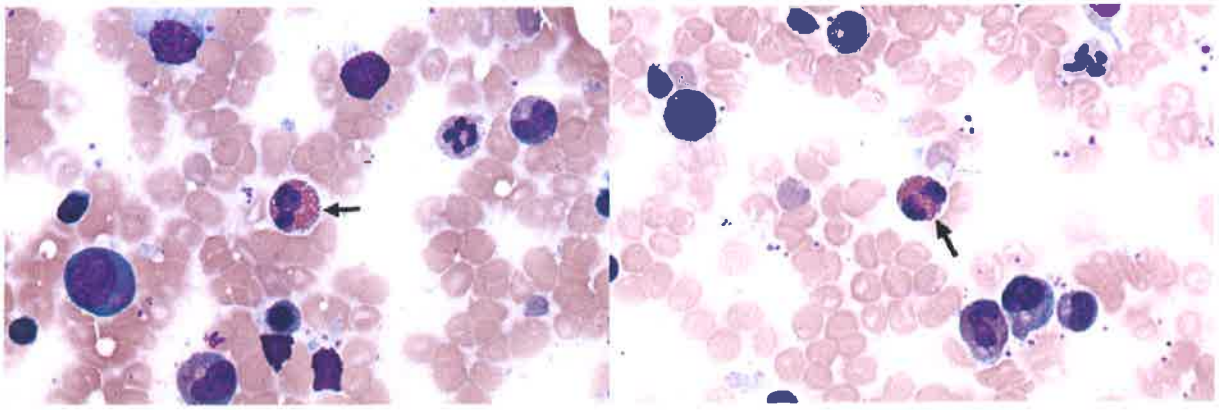
Cell Identification

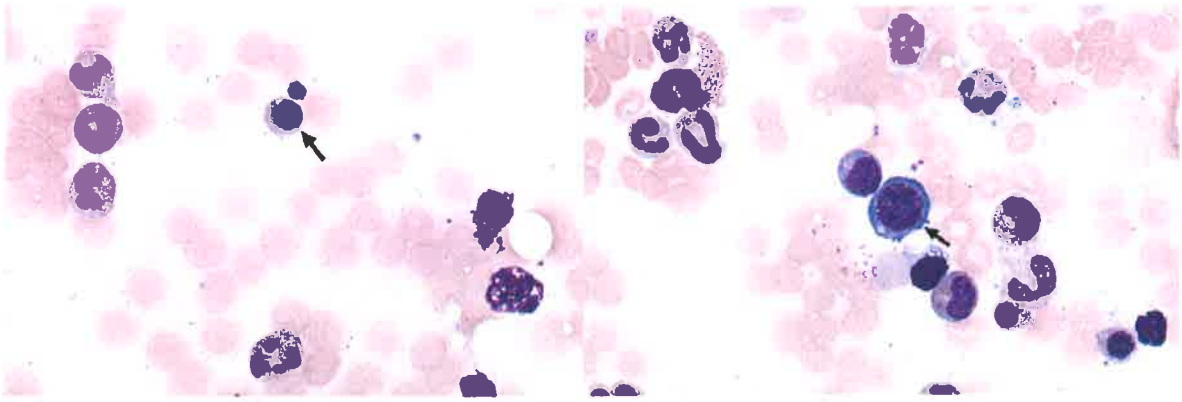
BMD-02



Identification	Participants No.	%	Evaluation
Eosinophil, any stage	282	97.9	Educational
Eosinophil, any stage with atypical/basophilic granulation	3	1.0	Educational
Basophil, any stage	1	0.3	Educational
Erythrocyte	1	0.3	Educational
Neutrophil, segmented or band	1	0.3	Educational

The arrowed cells are eosinophils, as correctly identified by 97.9% of participants. Eosinophils are recognizable by their numerous coarse orange-red granules, which have a refractile appearance under the microscope due to their crystalline structure. They are larger than neutrophil granules. Most mature eosinophils will have 2 nuclear lobes. However, about 20% will have 3 or more lobes. In the bone marrow immature eosinophils such as eosinophilic myelocytes can be seen. These may contain a few dark purple granules admixed with the normal orange-red granules.

