

Standards of Pathology in the Diagnosis of Systemic Mastocytosis: Recommendations of the EU-US Cooperative Group



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Abbreviations used

<i>AdvSM</i>	Advanced systemic mastocytosis
<i>AHN</i>	Associated hematologic neoplasm
<i>AIM</i>	American Initiative in Mast Cell Diseases
<i>AML</i>	Acute myeloid leukemia
<i>ASM</i>	Aggressive systemic mastocytosis
<i>BM</i>	Bone marrow
<i>BMM</i>	Bone marrow mastocytosis
<i>CM</i>	Cutaneous mastocytosis
<i>ECM</i>	Extracutaneous mastocytoma
<i>ECNM</i>	European Competence Network on Mastocytosis
<i>GI</i>	Gastrointestinal
<i>IHC</i>	Immunohistochemistry
<i>ISM</i>	Indolent systemic mastocytosis
<i>MCs</i>	Mast cell(s)
<i>MCL</i>	Mast cell leukemia
<i>MCS</i>	Mast cell sarcoma
<i>MDS/MPN</i>	Myelodysplastic/myeloproliferative neoplasm
<i>MLL</i>	Myelomastocytic leukemia
<i>NGS</i>	Next generation sequencing
<i>PB</i>	Peripheral blood
<i>SSM</i>	Smoldering systemic mastocytosis
<i>VAF</i>	Variant allele frequency
<i>WDSM</i>	Well-differentiated systemic mastocytosis
<i>WHO</i>	World Health Organization
<i>WoCo</i>	Working Conference
<i>WP</i>	Work package

Pathology plays a central role in the diagnosis of systemic mastocytosis (SM), its delineation from other neoplasms and reactive conditions, and in monitoring of SM under therapy. The morphologic hallmark of SM is the accumulation of spindle-shaped, hypogranulated mast cells (MCs) in bone marrow (BM) and other extracutaneous tissues. Four of the 5 World Health Organization—defined diagnostic criteria (ie, compact MC aggregates [=major criterion]; atypical MC morphology; activating *KIT* point mutations; aberrant expression of CD25 and/or CD2 and/or CD30 in MCs [=minor criteria]) can be addressed by the pathologist. The final classification of SM variants as either BM mastocytosis, indolent SM, smoldering SM, aggressive SM (ASM), SM with an associated hematologic neoplasm (SM-AHN), or MC leukemia (MCL) has important prognostic significance and requires the integration of certain morphological, clinical, radiological, and biochemical data, referred to as B- and C-findings. Substantial diagnostic challenges may be posed to the pathologist and clinician especially in the so-called advanced SM variants, that is, ASM, MCL, and SM-AHN. In this article, updated recommendations of the EU-US Cooperative Group regarding standards of pathology in the diagnosis of SM, presented during the year 2020 Working Conference held in September in Vienna, are reported. © 2022 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2022;10:1986-98)

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Mastocytosis comprises a heterogeneous group of hematopoietic stem cell—derived neoplasms characterized by an abnormal expansion and accumulation of clonal mast cells (MCs). In systemic mastocytosis (SM), neoplastic MCs carry activating *KIT* codon 816 point mutations, usually p.D816V, and aberrantly express CD25, but often also CD2 and/or CD30.¹⁻⁵ The key clinical and serological findings include typical maculopapular skin rash and an elevated baseline serum tryptase level. According to European Competence Network on Mastocytosis (ECNM) data, in aggressive SM (ASM), MC leukemia (MCL), and SM-associated hematologic neoplasm (AHN), between 50% and 75% of patients lack skin lesions.^{6,7} Inappropriate release of MC mediators may cause different clinical symptoms, impairing quality of life significantly, and may sometimes also lead to anaphylaxis. Patients with indolent forms of SM (ISM) have normal or near-normal life expectancies. In contrast, the rare aggressive or leukemic forms of SM belong to the most aggressive human hematologic neoplasms. SM may be associated with another AHN, most commonly of myeloid origin, such as myelodysplastic syndrome and myelodysplastic/myeloproliferative neoplasm (MDS/MPN) (eg, chronic myelomonocytic leukemia or MDS/MPN-unclassifiable [MDS/MPN-U], chronic eosinophilic leukemia, or acute myeloid leukemia [AML]).⁸⁻¹⁰ Recently, targeted therapies directed against *KIT* have been introduced for patients with advanced SM (AdvSM).¹¹⁻¹⁸

The year 2020 Working Conference (WoCo) on MC disorders was held in Vienna between August 30 and September 1, 2020. It was undertaken within an ECNM-AIM (American Initiative in Mast Cell Diseases) multicenter cooperative initiative to standardize parameters of disease and diagnostics for practice and clinical trials in MC disorders, including mastocytosis. Work packages (WP) were initiated several months before the venue of the WoCo to prepare consensus opinions for each WP. WP1 was devoted specifically to pathology in mastocytosis. The main questions that were addressed dealt with an updated definition of morphology-based diagnostic criteria for SM, diagnostic problems in SM with an AHN (SM-AHN), especially in so-called AdvSM, and the important issue of molecular studies in cytological preparations as well as paraffin-embedded formalin-fixed and paraffin-embedded (FFPE) tissues including bone marrow (BM) and extramedullary organs/tissues. In this report, consensus recommendations proposed during the WoCo and in postmeeting discussions are presented. These recommendations confirm and extend the previous recommendations of the ECNM.^{1,3,19-22}

DIAGNOSTIC CRITERIA FOR SM

According to the proposal of the year 2020 Vienna WoCo, the World Health Organization (WHO)-defined diagnostic criteria for SM shall largely remain unchanged, except for 2 modifications in the minor diagnostic criteria, namely, aberrant expression of CD30 by MCs and activating *KIT* point mutations not restricted to codon 816.³

The major diagnostic criterion for SM is the detection of extracutaneous infiltrates consisting of compact MC aggregates comprising ≥ 15 MCs (Figure 1). The 4 minor diagnostic criteria include (1) atypical spindle-shaped morphology of MCs with hypogranulated cytoplasm in $\geq 25\%$ of total MCs in tissue

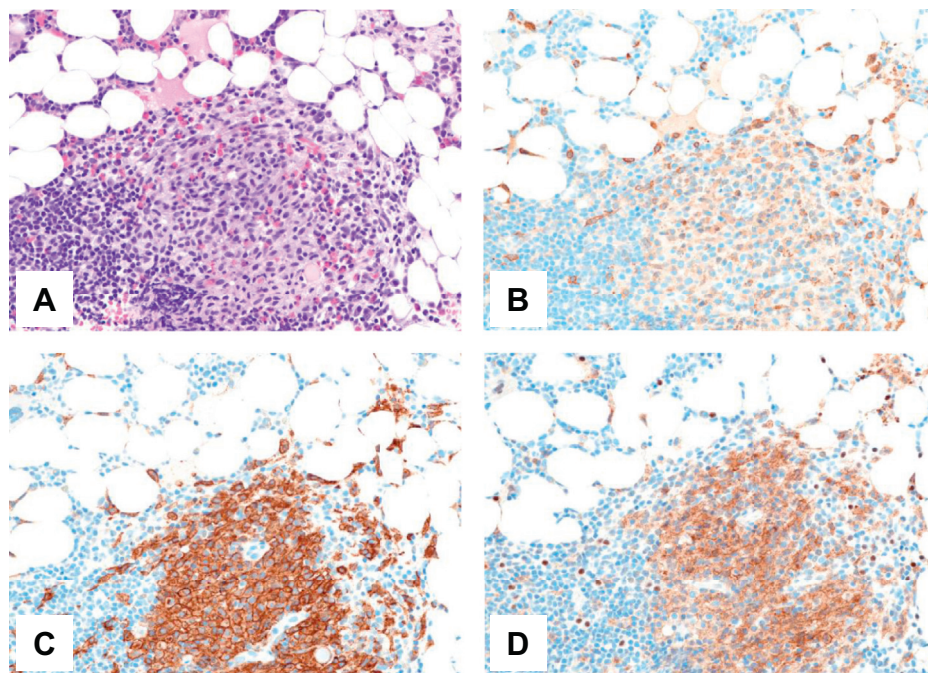


FIGURE 1. ISM with a mixed compact MC aggregate. (A) ISM with multifocal compact MC aggregates in a 54-year-old female patient. A typical mixed infiltrate containing lymphocytes (*lower left*) and a lot of eosinophilic granulocytes between histiocytoid MCs (H&E, $\times 200$). (B) In the present case, MCs show an only weak expression of tryptase ($\times 200$), (C) but a strong expression of KIT (CD117) and (D) also a strong aberrant expression of CD25 (=minor diagnostic criterion, $\times 200$). H&E, Hematoxylin and eosin; ISM, indolent systemic mastocytosis; MC, mast cell;

