

SOLVING CLINICAL PROBLEMS IN BLOOD DISEASES

A physician or group of physicians considers presentation and evolution of a real clinical case, reacting to clinical information and data (boldface type). This is followed by a discussion/commentary.

Myelomastocytic leukemia versus mast cell leukemia versus systemic mastocytosis associated with acute myeloid leukemia: A diagnostic challenge

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Case 1

A 35-year-old woman presented in September 2006 with progressive urticaria, which was refractory to increasing doses of antihistamines and prednisone. Features of urticaria pigmentosa were not present. She presented 11 months later with fatigue and petechiae. An initial bone marrow (BM) biopsy was hypercellular with left-shifted myelopoiesis, mild reticulin fibrosis, and diffuse infiltration by mast cells (MCs). No compact dense MC infiltrates were detected on BM biopsy.

Mastocytosis is currently defined as a clonal, neoplastic proliferation, and accumulation of MCs in one or multiple organs [1]. Clinical symptoms are caused by the release of chemical mediators and by infiltration of tissues by neoplastic MCs. In cutaneous mastocytosis, MC infiltration is limited to the skin and typically presents in childhood with urticarial symptoms. It has a benign clinical course and may regress spontaneously [2]. In adults, cutaneous disease is more frequently associated with indolent rather than aggressive forms of systemic mastocytosis (SM).

Although a diagnosis of SM was considered, the lack of compact dense aggregates was considered atypical, and full criteria for SM were not met. In the 2008 WHO classification, SM is diagnosed when one major criterion and one minor criterion, or at least three minor criteria are present (Table I). The major diagnostic criterion for SM is fulfilled by the presence of multifocal dense MC infiltrates (≥ 15 MCs per aggregate) detected histologically in the BM or in another extracutaneous organ(s). Minor criteria include: (1) $\geq 25\%$ of the MCs in the infiltrate are spindle-shaped or show atypical morphology or, $\geq 25\%$ are immature or atypical on BM aspirate smears; (2) a serum tryptase level persistently greater than 20 ng/mL (this parameter is not valid if there is an associated clonal myeloid disorder); (3) detection of the characteristic D816V *KIT* mutation in BM, blood, or another extracutaneous organ; and (4) expression of CD2, CD25, or both in neoplastic MCs [1–3]. Indolent forms of systemic MC disease may be characterized by one or more “B” findings (Table II). If two or more B findings are present the disease is called smoldering SM. Treatment of mediator-related symptoms, when present, is commonly employed in all SM variants, whereas cytoreductive agents are not prescribed. In contrast, aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL) are characterized by one or more “C” findings (Table II), which often require cytoreductive therapy. SM may also be associated with a clonal hematologic disease that is not of mast cell origin (SM-AHNMD), such as myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), acute leukemia, lymphoma, or

plasma cell myeloma. In SM-AHNMD, full WHO diagnostic criteria for both SM and the AHNMD are met [4].

A BM aspirate smear showed intermediate size blasts with open chromatin, single prominent nucleoli, and agranular basophilic cytoplasm and metachromatic blasts with large deeply basophilic granules (Fig. 1). An abdominal ultrasound showed no organomegaly.

MC morphology

Reactive tissue MC are small with round centrally located nuclei, indistinct to absent nucleoli, abundant cytoplasm, and faint cytoplasmic granules on H&E stained tissue sections; in Romanowsky stained smears, MCs contain tightly packed uniform metachromatic granules with round to oval shaped nuclei (Table III). Neoplastic MC morphology has been classified into three subtypes: (1) Metachromatic blasts with high nuclear to cytoplasmic ratio, fine nuclear chromatin, prominent nucleoli, and several metachromatic granules; (2) atypical MC type I with elongated cytoplasmic projections, oval eccentric nuclei, and hypogranulated cytoplasm; and (3) atypical MC type II with bi- or polylobed nuclei (Table III) [4,5]. Recognition of neoplastic MC morphology has been shown to correlate with clinical outcome [4]. Atypical MC type I are more commonly seen in SM with a clinically indolent course whereas atypical MC type II and metachromatic blasts are more common in patients with MML and MCL, which are associated with a poor prognosis and short survival [1,4,5].

In light of the similarities in morphology between MCs and basophils, the differential diagnosis of MML also includes

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TABLE I. 2008 World Health Organization Diagnostic Criteria for Systemic Mastocytosis^a

Major criterion	
Multifocal, dense infiltrates of MC (≥ 15 MC in aggregates) in BM and/or other extracutaneous organ(s)	
Minor criteria	
1.	>25% of MC in the infiltrate of biopsy sections are spindle-shaped or have atypical morphology or, of all MC in the BM aspirate smears, >25% are immature or atypical
2.	Activating point mutation at codon 816 of <i>KIT</i> in BM, blood, or another extracutaneous organ
3.	MC in BM, blood, or other extracutaneous organs express CD2 and/or CD25 in addition to normal MC markers
4.	Serum total tryptase persistently exceeds 20 ng/mL ^b

MC, mast cells; BM, bone marrow.

