Fig. 2. Bone marrow biopsy showed infiltration of T-cell/histiocyte-rich B-cell lymphoma (TC/HRBCL). (A) Scattered large atypical cells in a lymphohistiocytic background (H&E, ×1,000). (B) Scattered atypical B cells labeled with CD20 antibody, CD20 (×200). (C) Infiltration of reactive T cells with a nodular pattern labeled with CD3 antibody, CD3 (×200).

dominantly occurs in younger patients and men. Bone marrow and extra-nodal involvements are more frequent in this subtype than in other types of DLBCL [4].

Lymphoma misdiagnoses that delay the appropriate treatment can be catastrophic. The lack of response to infection treatments and alternative diagnoses should promptly improve differential diagnoses. Lymphoma should be considered in patients with bone and joint pain.

Tahsin Özpolat¹, Bulent Saka², Ipek Yonal-Hindilerden³, Cemil Tascioglu², Öner Dogan⁴, Meliha Nalcaci³

¹Bloodworks Research Institute, Seattle, WA, USA, ²Department of Internal Medicine, ³Department of Hematology, ⁴Department of Pathology, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey

Correspondence to: Tahsin Özpolat
Bloodworks Research Institute, 1551 Eastlake Ave E Suite 100, Seattle, WA 98102, USA
E-mail: tahsino@bloodworksnw.org

Received on Jan. 27, 2018; Revised on Feb. 28, 2018; Accepted on May 10, 2018
https://doi.org/10.5045/br.2018.53.3.250

Acknowledgments
H.T.O and M.N conceived the study, analyzed data, and wrote the manuscript. H.T.O., B.S, I.Y.H., C.T, O.D., and M.N followed-up, diagnosed, and cared for the patient during hospitalization. H.T.O and M.N followed-up the patient at an outpatient clinic.

Authors’ Disclosures of Potential Conflicts of Interest
No potential conflicts of interest relevant to this article were reported.

REFERENCES
1. Unni KK, Hogendoorn PCW. Malignant lymphoma. In: Fletcher CDM, Unni KK, Mertens F, eds. WHO classification of tumours: pathology and genetics of tumours of soft tissue and bone. Lyon, France: IARC Press, 2002:306-8.
2. Choi JY, Hahn JS, Suh CO, Yang WI. Primary lymphoma of bone—survival and prognosis. Korean J Intern Med 2002;17:191-7.
3. Cabanela ME, Sim FH, Beabout JW, Dahlin DC. Osteomyelitis appearing as neoplasms. A diagnostic problem. Arch Surg 1974;109:68-72.
4. El Weshi A, Akhtar S, Mourad WA, et al. T-cell/histiocyte-rich B-cell lymphoma: clinical presentation, management and prognostic factors: report on 61 patients and review of literature. Leuk Lymphoma 2007;48:1764-73.

Massive splenomegaly: flow cytometry as a diagnostic tool for systemic mastocytosis

TO THE EDITOR: Mastocytosis is a spectrum of disorders related to the proliferation and accumulation of mast cells in one or more organs. Systemic mastocytosis (SM) was classified by World Health Organisation (WHO) classification for hematolymphoid neoplasms in 2008 as a myeloproliferative neoplasm. However, in the 2016 update of the WHO classification, it has been regarded as a separate entity. SM is sub-classified into indolent SM, smoldering SM, SM with an associated hematological neoplasm (AHN), aggressive SM, and mast cell leukemia [1]. SM is characterized by involvement of at least one extracutaneous organ with or without evidence of skin lesions [2]. We are presenting a rare case of aggressive SM with massive splenomegaly, which could be diagnosed on bone marrow (BM) immunophenotyping.

Case Report
A 61-year-old man presented with a 2-year history of progressively increasing left hypochondriac lump, abdominal pain, and progressive weight loss of 25–30 kg in the last 3 months. There was no fever, night sweats,
blood in stools, hematemesis, bony pain, or bleeding from any other site. He was an occasional alcoholic and non-smoker. On physical examination, the patient was afebrile with normal vital signs. No pallor, icterus, clubbing, cyanosis, edema, and peripheral lymphadenopathy were found. Abdominal examination revealed massive splenomegaly (16 cm below the left coastal margin) and hepatomegaly (4 cm below the right coastal margin), with the rest of the systemic examination within normal limits. The complete blood count showed hemoglobin level of 10.2 g/dL, total leukocyte count of 5,480/μL, and platelet count of 282,000/μL. Differential count revealed neutrophils 70%, lymphocytes 11%, monocytes 4%, metamyelocyte 1%, basophil 1%, and eosinophils 13%. The absolute eosinophil count was 712/μL, and lactate dehydrogenase (LDH) level was 180 U/L (laboratory reference range is 91–180 U/L). HIV/HBsAg/HCV were negative. Liver and renal function tests were normal. Stool examination was unremarkable. Abdominal ultrasonography showed massive splenomegaly with hepatomegaly and normal echotexture of the liver.

