Case report

Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN with RS-T) complicated by hyperleukocytosis and gene analysis in relation to leukocytosis

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Myelodysplastic/myeloproliferative neoplasm (MDS/MPN) with ring sideroblasts and thrombocytosis (MDS/MPN with RS-T), which exhibits both an increased number of marrow ring sideroblasts and thrombocytosis, is a rare disorder classified as one of the newly established forms of MDS/MPN in the WHO 2016 classification. A 77-year-old female with marked thrombocytosis of 1,024×10⁹/L was tentatively diagnosed with essential thrombocythemia in 2011, and the thrombocytosis was controlled using hydroxycarbamide and low-dose busulfan. In 2016, the leukocyte count increased to a peak value of 68.8×10⁹/L (86.6% mature neutrophils) during platelet-reduction therapy. Bone marrow aspirate exhibited hypercellularity with ring sideroblasts comprising 41.5% erythroblasts without excess myeloblasts. Cytogenetic examination demonstrated the JAK2 V617F mutation and chromosomal abnormality of 46,XX,del(20)(q1?). Furthermore, dysplastic features of erythroid and granuloid precursors, as well as many large atypical megakaryocytes, were observed. Further genetic examinations revealed the SF3B1 K700E mutation, but not amplification of the JAK2 gene or pathogenic mutations in the 13 other genes examined. A diagnosis of MDS/MPN with RS-T was established and hyperleukocytosis was controlled using a higher dose of hydroxycarbamide. Although the patient maintained a stable disease state, she became RBC transfusion-dependent. Hyperleukocytosis, regardless of chemotherapy, is rare and may be novel in this disorder.

Keywords: MDS/MPN with RS-T, hyperleukocytosis, SF3B1 mutation, JAK2 mutation

INTRODUCTION

Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN with RS-T) is a rare subtype of MDS/MPN, which is characterized by thrombocytosis of greater than 450×10⁹/L, ring sideroblasts with greater than 15% marrow erythroblasts, and dysplastic features of erythroid and granuloid precursors with less than 5% blasts. In the WHO 2008 classification, MDS/MPN with RS-T was provisionally registered as refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T), and in the WHO 2016 classification, this provisional entity was established as MDS/MPN with RS-T. MDS/MPN with RS-T is negative for the bcr-abl chimera gene and has a characteristic genetic background in which 2 genes are often mutated; one mutation is JAK2 V617F, which promotes myeloid proliferation, and the other is SF3B1 mutation, which causes myelodysplasia with ring sideroblasts (MDS-RS). Therefore, MDS/MPN with RS-T exhibits clinical features of both MPN, especially essential thrombocythemia (ET), and MDS-RS. Although anemia is often present in patients with MDS/MPN with RS-T, in the early stage of this disorder, hemoglobin levels and leukocyte and platelet counts are slightly higher than those in MDS-RS patients, whereas patients with MDS/MPN with RS-T often have lower hemoglobin levels and leukocyte and platelet
counts than patients with ET. We report a patient with MDS/MPN with RS-T who developed marked leukocytosis of $68.8 \times 10^9/L$ during platelet-reduction therapy. Such hyperleukocytosis may be rare and novel in this disorder.

**MATERIALS AND METHODS**

**Mutational analysis of the SF3B1 gene and 14 other genes**

Genomic DNA was extracted from whole blood of the present patient in August 2017 when the white blood cell (WBC) count was $33.3 \times 10^9/L$, with 84.3% neutrophils, 2.0% monocytes, 5.7% eosinophils, 0.7% basophils, and 7.3% lymphocytes. Mutation analyses using Cancer Hot Panel v2 (CHPv2) targeting 15 genes, including SF3B1 and JAK2, were performed by next-generation sequencing using the IonProton™ system (Torrent server ver. 5.2 and Variant caller ver. 5.2) (Thermo Fisher Scientific, K.K., Tokyo, Japan). Whole exon and target sequencing was performed for SF3-B1 and the remaining genes, respectively. These gene analyses were performed at the Department of Genome Biology, Kindai University School of Medicine after obtaining written informed consent from the patient and the approval by the ethics committees of both Shinko Hospital and Kindai University School of Medicine.