^a Diagnosis of systemic mastocytosis requires the presence of the major criterion and one minor criterion or at least three minor criteria.^b Serum tryptase is not valid as a criterion if there is an associated clonal myeloid disorder.**TABLE II. "B" and "C" Findings Associated with Advanced Systemic Mast Cell Neoplasms**

B: Findings	
1. Increased MC burden	>30% MC infiltration on BM biopsy and/or total serum tryptase level >20 ng/mL
2. Dysplasia or myeloproliferation	Hypercellular marrow, signs of myelodysplasia or myeloproliferation, and normal or slightly deviated blood counts without sufficient criteria to diagnose an AHNMD
3. Organomegaly	Palpable hepatomegaly without ascites or signs of liver dysfunction, palpable or radiologic lymphadenopathy (>2 cm), or palpable splenomegaly without hypersplenism
C: Findings	
1. Bone marrow dysfunction	ANC < $1.0 \times 10^9/L$, Hb < 10 g/dL, or platelets < $100 \times 10^9/L$
2. Liver dysfunction	Palpable hepatomegaly with impaired liver function, ascites, and/or portal hypertension
3. Skeletal organopathy	Large osteolytic lesions and/or pathological fractures
4. Splenic dysfunction	Palpable splenomegaly with hypersplenism
5. GI dysfunction	Malabsorption with weight loss and/or hypoalbuminemia

MC, mast cell(s); BM, bone marrow; AHNMD, associated clonal hematologic nonmast cell lineage disease; ANC, absolute neutrophil count; Hb, hemoglobin; GI, gastrointestinal.

basophilic leukemia (BAL), a very rare neoplasm accounting for <1% of all AML cases [1]. Neoplastic cells are medium-sized blasts with round or oval bilobed nuclei, basophilic cytoplasm, and a variable number of metachromatic granules, and are morphologically indistinguishable from metachromatic blasts seen in MML [1]. Blasts typically express myeloid markers including CD13 and CD33 and are usually negative for CD117 and chymase, and express only low amounts of tryptase, which differentiates them from cells of the MC lineage [1]. Definitive diagnosis of BAL is now more easily undertaken with the introduction of basophil-specific immunohistochemical antibodies 2D7 and BB1 [6].

Two months later, she was admitted to the hospital for febrile neutropenia. A serum tryptase level was 156 ng/mL (reference range <12 ng/mL). A complete blood count (CBC) showed neutropenia with an absolute neutrophil count of $0.4 \times 10^9/L$ and thrombocytopenia with a platelet count of $42 \times 10^9/L$. A peripheral blood smear contained 10% blasts and <1% metachromatic blasts (Table IV). On BM aspirate smears, 9% blasts and 5% metachromatic blasts were enumerated and multilineage dysplasia was seen; however, aspirate smears lacked particles and appeared hemodilute (Table IV). BM biopsy showed 5% immature mononuclear cells resembling blasts that expressed MPO, and dim CD117, and lacked CD34 and tryptase. The marrow was also infiltrated in an

interstitial pattern by immature MCs (50%), expressing tryptase, CD25, and CD117, and lacking CD34 (Fig. 1). No dense MC aggregates were seen. Flow cytometry identified two distinct populations in the BM: a myeloblast population expressing CD13, CD33, dim CD45, CD117, and MPO, but lacking CD34, and a MC population expressing CD25 and CD117, and lacking CD34 and MPO. PCR was negative for the D816V *KIT* mutation by allele-specific PCR [7] and cytogenetics showed a normal karyotype (46,XX). The patient subsequently developed respiratory failure and was transferred to the intensive care unit where she received cladribine chemotherapy. Although recovering from respiratory failure, the patient continued to be pancytopenic after cladribine therapy and became febrile 2 months later with *Enterococcus* bacteremia. She was noted to have massive hepatomegaly and a biopsy of the liver showed diffuse infiltration by immature mononuclear cells, which expressed MPO but not CD34. A population of tryptase positive MCs was also identified (10%). A repeat BM biopsy was performed and was consistent with the liver biopsy, both suggestive of leukemic transformation.