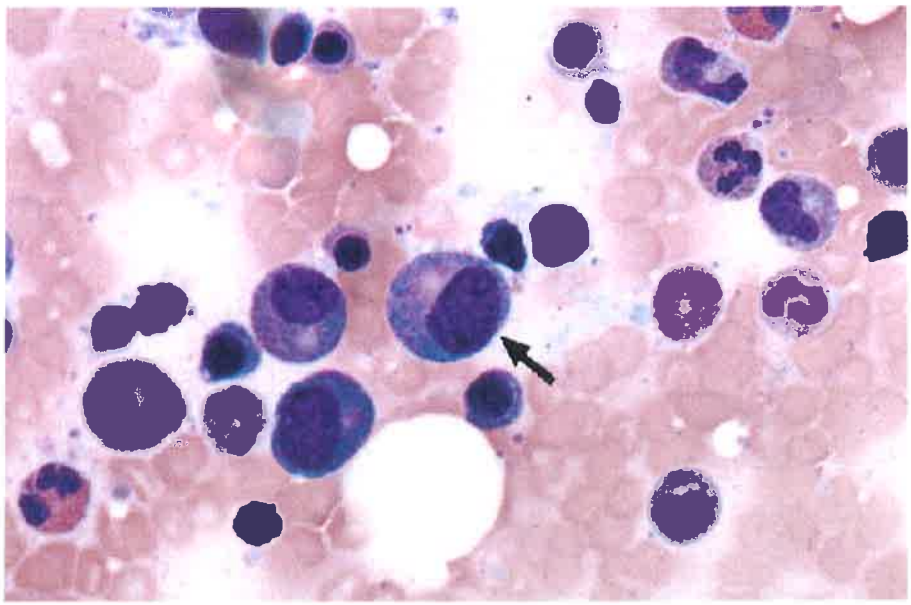
BMD-03



Identification	Participants No.	%	Evaluation
Erythrocyte precursor, normal (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	281	97.6	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	3	1.0	Educational
Erythrocyte precursor with megaloblastic changes/maturation	2	0.7	Educational
Erythrocyte	1	0.3	Educational
Erythrocyte precursor with vacuolated cytoplasm	1	0.3	Educational

The arrowed cells are erythrocyte precursors, normal (polychromatophilic and basophilc normoblasts), as correctly identified by 97.6% of participants. Polychromatophilic normoblasts are the next most mature of the nucleated erythroid precursors, characterized by well-rounded nuclei with condensed chromatin, producing a checkerboard or cartwheel appearance. In distinction to the more immature basophilic normoblast erythroid precursors, polychromatophilic normoblasts have a far less basophilic cytoplasm, owing to a greater degree of hemoglobinization, producing a gray colored cytoplasm. Orthochromatic normoblasts (the next most mature erythroid precursors), in contrast, have much more prominent hemoglobinization, resulting in a more characteristic erythroid (ergo orthochromatic) pink-colored cytoplasm.

