Therapy Related Mast Cell Leukemia Presenting In A Pediatric Patient:

A Case Report and Literature Review

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Abstract

Mastocytosis is a heterogenous group of disorders caused by the proliferation and accumulation of neoplastic mast cells. In the pediatric population, mastocytosis is typically limited to the skin (cutaneous mastocytosis) and follows an indolent clinical course with excellent prognosis. More aggressive disorders, such as mast cell leukemia and myelomastocytic leukemia, most commonly present in adult patients, with only a few cases reported in the pediatric population. We present a rare case of mast cell leukemia with myelodysplastic features, arising post chemotherapy in a 13 year old female with a history of rhabdomyosarcoma. The patient initially presented with sore throat, body aches, upset stomach and fever. A complete blood count and peripheral smear revealed thrombocytopenia and abnormal cells concerning for acute promyelocytic leukemia. Computed tomography reported hepatosplenomegaly, enhancing liver lesions and sclerotic lesions throughout the spine and sternum. A bone marrow biopsy was performed. Peripheral smear examination revealed occasional circulating large mast cells and myeloid blasts. Aspirate smears demonstrated 4-5% myeloid blasts and 25% atypical immature mast cells with focal clustering >15 cells per aggregate. Dysplastic features were identified in both the myeloid and erythroid cell lines. Flow cytometric analysis of the bone marrow aspirate identified two populations of cells: a population of myeloid blasts, comprising 2.4% of the total cells, (positive for CD34, HLA-DR, CD117, CD33, and CD11b and negative for CD13) and a population of mast cells, comprising 4% of total cells which showed co-expression of CD2 and CD117. Cytogenetic studies showed complex karyotype and PCR was negative for KIT Asp816Val mutation on two occasions. A bone marrow biopsy obtained 6 days later, confirmed an atypical spindled mast cell infiltrate with positive staining for tryptase and CD117. Serum tryptase level was markedly elevated at 804 ng/mL. We concluded, based on the combined aspirate and bone marrow biopsy findings, that our case characterizes a mast cell leukemia with myelodysplastic changes arising post chemotherapy treatment in a pediatric patient. This case is presented to highlight the important diagnostic criteria used in the evaluation of mastocytosis and the overlapping features that make this this group of disorders a diagnostic challenge.

Introduction

Mastocytosis is a clonal, neoplastic proliferation and accumulation of mast cells (MC) in one or more organ systems (1). Multiple studies have demonstrated that MCs are myeloid cells, originating from multipotent hematopoietic progenitor cells found in the bone marrow as well as in the peripheral blood (2-4). Terminal differentiation and maturation occur after MCs distribute in the blood and migrate into tissues. Clinical symptoms are the result of MC proinflammatory and vasoactive chemical mediators released in response to IgE. Most commonly the clinical symptoms represent a reactive process, but may also be the initial presentation of an infiltration of neoplastic cells into various organ sites. In pediatric patients, MC disorders most commonly are limited to the skin and present with urticarial skin lesions as a result of localized infiltration of MCs (1,5,6). Cutaneous mastocytosis (urticarial pigmentosa) typically follows a benign clinical course and may regress spontaneously. In adults, mastocytosis encompasses a heterogeneous group of disorders, whose clinical course range from highly aggressive neoplasms, such as mast cell leukemia (MCL), to indolent systemic mastocytosis with normal life expectancy (6). MC disorders may also present with advanced myeloid neoplasms in cases of myelomastocytic leukemia (MML) and systemic mastocytosis with an associated hematologic clonal non-mast cell lineage disease (AHNMD). Aggressive mast cell disorders rarely present in pediatric patients, with only a few cases reported (6-10). The objective of this article is to present a rare case of a therapy-related mast cell neoplasm presenting in a pediatric patient.