sections of extracutaneous organs or atypical MC morphology in BM smears (Figure 1, A; Figure 2, C and D; Figure 3, A, C, and D), (2) detection of *KIT* point mutations at codon 816 or other critical regions of the *KIT* gene, (3) aberrant expression of CD25 and/or CD2 and/or CD30 in MCs of BM (Figure 1, D; Figure 4, B; Figure 5, A and B), blood, or other extracutaneous tissues, as determined by immunohistochemistry (IHC) and/or flow cytometry, and (4) basal serum tryptase level >20 ng/mL (see limitations in Table I).^{1,3,8,19} The diagnosis of SM can be established when the major plus 1 minor diagnostic criterion or, in the absence of the major criterion, at least 3 minor criteria are fulfilled (Table I).^{1,3,8}

Because 4 of the 5 diagnostic criteria can be addressed in tissue biopsies and/or cytological specimens, conventional pathology and molecular pathology play a central role in the diagnosis (or exclusion) of SM. Although the morphology-based diagnostic criteria had been defined for BM MC infiltrates, they can likewise be applied to any other extracutaneous tissue.

The SM variants are defined in the presence or absence of so-called B- and C-findings, the presence or absence of an AHN, and the percentage of MCs in BM or peripheral blood (PB) smears (Table II). Of note, the diagnosis of MCL requires the investigation of a BM smear. B-findings are indicative of a huge disease burden, that is, (1) MC infiltrates $\geq 30\%$ of cellularity in BM sections and/or serum tryptase ≥ 200 ng/mL and/or *KIT* p.D816V variant allele frequency (VAF) $\geq 10\%$ in BM or PB leukocytes, (2) signs of myeloproliferation and/or myelodysplasia in BM studies (not fulfilling diagnostic criteria for a WHO-defined myeloid neoplasm), and (3) hepato-/splenomegaly and/or lymphadenopathy without signs of organ dysfunction. In contrast, C-findings reflect signs of organ damage (requiring

cytoreductive therapy in most patients). C-findings include substantial cytopenias due to BM MC infiltration, impaired liver function (with ascites and/or portal hypertension), palpable splenomegaly with hypersplenism, malabsorption (with hypoalbuminemia), and large-size osteolytic lesions (with pathologic fractures not caused by osteoporosis but related to MC infiltrates).^{3,8} In each case, the local MC infiltration in the affected organ must be documented as causative for organ damage to count as a C-finding. Therefore, both the pathologist and clinician are involved when the conclusion is drawn that an organopathy (organ damage) should count as a C-finding.

CLASSIFICATION OF MASTOCYTOSIS

The WHO classification of mastocytosis remains mainly unchanged since its first publication in 2001.^{1,8,23} In this classification, 3 major disease categories are defined: cutaneous mastocytosis (CM), systemic mastocytosis (SM), and a very rare category of localized MC tumors.^{3,8} CM comprises the variants maculopapular CM (MPCM) previously termed urticaria pigmentosa, diffuse CM, and cutaneous mastocytoma. SM is further divided into BM mastocytosis (BMM), ISM, smoldering SM (SSM), ASM, SM-AHN, and MCL.^{3,20,24} The rare forms of localized MC neoplasms encompass MC sarcoma (MCS) and the reintroduced benign extracutaneous mastocytoma (ECM).^{25,26} WHO-approved variants of SM are shown in Table II.

Most pediatric patients with mastocytosis present with (pure) CM characterized by involvement of just the skin, but no other organs. The diagnosis of (pure) CM requires the presence of typical skin lesions with a positive Darier's sign and the absence of SM. In an adult patient, typical skin lesions with a positive

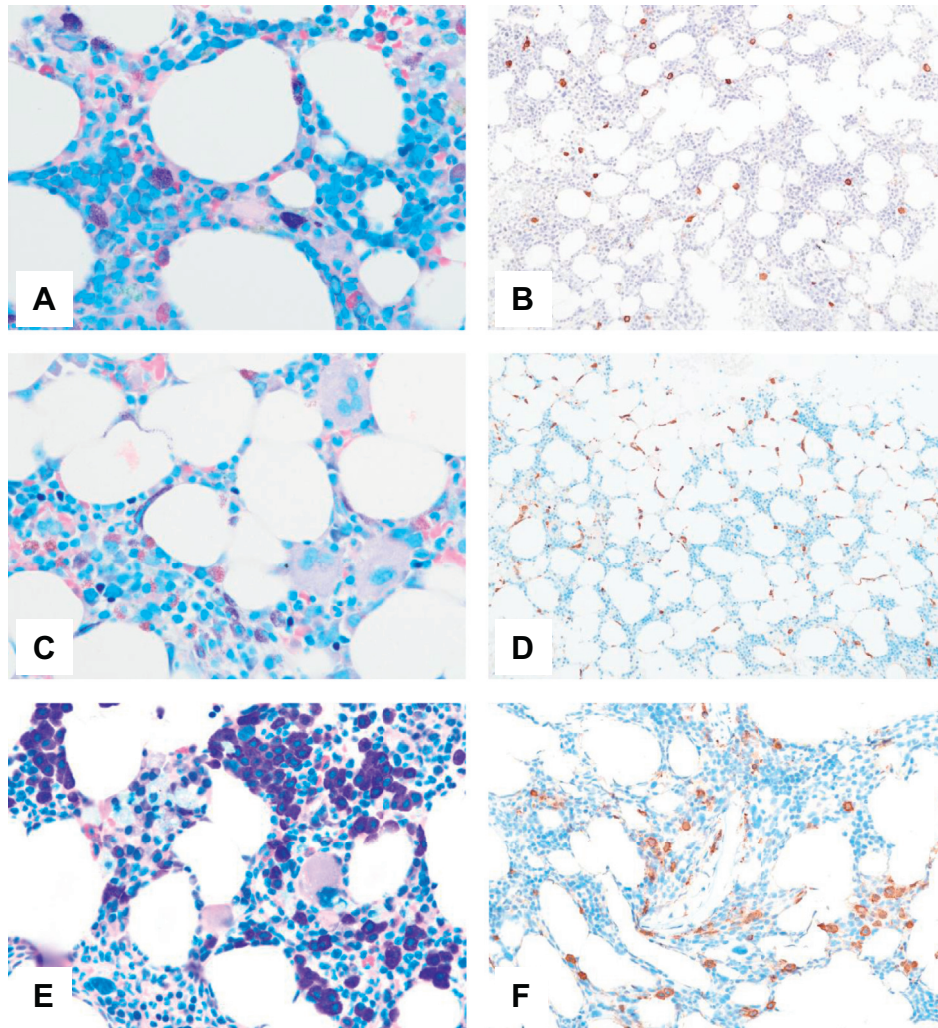


FIGURE 2. MC histomorphology in SM and MCH. (A) MCH in a staging BM biopsy of an 80-year-old male patient with follicular lymphoma (Giemsa, $\times 400$). (B) Antitryptase immunohistochemistry demonstrates a slight diffuse increase of very predominantly round MCs ($\times 400$). The MCs were CD25-negative and no KIT p.D816V mutation was detectable by digital droplet PCR. (C) ISM in a 54-year-old female patient with known cutaneous lesions. A moderate increase in spindle-shaped and slightly hypogranulated MCs is recognizable in higher magnification (Giemsa, $\times 400$), (D) but may be much easier appreciated in IHC stains against tryptase even at lower magnification, also demonstrating that most MCs are atypically spindle-shaped (=minor diagnostic criterion). The KIT p.D816V variant allele frequency was 2% (tryptase, $\times 100$). (E) WDSM in a 70-year-old male patient with compact and diffuse infiltrates of mature-appearing round and hypergranulated MCs (Giemsa, $\times 400$). (F) Antitryptase IHC demonstrates the round phenotype of MCs in the same patient (tryptase, $\times 100$). *BM*, Bone marrow; *IHC*, immunohistochemistry; *ISM*, indolent systemic mastocytosis; *MC*, mast cell; *MCH*, MC hyperplasia; *PCR*, polymerase chain reaction; *SM*, systemic mastocytosis; *WDSM*, well-differentiated systemic mastocytosis.