Myelomastocytic leukemia

Myelomastocytic leukemia (MML) is a rare disease that has previously been described in patients with advanced myeloid neoplasms (most commonly refractory anemia with excess blasts, a subtype of MDS, or AML) with elevated numbers of immature atypical MCs who do not meet full criteria for SM [8]. MML typically presents with an increase in myeloblasts (>5%) as well as >10% metachromatic blasts in the peripheral blood and/or BM but without focal dense MC infiltrates, without MC expression of CD2 or CD25, and without evidence of the D816V *KIT* mutation by PCR [2,3,8,9]. Similar to patients with MDS or AML without MML, dysplastic features are seen in one or more myeloid cell lineages [3]. Mature MCs and basophils may also be observed. In Case 1, the finding of numerous metachromatic blasts together with the diffuse infiltration pattern suggested either MML or MCL, as metachromatic blasts are rarely observed in SM or SM-AHNMD (Table III). Although CD25 expression was detected on MCs and MCs were atypical (2 minor criteria), the patient lacked the following findings: (1) focal dense MC aggregates; (2) an activating codon 816 *KIT* mutation; and (3) serum tryptase level was excluded from consideration given that a clonal myeloid cell population was present. Therefore, full criteria for SM were not met which also excluded MCL from the differential diagnosis. Metachromatic blasts were clearly MCs, not basophils, as these cells expressed high levels of KIT and tryptase. Histopathologic features included: (1) 5% metachromatic blasts in BM smears; (2) greater than 5% blasts in peripheral blood/BM smears; and (3) an interstitial MC infiltrate on biopsy. Immunophenotyping confirmed both myeloblast and MC populations, of which the latter appeared to be greater than 30% on the BM biopsy; this discrepancy with the percentage of metachromatic blasts in the BM smears appears due to the lack of particles present on the smears. Thus, Case 1 had findings diagnostic of MML with the unusual finding of CD25 expression and a normal karyotype, findings not described previously in MML.

Our review of the literature identified seven cases of MML [9–12]. Three of these cases were published in 1994, before the term "myelomastocytic leukemia" was adopted, but the reported clinical and histopathologic features were highly suggestive of MML. Two patients had MDS/RAEB and one patient had blast crisis of chronic myeloid leukemia. All patients had increased blasts and metachromatic cells, complex karyotypes, a poor prognosis and did not

