Systemic mastocytosis (SM) with associated clonal nonmast cell lineage disease is seen in up to 20% cases of SM. SM is uncommon in the pediatric population. T (8; 21) (q22; q22) is a good prognostic factor in acute myeloid leukemia (AML). However, the presence of SM confers poor prognosis in t (8; 21) (q22; q22) associated AML. We report the case of a child with t (8; 21) (q22; q22) associated AML with SM and her minimal residual disease status over the course of her treatment. In our case, the abnormal mast cells, showing co-expression of CD25 and CD2, persisted even after the marrow showed no evidence of residual AML.

**KEY WORDS:** Acute myeloid leukemia, mastocytosis, minimal residual disease

**INTRODUCTION**

Childhood acute myeloid leukemia (AML) is uncommon and childhood AML associated with clonal mast cells is extremely rare. Translocation (8; 21) associated AML is seen in 10–20% of all AML cases and the frequency is similar in pediatric and adult population. The current WHO classification divides mastocytosis into two major categories: Cutaneous and systemic. Bone marrow (BM), liver, and spleen are commonly involved in the systemic variant. Systemic mastocytosis (SM) with associated clonal nonmast cell lineage disease (SM-AHNMD) occurs in approximately 20% of cases of SM and shows the presence of an associated clonal myeloid malignancy. Myelodysplastic syndromes, chronic myelomonocytic leukemia, AMLs, and particularly AML associated with t (8; 21) (q22; q22) are commonly seen. However, t (8; 21) (q22; q22) associated AML with SM in the pediatric population is extremely rare with few case reports in literature describing this entity.

**CASE REPORT**

A 7-year-old girl presented with complaints of fever, abdominal distention, and weakness for a month. Examination revealed the presence of pallor, petechial rash, and splenomegaly. The patient did not show evidence of skin involvement or mast cell mediator release symptoms. Complete blood count revealed low hemoglobin (7.2 g/dL) and thrombocytopenia (platelets of 30 × 10^9/L) and leukocytosis (total leukocyte count of 33.89 × 10^9/L). Peripheral smear examination revealed 24% blasts which expressed cytochemical myeloperoxidase (MPO). BM aspirate was hypercellular and showed dyspoiesis along with relative suppression of erythroid and megakaryocyte series. BM showed the presence of 70% blasts with few showing Auer rods. In addition, it revealed dense aggregates (>15 cells) of round to ovoid shaped, densely granular mast cells, some having spindle-shaped morphology (Figure 1). Toluidine blue stain highlighted metachromatic granules. BM biopsy also revealed similar findings with aggregates of mast cells as highlighted by c-KIT immunostain. The facility to determine serum tryptase levels was not available at our institution, and hence tryptase levels are not provided here. Flow cytometric immunophenotyping (10 color antibody panel, BC Navios) was done including a specially designed tube for detecting an abnormal mast cell phenotype. BM aspirate sample was processed by bulk lysis-stain-wash method and analyzed using Kaluza analysis software (Beckman Coulter). Abnormal blasts gated out on CD45 versus side scatter plot revealed a myeloid phenotype. Blasts expressed dim CD45, moderate CD34 and HLADR, heterogeneous.
CD13 and CD19, and moderate CD33 and CD38 along with cytoplasmic MPO [Figure 2, Row A] The blasts were negative for CD117, CD11b, CD15, CD16, CD10, CD20, CD22, CD79a, CD66c, CD123, CD58, CD3, CD7, CD5, CD4, and CD8. CD56, known to be associated with KIT mutations in AML, was weakly expressed. Mast cells were isolated using their bright CD117 expression and high side scatter. They also expressed bright CD33, bright CD25 with dim CD2 [Figure 2, Square D]. Mast cells were negative for HLADR and CD123. Fluorescence in situ hybridization (FISH) with the dual color dual fusion AML1/ETO probe was performed on interphase and metaphase cells from the BM aspirate. The signal pattern revealed AML1/ETO (RUNX1T1/RUNX1) fusion with an additional copy of AML1/ETO (duplication of derivative 21). There was no evidence of 9q deletion. Analysis for FLT3-ITD (FMS-related tyrosine kinase 3), NPM1 (nucleophosmin 1), and CCAAT-binding enhancer protein alpha mutations using multiplex polymerase chain reaction (PCR) in combination with fluorescence-based capillary electrophoresis was negative. The c-KIT D816V mutation was tested on whole BM by allele-specific PCR was also negative. The final diagnosis of SM with t (8; 21) (q22; q22) associated AML (SM-AHNMD) was rendered.