Darier's sign are usually sufficient to establish the cutaneous involvement. In case the skin lesions are however equivocal, increased MC numbers and/or an (activating) *KIT* mutation in a lesional skin biopsy is required to confirm the involvement of the skin. In contrast to children, a complete BM workup is required in adult patients with skin involvement to additionally sort out systemic MC infiltration, as most of these patients will ultimately be found to exhibit SM.^{19,27,28} Until complete staging has been performed, a provisional diagnosis of "mastocytosis in the skin" can be made.^{6,19,28} In contrast, pediatric patients with typical skin lesions and a positive Darier's sign as well as no features of systemic involvement can be classified as CM without performing a BM workup.

Based on a better progression-free survival in patients without B-findings, tryptase levels <125 ng/mL, and no detectable dense SM infiltrates in extramedullary organs, BMM has been proposed as a new full SM variant, with distinct biological and clinical features as compared with ISM, by the year 2020 Vienna WoCo.²⁴ In ISM, a maximum of 1 B-finding is allowed. Per definition, SSM is diagnosed in the presence of ≥ 2 B-findings, whereas ASM can be diagnosed in the presence of ≥ 1 C-finding.^{1,29} MCL requires the detection of $\geq 20\%$ MCs in BM or blood smears.¹⁵ All SM variants can be associated with another non-MC (myeloid or more rarely lymphoid) hematologic neoplasm (SM-AHN). ASM, MCL, and SM-AHN are collectively termed AdvSM.¹¹

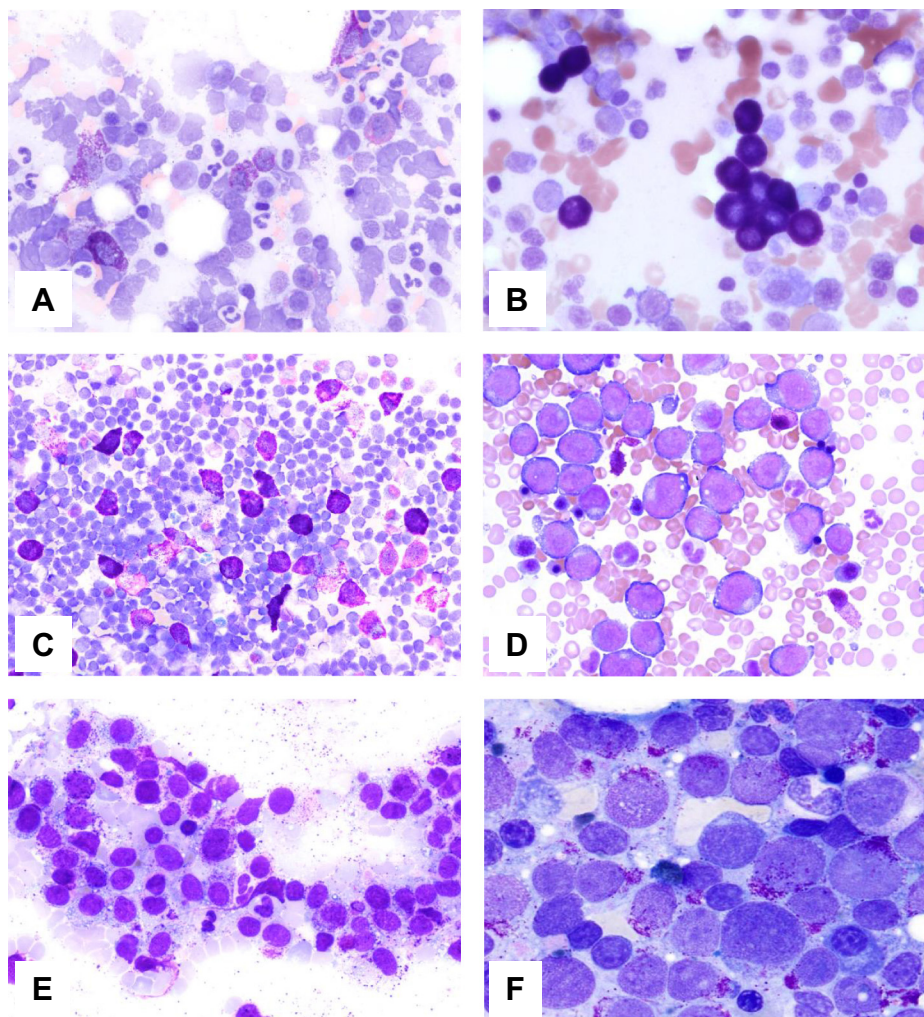


FIGURE 3. Cytomorphology of SM and MML. (A) SSM with atypical (spindle-shaped) MC type I and 2 atypical bi-/multilobated MC type II (center) in a BM smear (Wright-Giemsa, $\times 400$). (B) WDSM, with round and hypergranulated MCs (Wright-Giemsa, $\times 650$). (C) ASM-T, with up to 10% partly spindle-shaped MCs (atypical MC type I, Wright-Giemsa, $\times 400$). (D) SM-AML, myeloid blast cells with intermingled spindle-shaped MC (atypical MC type I, Wright-Giemsa, $\times 400$). (E) MCL, hypogranulated metachromatic blastoid mast cells in a BM smear (Wright-Giemsa, $\times 650$). (F) MML, metachromatic blasts in a BM smear of a patient with myelomastocytic leukemia (MML; Wright-Giemsa, $\times 650$). *AML*, Acute myeloid leukemia; *ASM*, aggressive systemic mastocytosis; *BM*, bone marrow; *MC*, mast cell; *MCL*, mast cell leukemia; *SM*, systemic mastocytosis; *SSM*, smoldering systemic mastocytosis; *WDSM*, well-differentiated systemic mastocytosis.

HISTOMORPHOLOGY

The diagnosis and subtyping of SM is usually based on the histopathologic investigation of a BM trephine biopsy specimen of the iliac crest of adequate length (≥ 1.5 cm marrow without cortex), fixed in neutral-buffered formalin, decalcified, and embedded in paraffin.³⁰ Of note, decalcification in ethylenediaminetetraacetic acid, in comparison to hydrochloride-based decalcificants, results in a much better preservation of nucleic acids and allows the successful application of molecular techniques like polymerase chain reaction and next generation sequencing (NGS) in almost all cases (own unpublished observations).