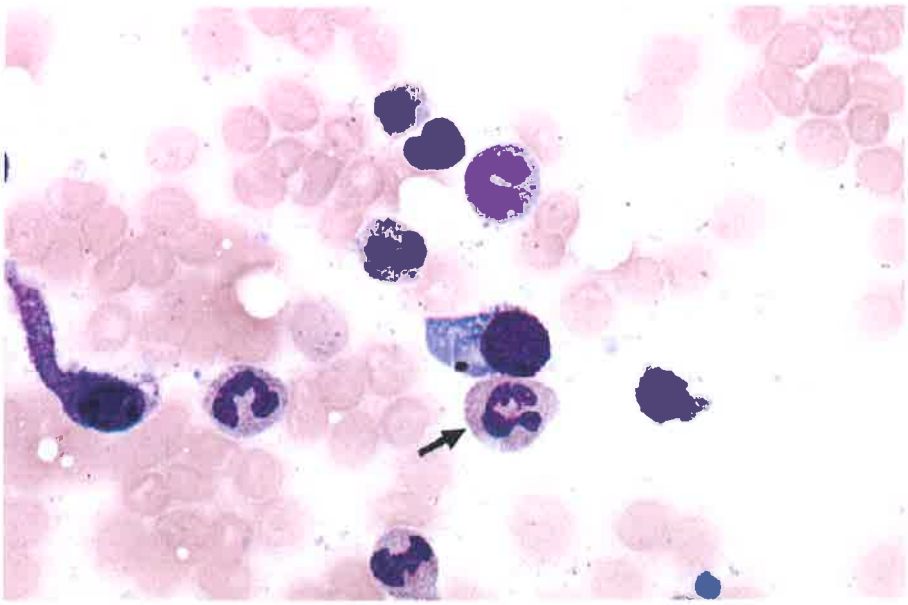
BMD-04



Identification	Participants No.	%	Evaluation
Neutrophil, myelocyte	248	86.1	Educational
Neutrophil, promyelocyte	27	9.4	Educational
Plasma cell (to include morphologically mature, abnormal, and containing inclusion, eg, Dutcher body, Russell body, etc)	7	2.4	Educational
Neutrophil, metamyelocyte	6	2.1	Educational

The arrowed cell is a neutrophil, myelocyte, as correctly identified by 86.1% participants. Myelocytes are usually confined to the bone marrow but may be seen in the blood as part of a left shift or other pathological states. They comprise approximately 10% of bone marrow cells and are 10 - 18 microns with a round nucleus that may be flattened on one side. The nuclear to cytoplasmic ration is 1:1 – 2:1 and is eccentrically placed. The chromatin is slightly condensed and a nucleolus is absent. The cytoplasm contains specific granules that are admixed with primary granules and a perinuclear hof or clearing can be seen. Specific granules increase and primary granules decrease as the cells mature through this stage. Although one may consider the arrowed cell as a promyelocyte, it is beginning to demonstrate specific granules, has a lower N:C ratio, and oval-shaped nucleus - all of which are in keeping with a myelocyte.