In view of marked hepatosplenomegaly, BM aspiration and biopsy were done. BM aspiration showed hypercellular marrow with myeloid predominance and prominent eosinophils and their precursors constituting 23% of the myelogram. Blasts were not increased. Mast cells were increased in the aspirate and imprint smears and highlighted by toluidine blue staining (Fig. 1A). Some mast cells exhibited hypogranularity and oval nuclei, instead of normal round (Fig. 1A inset). The overall mast cell percentage was -8%. The flow cytometric (FCM) analysis of the BM aspirate showed aberrant immunophenotype in mast cells. The CD117-positive mast cells showed aberrant expression of CD2 and CD25 in addition, they exhibited slight downregulation of CD117 (Fig. 1B–E). BM biopsy showed 80–90% cellularity with marked eosino-
philic hyperplasia and increased megakaryocytes. There were multiple large aggregates of mast cells found mostly around the perivascular and paratrabecular regions. The paratrabecular aggregates were causing new bone formation (Fig. 2A). Each aggregate comprised more than 15 cells, with >25% exhibiting atypical morphology (Fig. 2B). The reticulin staining showed grade 2 fibrosis. Toluidine blue staining was performed on BM aspiration and biopsy, which highlighted the mast cell hyperplasia (Fig. 2C). Immunohistochemistry (IHC) for CD117 was performed, and mast cells were found to be positive (Fig. 2D). The reverse transcriptase-polymerase chain reaction (RT-PCR) for FIP1L1-PDGFRα was negative. However, KITD816V mutation and serum tryptase could not be performed as the patient was hesitant. Further, a history of diarrhea was elicited from the patient.

Therefore, the patient fulfilled the major criteria of the presence of dense mast cell aggregates and the minor criteria of atypical morphology and abnormal immunophenotype and was, hence, classified as having SM. Further, he had three “B” findings in the form of presence of splenomegaly without hypersplenism, hepatomegaly without ascites, and myeloproliferation. Moreover, the history of diarrhea and weight loss pointed toward the presence of one C finding of malabsorption.

Considering all the above findings and investigations, we diagnosed our patient with aggressive SM. The patient was initially administered with interferon alpha but was lost to follow-up.

**Discussion**

Mastocytosis is an infrequent condition characterized by abnormal growth and accumulation of mast cells in various organs. This condition is subdivided into cutaneous mastocytosis (CM) and SM [2]. CM is more frequent during childhood and usually presents at an early age usually in the first year of life. SM involves at least one extracutaneous organ and may follow an indolent course or may be associated with life-threatening symptoms. The BM is the most frequently involved organ in SM [3]. The diagnosis of SM requires one major and one minor criteria of classification of WHO 2008. Further sub-classification of SM requires the presence of “B” and “C” findings. One of the minor WHO 2008 criteria for the diagnosis of SM is aberrant expression of CD2 and/or CD25 by neoplastic mast cells [2]. These diagnostic criteria have remained the same in the WHO 2016 classification [1].

In the past few years, immunophenotyping of mast cells has become a useful adjunct in the evaluation of SM. The normal mast cells are positive for CD117 and consistently negative for CD2 and CD25. In SM, the mast cells are commonly positive for CD2, CD25, or both. Although both IHC and FCM analysis can be performed, IHC assessment for CD2 and/or CD25 is difficult, because lymphocytes and few other activated cells also express these markers. However, FCM uses a multiparametric approach that allows the aberrant expression of the above markers on specifically gated mast cells [4], and this was applied in our case. Thus, these markers distinguish neoplastic mast cells from normal and reactive mast cells as they both do not express CD2.
and CD25 [5].

Being an extremely sensitive method, FCM is useful in detecting neoplastic mast cells even with a low burden of the disease and expected to identify even those cases which do not fulfill the diagnostic criteria for SM [4]. It proved to be an important diagnostic tool in the present case as mast cells may be increased in many reactive conditions as well.