Clinical History

A 13-year-old white female with a history of rhadomyosarcoma, treated with surgical resection and chemotherapy eleven years prior to admission, presented to her primary care physician with sore throat and fever for 3 weeks duration. Initial mono and rapid strep tests were negative. Over the course of the next several days the patient continued to complain of body aches, upset stomach and fever. Further clinical evaluation with a complete blood count (CBC) was significant for severe thrombocytopenia with a platelet count of 40 K/μl. The patient was sent to the emergency room where a repeat CBC and peripheral smear reported a platelet count of 20 K/μl and abnormal

cells concerning for acute promyelocytic leukemia. She was subsequently transferred to our institution

On physical examination, the pulse was 60 beats/min and blood pressure was 112/62. No hepatosplenomegaly was identified on clinical exam. However, computed tomography imaging studies reported sclerotic lesions throughout the spine and sternum, moderate hepatosplenomegaly, numerous enhancing liver lesions, extensive periportal soft tissue thickening, and mild ascites. Laboratory studies revealed a white blood cell count of 10.7 K/µl, a normocytic anemia (hemoglobin 9.5 g/dl), and severe thrombocytopenia (22 K/µl). Other significant laboratory values included an elevated D-dimer level (2374 ng/mL), alkaline phosphatase (475 U/L), and lactate dehydrogenase (647 U/L). The patient's liver enzymes were within normal limits and viral, hepatitis and autoimmune panels were negative or non-reactive. Over the course of the next several days the patient developed massive ascites and a pleural effusion. A bone marrow biopsy was performed.

Materials and Methods

Two separate bone marrows, submitted 6 days apart, where received fresh

Marrow aspirate smears were stained with Wright-Giemsa according to the standard operating procedures. Marrow biopsies are stained with Hematoxylin and Eosin (H&E) after fixation in acid zinc formalin and decalcification. A marrow clot section was stained with H&E after fixation with 10% neutral formalin.

Flow cytometry

The specimens were analyzed on a Beckman Coulter FC500 flow cytometer (Fullerton, CA) using a five-color platform. All antibodies were purchased from Beckman Coulter. Blasts were gated based on dim CD45 intensity and low side scatter intensity. The following antibody combinations were used: 34/33/DR/117/45, 14/11b/34/13/45, 34/10/19/20/45, 2/7/3/5/45, 8/4/3/56/45, 34/123/4/56/45, MPO/3/45.

Immunohistochemistry

Immunohistochemistry was performed on either marrow core biopsy or clot section. The following antibodies were used: CD2, CD25, CD117, and mast cell tryptase.

Molecular cytogenetics by fluorescence in situ hybridization (FISH)

FISH with DNA probes specific for the ABL1 oncogene (9q34) and BCR region (22q11.2) (Vysis,Inc.), PML (15q24) and RARA (17q21.1) genes, chromosome 8 centromere and chromosome X centromere (Vysis,Inc.), and RUNX1T1 (ETO) at 8q22 and RUNX1 (AML1) at 21q22 regions (Vysis, Inc.) were analyzed on paraffin embedded bone marrow clot and biopsy specimens.

Mutational analysis of C-Kit

Bone marrow aspirates were evaluated for an activating point mutation, resulted from a change of aspartic acid (D) to valine (V) at codon 816 (D816V) in the kinase domain of c-kit using allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) with fragment analysis on an ABI3100 genetic analyzer. Briefly, DNA was extracted from whole bone marrow and PCR was used to amplify across the mutation site in 2 separate tubes; 1 contained a reverse primer complementary to the unmutated sequence and the other contained a reverse primer complementary to the mutated sequence. Each of these reverse primers was labeled with a