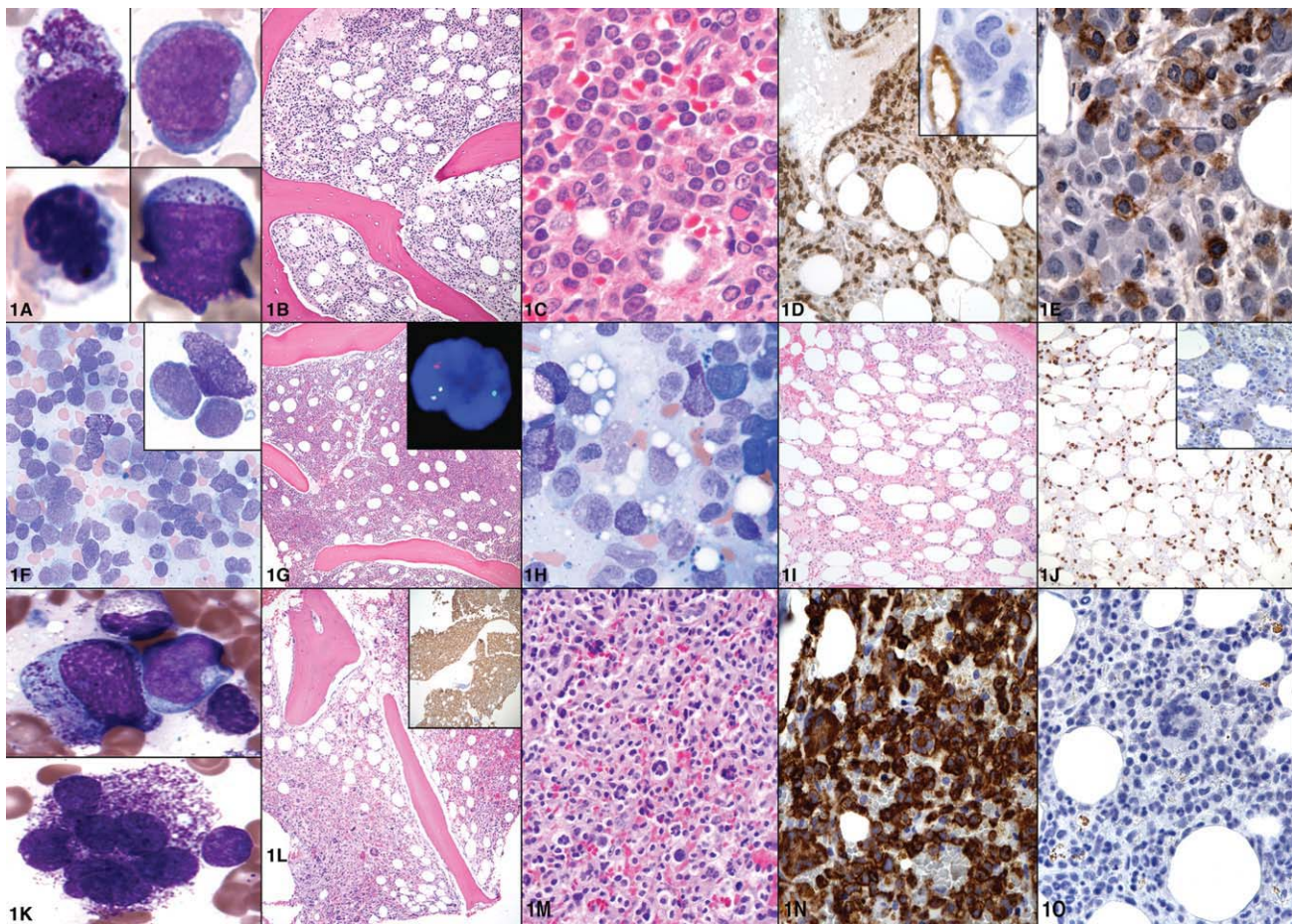


Figure 1. Top row: Case 1, myelomastocytic leukemia; (A) BM aspirate demonstrates a metachromatic blast with abundant metachromatic granules, immature nuclear chromatin, and high N:C ratio (top left), a myeloblast with high N:C ratio, fine nuclear chromatin, and scant basophilic cytoplasm (top right), a dysplastic erythroid precursor with an enlarged nucleus, irregular nuclear contours, and pale basophilic cytoplasm (bottom left), and another metachromatic blast with an enlarged nucleus, fine nuclear chromatin, scant basophilic cytoplasm, and azurophilic granules (bottom right). (B) $\times 100$ view of BM biopsy demonstrating no focal lesions (C) $\times 400$ H&E BM biopsy demonstrating immature mononuclear cells with some cells showing granulocytic differentiation. (D) $\times 400$ BM biopsy with IHC staining for tryptase highlighting the interstitial pattern of MC infiltration, an immunohistochemical stain for CD34 was negative (inset); (E) $\times 400$ BM biopsy with IHC staining for CD25 showing cytoplasmic staining in scattered interstitial MC and no staining in adjacent blasts. Middle row: Case 2, myelomastocytic transformation in AML; (F) diagnostic BM aspirate showing numerous enlarged myeloblasts with high N:C ratio, fine nuclear chromatin, and scant basophilic cytoplasm along with scattered atypical MC (inset). (G) $\times 100$ H&E diagnostic BM biopsy showing hypercellularity, FISH analysis demonstrated interphase nuclei with 1 red, 1 green, and 2 fusion signals, consistent with the t(8:21)(q22;q22) translocation fusion product (inset). (H) $\times 400$ postinduction BM aspirate showing numerous MC with enlarged bilobed nuclei and abundant cytoplasmic granules without increased blasts. (I) $\times 200$ H&E postinduction BM biopsy showing a marked decrease in cellularity from the previous biopsy. (J) $\times 200$ postinduction BM biopsy with IHC staining for mast cell tryptase which highlights the interstitial pattern of MC infiltration, an immunohistochemical stain for CD25 showed absent expression in mast cells and blasts (inset). Bottom row: Case 3, MCL; (K) $\times 100$ BM aspirate showing metachromatic blast with enlarged nucleus, fine nuclear chromatin, basophilic cytoplasm, and scattered metachromatic granules (top) and multinucleated MC with small round nuclei, fine nuclear chromatin, and abundant cytoplasmic granules (bottom). (L) $\times 100$ H&E BM biopsy showing hypercellularity and scattered giant cells, $\times 100$ BM biopsy with IHC staining for mast cell tryptase, which highlights the diffuse pattern of MC infiltration (inset). (M) $\times 400$ H&E BM biopsy showing numerous immature mononuclear cells and occasional multinucleated cells. (N) $\times 400$ BM biopsy with IHC staining for CD117 showing strong membranous and cytoplasmic staining. (O) $\times 200$ BM biopsy with IHC staining for CD25 showing lack of expression in MC. BM, bone marrow; N:C, nuclear to cytoplasmic; H&E, hematoxylin and eosin; IHC, immunohistochemical; MC, mast cell(s); FISH, fluorescence in situ hybridization; MCL, mast cell leukemia.

TABLE III. Mast Cell Morphology

MC type	Size/shape	Nucleus	Nuclear chromatin	Cytoplasm	Nuclear to cytoplasmic ratio	Associated disorders
Reactive MC	Small-medium, round or oval	Central, round or oval	Condensed	Well granulated, may be hypo-/degranulated	Low	Reactive mastocytosis
Atypical MC type I	Elongated cytoplasmic extensions (spindle shaped)	Central or eccentric, oval	Condensed	Hypogranulated, focal granule accumulation without degranulation	Variable	SM, SM-AHNMD
Atypical MC type II	Variable	Bi-/polylobed	Fine or condensed	Hypogranulated without degranulation	Variable	MCL, MML
Metachromatic ^a blast	Medium-large, round or oval	Prominent nucleoli	Fine	Few metachromatic granules	High	MCL, MML

ASM, aggressive systemic mastocytosis; SM-AHNMD, systemic mastocytosis with an associated clonal hematological nonmast cell lineage disease; MCL, mast cell leukemia; MML, myelomastocytic leukemia; MC, mast cell(s); BM, bone marrow.