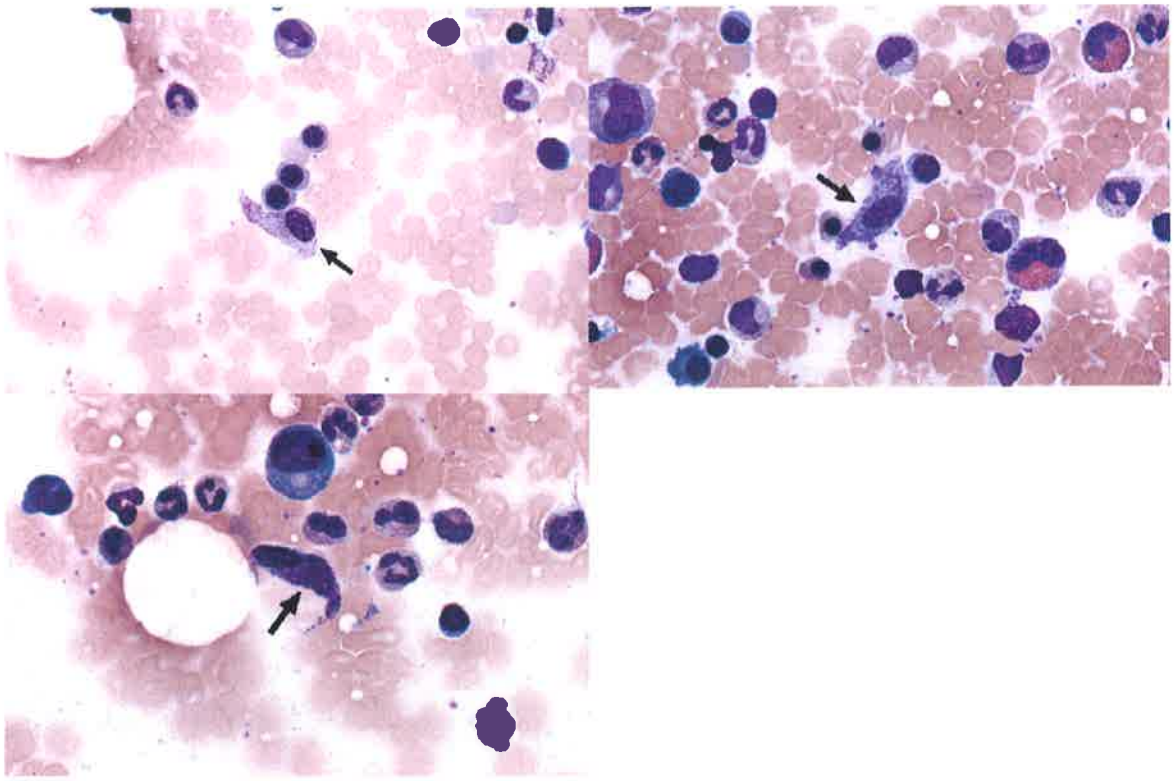
BMD-05



Identification	Participants No.	%	Evaluation
Neutrophil, segmented or band	287	99.7	Educational
Eosinophil, any stage with atypical/basophilic granulation	1	0.3	Educational

The arrowed cell is segmented neutrophil, as correctly identified by 99.7% participants. Segmented neutrophils are the most mature form of neutrophil and represent 12 - 25% of bone marrow cells. They are 10 - 15 microns in size and show segmented nuclei containing condensed chromatin, with 2 - 5 nuclear lobes in most instances. The lobes are connected by a thin filament of chromatin. The N:C ratio is approximately 1:3 and the cytoplasm is pink due to the numerous specific granules present. The presence of thread-like filaments between nuclear lobes is the basis for distinguishing the segmented neutrophil from its precursor, the band neutrophil. However, in repeated proficiency testing studies, it has not been possible to achieve consistent differentiation between bands and segmented neutrophils. Therefore, for the purposes of proficiency testing it is not required that they are differentiated.

BMD-06



Identification	Participants No.	%	Evaluation
Mast cell, atypical, spindled	222	77.1	Educational
Mast cell	44	15.3	Educational
Macrophage (histiocyte)	8	2.8	Educational
Squamous epithelial cell/endothelial cell	3	1.0	Educational
Megakaryocyte or precursor, normal	2	0.7	Educational
Osteoclast	2	0.7	Educational
Stromal cell	2	0.7	Educational
Basophil, any stage	1	0.3	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polychromatophilic, and orthochromic normoblasts)	1	0.3	Educational
Metastatic tumor cell or tumor cell clump	1	0.3	Educational
Neutrophil, segmented or band	1	0.3	Educational
Neutrophil, metamyelocyte	1	0.3	Educational
Osteoblast	1	0.3	Educational

The arrowed cell is a mast cell, as correctly identified by 77.1% participants. The normal mast cell is a large (15 - 30 µm) round or elliptical cell with a small, round nucleus and abundant cytoplasm packed with black, bluish black, or reddish purple metachromatic granules. Normal mast cells are differentiated from blood basophils by the fact that they are larger (often twice the size of blood basophils), have more abundant cytoplasm, and have round rather than segmented nuclei. The cytoplasmic granules of mast cells are smaller, more numerous, and uniform in appearance compared to a basophil. Mast cell granules often obscure the nucleus. Although both mast cells and basophils are primarily involved in allergic and anaphylactic reactions via release of bioactive substances through degranulation, the content of their granules is different. In this case, the mast cells are abnormal in that the nucleus of this example and many others in the smear is ovoid or spindled and cytoplasmic granulation is less than the dense granulation seen in normal mast cells.

Case Presentation:

This bone marrow aspirate smear is from a 59-year-old woman with systemic mastocytosis. Laboratory data include: WBC = 5.4 x 10E9/L; RBC = 4.38 x 10E12/L; HGB = 13.1 g/dL; HCT = 39.1%; and PLT = 163 x 10E9/L.