fluorescent tag with both tubes containing an identical, non-labeled forward primer. Both primer sets amplified a 200 base pair fragment that differs only at the mutation site. The unmutated fragment should be amplified in all samples. Samples negative for *KIT* Asp816Val will not have an amplified fragment in the mutated reaction tube. Positive samples will have amplified fragments in both the unmutated and mutated tubes. The test gives a qualitative (positive or negative) result only, as the end point PCR used is not reliable for quantification (Mayo Clinic, 2012).
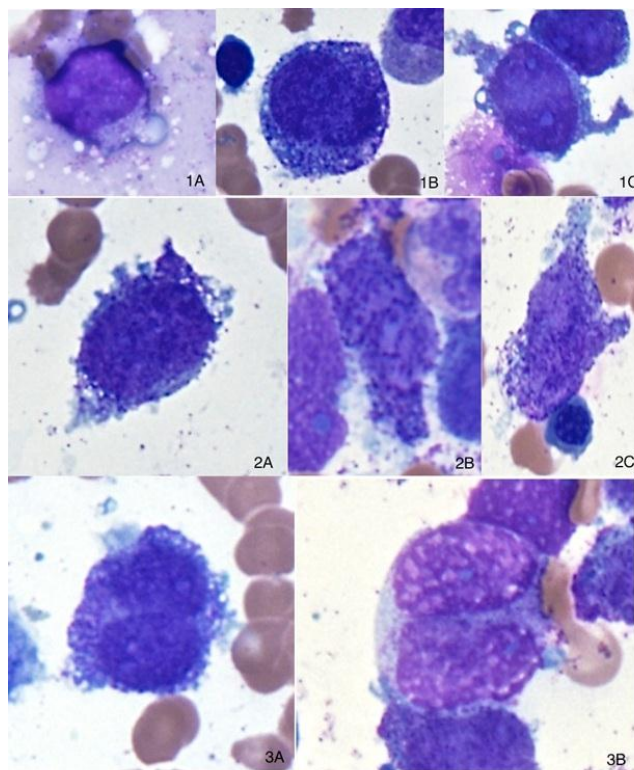

Figure 1. Examination of the bone marrow aspirate smear revealed a polymorphous population of atypical mast cells which comprised 25-30% of the total counted cells. Metachromatic blasts (1A,1B,1C, Wright-Giemsa 1000x magnification), type I atypical mast cells with oval nuclei, cytoplasmic extensions and hypogranulation (2A,2B,2C, Wright-Giemsa 1000x magnification) and type II atypical mast cells also known as promastocytes (3A,3B, Wright-Giemsa 1000x magnification) with binucleation, prominent nucleoli and granulation were seen throughout the smears.

Results

The peripheral blood smear showed a decreased number of red cells with anisopoikilocytosis including target cells and dacrocytes. Occasional circulating large mast cells and myeloid blasts with no overt myelodysplastic features were identified. Platelets were decreased in number with normal morphology. A bone marrow aspirate showed 4-5% myeloid blasts with 25% immature mast cells (metachromatic blasts, type I and type II atypical mast cells), a subset of hypogranular myeloid cells with abnormal nuclear segmentation, and erythroid precursors with megaloblastic change (Fig. 1). A bone marrow biopsy demonstrated a population of atypical spindle cells in viable areas with aggregation in a paratrabecular pattern (Fig. 2). Immunohistochemical stains on the biopsy and clot sections show a subset of cells with positive staining for tryptase and CD117. A few clusters (>15 cells) were positive for tryptase (Fig. 3). Flow

cytometric analysis of the bone marrow aspirate identified two populations of cells: a population of myeloid blasts, comprising 2.4% of the total cells, was positive for CD34, HLA-DR, CD117, CD33, and CD11b and negative for CD13. A second a population of cells, comprising 4% of total cells showed co-expression of CD2 and CD117. Cytogenetic studies showed a hyperdiploid complex karyotype with six of fourteen metaphases with extra copies of chromosomes 1, 8, 9, 19, 20, 22, and X and loss of chromosome 13 and 14. In addition there were structural abnormalities in 4p and 21q and several marker chromosomes, which was consistent with a neoplastic disorder but no specific abnormalities to classify the lesion diagnostically. FISH with DNA probes specific for the ABL1 oncogene (9q34) and BCR region (22q11.2) and for the PML (15q24) and RARA (17q21.1) genes were within the laboratory's normal limits. The bone marrow biopsy specimen was negative for a KIT Asp816Val mutation on two occasions.
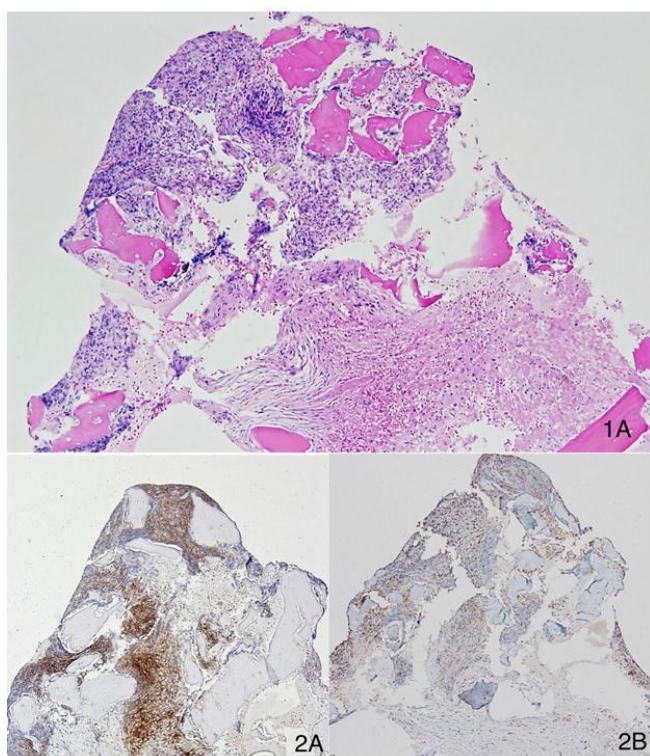