^a Metachromatic: a cell component that characteristically takes on a color different from that of the dye with which it is stained.

TABLE IV. Clinical, Laboratory and Pathologic Data of 3 Patients with Advanced Mast Cell Neoplasms

Case	1	2	3
Age/sex	32 F	64 M	61 F
Blast %, PB/BM smears	10%/9% ^a	80%/78%	0%/2%
MC %, PB/BM smears	<1%/5% ^a	0%/5%, 20% ^b	3.5%/68%
MC morphology	Metachromatic blasts	Atypical MC type II	Metachromatic blasts, giant multinucleated MC
BM biopsy MC pattern	Interstitial (diffuse)	Interstitial (diffuse)	Diffuse with focal dense aggregates
Serum tryptase (ng/mL) ^c	156	55	467
CD25 ^d	+	—	—
Cytogenetic results	46,XX	t(8;21)(q22;q22)	46,XX
Activating D816V <i>KIT</i> mutation	Absent	Absent ^e	Absent
Proposed diagnosis	MML	Transformation to myelomastocytic leukemia (MML) in the setting of AML	MCL

PB, peripheral blood; BM, bone marrow; MC, mast cell(s); MCL, mast cell leukemia; MML, myelomastocytic leukemia.

Percentage of blasts and mast cells reflect percentage of all nucleated cells.

^a BM aspirate smears lacked particles and appeared hemodilute.

^b 5% MC enumerated on initial BM aspirate smears; 25% MC on subsequent postinduction BM aspirate smears.

^c Reference range <11.5 ng/mL.

^d All MCs were CD2 negative.

^e N822K *KIT* mutation detected.

meet criteria for SM [12]. The remaining four cases were described between 1999 and 2006 [9–11]. All cases described the presence of metachromatic blasts. Two cases exhibited elevated blasts, diffuse MC infiltrates, and the absence of the D816V *KIT* mutation [9,10]. One patient had a diagnosis of MDS/RAEB with complex karyotype [10]. The other patient had AML with t(8;21) variant and was shown to have *AML1/ETO* fusion genes in both myeloblasts and MC [9]. The remaining two cases were reported to have findings of MML and did not meet criteria for SM; however, specific data was not provided by the authors [11].

Her clinical status rapidly deteriorated and she expired shortly thereafter, ~2 years after initial presentation. The diagnosis of MML was made posthumously.

Advanced MC neoplasms are notoriously difficult to manage and no curative treatments are currently available. Treatment for advanced SM may include standard therapy for mediator symptoms [4] along with cytoreductive therapy including interferon- α with or without corticosteroids, and cladribine in those who have slowly progressing ASM [6]. However, major and partial responses are observed in only a subset of patients [4,6]. The initial three cases of MML described had complex karyotypes and poor prognosis [12]. In the more recent literature, one patient with MML went into complete remission with polychemotherapy [10], and another patient went into complete remission following myeloablative stem cell transplantation [9].

Case 2

A 64-year-old man with a medical history of diabetes mellitus, coronary artery disease, and hypertension presented to an outside hospital with fever, fatigue, and left flank pain in late August 2007. A BM biopsy showed a normocellular marrow with increased myeloblasts. He was initially diagnosed with myelodysplastic syndrome and was transfused four units of packed red blood cells. He was subsequently evaluated at Stanford Hospital where a CBC revealed a white blood cell (WBC) count of $32.5 \times 10^9/L$, hemoglobin 8.6 g/dL, and

platelet count $12 \times 10^9/L$. The peripheral blood smear showed 80% blasts, and no circulating MCs (Table IV). Blasts were intermediate to large in size with slightly irregular nuclear membranes, sparse cytoplasmic granulation, occasional large cytoplasmic salmon granules, and smooth chromatin. A BM biopsy exhibited hypercellularity and 78% blasts were enumerated on aspirate smears (Fig. 1, Table IV). Blasts expressed myeloid markers (CD15, dim CD33, CD34, CD38, CD117, and MPO) as well as HLA-DR and CD19.

A diagnosis of AML was rendered. Cytogenetic analysis showed t(8;21)(q22;q22), representing the *RUNX1-RUNX1T1* rearrangement. Induction chemotherapy was initiated with cytarabine, daunorubicin, and p-glycoprotein inhibitor zosuquidar on a clinical trial basis.