(BONE MARROW, WRIGHT-GIEMSA)

Case Discussion: Systemic mast cell disease

The bone marrow aspirate smear from this 59-year-old woman shows tri-lineage hematopoiesis with full maturation of the erythroid and myeloid lines. Dysplastic features are not present and blasts are not increased. Notably, the majority of mast cells present are atypical and demonstrated ovoid/spindled nuclei and decreased granulation. This morphologic finding is suspicious, but not diagnostic in isolation, for systemic mastocytosis (SM). Although not shown, the trephine biopsy for this case showed multiple large aggregates of spindled mast cells.

The definition of SM in the 2016 WHO Classification update requires the presence of the major criterion: multifocal dense infiltrates of mast cells (≥ 15 cells/aggregate) in the bone marrow biopsy and/or other extracutaneous organs. However, one of the following minor criteria must also be present:

1. > 25% of the mast cells in the bone marrow biopsy are spindled or have other abnormal
2. Presence of an activating mutation of *KIT* at codon 816 in bone marrow, blood, or other extracutaneous site.
3. Demonstration of CD25 with or without CD2 on mast cells in addition to normal mast cell markers in blood, bone marrow, or other extracutaneous site.
4. Serum tryptase persistently elevated (> 20 ng/mL) in the absence of associated myeloid neoplasm.

In the absence of the major criterion, 3 or more minor criteria must be present for a diagnosis of SM.

Question 1: A diagnosis of systemic mastocytosis

A. cannot be made in this case given current information because the major criterion is not met.
B. cannot be made in this case given current information because results of investigation for the presence of each of the minor criteria has not been revealed.
C. can be made in this case given the aspirate smear morphology and information provided above.
D. can be made in this case given current information, provided mutational studies on the bone marrow shows a *KIT* 816 mutation.

Despite incomplete information on the presence or absence of activating *KIT* mutations, immunophenotype of mast cells, or serum tryptase levels, the abnormal morphology of the mast cells and the provided information on the abnormal multiple spindled mast cell aggregates in the trephine biopsy allows a diagnosis of SM. Patients with SM present with a variety of symptoms that are categorized into 4 groups:

1. constitutional (fatigue, weight loss, fever, sweats)
2. cutaneous (flushing, urticarial, dermatographism)
3. mediator-related systemic symptoms (abdominal pain, gastrointestinal symptoms, headache, tachycardia, hypotension, syncope)
4. musculoskeletal (muscle and bone pain, fractures, osteopenia/osteoporosis)

Physical findings include splenomegaly, lymphadenopathy, and hepatomegaly, particularly in advanced disease. The symptoms in SM can range in severity making diagnosis difficult and a high index of suspicion is needed to direct appropriate testing. Adherence to diagnostic criteria allows proper diagnosis. Hematologic abnormalities may be seen since SM is associated with hematologic neoplasms such as myelodysplastic and myeloproliferative neoplasms. Myelodysplastic/myeloproliferative (MDS/MPN) overlap neoplasms such as chronic myelomonocytic leukemia and MDS/MPN neoplasm, unclassifiable, are most common. Indeed, the SM may sometimes be initially overlooked because of the dominant features of the associated hematologic malignancy masks the mast cell proliferation.

Question 2: Systemic mast cell disease

A. does not involve lymph nodes
B. is a diagnosis of exclusion
C. may be missed due the presence of an associated other hematologic malignancy
D. must demonstrate cutaneous manifestations

Pathologically, abnormal mast cells may form loose or compact aggregates in bone marrow or other tissues. They may acquire a spindled shape. Romanowsky-stained smears show that mast cells are abnormal. As opposed to normal mast cells with round to slightly oval nuclei and abundant cytoplasm containing densely packed with granules, the abnormal mast cells of SM may become elongated and hypogranular. Bi- or multilobated mast cells can be seen uncommonly and are associated with aggressive disease. Immunostaining or flow cytometry demonstrates abnormal expression of CD25 and less often CD2 on mast cells. Genetically, as noted above, activating mutations in the tyrosine kinase *KIT* at codon 816 are seen. However, patients with this mutation are usually not responsive to treatment with the tyrosine kinase inhibitor imatinib. Other kinase inhibitors such as midostaurin have recently been shown to be effective in patients with advance/refractory SM.