Figure 2. Examination of the bone marrow biopsy revealed a population of atypical spindled cells arranged in a paratrabecular pattern (1A, Hematoxylin & Eosin, 400x magnification). These cells stained strongly positive for CD117 (2A, CD117 immunohistochemistry, 400x magnification) and focally positive for mast cell tryptase (2B, mast cell tryptase immunohistochemistry, 400x magnification).

Discussion

MCL and MML are distinct entities, based on clearly defined parameters, with important clinical implications. Primary MC diseases, such as aggressive SM and MCL are considered relatively insensitive to conventional chemotherapies, while several studies have shown MML may have a significantly better chance of cure by aggressive chemotherapy and stem cell transplantation (11,12).

Table 1 WHO diagnostic criteria for systemic mastocytosis

Major Criterion	(1) Multifocal dense mast cell (MC) infiltrates (≥15 MC/infiltrate) in the bone marrow and/or other extracutaneous organs
Minor Criterion	(1) >25% of MC are spindle-shaped cells in MC-infiltrates; or >25% of all MC are atypical MC in bone marrow smears
	(2) Expression of CD2 and/or CD25, in addition to normal MC markers
	(3) Serum tryptase level >20 ng/ml (not valid in cases with an AHNMD)
	(4) c-kit point mutation at codon 816 (most commonly D816V) in bone marrow or in another extracutaneous organ

* The diagnosis is established if at least one major and 1 minor or 3 minor criteria are fulfilled.
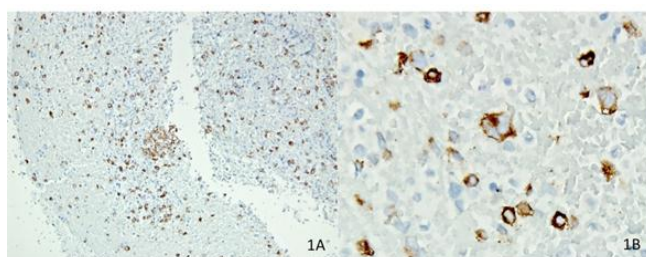

Figure 3. Examination of the bone marrow clot revealed focal clusters of greater than fifteen cells staining positive for mast cell tryptase (1A, mast cell tryptase immunohistochemistry, 400x magnification). However, most of the positive cells stained in a diffuse, interstitial pattern (1B, mast cell tryptase immunohistochemistry, 1000x magnification).

Mast cell leukemia is a rare, highly aggressive, subtype of mastocytosis. As defined by the 2008 WHO criteria, MCL cases meet the criteria for systemic mastocytosis (Table 1) with additional findings including (1) ≥20% MCs upon BM aspirate examination, (2) neoplastic MC infiltration of the BM and other extracutaneous organs, and (3) high-grade cytologic findings (1,13). The BM reveals a diffuse compact infiltrate of MC with focal clusters with greater than fifteen cells with marked reduction of fat cells and normal hematopoietic elements. The presence of at least 10% circulating MC in the blood separates MCL from the more common aleukemic subvariant (1,5). Cases of MCL may present with associated myelodysplastic syndromes allowing for the diagnosis of MCL-MDS (14).