SM is characterized by an accumulation of neoplastic and usually atypical, that is, spindle-shaped and hypogranulated,

MCs in various organs and tissues, most frequently the BM. Besides an interstitial (ie, loosely scattered) increase in MCs, the diagnostic hallmark and (therefore) the major diagnostic criterion of SM is the demonstration of (multifocal) compact MC infiltrates in BM and/or other extracutaneous tissues (Figures 1 and 2; Table E1, available in this article's Online Repository at www.jaci-inpractice.org).^{1,31,32} Compact MC infiltrates are dense aggregates of ≥ 15 MCs typically admixed with other cell types, especially eosinophils, lymphocytes, histiocytes, and fibroblasts, in varying numbers (Figure 1).³³ Thus, the appearance and cellular distribution of (diagnostic) compact MC aggregates may vary in serial sections, between lesions of the same patient, between lesions of different patients, and between SM subtypes. Depending on the SM subtype, MC

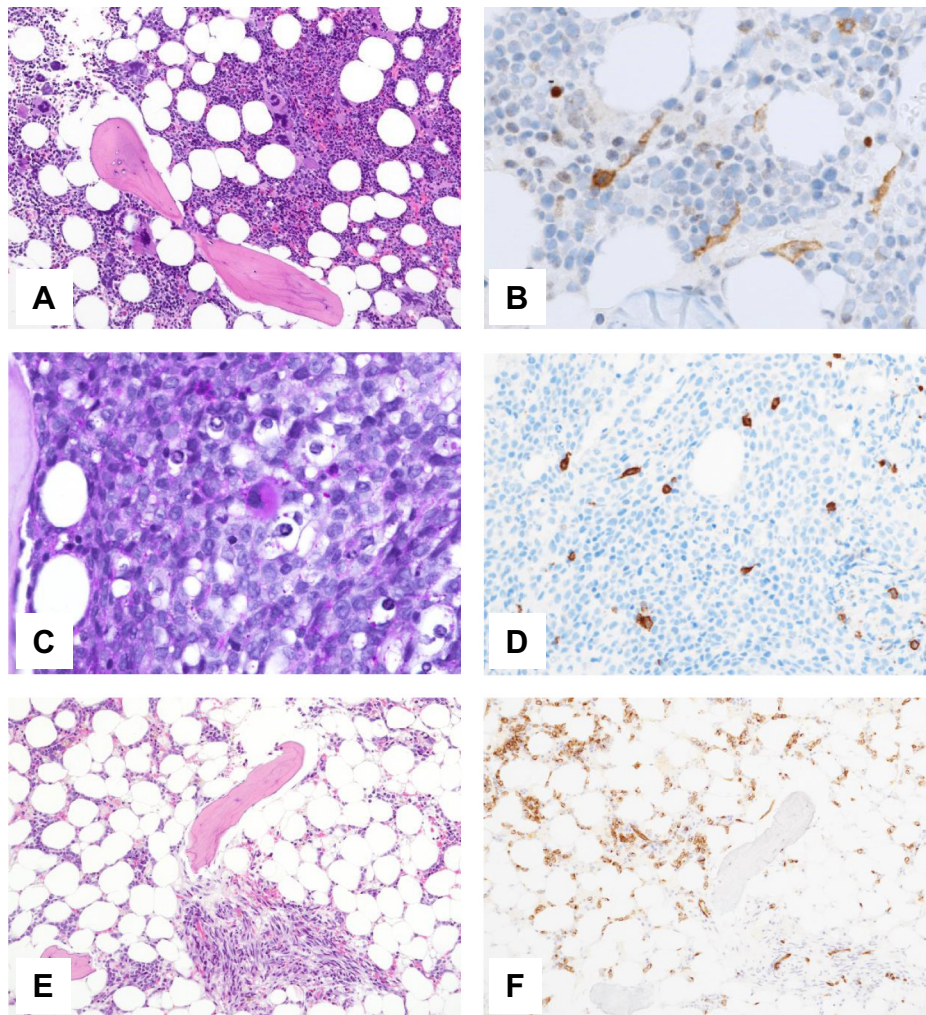


FIGURE 4. Diagnostic challenges in SM. (A, B) BMM-PMF in a 71-year-old female patient with leukocytosis and thrombocytosis. At medium power, a hypercellular BM with some megakaryocyte clusters points to the obvious diagnosis of a chronic myeloproliferative neoplasm (A, H&E, $\times 100$). Antitrypsase and anti-CD117 IHC uncovered a slight interstitial infiltrate of mostly spindle-shaped MCs ($<1\%$ of cellularity), which then also showed an aberrant expression of CD25 (B, CD25, $\times 400$). The *KIT* p.D816V mutation was detected by digital droplet PCR (VAF 0.1%). On request, the patient had no skin lesions and the serum tryptase was not elevated. Thus, the diagnosis of SM in this patient was based on the presence of 3 minor diagnostic criteria. (C, D) ASM-MDS/MPN-U under avapritinib therapy in an 83-year-old male patient with transformation to BMM-sAML. (C, periodic acid-Schiff [PAS], $\times 400$; D, CD117, $\times 100$) The *KIT* p.D816V targeted therapy resulted in an improvement of clinical symptoms and, obviously, caused a dramatic reduction of the MC burden from $>60\%$ to $1\%-2\%$ cellularity. Molecular analyses by NGS and digital droplet PCR detected mutations in *NF1* (VAF 14%), *NPM1* (VAF 34%), *SRSF2* (VAF 43%), *TET2* (VAF 85%), and *KIT* p.D816V (VAF 0.7%). (E, F) AML with initially occult SM in a 70-year-old male patient. A compact aggregate of spindle-shaped MC is easily recognizable even in an H&E stain (E, H&E, $\times 100$) approximately 1 month after initiation of cytoreductive therapy. Still, CD34-positive blasts make up most nucleated BM cells (F, CD34, $\times 100$). AML, Acute myeloid leukemia; ASM, aggressive systemic mastocytosis; BM, bone marrow; BMM, bone marrow mastocytosis; H&E, hematoxylin and eosin; MC, mast cell; MCL, mast cell leukemia; MDS, myelodysplastic syndrome; MPN-U, myeloproliferative neoplasm—unclassifiable; NGS, next generation sequencing; PCR, polymerase chain reaction; PMF, primary myelofibrosis; SM, systemic mastocytosis; SSM, smoldering systemic mastocytosis; VAF, variant allele frequency; WDSM, well-differentiated systemic mastocytosis.

aggregates may show varying degrees of fibrosis, which is especially pronounced in SSM and ASM (Figure 5, E and F). Care must be taken not to misinterpret (peritrabecular) scar tissues unrelated to SM as predominantly fibrotic MC aggregates, because these scars often contain MCs that became flattened beneath the collagen fibers.

In hematoxylin and eosin–stained tissue sections, individual normal or reactive MCs cannot reliably be differentiated from other cells like histiocytes. Except for MCs in scars or fibrotic tissues, MCs have round to oval nuclei with clumped chromatin and abundant cytoplasm. Nucleoli are absent. In contrast, neoplastic MCs in SM often have a more spindling appearance and therefore