A postinduction BM unmasked a previously unrecognized interstitial (diffuse) MC infiltrate (20% MCs) and <5% blasts (Table IV). MCs were round with bilobed nuclei and fine granules (Fig. 1). MCs expressed CD117 and tryptase, and lacked expression of CD25 and CD2 (Fig. 1). PCR revealed the N822K *KIT* mutation [7], previously reported to be imatinib-sensitive [13]. A serum tryptase level was elevated at 55 ng/mL (reference range <11.5 ng/mL). Complete remission of AML was achieved with induction chemotherapy. At this time, <1% blasts and 5% MCs were enumerated on the aspirate, and the *RUNX1-RUNX1T1* rearrangement was detected by FISH in cells with bilobed nuclei (data not shown). He received three cycles of high-dose cytarabine consolidation chemotherapy with concurrent imatinib. Surveillance BM biopsies at 1 and 7 months postinduction were free of leukemia and contained <5% MCs. At 11 months postinduction, he was found to have 25% blasts on a subsequent peripheral blood smear and a BM aspirate and biopsy showed recurrent AML with 33% BM blasts and 5% MCs. Cytogenetic analysis again showed t(8;21)(q22;q22) with *RUNX1-RUNX1T1* rearrangement. A repeat serum tryptase level was normal at 6.47 ng/mL.

Systemic mastocytosis with acute myeloid leukemia

Approximately 20–30% of patients with SM will develop a second hematologic disease, which is usually a clonal myeloid disorder [6,14]. The pathogenesis of solitary AML is a multistep process that involves impaired myeloid differentiation which leads to acute leukemia when other mutations, commonly involving *KIT* and other receptor tyrosine kinases, are acquired over time [15]. Dash and Gilliland have proposed a two-step theory for the pathogenesis of AML: (1) a constitutively activated tyrosine kinase results in proliferative and/or antiapoptotic activity to hematologic cells with no affect on cellular differentiation and (2) acquired transcription factor fusion occurs which has limited effect on proliferation but impairs normal hematologic differentiation [16]. *KIT* mutations are associated with enhanced proliferation of MCs and are frequently detectable in a group of patients with core-factor binding leukemias, such as AML with inv(16) or t(8;21)(q22;q22). In such cases, D816V *KIT* is the most common mutation found [2,13,17].

Case 2 was a diagnostic challenge because it appeared to be a case of t(8;21) AML with unmasking of SM after induction chemotherapy. However, it lacked the following key findings of SM: (1) focal dense MC infiltrates on BM biopsy, (2) the activating codon D816V *KIT* mutation, (3) expression of CD2 and/or CD25 on MC, and (4) serum tryptase level could not be considered due to the presence of the associated clonal myeloid neoplasm, AML. Therefore, this case did not meet criteria for the diagnosis of SM. The observed *KIT* mutation, N822K, has been reported in

the context of AML but so far has not been reported to occur in patients with SM. Whether this *KIT* mutation was expressed in neoplastic MC and/or played a role in the myelomastocytic transformation in this patient remains unknown. The initial BM smears showed 5% MCs, with 20% MCs seen on postinduction smears. At the time of first relapse, the patient presented with both increased blasts and MCs. All these results suggest that the patient developed a myelomastocytic transformation occurring in AML, with eventual development of frank MML.

Although Case 2 is most representative of transformation to MML in the setting of AML, SM-AML was strongly considered. A review of the literature identified seven cases of SM-AML with t(8;21)(q22;q22), all of which met full WHO criteria for SM [15,17–21]. In only one patient did mastocytosis chronologically precede the AML. In four cases, the presence of MC was masked by excessive blast accumulation at the time of diagnosis, and in two cases the diagnoses were concurrent. Complete remission of AML after polychemotherapy was achieved in four patients. Response of the SM component to polychemotherapy was variable. Stem cell transplantation was employed and resulted in complete remission in two patients, and four patients died of their disease despite treatment; two patients at 3 years, one at 9 years, and 1 at 6 months after the initial diagnosis was made. There is also one published report on a patient with AML with t(8;21) with myelomastocytic leukemia [9]. This patient also received a stem cell transplant. Interestingly, in contrast to cases with SM-AML, this particular patient went into complete continuous remission for both AML and the MC disease component [9].