The most important differential diagnosis of MCL is MML. MML is a rare entity described in patients with advanced myeloid neoplasms with increased numbers of atypical immature MCs. MML typically presents as an increase in myeloblasts (>5%) as well as >10% metachromatic blasts in the peripheral blood and/or BM (14). However, the criteria for SM is not met, as evidenced by the lack of focal dense MC infiltrates, expression of CD2 and/or CD25 or identification of the D816V KIT mutation by PCR (1,13-15). Similar to patients with MDS or AML without MML, dysplastic features are seen in one or more myeloid cell lineages.

To fulfill the criteria for SM, one major and one minor criterion, or three minor criteria must be present (Table 1) (1). The major criterion specifically requires multifocal, dense infiltrates of MC (≥15 MC in aggregates) in BM sections and/or extra cutaneous

organs. This requirement seems to stand in opposition to the diffuse BM infiltrate typically described in MCL. This point of confusion has been noted in several studies with the conclusion that it is necessary to identify a mixed pattern, with both a dense, multifocal and diffuse infiltrate of MCs (13). In our case, initial review of the BM was limited to aspirate and cell clot sections, due to the patient's inability to tolerate the procedure. Six days later a BM biopsy was successfully performed and although changes consistent with high dose steroid treatment were noted, the results were combined in an effort to classify the disease. The primary reviewers of the case felt that a few small clusters (>15) of atypical mast cells, identified on the aspirate smears, as well as a diffuse spindle cell infiltrate identified in the core biopsy, warranted a mixed pattern of MC infiltration, fulfilling the major criterion for SM. Furthermore, MC comprised 25% of all nucleated cell in the aspirate smear and less than 10% in the peripheral smear; thus a final diagnosis of MCL, aleukemic variant with myelodysplastic changes was proposed. The finding of a small cluster of atypical MCs on aspirate smear was not noted by secondary review, therefore not fulfilling the major criteria for SM and prompting the consideration of MML.

Arredondo et al. notes that even in the absence of multifocal, dense infiltrates, most cases of MCL would meet the requirements for SM by fulfilling minor criteria, since MCL typically presents with marked cytologic atypia, expression of CD2 and/or CD25 and >50% of cases are found to have the D816V KIT mutation (13). While this case certainly fulfills the criteria for marked atypia, the remaining criteria are a topic of debate. Serum tryptase levels in MML and MCL are typically above 100 ng/ML (16). However, tryptase levels can be elevated in several other myeloid neoplasms (15). In our case 4% of cells demonstrated co-expression of CD2/CD117 by flow cytometry and would favor a diagnosis of MCL since MML typically lacks CD2 and CD25 expression. However, the absence of the D816V KIT mutation provides no further support for the diagnosis of MCL. KIT is a tyrosine kinase type receptor encoded by the c-kit proto-oncogene and is one of the critical molecules of MC development, proliferation and survival (4,17). Gain of function mutation in the c-kit gene is associated with enhanced growth of MC, particularly c-kit D816V (Asp-816-Val). In a review of 10 cases of MCL by Valentini et al., molecular studies identified the D816V mutation is six patients and one patient with a silent D816V KIT mutation (5). While some reported cases of MCL will not harbor the D816V KIT mutation, MML by definition lacks the D816V KIT mutation.

Cytogenetic studies are another resource that may aid in the distinction between MCL and MML. In the same study of 10 patients with MCL by Valentini et al., cytogenetic analysis in all but one patient resulted in a normal karyotype (5). MML, by contrast typically presents with a complex karyotype, as was seen in our patient.

Advanced MC neoplasms are extremely rare in pediatric patients. In our review of the literature, only five cases of mast cell neoplasms in pediatric patients have been reported (6-10), none of which resulted from prior chemotherapy or radiation therapy. To the best of our knowledge, this is the first reported case of therapy-related mast cell leukemia in a pediatric patient. As so few cases of mast cell neoplasms have been reported in the pediatric population, no adequate studies have been performed to predict prognosis and effective treatment strategies in these patients (18).

MC disease is a heterogeneous group of disorders with overlapping clinical features that make the pathologic findings critical for classification. Challenging cases, such as the one described, and variants such as MML do not fit neatly into the current WHO classification scheme. Careful consideration of diagnostic criteria and thorough review of histologic and molecular findings are essential in establishing the correct diagnosis.

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