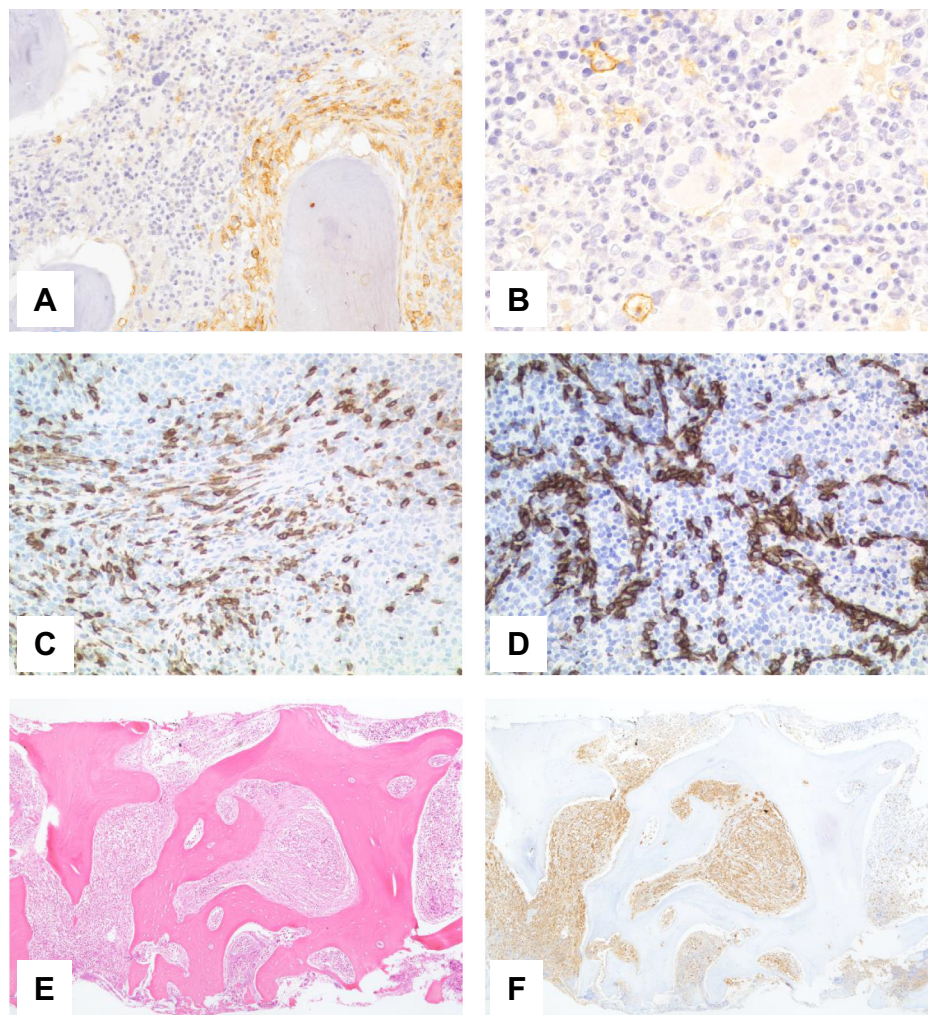


FIGURE 5. Diagnostic challenges in SM, continued 1. (A, B) Aleukemic MCL-MDS/MPN-U in a 75-year-old male patient with aberrant expression of CD30 in a peritrabecular compact MC aggregate (C, $\times 100$) and in single MC of the interstitial infiltrate (D, $\times 200$). (C, D) FIP1L1-PDGFRa positive myeloid neoplasm with eosinophilia. Patients typically show a marked increase in (spindle-shaped) MCs (C, tryptase, $\times 100$) that tend to form (usually) subdiagnostic MC aggregates (D, CD117, $\times 100$). In addition, CD25 can be expressed. With only a few exceptions, no *KIT* p.D816V mutation is detected. (E, F) ASM-CMML-1 in a 52-year-old male patient with marked monocytosis. Typical findings in ASM are osteosclerosis (E, H&E, $\times 10$) and large confluent compact tryptase-positive MC aggregates with a marked fibrosis (F, tryptase, $\times 10$). In the present case, SM is covering 90% of the BM biopsy area, thus histomorphologically obscuring the AHN component of the disease. *AHN*, Associated hematologic neoplasm; *ASM*, aggressive systemic mastocytosis; *CMML*, chronic myelomonocytic leukemia; *H&E*, hematoxylin and eosin; *MCL*, mast cell leukemia; *MDS*, myelodysplastic syndrome; *MPN-U*, myeloproliferative neoplasm—unclassifiable; *SM*, systemic mastocytosis.

may mimic fibroblasts. These spindle cells may also be arranged along fat cells or along the surface of individual bone trabeculae, making them even more difficult to recognize. Compact micro-nodular MC aggregates in SM often appear granulomatoid, depending on their size and cellular composition. Appropriate special stains (eg, basic dyes like Giemsa and toluidine blue) for metachromatic granules allow the proper identification of MCs.^{1,34,35} The only other metachromatic cells are basophils (especially in basophilic leukemia) and metachromatic blast cells in myelomastocytic leukemia (MML).^{20,21,36} However, metachromatic granules may be reduced or even absent in the neoplastic MCs of SM, and the quality of Giemsa and toluidine blue stainings

may differ between different laboratories. Therefore, IHC using antibodies against tryptase and KIT (CD117) is the most specific and reliable method for the identification and enumeration of MCs in general and of (highly) atypical and immature MCs, particularly when only interstitial MC infiltrates are present. Thus, IHC should be applied in every case of suspected SM, and, in addition to MC morphology, the MC cellularity and BM area covered by MC infiltrates should be reported as a possible B-finding and for comparison with follow-up investigations. So far, little is known about histomorphological findings in BM and extracutaneous tissues in pediatric patients. A recent study described a substantial subset of patients with hypocellularity for age (approximately 44%)

TABLE I. Diagnostic criteria for systemic mastocytosis

Major criterion:

Multifocal dense infiltrates of mast cells (≥ 15 mast cells in aggregates) in BM biopsies and/or in sections of other extracutaneous organ(s)

Minor criteria:

1. $>25\%$ of all mast cells are atypical cells (type I or type II) in BM smears or are spindle shaped in dense and diffuse mast cell infiltrates in sections of BM or other extracutaneous organ(s)*
2. Activating *KIT* point mutation(s) at codon 816 or in other critical regions of *KIT*† in BM or another extracutaneous organ(s)
3. Mast cells in BM, blood, or another extracutaneous organ(s) aberrantly express 1 or more of the following antigens: CD2, CD25, and CD30‡
4. Baseline serum tryptase concentration >20 ng/mL in the absence of a myeloid AHN§. In the case of H α T, the tryptase level should be adjusted||

If at least 1 major and 1 minor or 3 minor criteria are fulfilled, the diagnosis is SM.

AHN, Associated hematologic neoplasm; BM, bone marrow; H α T, hereditary alpha-tryptasemia; SM, systemic mastocytosis.

*In tissue sections, an abnormal mast cell morphology counts in both a dense infiltrate and a diffuse mast cell infiltrate. In the bone marrow smear, an atypical morphology of mast cells does not count as an SM criterion when mast cells are located within or adjacent to bone marrow particles. Morphologic criteria of atypical mast cells have been described.

†Any type of *KIT* mutation counts as the minor SM criterion when published solid evidence for its transforming behavior is available.

‡Expression must be confirmed by either flow cytometry or immunohistochemistry.

§Myeloid neoplasms can lead to an increased serum tryptase level. Therefore, this criterion does not count in cases of SM-AHN.

||A possible mode for adjustment has been proposed by Valent et al.³ The basal tryptase level may be divided by 1 plus the extra number of the alpha-tryptase encoding gene copies. For example, when the tryptase level is 30 and 2 extra copies of the alpha-tryptase encoding gene are found in a patient with H α T, the H α T-corrected tryptase level is $10 (30/3 = 10)$ and thus is not a minor SM criterion. Please note that this "correlation approach" is still under discussion within the ECNM-AIM group.

and a significant increase in hematogones in cutaneous and systemic disease (approximately 50%).³⁷

CYTOMORPHOLOGY

In smear preparations, normal MCs are round, medium size cells with abundant metachromatic granules in basic dyes like May-Grünwald-Giemsa (=Pappenheim; Wright-Giemsa in the United States) or toluidine blue. MCs react strongly with naphthol AS-D chloroacetate esterase but do not express myeloperoxidase.³² MCs usually account for substantially less than 1% of all nucleated BM cells. Enumeration of MCs must be performed outside crushed BM particles. The percentage of MCs in BM smears must be reported in every patient with SM. A (reactive) MC hyperplasia can be observed in myeloid neoplasms, lymphoplasmacytic lymphoma, hypoplastic or aplastic marrow, or when stem cell factor is administered. Neoplastic MCs in SM show various degrees of cellular atypia including hypogranulation.^{1,20,38} The following 4 MC types have been defined in BM smears of patients with SM: normal-appearing MCs (eg, in well-differentiated systemic mastocytosis [WDSM]); atypical MC type I, atypical spindle-shaped MCs; atypical MC type II bi-/multilobated MCs (promastocytes); and metachromatically granulated blast cells (indistinguishable from basophil precursors [Figure 3; Table E2, available in this article's Online Repository at www.jaci-inpractice.org]).^{20,38,39} Normal-appearing MCs in

WDSM appear bigger and hypergranulated compared with reactive MCs in a non-SM patient. MC morphology also should be described in every BM report.

