Immunohistochemical Staining to Identify Concomitant Systemic Mastocytosis in Acute Myeloid Leukemia with \textit{RUNX1::RUNX1T1}

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Systemic mastocytosis with associated hematological neoplasm (SM-AHN) poses diagnostic challenges because of the coexistence of non-mast cell lineage hematological malignancies, including myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN), and acute myeloid leukemia (AML), which mask the SM components [1–4]. We evaluated SM-AHN in patients with AML with \textit{RUNX1::RUNX1T1} as they are known to have a high prevalence of \textit{KIT} variants, which are one of the di-

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agnostic criteria for SM-AHN.

SM diagnosis depends on histopathological findings and the identification of mast cell aggregates, especially via immunohistochemical (IHC) staining, where mast cell aggregates are identified by CD117 staining and aberrant CD25 with or without CD2 expression indicate SM [3, 5]. KIT variants detected in SM are often simultaneously found in AHN, especially in core-binding factor (CBF)-AML: 35% in AML with inv(16)(p13.1q22); CBFB::MYH11 and 25% in AML with RUNX1::RUNX1T1 have KIT variants detected in BM aspirates by Sanger sequencing and/or next-generation sequencing (NGS). NGS was performed on BM aspirates of patients when requested by the clinician, using the Oncomine Myeloid Research Assay (Thermo Fisher, Waltham, MA, USA), IonS5 XL (Thermo Fisher), or a customized panel with the NextSeq 550 platform (Illumina, San Diego, CA, USA).

The KIT D816V variant burden in different samples, including BM aspirates, smear slides, and trephine biopsy sections, was evaluated at diagnosis and follow-up using droplet digital PCR (ddPCR). DNA was extracted using a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) and QIAcube (Qiagen). ddPCRs were run in a QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA) according to the manufacturer’s instructions. The thermal cycles were as follows: enzyme activation at 95°C (10 minutes); 40 cycles of denaturation at 94°C (30 seconds), annealing and extension at 55°C (1 minute), and enzyme inactivation at 98°C (10 minutes); and holding at 4°C. Gating was performed based on positive and negative controls and analyzed using the QuantaSoft software v1.7.4 (Bio-Rad). Data were considered valid when at least 15,000 droplets were available.

Survival analysis was performed using the Kaplan-Meier method to compare overall survival (OS) in AML with RUNX1::RUNX1T1 with or without SM-AHN. OS was defined as the time from the date of diagnosis to the date of death or last follow-up. Categorical values were compared using Fisher’s exact test, and continuous variables were compared using the Mann-Whitney test. Statistical analyses were performed using SPSS version 25 (IBM Corporation, Armonk, NY, USA) and Prism 9.3.1 (GraphPad, San Diego, CA, USA).

Twenty-three patients were diagnosed as having AML with RUNX1::RUNX1T1 during the study period, four of whom (17.4%) were also diagnosed as having SM-AHN. The clinical characteristics of the patients with or without SM-AHN are summarized in Table 1. No significant differences in clinical characteristics were observed between the two groups, except for the presence of KIT variants (P = 0.040). KIT variants were present in six (26.1%) AML patients with RUNX1::RUNX1T1, in three out of four patients with SM-AHN (75%); two D816V and one N822K) and in three patients without SM-AHN (15.8%; D816V, A820G, Y418delinsSVYIYIH). Two (50%) patients with D816V KIT variants were diagnosed as having SM-AHN. Patients with or without SM-AHN did not show a significant difference in OS (P = 0.565).

We evaluated whether SM-AHN and AML diagnoses were concurrent. All patients were diagnosed as having SM-AHN based on follow-up BM tests. Patients with SM-AHN at the time of AML diagnosis did not show evident mast cell aggregation on hematoxylin-eosin slides. Thus, IHC staining for CD117 and CD25 was performed for diagnostic and follow-up BM samples (Fig. 1). In retrospective analysis, CD117 expression was observed in mast cell aggregates at diagnosis in two patients, whereas it was not evident in the other two patients, with diffuse CD117-positive staining observed on the blasts at diagnosis of SM-AHN.
Table 1. Characteristics of patients with AML with RUNX1::RUNX1T1

| Characteristics          | Without SM-AHN (N = 19) | With SM-AHN (N = 4) | P    |
|--------------------------|-------------------------|---------------------|------|
| Age (yr)                 |                         |                     |      |
| Median (range)           | 43 (4–74)               | 38.5 (8–56)         | 0.683|
| Sex, N (%)               |                         |                     |      |
| Male                     | 12 (63.2)               | 2 (50)              | 1.000|
| Female                   | 7 (36.8)                | 2 (50)              |      |
| Hb (g/L)                 |                         |                     |      |
| Median (range)           | 84 (52–116)             | 86 (69–101)         | 0.892|
| Leukocytes (×10⁹/L)      |                         |                     |      |
| Median (range)           | 5,810 (750–26,610)      | 10,540 (4,690–29,930)| 0.366|
| Absolute neutrophil count (×10⁹/L) |               |                     |      |
| Platelets (×10⁹/L)       |                         |                     |      |
| Median (range)           | 1,243 (66–13,017)       | 730 (125–1,373)     | 0.457|
| KIT variant, N (%)       |                         |                     |      |
| D816V                    | 3 (15.8)                | 3 (75)              | 0.040|
| Other                    | 1 (5.3)                 | 1 (25)              |      |
| Follow-up, months, median (range) | 22 (2–68)             | 33 (11–70)         | 0.494|
| Hematopoietic stem cell transplantation, N (%) | 11 (57.9)               | 4 (100)              | 0.257|
| Death, N (%)             | 2 (11.1)                | 1 (25.0)            | 0.470|

Abbreviations: AML, acute myeloid leukemia; SM-AHN, systemic mastocytosis with associated hematological neoplasm.

