone marrow biopsy sections should be stained for tryptase, CD117 and CD25 in addition to routine hematoxylin-eosin stains. In normal bone marrow, mast cells are distributed interstitially and singly, with a predominantly round shape. Although even a small clustering of mast cells should raise suspicion for mastocytosis, to meet the major criterion, the clusters should contain at least 15 mast cells. Mast cell clusters in mastocytosis occur paratrabecularly, perivascularly, or interstitially. Sometimes, a spindle-shaped infiltrate is observed to line the bone trabeculae. Eosinophils are frequently found in increased numbers around the infiltrate. Some samples may have a polytypic lymphocytic infiltrate around or within the lesions.^{21,22} Patients with smoldering or ASM or MCL often have a high degree of involvement in bone marrow biopsy (>30%), with associated marked fibrosis. The cells have spindle shapes, oval and eccentric nuclei with pale cytoplasm in hematoxylin-eosin staining. Tryptase stain confirms the nature of the infiltrate as mast cells.

Minor Criteria

- 1. More than 25% of the cells have morphologic abnormalities such as spindle shapes, hypogranulation, eccentric nuclei, cytoplasmic projections, and multilobed

Box 1
Diagnostic criteria of SM

Major

Multifocal dense aggregates of mast cells with more than 15 cells per aggregate in an extracutaneous tissue

Minor

- 1. Greater than 25% of mast cells have morphologic abnormalities such as spindle shapes, cytoplasmic projections, hypogranulation (see text)
- 2. Expression of CD25 with or without CD2 by mast cells
- 3. Detection of a codon 816 c-kit mutation by a sensitive technique in lesional tissue or peripheral blood
- 4. Serum baseline tryptase greater than 20 ng/mL^a

^a Not valid if there is AHNMD.

Data from Refs. ^{18–20}; see text for more detailed discussion; major +1 minor, or 3 minor, criteria are required.

or clefted nuclei.^{23,24} Normal bone marrow mast cells have round cytoplasm, with a centrally located nucleus and dense granulation. In bone marrow aspirate smears, mast cells are usually located in or near bone spicules. The percentage of mast cells in bone marrow aspirate smears has been shown to correlate with advanced mastocytosis and prognosis. If mast cell percentage is greater than 20% in an aspiricular area, the diagnosis is MCL. Mast cells of less than 5% are generally seen in ISM, whereas mast cells between 5% and 19% signify an intermediate prognosis,²³ which is often the case in ASM, and termed ASM in transformation (ASM-t), because these cases often progress to MCL.¹⁵

2. Mast cells aberrantly express CD25 and CD2.^{25,26} Normal or reactive mast cells usually do not express these markers. CD25 is more sensitive and specific than CD2.^{27,28} Expression of these markers can be detected by immunohistochemistry (IHC) or flow cytometry. IHC has the advantage of being applicable to archived paraffin-embedded samples.²⁹ Serial sections should be stained for tryptase to detect coexpression of CD25 in mast cells. CD2 staining does not always yield positive results, especially in advanced disease. Flow cytometry is also a sensitive and reliable method of detecting aberrant mast cell marker expression, if it is performed in reference centers with expertise on this technique. Because the mast cell percentage of the marrow aspirate is usually low (in many cases <1%), special gating techniques and acquisition of many events (at least 500,000 or preferably higher) are required for an accurate analysis.^{26,30}
3. Using a sensitive technique, c-kit point mutation at codon 816 is detectable in peripheral blood or lesional tissue.^{31–34} Detailed accounts of c-kit mutations in mastocytosis in pathogenesis of mastocytosis and their roles in altered signal transduction are discussed in other articles by Cruse and colleagues, Arock and colleagues in this issue. D816V c-kit mutation is detectable in more than 80% of patients with SM in bone marrow mast cells^{34,35} and in approximately 40% of children with cutaneous mastocytosis in lesional skin.³⁶ Analysis of peripheral blood may yield false-negative results in most commercially available tests, because of the somatic nature of the mutation, although recently introduced polymerase chain reaction–based sensitive tests approach the sensitivity in bone marrow sorted mast cells.³³ Mutation may be confined to mast cells in patients with indolent disease, whereas it may involve multiple other myeloid and even lymphoid lineages in patients with advanced disease.^{35,37,38} Patients with multilineage involvement were shown to have a higher risk of progression to aggressive mastocytosis and carry a poorer prognosis. c-kit D816V mutation renders the mast cells resistant to the currently available tyrosine kinase inhibitors, including imatinib.^{39–41}
4. Serum tryptase greater than 20 ng/mL.^{42–46} Normal median tryptase level in the healthy population is 5 ng/mL. Mast cells are the major source of the neutral protease tryptase. Basophils and myeloid progenitors also produce smaller amounts of this enzyme. Therefore, tryptase levels may be increased in other myeloid neoplastic processes and do not count as a minor criterion in SM-AHNMD.⁴⁷ Commercially available tryptase assay uses an enzyme-linked immunosorbent assay method to measure a combination of mature tryptases and protryptases. Mature tryptase (β tryptase) is stored in mast cell granules and is released after a mast cell degranulation or anaphylactic event, with peak levels found in approximately 1 hour after the degranulation. β Tryptase levels are undetectable in sera from healthy individuals. In contrast, protryptases (mainly encoded by α tryptase) are targeted to cell membrane and constitutively secreted out of the mast cells. Therefore, the total tryptase level at baseline reflects α (pro) tryptase and correlates with total body mast cell burden. A basal tryptase level less than 20 ng/mL does not