IMMUNOHISTOCHEMISTRY

Virtually all reactive and neoplastic MCs express tryptase and KIT (CD117), thus allowing the distinction from neoplastic basophils, which may produce small amounts of tryptase but usually do not express KIT (CD117). Aberrant expression of CD25, CD2, and/or CD30 in neoplastic MCs, detected by IHC or flow cytometry, underlines the neoplastic nature of MCs and therefore serves as a minor diagnostic criterion of SM (Figures 1 and 2).^{2,4,40} Although CD25 can be detected in almost every case of SM, the expression of CD2 and CD30 is less consistently detected, in particular using IHC only and no flow cytometry.^{2,4,41} In extramedullary organs with prominent lymphatic tissues (gut, spleen, lymph nodes), T-lymphocytes expressing CD2 with/without CD25 may cause diagnostic problems in suspected SM.⁴¹ The aberrant expression of CD30 in neoplastic MCs may lead to misinterpretation as neoplastic cells from Hodgkin lymphoma and anaplastic large cell lymphoma, as well as other lymphomas, also display CD30.⁴²⁻⁴⁴ This may especially be the case in MCS with type II MCs with large multilobated nuclei and prominent nucleoli.

In gastrointestinal (GI) tract mucosa, MCs often show only weak expression of tryptase leading to an aberrant-incomplete immunophenotype (CD117++, CD25+, tryptase -/+).⁴² This may lead to an erroneous diagnosis of CD117-positive, CD34-negative forms of AML (PK, own unpublished observations).

Normal and neoplastic MCs may express several additional immunohistochemical markers applied in hematopathology that easily may lead to misinterpretation (Table III). These markers may also be expressed in basophils or other cell types and include, among others: CD9, CD14, CD26, CD45, CD52, CD68, CD99, CD123, CD131, and 2D7.⁴²

MOLECULAR PATHOLOGY

Mutations in the *KIT* gene, especially *KIT* p.D816V, leading to constitutive activation of the KIT receptor, are the molecular hallmark of SM and can be detected in $>80\%$ of all cases with SM. The prevalence of *KIT* p.D816V is highly dependent on the disease subtype (typical ISM $>90\%$, SSM $>90\%$, SM-AHN $>90\%$, ASM $>80\%$, MCL $<70\%$, MCS $<50\%$, WDSM $<50\%$).^{45,46} The detection of *KIT* p.D816V outside the MC lineage (so-called multilineage involvement) results in higher detectable allele frequencies and is of adverse diagnostic importance. Multilineage involvement by *KIT* p.D816V is frequently detected in SSM and especially in ASM. In SM-AHN, depending on the AHN subtype, *KIT* p.D816V and other mutations have been demonstrated to occur in both disease compartments with varying frequencies, thus demonstrating a clonal relationship and a common hematologic progenitor.¹⁰ In contrast, a high mutation burden is rarely seen in pure ISM. Thus, we proposed to include a *KIT* p.D816V VAF $\geq 10\%$ in BM aspirate or PB as a B-finding for SSM.³ Detection of mutations in non-*KIT* genes by NGS methods is indicative of the presence of a (myeloid) AHN and may have adverse prognostic significance, especially when mutations in *SRSF2*, *ASXL1*, *RUNX1*, and/or *DNMT3A* are detected.^{4,47-49}

TABLE II. Diagnostic criteria, and morphologic and molecular features for SM variants

Variant	Diagnostic criteria	Morphologic features	Other features
BMM	- No skin lesions - No B-finding(s) - Basal serum tryptase <125 ng/mL - No dense SM infiltrates in an extra-medullary organ	- Low to very low MC burden in the BM - Compact MC infiltrates >70% of cases - Morphologic features otherwise as in ISM - WD MC morphology possible	
ISM	- Typical skin lesions ≤1 B-finding - ISM without skin lesions: ≤1 B-finding* and/or basal serum tryptase ≥125 ng/mL and/or dense SM infiltrates in any extramedullary organ	- Low MC burden in the BM (usually <5%-10% of section area) - Compact MC infiltrates >70% of cases, typically of mixed morphology, containing numerous eosinophils, lymphocytes, fibroblasts, and histiocytes in addition to neoplastic MCs - Usually >25% spindle-shaped MCs - Hemopoiesis usually normal, mild reactive changes possible - Paratrabeular compact MC infiltrates often with prominent fibrosis and osteosclerotic changes in adjacent trabeculae - In BM smears atypical MCs (usually type I) - WD MC morphology possible	<i>KIT</i> p.D816V mutation in 90%-95% - In patients with WD morphology mutational analysis in all other relevant regions of the <i>KIT</i> gene may be considered
SSM	≥2 B-findings† - No C-finding(s)‡	- High MC burden in the BM (usually >30% of section area) - Diffuse-compact MC infiltration pattern - Usually spindle-shaped MCs embedded in a dense fibrotic stroma with pronounced osteosclerosis - Hemopoiesis often with mild dysplastic changes (not fulfilling WHO criteria for AHN) - WD MC morphology rarely observed	<i>KIT</i> p.D816V almost always present, usually with multilineage involvement - High tryptase levels and organomegaly in most cases - Skin lesions in most cases
ASM	≥1 C-finding	- High to very high MC burden in the BM (up to 80% of section area) - Diffuse-compact MC infiltration pattern - Usually spindle-shaped MCs embedded in a dense fibrotic stroma with pronounced osteosclerosis - Pure ASM less frequent than ASM-AHN - MCs in BM smears between >5% and <20%, indicating ASM in transformation (ASM-t) - Hemopoiesis often with mild dysplastic changes not fulfilling WHO criteria for AHN	<i>KIT</i> p.D816V almost always present, usually with multilineage involvement - High tryptase levels - Skin lesions frequently absent
SM-AHN	Criteria for a WHO-defined hematologic neoplasm fulfilled	- Any subtype of SM and any type of WHO-defined myeloid§ and lymphoid† neoplasm can occur - In most cases, AHN obscures SM (IHC for tryptase and <i>KIT</i> [CD117] required) - In ASM or MCL, an AHN may be difficult to detect - In every case of suspected SM-AHN, immunostains for neoplastic MCs (tryptase, <i>KIT</i> [CD117], CD25, CD30) and for myeloid neoplasms (including CD14, CD34, CD42b, and CD71) are highly recommended - WD MC morphology possible	<i>KIT</i> p.D816V usually present when sensitive techniques are applied - Mutations in <i>SRSF2</i>, <i>ASXL1</i>, <i>RUNX1</i>, and others indicate a poor prognosis

(continued)

TABLE II. (Continued)

Variant	Diagnostic criteria	Morphologic features	Other features
MCL	• $\geq 20\%$ MCs in BM smears – Classic MCL: $\geq 10\%$ MCs – Aleukemic MCL: $<10\%$ MCs in PB smears – Acute MCL (≥ 1 C-finding(s)) – Chronic MCL (no C-findings)	• High MC burden in the BM (usually $>50\%$ of section area) • Diffuse-compact MC infiltration pattern • Round and hypogranulated MCs in most cases • Rarely highly pleomorphic MCs • Fibrosis content usually much lower than in SSM/ASM • Pure MCL is a diagnosis of exclusion of AHN • WD MC morphology rarely observed (chronic MCL)	• <i>KIT</i> p.D816V in approximately 50%-70% • Other <i>KIT</i> codon 816 mutations and mutations in other regions of <i>KIT</i> more frequent than in other SM subtypes • Skin lesions absent in most patients

ANC, Absolute neutrophil count; ASM, aggressive systemic mastocytosis; BM, bone marrow; BMM, bone marrow mastocytosis; Hb, hemoglobin; *HaT*, hereditary alpha-tryptasemia; IHC, immunohistochemistry; ISM, indolent systemic mastocytosis; MCL, mast cell leukemia; MCs, mast cells; PB, peripheral blood; PLT, platelet count; SM-AHN, systemic mastocytosis with an associated hematologic neoplasm; SSM, smoldering systemic mastocytosis; VAF, variant allele frequency; WD, well-differentiated; WHO, World Health Organization;

*Serum tryptase levels may exceed 200 ng/mL (if no other B-finding is detected) or below 200 ng/mL (in which case 1 B-finding may be detected).