She was started on induction chemotherapy with high dose cytarabine in view of t (8:21). However, postinduction BM was not in remission and revealed 75% blasts. A high sensitivity multicolor flow cytometric assay was used to detect the presence of minimal residual disease (MRD). Postinduction flow analysis showed 58% residual disease. Abnormal mast cells also persisted along with the abnormal myeloid blasts. FISH with dual color dual fusion AML1/ETO probe performed on interphase and metaphase cells from the BM aspirate also showed the persistence of AML1/ETO (RUNX1T1/RUNX1) fusion in 90% of the cells. Induction phase was followed by two cycles of cytosine arabinoside, daunorubicin, and etoposide (ADE). After the first cycle [Table 1], BM morphology showed 6% blasts and revealed 0.6% MRD positive on FCI [Figure 2, Row B]. AML1/ETO (RUNX1T1/RUNX1) fusion by FISH also persisted in 3% of the BM cells. Although the BM showed 7% blasts on morphology after the second cycle of
Table 1: Trend showing presence of minimal residual disease in the bone marrow using flow cytometric immunophenotyping

| Duration of chemotherapy | Blasts in bone marrow | Residual disease by FCI MRD assay | Abnormal mast cells present | FISH for AML1/ETO fusion |
|--------------------------|-----------------------|----------------------------------|-----------------------------|--------------------------|
| 1 month (induction)      | 75%                   | 58%                             | Present                     | Positive                  |
| January 29, 2015 (%)     | February 27, 2015     |                                  |                              |                          |
| 2 months (1st ADE)       | 6%                    | 0.6%                            | Present                     | Positive                  |
| March 17, 2015 (%)       | April 20, 2015        |                                  |                              |                          |
| 3 months (2nd ADE)       | 7%                    | Negative                         | Present                     | Not done                  |
| April 27, 2015           | May 27, 2015          |                                  |                              |                          |

† Cycle, FCI: Flow cytometric immunophenotyping; ADE: Arabinoside, daunorubicin, and etoposide; FISH: Fluorescence in situ hybridization; MRD: Minimal residual disease; AML: Acute myeloid leukemia

A DE (i.e., after 3 months of chemotherapy, Table 1), there was no MRD by FCI [Figure 2, Row C]; however, the abnormal mast cells continued to persist [Figure 2, Square D]. She received her third consolidation cycle with cladribine with cytarabine and is planned for allogenic stem cell transplantation as soon as a donor is identified. Interestingly, abnormal mast cells persisted throughout the course of treatment. The level of persistence of disease over the course of treatment is shown in Table 1.

**DISCUSSION**

SM-AHNMD is seen in approximately 20% cases of SM. Although WHO 2008[2] mentions the expression of CD25 and/or CD2 as diagnostic criteria, the CD25 expression has been reported to be more specific than CD2 expression in neoplastic mast cells.[10] Mast cells in this case showed strong CD25 but weak CD2 expression. Bright CD117 expression and lack of CD123 can help distinguish mast cells from basophils. 9q deletion has been reported as the most common additional cytogenetic abnormality in t (8; 21)(q22;q22) associated AML especially in myelomastocytic leukemia (AML with increased BM mast cells not meeting criteria for SM). This additional cytogenetic abnormality seen in 15–35% of t (8; 21)(q22;q22) cases and up to 14% of pediatric t (8; 21)(q22;q22) associated AML[1,3] was not seen in our case.