Question 3: Molecular and pathologic features of SM include:

A. expression of CD25 on mast cells by flow cytometry
B. *KIT* loss of function mutation at codon 816
C. loss of CD2 expression on mast cells by flow cytometry
D. round mast cells

Five clinical variants of SM are now recognized (indolent SM, smoldering SM, SM with an associated hematologic malignancy, aggressive SM, and mast cell leukemia). While it is beyond the scope of this exercise to detail all diagnostic criteria and features for these variants as listed in the WHO classification, all variants must meet the basic criteria above for SM. Discrimination between the clinical variants is based on factors that assess tumor burden (so called "B" findings) and factors that assess organ dysfunction that dictate need for cytoreductive therapy (so called "C" findings). Indolent systemic mastocytosis has low tumor burden and no "C" findings. Smoldering SM has some "B" findings but no "C" findings. SM with associated hematological malignancy (AHM), as the name implies, has an AHM which is usually a myeloid malignancy. Lymphoid malignancies such as lymphoma or myeloma are uncommon. The prognosis is usually driven by the underlying AHM. Aggressive SM must have more than 1 "C" finding and the patient must not meet criteria for mast cell leukemia. Mast cell leukemia is present when ≥ 20% of the marrow cells are mast cells, which usually have atypical and immature cytologic features such as fine chromatin. Atypical *KIT* mutations (non-codon 816) are often present and multiple secondary mutations may be detected.

Question 4: Clinical variants of SM:

A. are defined by factors that assess tumor burden and organ dysfunction
B. are defined by histopathologic findings in bone marrow
C. are defined by molecular genetic variants
D. include mast cell sarcoma

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References:

Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press, 2017.

Answers to Questions:

Answer to question 1. A diagnosis of systemic mastocytosis:
C. can be made in this case given the aspirate smear morphology and information provided above.

The aspirate smear demonstrates many atypical mast cells and it is mentioned above that the trephine had multiple aggregates of mast cells with spindled mast cells. Thus, the major and first minor criteria are present, allowing for a diagnosis of SM with current information. According to the 2016 WHO update, SM may be diagnosed when the major criterion is satisfied along with one minor criterion. In the absence of the major criterion, one must have 3 or more minor criteria.

Answer to question 2. Systemic mast cell disease:
C. may be missed due the presence of an associated other hematologic malignancy

SM has specific diagnostic criterion and thus it is not an exclusionary diagnosis. While skin manifestations are often present in SM, they are not mandatory for an SM diagnosis. While not common, SM can involve lymph nodes, particularly in aggressive phases of the disease.

Answer to question 3. Molecular and pathologic features of SM include:
A. expression of CD25 on mast cells by flow cytometry

The pathologic features of SM include cytologically abnormal mast cells, often with elongated or spindled nuclei and hypogranulation that occur in clusters/aggregates of > 15 cells. These mast cells are immunophenotypically abnormal. Although they express pan-mast cell markers such as mast cell tryptase and CD117, they aberrantly coexpress CD25 or CD2. This can be assessed by flow cytometry or immunohistochemistry. *KIT* mutations are typically present at codon 816 that activates the *KIT* tyrosine kinase.

Answer to question 4. Clinical variants of SM:
A. are defined by factors that assess tumor burden and organ dysfunction

Five clinical variants of SM are now recognized (indolent SM, smoldering SM, SM with an associated hematologic malignancy, aggressive SM, and mast cell leukemia). The different variants are characterized by factors that assess tumor burden and organ. They are not defined by molecular genetic abnormalities or histopathologic features. Mast cell sarcoma is not a clinical variant of SM.