Conclusion

The diagnosis of SM requires a cognisance of this rare entity along with a high index of suspicion. It should be considered as one of the differential diagnoses in cases with massive splenomegaly especially when the usual causes have been excluded. The assessment of the immunophenotype of mast cells is helpful in establishing the diagnosis of SM. This case also highlights the role of FCM in identifying abnormal mast cells based on routinely available markers in laboratories performing leukemia FCM.

Loveena Rastogi1, Jasmita Dass1, Gaurav Dhingra1, Nitin Gupta2, Jyoti Kotwal1

1Department of Hematology, 2Department of Clinical Hematology, Sir Ganga Ram Hospital, New Delhi, India

Correspondence to: Jasmita Dass
Department of Hematology, Sir Ganga Ram Hospital, New Delhi 110060, India
E-mail: drjasmita@gmail.com

Received on Feb. 2, 2018; Revised on Mar. 27, 2018; Accepted on May 10, 2018
https://doi.org/10.5045/br.2018.53.3.251

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391-405.
2. Horny HP, Metcalfe DD, Bennett JM, et al. Mastocytosis. In: Swerdlow SH, Campo E, Harris NL, et al, eds. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: IARC Press, 2008:54-63.
3. Arredondo AR, Gotlib J, Shier L, et al. Myelomastocytic leukemia versus mast cell leukemia versus systemic mastocytosis associated with acute myeloid leukemia: a diagnostic challenge. Am J Hematol 2010;85:600-6.
4. Pozdnyakova O, Kondratiev S, Li B, Charest K, Dorfman DM. High-sensitivity flow cytometric analysis for the evaluation of systemic mastocytosis including the identification of a new flow cytometric criterion for bone marrow involvement. Am J Clin Pathol 2012;138:416-24.
5. Jabbar KJ, Medeiros LJ, Wang SA, et al. Flow cytometric immunophenotypic analysis of systemic mastocytosis involving bone marrow. Arch Pathol Lab Med 2014;138:1210-4.

Emergence of chronic myeloid leukemia following autologous stem cell transplantation in a young woman with multiple myeloma

TO THE EDITOR: We read with great interest the recent paper “A case of synchronous multiple myeloma and chronic myeloid leukemia” published by Lee et al. [1] and report here a similar case that further highlights the management challenges in such complex cases. A 39-year-old female presented with left hip pain 5 years ago and a plain film showed a large lytic lesion in the neck of the femur. Her complete blood count revealed a hemoglobin level of 12 g/dL, platelet count of 233×10⁹/L and total white blood cell (WBC) count of 10.8×10⁹/L with normal serum calcium and creatinine levels. A bone marrow biopsy showed 40% bone marrow plasma cells (Fig. 1), lambda chain levels were elevated at 2,760 mg/L with a free light chain ratio of 0.0004, resulting in a diagnosis of multiple myeloma (MM). She completed 6 cycles of cyclophosphamide, bortezomib and dexamethasone chemotherapy in conjunction with radiation therapy to the fracture site and received monthly bisphosphonate infusions. This was consolidated with a melphalan-conditioned autologous stem cell transplant. She did not receive maintenance lenalidomide therapy given a previous history of pulmonary embolism. Two and a half years following her initial diagnosis, despite a normal serum free light chain ratio, it was noted that she had a rising WBC count with a peak level of 40.2×10⁹/L, and a platelet count of 204×10⁹/L, neutrophil count of 32.2×10⁹/L, monocyte count of 2.7×10⁹/L and basophil count of 0.59×10⁹/L. Molecular analysis of the peripheral blood did not identify a JAK-2 mutation, however the BCR-ABL ratio, measured by real-time quantitative polymerase chain reaction, was elevated at 140.6%. Bone marrow (BM) sampling showed marked granulocytic hyperplasia without excess blasts consistent with chronic-phase chronic myeloid leukemia (CML) (Fig. 2). There was no increase in plasma cells and BM karyotype confirmed the presence of an abnormal clone that contained a translocation between the long arms of chromosomes 9 and 22 with breakpoints at 9q34 and 22q11.2. This BCR-ABL rearrangement was analyzed in 192 out of 200 cells and the results were consistent with CML. The patient initially commenced imatinib therapy at 400 mg daily, but at 6 months, her disease control was suboptimal with a BCR-ABL ratio of 23%. She subsequently started nilotinib 400 mg twice daily resulting in an improved molecular response with a most recent transcript ratio of 1.4%. Her most recent BM aspirate showed CML in morphological remission and her free light chain ratio remained normal in line with ongoing MM remission. The co-diagnosis of MM and CML is a vanishing rare