**CASE REPORT**

A 77-year-old female was referred to our hospital because of thrombocytosis of $1,150 \times 10^9/L$ in 2011. Mild thrombocytosis had been observed from 2004 and it gradually progressed. Mild leukocytosis was also observed from 2007, and periodic checkups had been performed regarding these increased blood cell counts at a clinic. Regarding her past medical history, she had pulmonary tuberculosis, sick sinus syndrome, and cerebral lacunar infarction in 1996, 2005, and 2010, respectively. She did not have specific family or life history.

At presentation at our hospital in 2011, she was physically normal, with no hepatosplenomegaly or superficial lymphadenopathy. Laboratory examination revealed a WBC count of $12.7 \times 10^9/L$, with 56.8% neutrophils, 8.8% eosinophils, 2.0% basophils, 5.3% monocytes, and 25.6% lymphocytes, a hemoglobin concentration of 11.6 g/dL, and a platelet count of $1,024 \times 10^9/L$. Fluorescence in situ hybridization (FISH) for the bcr-abl chimera gene on circulating neutrophils was negative. The neutrophil alkaline phosphatase (NAP) score was 329, being within normal limits (normally 170 to 330). Serum concentrations of lactic dehydrogenase and vitamin 12 were elevated to 262 IU/L (normally 115 to 245 IU/L) and higher than 1,500 pg/mL (normally 180 to 914 pg/mL), respectively. Other blood chemical and serological tests were normal. Although bone marrow examination was not performed, a tentative diagnosis of ET was made based on these findings.

As she had a past history of cerebral lacunar infarction, the thrombocytosis was controlled using hydroxycarbamide (250-500 mg/day) with subsequent normal platelet counts until November 2014 when the platelet count was elevated to $827 \times 10^9/mL$ (Figure 1). Then, hydroxycarbamide was switched to busulfan (2 mg/day) with a subsequent platelet count of less than $300 \times 10^9/L$ until December 2015. In July 2015, the WBC count began to increase to a peak value of $68.8 \times 10^9/L$ (86.6% mature neutrophils and 0% blasts)
Hyperleukocytosis in MDS/MPN with RS-T regardless of platelet-reduction therapy with busulfan. Therefore, further examinations were performed in June 2016. Bone marrow aspirate revealed slight hypercellularity with a nucleated cell count (Ncc) of 123.2×10^9/L and a megakaryocyte count of 32/μL. Myeloblasts and ring sideroblasts (Figure 2A) comprised 0.3 and 41.5% of marrow nucleated cells and erythroblasts, respectively. The ratio of myeloid to erythroid cells was 4.1. Dysplastic features of marrow cells, such as erythroblasts with a megaloblastoid chromatin network, abnormal nuclear lobulation (Figure 2B), giant metamyelocytes (Figure 2C), and neutrophils with hypolobulated nuclei (Figure 2C), were observed. Chromosomal analysis of the marrow cells demonstrated an abnormal karyotype of 46,XX,del(20)(q1?) in 20 of 20 dividing cells analyzed. PCR analyses revealed the JAK2 V617F mutation but not calreticulin (CALR) type 1 or 2 mutation. Furthermore, bone marrow biopsy was performed in February 2017. As shown in Figure 3, the bone marrow was highly hyperplastic with many large atypical megakaryocytes. Many ring sideroblasts were histologically noted when stained with potassium ferrocyanide solution (data not shown). Bone marrow fibrosis was not observed. Based on these results, MDS/MPN with RS-T was strongly suspected, and the patient was treated for hyperleukocytosis using a higher dose of hydroxycarbamide (1,500 to 1,000 mg/day), which subsequently improved the WBC count; however, she became RBC transfusion-dependent (Figure 1).