The clonal origin of neoplastic MCs

A few studies have evaluated the clonal origin of MCs in advanced MC neoplasms such as MML and SM-AHNMD and their relationship to the myeloid leukemic blasts [9,15,17,19,21]. Sperr et al. addressed the clonal origin of MCs in MML using FISH analysis with probes specific for *AML1/ETO (RUNX1-RUNX1T1)*. FISH positivity was found in both tryptase-negative blasts and in tryptase-positive MCs, supporting the idea that MCs and myeloblasts originated from the same clone in this patient [9]. Published data in SM-AML has been mixed with respect to the clonal origin of MC. In SM-AML, Sperr et al. demonstrated the presence of the D816V *KIT* mutation in MCs but not in blasts from the same patient, suggesting either different clonal origins of SM and AML or two separately developed subclones in these patients [15]. Another case report confirmed the presence of t(8;21)(q22;q22), representing the *RUNX1-RUNX1T1* rearrangement, in blasts but not in aberrant MCs, and concluded that no definite statement regarding the relationship between blasts and MCs could be made [17]. Pullarkat et al. used FISH on MCs identified using either a fluorescence sorting or image analyzer to determine the clonal origin of MCs in a patient with SM-AML who underwent hematopoietic stem cell transplant [19]. Evaluation of BM samples before HSCT and at 40 days, 3 months, and 1 year thereafter revealed the *RUNX1-RUNX1T1* rearrangement, in 82%, 95%, 71%, and 86% of MCs, respectively, but not in other cell lines, consistent with a common origin of MCs with the leukemic clone [19]. While these studies provide some insight into the clonal origin of neoplastic MCs, there is no consensus about whether MCs and myeloblasts are derived from a common or distinct hematopoietic progenitor. Techniques such as single cell laser capture microdissection and more detailed molecular studies may provide additional insights and technical inroads in this regard [22].

He underwent re-induction chemotherapy with one cycle of cytarabine and clofarabine on a clinical trial

protocol. A BM obtained after reinduction showed no evidence of residual leukemia and no elevated MCs. He received a stem cell transplantation from an HLA-matched sibling, then relapsed with acute leukemia and went to hospice; BM sampling was not performed at the time of relapse.

SM-AHNMD is treated in a dichotomous fashion with the AHNMD being addressed as if no SM were present, and vice versa [4]. Ustun et al. recently described a patient with SM-AML who was initially treated with chemotherapy and high-dose cytarabine resulting in hematologic complete remission with persistent MCs in the BM, and persistent lytic lesions on radiographic evaluation [23]. Because of the unavailability of allogeneic stem cell transplantation, the patient was consolidated with dasatinib and four consolidation cycles of high-dose cytarabine, resulting in a decrease in MC numbers by 50%, improvement in lytic lesions, and absence of the D816V *KIT* mutation detection on follow-up BM evaluations [23]. If the D816V *KIT* mutation can be identified in both the SM and AHNMD clones, there may be a role for these newer *KIT* inhibitors for treating both disease components. However, as single agent therapy with tyrosine kinase inhibitors (TKI) does not seem to eradicate neoplastic cells, future treatment approaches are likely to combine these TKI with standard treatment such as cladribine or polychemotherapy.

Case 3

A 61 year-old woman presented with right upper quadrant abdominal pain, nausea, and vomiting. An abdominal ultrasound showed hepatomegaly and a CBC was notable for a WBC count of $2 \times 10^9/L$, hemoglobin 9.6 g/dL, and platelet count of $18 \times 10^9/L$, with circulating MCs (3.5%) on peripheral smear (Table IV). A BM biopsy showed a hypercellular marrow with 68% MCs and 2% blasts on aspirate smears (Table IV). BM touch preparation showed immature MCs with smooth nuclear chromatin and oval nuclei with occasional nuclear irregularities as well as multinucleate forms (Fig. 1). MCs expressed tryptase, CD68, and CD117 and lacked expression of S100, CD1a, CD2, and CD25 (Fig. 1). Cytogenetic analysis showed a normal (46,XX) karyotype. BM was negative for the KIT D816V mutation by allele-specific PCR [7].

A presumptive diagnosis of MCL was made. MCL meets WHO criteria for SM, and is characterized by $\geq 20\%$ MCs in the BM aspirate smears, with dense focal and diffuse infiltrates of atypical immature MC in the BM biopsy [2].

Ten days after diagnosis, a 7-day course of cladribine was initiated. She remained persistently pancytopenic without circulating MCs. A follow-up BM biopsy after 8 weeks demonstrated 80% MCs and scattered multinucleated giant MCs with an immunophenotype showing tryptase and CD117 positivity, but lacking expression of CD2 and CD25. MCs showed an interstitial and focally diffuse pattern on BM biopsy. She was treated with induction chemotherapy consisting of daunorubicin and cytarabine. A postinduction BM biopsy showed persistent hypercellularity with $>95\%$ MCs. She was started on imatinib and prednisone for 2 weeks without clinical or laboratory improvement. She was subsequently initiated on a phase 2 clinical trial of the *KIT* tyrosine kinase inhibitor midostaurin. At that time, a serum tryptase level was elevated at 467 ng/mL. A peripheral blood smear after a 1-month cycle of midostaurin showed $>10\%$ circulating MCs and a BM specimen showed a hypercellular marrow with diffusely infiltrating MCs (90%) with a stable expression pattern from previous biopsies. A repeat serum tryptase level was markedly increased at 1913 ng/mL.