†B-findings: (1) high MC burden: MC infiltration grade in BM histology $\geq 30\%$ and/or serum tryptase ≥ 200 ng/mL and/or *KIT* p.D816V VAF $\geq 10\%$ in BM or PB leukocytes, (2) signs of myeloproliferation and/or myelodysplasia not sufficient for the diagnosis of a WHO-defined hematologic neoplasm. (3) organomegaly: palpable hepatomegaly without ascites or other signs of organ damage or/and palpable splenomegaly without hypersplenism and without weight loss or/and lymphadenopathy palpable or visceral lymph node enlargement (>2 cm).

‡C-findings: (1) cytopenia/s (ANC $< 1 \times 10^9/L \pm Hb < 10$ g/dL $\pm PLT < 1.0 \times 10^9/L$); (2) hepatopathy: ascites and elevated liver enzymes $\pm$ hepatomegaly or cirrhosis $\pm$ portal hypertension; (3) palpable splenomegaly with hypersplenism $\pm$ weight loss $\pm$ hypoalbuminemia; (4) malabsorption with hypoalbuminemia $\pm$ weight loss; (5) large-sized osteolysis (≥ 2 cm) with pathologic fracture $\pm$ bone pain.

§In lymphoid AHNs, the diagnosis of both neoplasms in the same organ is required.

TABLE III. Immunohistochemical markers in the diagnosis of SM

Antigens expressed in MCs	
Antigen	Differential diagnosis
Tryptase	Tryptase-positive AML, MML
CD117	Hematopoietic blast cells
CD25	HCL
CD30	CHL, ALCL
CD2	T cells, T-NHL
CD14	Monocytic/histiocytic neoplasms
CD33	Myeloid neoplasms
CD68	Monocytic/histiocytic neoplasms
CD99	Ewing sarcoma, ALL
CD123	Basophilic leukemia, plasmacytoid dendritic cell neoplasm
Antigens used in the diagnosis of AHNs that are never detected in MCs by IHC	
Antigen	Target cells
CD34	Blast cells
CD42b, CD61	Megakaryocytes
CD71	Erythropoietic cells
E-cadherin	Early erythropoietic cells
MPOX	Granulocytic cell neoplasms
CD20, CD79a	B-cell neoplasms
CD38, CD138	Plasma cell neoplasms
CD3, CD5, CD7	T-cell neoplasms

AHN, Associated hematologic neoplasm; ALCL, anaplastic large cell lymphoma; ALL, acute lymphatic leukemia; AML, acute myeloid leukemia; CHL, classical Hodgkin lymphoma; HCL, hairy cell leukemia; IHC, immunohistochemistry; MCs, mast cells; MML, myelomastocytic leukemia; MPOX, myeloperoxidase; NHL, non-Hodgkin lymphoma; SM, systemic mastocytosis.

DIAGNOSTIC CHALLENGES

In typical cases of BMM and ISM, a combination of compact and interstitial MC infiltrates is usually observed. In approximately

20% to 25% of patients with BMM/ISM (with or without AHN), dense or compact MC aggregates are absent (or missed in the present BM biopsy specimen) and only an interstitial infiltrate of loosely scattered MCs is present, which is best appreciated when IHC stains for tryptase and *KIT* (CD117) are applied (Figure 2, C and D). In these patients, the diagnosis of SM can only be established by documenting the presence of 3 minor diagnostic criteria.^{27,50} Because in this situation the MC burden and, therefore, the serum tryptase level is usually low, the positive diagnostic criteria are “atypical morphology,” *KIT* p.D816V, and an aberrant expression of CD25 (and/or CD2 and/or CD30). Especially in SM-AHN without compact MC aggregates, interstitial MC infiltrates may easily be overlooked unless special immunostains for tryptase and CD117 are applied, or Giemsa stains are performed and very carefully inspected (Figure 4, A and B).⁵¹

In (SM)-AML, an underlying SM may only be detected after (successful) cytoreductive therapy.⁵² Thus, immunostains (ie, tryptase and *KIT* [CD117] as well as CD25 and CD30) should be repeated in control marrows after (successful) cytoreductive therapy, where initially occult compact MC infiltrates may then be visualized (Figure 4, C and D). Likewise, in all patients with *KIT* p.D816V-positive AML, a meticulous investigation of the BM should be performed in order not to overlook (occult) SM.^{9,52,53}

Patients with AdvSM under cytoreductive and/or targeted therapy should receive repeated follow-up BM biopsy investigations with meticulous morphologic, immunohistochemical, and molecular workup to monitor therapy responses and possible disease progressions/transformations (Figures 4, E and F, and 5, A and B).^{12,14}

In patients with myeloid/lymphoid neoplasms and eosinophilia, MCs may be increased, even forming dense infiltrates (typically without the mixed cellular microenvironment described above), and are often spindle-shaped and express CD25 (Figure 5, C and D). Thus, in these cases, the diagnosis of