rule out mastocytosis, whereas slight tryptase increases greater than 20 ng/mL can be seen in individuals other than those with mastocytosis (including those with renal disease, myeloid neoplasms, and idiopathic) and even in apparently healthy controls. Therefore, tryptase levels alone should not be used as the sole criterion to diagnose mastocytosis.

Some patients with mast cell activation symptoms and lower tryptase levels may not meet the full WHO criteria for the diagnosis of mastocytosis but may have CD25+ mast cells in bone marrow biopsies or have the c-kit D816V mutation detectable.^{48–50} These patients meeting only 1 or 2 minor criteria are termed to have monoclonal mast cell activation syndrome (MMAS). It is not clear whether patients with MMAS progress to full-blown mastocytosis with time.

A rare histopathologic variant of SM termed well-differentiated SM (WDSM) is characterized by aggregates of mast cells with round shapes and fully granulated morphology with no CD25 expression.⁵¹ Aberrant CD30 expression may be a useful marker for these patients, who generally have childhood-onset mastocytosis with skin lesions and progress to have adult-onset systemic disease.^{52,53} Most patients with WDSM do not carry the D816V c-kit mutation and thus may respond to imatinib.⁵⁴ The WDSM morphology may be found in patients with ISM but can sometimes also be found in patients with advanced SM requiring therapy. Therefore also, the term WDSM should be used as a descriptive appendix to the final diagnosis, for example, ISM-WDSM, ASM-WDSM, or ASM with WDSM morphology. Sometimes, it is nearly impossible to differentiate between WDSM and reactive mast cell hyperplasia.

CLASSIFICATION

The WHO classification divides mastocytosis into 7 categories (Box 2). A major check-point in classification of mastocytosis is to determine whether the patient has cutaneous mastocytosis or SM. This determination correlates with the age of onset of disease and is defined by histopathologic criteria, as discussed earlier. If a diagnosis of SM is made, the next step is to classify the disease into 1 of 4 categories of SM.

- 1. ISM. Most patients with adult-onset disease fall into this category. Patients with ISM satisfy the WHO diagnostic criteria for SM but do not have other hematologic

Box 2

Classification of mastocytosis

- Cutaneous mastocytosis
- ISM
- Smoldering SM^a
- SM-AHNMD
- ASM
- MCL
- MCS
- Extracutaneous mastocytoma

^a The smoldering subtype of SM has recently been accepted as a separate category by the consensus group, but was not implemented as such in the WHO classification of 2008.

disorders or evidence of tissue dysfunction caused by mast cell infiltration. Prognosis of ISM is good, with chance of progression to one of the more advanced categories being less than 5%. Patients with ISM often have chronic symptoms of mast cell degranulation, such as flushing, pruritus, abdominal cramps, and diarrhea, and usually require symptomatic management with antimediator therapy. The risks of hypotensive anaphylaxis^{55–57} and osteoporosis⁵⁸ are increased. A substantial subset of patients who experience systemic allergic reactions to Hymenoptera stings have been described as having ISM or MMAS.^{59,60}

A WHO subvariant of ISM called smoldering SM is characterized by increased mast cell burden (>30% infiltration in bone marrow biopsy and tryptase levels >200 ng/mL) and may have splenomegaly or hepatomegaly without significant organ impairment and subtle dysplastic changes not meeting the full criteria of another hematologic disease or ASM (B findings).^{61–63} These patients may be at a higher risk of progression to a more advanced category. WHO has included this variant as a subcategory of ISM. However, more recently, the smoldering type of SM has been recognized as a major defined category of SM, with unique clinical and molecular features. The key molecular feature is the clonal multilineage involvement, which can be shown by the presence of KIT D816V in various hematopoietic lineages.