Fig. 1. BM biopsy section and IHC staining results at diagnosis showing an increase in blasts and scattered mast cells (A, B, C) and evident mast cell aggregates post induction (D, E, F). (A, D) Hematoxylin-eosin stain (×400). (B, E) CD117 positivity in mast cells (×400). (C, F) Aberrant CD25 expression in atypical mast cells (×400).

Abbreviations: BM, bone marrow; IHC, immunohistochemical.
Table 2. ddPCR results for KIT D816V in BM aspirates, smear slides, and tissue sections

| Sample number | Sampling time | Sample type | Blast count (%) | Mutant allele burden (%) |
|---------------|---------------|-------------|-----------------|--------------------------|
| S1            | Diagnosis     | BM smear slide | 52.2            | 46.5                     |
|               |               | BM aspirate   |                 | 46.9                     |
|               |               | BM tissue     |                 | 47.3                     |
|               | Post-induction| BM smear slide | 0.5             | 1.3                      |
|               |               | BM aspirate   |                 | 0.6                      |
|               |               | BM tissue     |                 | 1.2                      |
| S2            | Diagnosis     | BM smear slide | 80.7            | 32.1                     |
|               |               | BM aspirate   |                 | 31.8                     |
|               |               | BM tissue     |                 | 50.0                     |
|               | Post-induction| BM smear slide | 2.6             | 3.2                      |
|               |               | BM aspirate   |                 | 0.1                      |
|               |               | BM tissue     |                 | 3.2                      |

Abbreviations: ddPCR, droplet digital PCR; BM, bone marrow

AMLAbl1. Aberrant CD25 expression on mast cells at diagnosis was evident in only one patient. However, IHC staining of BM samples after induction revealed the presence of mast cell aggregates with CD117 expression and aberrant CD25 expression in all patients.

The KIT D816V variant burden in two patients with SM-AHN who had KIT D816V was assessed using diagnostic and follow-up samples (Table 2). The variant allele burden decreased with decreasing blast count and was similar in all three sample types. The variant burden was low in post-induction samples and lower in BM aspirates than in smear slides and tissue sections for both patients. However, the KIT D816V variant persisted after induction. Targeted sequencing results were obtained for two patients with SM-AHN and revealed additional variants in RUNX1 (N=2), FLT3 (N=1), TP53 (N=1), and NRAS (N=1) at diagnosis.

Concomitant SM-AHN was diagnosed in 17.4% of the AML patients with RUNXI::RUNXIT1 via CD117 and CD25 IHC staining in post-induction BM sections. Many studies have reported SM-AHN diagnosis in patients with AML [4, 8, 12, 13]; however, the clinical characteristics of patients with and without SM-AHN have not been compared. The OS and clinical characteristics did not differ significantly, but KIT variants were more prevalent in patients with SM-AHN. SM-AHN is difficult to diagnose in AML with RUNXI::RUNXIT1 because of a marked expansion of blasts and CD117 expression in blasts [4]. As NGS is routinely performed as a diagnostic test for myeloid malignancies, the presence of KIT D816V has been reported to predict concurrent SM-AHN in MDS, MPN, and AML [9]. A higher KIT variant burden in tissue samples than in liquid samples has been reported in mastocytosis, including advanced SM [11]. In our study, although only two patients were tested, the variant allele frequency in the BM smear slides was similar to that in the BM tissues, but greater than that in the BM aspirates, because BM components are enriched in tissues and smear slides. Thus, when fresh BM aspirate is not available, archived BM smear slides can serve as a reliable source of DNA for variant analysis [14]. The genomic analysis of AML with RUNXI::RUNXIT1 showed that the KIT D816V variant was present in 17.4% of patients (N=4). As these patients tested positive for one minor criterion, it is advisable to screen for mast cell collections. However, according to the European Competence Network on Mastocytosis (ECNM), non-D816V KIT variants are present in <10% of patients with mastocytosis, and therefore, the ECNM recommends whole KIT gene sequencing when KIT variant is not detected by codon 816-targeted assays, and non-D816V KIT variants may show a different response to treatment [15-17]. The persistence of KIT variants after induction therapy may be attributed to the SM or AHN component. Further, neoplastic mast cells of SM associated with AML with t(8;21) also carried RUNXI::RUNXIT1 as well as KIT variant as indicated by targeted FISH and single-cell analysis [10, 18]. Thus, it is difficult to predict the SM burden or use KIT variant burden as a minimal residual disease marker for SM-AHN [9]. Since therapies targeting the SM component of SM-AHN are available, confirmation of the diagnosis is clinically significant, especially for advanced SM [17, 19]. SM-AHN diagnosis requires additional testing with IHC staining after induction, and testing for KIT variants may be performed using various BM samples with high mutation burdens, such as tissues or smear slides. Although a previous report has suggested that SM-AHN is uncommon in patients with CBF-AML, in this study, SM-AHN diagnosis was confirmed in 50% of the patients with KIT-mutated AML with RUNXI::RUNXIT1 [20]. Therefore, it is necessary to screen mast cell aggregates for CD117 expression and subsequently for CD25 expression through IHC staining of samples collected from AML patients with RUNXI::RUNXIT1 after induction.

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AUTHOR CONTRIBUTIONS

Hwang SM, Lee JS, Seong MW, Seo SH, Paik JH, Kim SA, Lee JY, Lee JO were involved in study organization, data collection and revision of the draft. Hwang SM, Kim BJ, Lee JS, Seong MW, Seo SH, Paik JH, Chang YH interpreted the data. Hwang SM, Kim BJ wrote the first draft of the manuscript. Hwang SM, Chang YH, Bang SM designed the study and composed the final draft of the manuscript. All the authors have read the final manuscript and approved the submission.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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