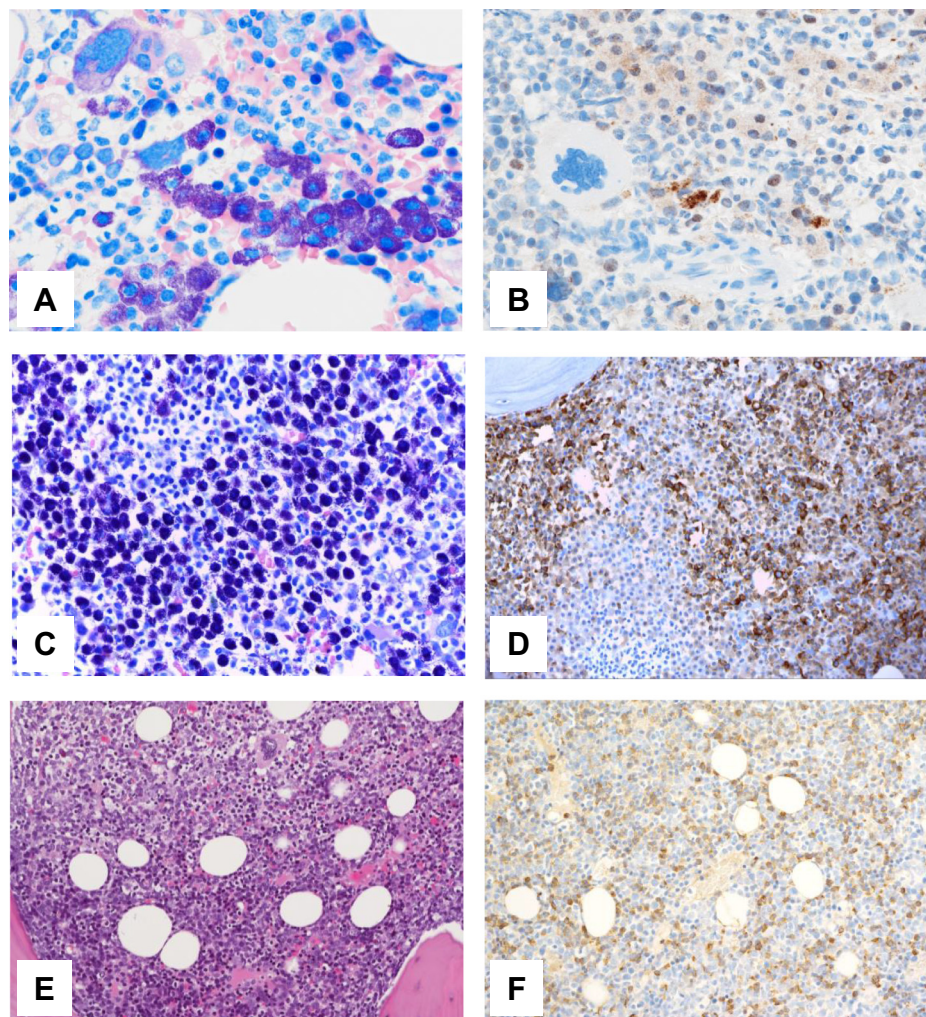


FIGURE 6. Diagnostic challenges in SM, continued 2. (A, B) WDSM in a 70-year-old male patient (see also Figure 2, E and F) with normal-appearing round and hypergranulated MC forming compact MC infiltrates. Of note, compact MC aggregates typically do not contain other cell types like eosinophils, lymphocytes, and fibroblasts and do not show a fibrotic background (Giemsa, $\times 400$). (B) Typically, there is no aberrant (membranous) expression of CD25 in MC in WDSM (CD25, $\times 400$). (C, D) aMCL-AML in a 62-year-old female patient with an MCL component of WD phenotype (C, Giemsa, $\times 200$) and myeloid blasts expressing myeloperoxidase, which is not expressed in neoplastic MC (center/left; D, MPOX, $\times 200$). (E, F) MML in a 53-year-old female patient with marked hypercellular BM with dysplastic changes (E, H&E, $\times 100$) and dense interstitial infiltrates of blastoid cells with mastocytic differentiation and expression of tryptase (F, tryptase, $\times 100$) and CD117 (not shown). True compact MC aggregates were not detectable; there was no aberrant expression of CD25, CD2, and CD30 and no detectable *KIT* mutation. *AML*, Acute myeloid leukemia; *BM*, bone marrow; *H&E*, hematoxylin and eosin; *MC*, mast cell; *MCL*, mast cell leukemia; *MML*, myelomastocytic leukemia; *MPOX*, myeloperoxidase; *SM*, systemic mastocytosis; *WD*, well-differentiated; *WDSM*, well-differentiated systemic mastocytosis.

SM-AHN may be established when SM criteria are met despite the absence of *KIT* p.D816V;^{54,55} detection of *PDGFRA*, *PDGFRB*, and *FGFR1* rearrangements is important in such cases. In the rare cases of MCL, a so-called diffuse-compact or packed MC infiltrate, significantly altering the pre-existing BM architecture, is present. Of note, ASM-AHN and MCL-AHN are much more frequent than pure ASM or pure MCL. Therefore, in cases with extensive BM MC infiltrates possibly obscuring the AHN component, blood findings like monocytosis or eosinophilia, or signs of myeloproliferation, must be recognized and may be an indication for the presence of a concomitant AHN (Figure 5, E and F).^{56,57} In these cases, extensive molecular and

cytogenetic studies also need to be performed. The association of MCL and AML represents a maximum diagnostic challenge for the hematologist and hematopathologist. Differential diagnoses include tryptase-positive AML and MML (Figure 6, C-F).²⁰ In contrast to ASM, almost always carrying the *KIT* p.D816V mutation, this mutation is present in less than 50% of patients with MCL.⁵⁸

In a minority of cases (<5%) exclusively round and well-granulated, mature-appearing MCs are present (Figure 6, A-D). Because this morphological phenotype of MCs can be present in any SM variant, it is regarded a morphological pattern and not a separate (new) variant.³ Notably, neoplastic MCs in WDSM

typically do not show aberrant CD25 expression and usually do not carry *KIT* codon 816 mutations, especially *KIT* p.D816V (Figure 6, B).⁵⁹ However, MCs in WDSM frequently show aberrant expression of CD30.^{5,60}

In some cases with aggregates of MCs and lymphocytes, this may pose a diagnostic challenge because immunophenotyping may reveal that the lymphocytic aggregates may represent a mixture of B cells and T cells, mimicking a lymphoplasmacytic lymphoma with an increase in MCs.

Although the morphology-based diagnostic criteria for SM were defined for the BM, they may be applied to SM infiltrates in other extracutaneous organs as well. At least, for MC infiltrates of the GI tract, a 5-tiered grading system with differing infiltration patterns has been proposed (Table E3, available in this article's Online Repository at www.jaci-inpractice.org). In biopsy samples of the GI tract, numerous loosely scattered MCs can be appreciated in the lamina propria mucosae when IHC with antibodies against tryptase and/or KIT (CD117) is applied and must not be confused with SM. Neoplastic MCs may, however, show an incomplete aberrant immunophenotype with variable and reduced expression of tryptase in up to 20% of cases.^{61,62}

CONCLUSIONS

Consensus diagnostic criteria and a global classification of mastocytosis have initially been developed during the year 2000 WoCo in Vienna and were then accepted and published by the WHO in its 2nd, 3rd, and 4th editions on the "Classification of Tumors of Haematopoietic and Lymphoid Tissues." Important issues related to BM pathology were presented during the Vienna 2020 WoCo on mastocytosis and were further addressed in pre- and post-conference discussions. Notably, BMM has been proposed as a new full SM subtype, ECM as a reintegrated disease variant, and 2 of the 4 minor diagnostic criteria have been updated, now allowing the (immunohistochemical and/or flow cytometric) detection of CD30 in MCs and the detection of activating *KIT* mutations outside codon 816 as minor criteria. Still diagnosis and subtyping of SM requires a thorough examination of BM specimens (trephine biopsy and aspirate from the iliac crest) by histo-/cytopathology, IHC, and molecular pathology and the integration of biochemical (tryptase) and clinical (B- and C-) findings. By its ability to address 4 of the 5 diagnostic criteria, pathology plays an integral part in the diagnosis and subtyping of SM, especially in AdvSM. In addition, pathology plays an important role in the histopathologic confirmation of the presence of B- and C-findings. Repeated follow-up BM biopsy investigations should be part of disease monitoring in patients with AdvSM.

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TABLE E1. MC infiltration pattern in SM^{E1}

Infiltration pattern	Description	SM variant(s)
(I) Focal/multifocal	Disseminated dense/compact MC infiltrates (usually associated with pattern II).	BMM, ISM, SM-AHN
(II) Diffuse-interstitial	Increase in loosely scattered MCs; usually also present in patients with multifocal infiltration; may occur in up to one-third of patients without compact infiltrates	BMM, ISM, SM-AHN
(III) Packed/diffuse-compact	Complete effacement of the pre-existing bone marrow	MCL, SSM, ASM
(IV) Mixed	Focal/multifocal and diffuse-interstitial	Any SM variant

AHN, Associated hematologic neoplasm; ASM, aggressive SM; BMM, bone marrow mastocytosis; ISM, indolent systemic mastocytosis; MCL, mast cell leukemia; SM, systemic mastocytosis.

TABLE E3. Grading of MC infiltration of the GI tract mucosa in SM^{E5}

Grade	Description
1	Loosely scattered clonal <i>KIT</i> p.D816V-positive MCs not increased in numbers
2	Slight increase in loosely scattered MCs, sometimes with tendency to form small groups (no compact MC infiltrates); most frequently encountered grade
3	Micronodular or band-like MC infiltrates in the (upper) mucosa; SM can be diagnosed
4	Diffuse-compact MC infiltration of the lamina propria mucosae; SM can be diagnosed with certainty
5	Sarcomatoid destructive (macroscopically visible) MC infiltration

ECNM, European Competence Network on Mastocytosis; GI, gastrointestinal; MCs, mast cells; SM, systemic mastocytosis.

TABLE E2. Morphologically defined mast cell subsets in bone marrow smears^{E2-E4}

MC type	Description	SM variant(s)
(A) Normal/reactive-appearing MCs	Mature-appearing MCs (in WDSM larger in size), round MCs, round to ovoid nucleus, no granulation defects	Normal/reactive MCs, BMM, ISM, WDSM
(B) Atypical MC type I	Spindle-shaped MCs Ovoid nucleus Variably hypogranulated	BMM, ISM, SSM, ASM, SM-AHN, (chronic) MCL
(C) Atypical MC type II (promastocytes)	Bi-/multilobated MCs, variably hypogranulated	SSM, ASM, (acute) MCL
(D) Metachromatic blast cell	Blastoid cell, high nuclear:cytoplasmic ratio, metachromatic granules, hypogranulation; indistinguishable from basophil precursors	(acute) MCL (rarely and only a minority of MCs), MML

AHN, Associated hematologic neoplasm; ASM, aggressive SM; BMM, bone marrow mastocytosis; ISM, indolent SM; MCL, mast cell leukemia; MCs, mast cells; MML, myelomastocytic leukemia; SM, systemic mastocytosis; SSM, smoldering SM; WDSM, well-differentiated SM.

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