A point mutation leading to substitution of valine for aspartic acid in (ASP816VAL or D816V) the catalytic domain of c-KIT is also a part of the WHO criteria to define SM. This mutation, first described by Nagata et al. in SM, is also seen in 10–25% cases of AML t (8; 21)(q22;q22) and is associated with a dismal prognosis.[3,9] The expression of CD56 by leukemic blasts correlates with the presence of KIT D816V mutation[10] was not evident in our case. It is believed that the abnormal mast cells and leukemic blasts arise from the same clone.[4] This has been proven by detecting the presence of RUNX1-RUNX1T1 or c-KIT mutations in both mast cells and leukemic blasts in those cases of AML associated with mastocytosis.

Some studies have reported that the mast cells become more prominent following the clearance of blasts after initiation of therapy.[1,10] Our case showed dense aggregates of mast cells right from the outset which persisted despite aggressive therapy. Although AML with t (8; 21)(q22;q22) has been categorized in the good prognostic group, the presence of a KIT mutation and/or associated SM indicates dismal prognosis.[1,3,9,10] This may be partially due to the chemoresistance of mast cells as evidenced by their persistence post-therapy and in some cases even post stem cell transplantation.[3,10] Although the patient’s BM showed 7% blasts on morphology after 2# of ADE on FCI, they did not show any leukemia-associated immunophenotypes and showed maturation patterns that are consistent with normal maturing myeloid blasts.

We present this report of SM associated with t (8; 21)(q22;q22) as a rare disease, more so in the pediatric population. Its presence abrogates the good prognostic impact of the recurrent t (8; 21)(q22;q22) translocation and hence we would like to emphasize the importance of appropriate work-up for SM in patients with AML with t (8; 21)(q22;q22).

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Reikvam H, Hatfield KJ, Kittang AO, Hovland R, Bruserud Ø. Acute myeloid leukemia with the t(8;21)(q22;q22) translocation: Clinical consequences and biological implications. J Biomed Biotechnol 2011;2011:104631.
2. Swerdlow SH, Campo E, Harris NL, Pileri SA, Stein H, Thiele J, et al. WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues. Lyon: IARC; 2008.
3. Pullarkat ST, Pullarkat V, Kroft SH, Wilson CS, Ahsanuddin AN, Mann KP, et al. Systemic mastocytosis associated with t(8;21)(q22;q22) acute myeloid leukemia. J Hematop 2009;2:27-33.
4. Nagai S, Ichikawa M, Takahashi T, Sato H, Yokota H, Oshima K, et al. The origin of neoplastic mast cells in systemic mastocytosis with AML1/ETO-positive acute myeloid leukemia. Exp Hematol 2007;35:1747-52.
5. Gogia A, Sharawat SK, Kumar R, Sarkar C, Bakhshi S. Systemic mastocytosis associated with childhood acute myeloid leukemia. J Pediatr Hematol Oncol 2013;35:163-4.
6. Mahadeo KM, Wolgast L, McMahon C, Cole PD. Systemic mastocytosis in a child with t(8;21)(q22;q22) acute myeloid leukemia. Pediatr Blood Cancer 2011;57:684-7.
7. Yabe M, Masukawa A, Kato S, Yabe H, Nakamura N, Matsushita H. Systemic mastocytosis associated with t(8;21)(q22;q22) acute myeloid leukemia in a child: Detection of the D816A mutation of KIT. Pediatr Blood Cancer 2012;59:1313-6.
8. Morgado JM, Sánchez-Muñoz L, Teodósio CG, Jara-Acevedo M, Alvarez-Twose I, Mattio A, et al. Immunophenotyping in systemic mastocytosis diagnosis: ‘CD25 positive’ alone is more informative than the ‘CD25 and/or CD2’ WHO criterion. Mod Pathol 2012;25:516-21.
9. Schnittger S, Kohl TM, Haferlach T, Kern W, Hiddemann W, Spiekermann K, et al. KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. Blood 2006;107:1791-9.
10. Bernd HW, Sodlar K, Lorenzen J, Osieka R, Fabry U, Valenti P, et al. Acute myeloid leukemia with t(8;21)(q22;q22) associated with “occult” mastocytosis. Report of an unusual case and review of the literature. J Clin Pathol 2004;57:324-8.