TABLE V. Histopathologic and Laboratory Criteria for Advanced Systemic Mast Cell Neoplasms

	ASM	SM-AHNMD	MCL	MML
Peripheral blood				
MC	—	—	+/- ^a	+
Blasts	—	+/-	—	+
Bone marrow				
Aspirate smear ^b		AHNMD findings	>20% Immature or atypical MC ^c <5% blasts	>10% MC ^c >5% blasts
>25% MC of total MC immature/atypical in smears or >25% MC of total MC spindled/atypical in biopsy	+	+	+	+
MC infiltrate in biopsy	Focal dense aggregates	Focal dense aggregates	Dense, interstitial and/or diffuse	Interstitial
Molecular genetic findings				
D816V activating <i>KIT</i> mutation in blood, BM, or other extracutaneous organ	+	+	+	—
MC immunophenotype				
Tryptase and CD117	+	+	+	+
CD25 and/or CD2	+	+	+	—
Serum markers				
Serum tryptase (ng/mL)	>20	>20	Usually >100	Usually >100

ASM, aggressive systemic mastocytosis; SM-AHNMD, systemic mastocytosis with an associated clonal hematological nonmast cell lineage disease; MCL, mast cell leukemia; MML, myelomastocytic leukemia; MC, mast cell(s); BM, bone marrow.

^a Less than 10% circulating MC is classified as aleukemic MCL.

^b Percentages in aspirate smears refer to percentage of all nucleated cells.

^c MC may include metachromatic blasts.

She was deemed to have progressive disease, was discontinued from the trial, and expired 5 months after initial presentation.

MC leukemia

MCL is a rare subtype of SM characterized by leukemic spread of MC and an aggressive rapidly progressing clinical course [24]. MCL, by definition, meets criteria for SM with additional features including (1) leukemic infiltration of the BM and other extracutaneous organs by neoplastic MCs, (2) at least 20% MCs in BM and/or blood smears, and (3) high-grade cytologic findings [1,18]. Case 3 met criteria for SM given that diffuse and multifocal dense MC infiltrates were present, >25% of all MC were immature/atypical and serum tryptase was persistently elevated, and no associated clonal myeloid disorder was found. "C" findings are commonly associated with MCL and in this case included BM dysfunction characterized by persistent pancytopenia and transfusion dependence, as well as hypalbuminemia.

MCL is the only advanced mastocytic disorder for which a cytologic diagnosis on an aspirate smear preparation is required to make the diagnosis [25]. A review of the literature by Noack et al. in 2004 identified <50 cases of MCL, which were reduced to 17 cases when strict WHO criteria were applied [24]. The clinical presentation and median overall survival in these cases was similar to that of our patient. Our review of the literature identified a more recent report of 10 cases of MCL, which met WHO criteria for MCL [25]. Three patients had typical MCL and seven patients had the aleukemic variant, as defined by <10% circulating MCs. Molecular biology studies identified the D816V *KIT* mutation in six patients and one patient had a silent C2415T *KIT* mutation.

Nine patients had MCs that expressed CD2 and/or CD25. This contrasts with our Case 3 which lacked both CD2 and CD25 expression. Various types of treatments were employed including corticosteroids, imatinib, cytarabine, α -interferon, polychemotherapy, and stem cell transplantation with complete remission being achieved in only one patient who underwent allogeneic stem cell transplantation after failure of two previous treatments [25].

MCL is a WHO defined entity; however, its description in this classification may lead to confusion. Specifically, to satisfy the major WHO criterion for SM, multifocal dense infiltrates must be present, whereas MCL is defined by diffuse MC infiltrates [1]. Thus, in MCL it is important to note that the MC infiltrate is dense and multifocal but also has an additional diffuse component (mixed pattern). MCs typically show marked atypia, serum tryptase levels are persistently elevated, and MCs often express CD2 and/or CD25. Moreover, in >50% of all cases, the *KIT* mutation D816V is detectable. Therefore, even in the absence of multifocal dense infiltrates, most cases of MCL would likely meet three of the minor criteria as is required for the WHO diagnosis of SM.

Although the aggressive nature of MCL commands early consideration of polychemotherapy and/or BM transplantation, the experience with these therapeutic modalities is limited in scope and has generally not been successful. Novel tyrosine kinase inhibitors, including midostaurin and dasatinib, have exhibited partial remitting activity in patients with advanced SM characterized by improvement in one or more C findings [26–28].

Advanced MC neoplasms are a group of heterogeneous disorders that often share a similar clinical presentation, which does not allow them to be distinguished on the basis of clinical findings alone. Therefore, histopathology and molecular genetic studies play a key role in the diagnosis of these neoplasms (Table V) and can provide useful information about the disease prognosis and potential treatment options. The diagnosis of any MC neoplasm requires a high degree of clinical suspicion and collaboration with a pathologist with specific expertise in evaluating the histopathology of these rare tumors. The current diagnostic criteria are helpful in assisting with the diagnosis of these myeloproliferative neoplasms. However, as with our experience, some challenging cases and more recently described variants such as MML do not fit into the current WHO classification scheme.

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