2. SM-AHNMD. These patients meet the criteria for SM as well as a second hematologic disorder. The associated hematologic disorder is usually myeloid: myelodysplastic syndromes, CMML, myeloproliferative disorders (including those associated with JAK2 V617F mutation), and AML have all been described.^{10,64} Lymphoproliferative disorders such as non-Hodgkin lymphoma and myeloma have also been occasionally described.^{65,66} The SM component in SM-AHNMD may be indolent or aggressive, and each component should be diagnosed and treated according to its own treatment guidelines.
3. ASM. This category of mastocytosis has a clinical course similar to other malignancies leading to organ damage and is associated with poor prognosis. The symptoms are caused by extensive mast cell infiltration, causing tissue dysfunction (C findings).^{18–20} Tissues involved in ASM include hematopoietic, gastrointestinal, and skeletal systems.¹² Pulmonary, renal, and central nervous system involvement is rare. C findings include cytopenias (absolute neutrophil count <1000/ μ L, hemoglobin <10 g/dL, platelets <100,00/ μ L) with a high degree of bone marrow involvement (usually >30%) or splenomegaly, increased levels in liver function tests with evidence of portal hypertension or ascites, extensive gastrointestinal infiltration documented by biopsy accompanied by malabsorption, hypoalbuminemia, weight loss and chronic diarrhea, or large osteolytic lesions with pathologic bone fractures. The last finding should not be confused by osteoporosis and resulting vertebral compression fractures, which are common in indolent mastocytosis. The local mast cell infiltration should be documented whenever possible. Tryptase levels in ASM are usually higher than 100 ng/mL and often increase rapidly over time.⁶⁷ In some of these patients, progression to MCL is seen within a few months. These cases may suffer from ASM-t, a condition defined by a mast cell count of 5% to 19% in bone marrow smears. Patients with ASM are candidates for mast cell cytoreductive or investigational therapies. In this regard, many patients with ASM have concomitant AHNMD (ASM-AHNMD). In these cases, the overall treatment plan has to be adapted to both conditions.
4. MCL. Mast cells are not components of normal peripheral blood. MCL is defined by a mast cell count of 20% or greater in bone marrow smears.¹⁵ Although some mast cells in circulation can be detected in ASM, presence of 10% or more mast cells in

peripheral blood is highly suspicious (diagnostic) for MCL. In aleukemic variants of MCL, bone marrow aspirate smears contain 20% or more mast cells in aspicular areas, but circulating mast cells are less than 10%. Mast cells in MCL have high-grade morphology, with significant degranulation, multilobular or clefted nuclei, and may even show mitotic figures. Serum tryptase is often significantly increased and may be found greater than 500 or even greater than 1000 ng/mL. MCL may occur de novo or occasionally develop secondary to other mast cell disease categories, such as ASM. MCL has a poor prognosis, and patients often have C findings at diagnosis or develop them shortly thereafter, although a chronic form with a relatively stable course over several years has been recognized.¹⁴ Similar to ASM, patients with MCL require cytoreductive and investigational therapies. In many cases, polychemotherapy is recommended, and in those who show a good response, stem cell transplantation has to be considered.

MCS is a rare malignant form of solid mast cell tumor with destructive infiltration and metastatic potential.¹⁶ This issue contains an excellent review article by Weiler and Butterfield on all cases of this tumor published in the literature. MCS may progress to MCL within a short period. Skin involvement has also been described and was reported to develop in the background of UP. Extracutaneous mastocytoma is an exceedingly rare benign mast cell tumor, and the clinical experience is limited to a few case reports.⁶⁸

SUMMARY

The current diagnostic criteria and classification of mastocytosis are well validated by clinical studies and have been shown to correlate with prognosis and to guide in the selection of therapy. As new knowledge is gained regarding clinical course, molecular pathogenesis and therapy for disease, evidence-based revisions to improve the current criteria should be considered by international consensus. Using the guidelines in this article, it should be straightforward to establish a diagnosis of mastocytosis in most cases. Cases with low mast cell burden or uncertain diagnostic interpretation should be referred to centers of expertise. In Europe, centers of excellence have been organized under the umbrella of the European Competence Network on Mastocytosis (<http://www.ecnm.net>). Although no similar organization exists in the United States, there are several centers of excellence, including the Brigham and Women's Hospital, Stanford, Mayo Clinic, MD Anderson, and National Institutes of Health. It is the hope for the future that these centers will develop and merge as a robust collaborative competence network in the United States. The patient support groups, such as the Mastocytosis Society, also provide valuable education and organize yearly meetings (<http://www.tmsforacure.org/>) and should thereby support academic studies and networking in this emerging field of science.

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