As shown in Table 1, gene analyses using genomic DNA extracted from the whole blood in August 2017 revealed the SF3B1 K700E mutation. The mutation was heterozygous and its frequency was 47.5% among the total DNA analyzed, indicating that almost all leukocytes at that time point had this mutation because the SF3B1 K700E mutation inevitably occurs heterozygously. The JAK2 V617F mutation was also detected, being homozygous with a frequency of 94.5%. No amplification of the JAK2 gene was observed. In the other genes examined, neither a pathogenic single nucleotide variant (SNV) nor gene amplification was noted (Table 1).

Based on the SF3B1 K700E mutation, marrow ring sideroblasts, dysplastic features of marrow hematopoietic cells, and the thrombocytosis, a diagnosis of MDS/MPN with RS-T was established. The treatment with hydroxycarbamide
(1,000 to 1,500 mg/day) was continued with leukocytosis of 16.3 to 27.3×10^9/L, normal platelet counts, and anemia requiring RBC transfusion (Figure 1); however, the disease was stable without blasts in the peripheral blood as of October 2018.

**DISCUSSION**

Regarding the diagnosis of the present patient, all diagnostic criteria, such as greater than 15% sideroblasts in the marrow cells, less than 1 and 5% blasts in the peripheral blood and bone marrow, respectively, thrombocytosis of greater than 450×10^9/L, SF3B1 gene mutation, and absence of the bcr-abl fusion gene, were fulfilled. The bone marrow examination was performed after platelet-reduction therapy, which had been continued for approximately 5 years; therefore, the ring sideroblasts in the present patient may have been a consequence of the hydroxycarbamide or busulfan treatment. However, there has been no report that these 2 cytotoxic agents induce ring sideroblasts. Furthermore, the presence of SF3B1 mutation may rule out the secondary ring sideroblasts.

MDS/MPN with RS-T is a rare disorder, whereas ET is common and can be easily diagnosed based on thrombocytosis, JAK2 or CALR mutation, and bone marrow examination. Although iron staining of the marrow preparation is required to differentiate MDS/MPN with RS-T, this staining is not always routinely performed during the diagnostic process for ET. The indicative factors for iron staining in a suspected ET case may include anemia and many dark blue granules in the cytoplasm of erythroblasts or erythrocytes, which can be observed by Wright-Giemsa staining.

Regarding leukocytosis in MDS/MPN with RS-T, Broseus et al. reported that the WBC count in MDS/MPN with RS-T is higher and lower than that in MDS-RS and ET, respectively. The leukocytosis in ET is usually mild to moderate, and to the best of our knowledge, marked leukocytosis in MDS/MPN with RS-T has not been reported. Therefore, hyperleukocytosis of 68×10^9/L, which developed during platelet-reduction therapy, may be novel. The JAK2 V617F mutation observed in the present patient was considered to play a role in the leukocytosis because it was homozygous, as observed in polycythemia vera, in which moderate leukocytosis around 30×10^9/L is occasionally observed. In addition, a homozygous state of JAK2 V617F in ET is rare, comprising 2 to 4% of ET patients carrying this mutation. This homozygosity may have occurred prior to the leukocytosis due to additional gene abnormalities, but not at the initial stage of the disease. Furthermore, a high allele frequency (94.5%) (Table 1) may also have led to the leukocytosis because a previous study demonstrated a significant but weak correlation between the JAK2 V617F allele burden and leukocytosis in ET. However, some additional factor may have been required to cause the marked leukocytosis in the present patient. We thus examined the amplified status of the JAK2 gene, which was negative, and the 13 other genes examined also did not have pathogenic mutations that may cause leukocytosis (Table 1). The chromosomal abnormality of del(20q) noted in the present patient is observed in 5 to 8% of MDS patients. MDS with del(20q) is associated with thrombocytopenia, but not leukocytosis. Moreover, the role of genes present in 20q in the pathogenesis of MDS remains unclear. Collectively, the mechanism of hyperleukocytosis in the present patient is not completely understood, and further investigation of other genes, such as ASXL-1, TET2, CBL, NRAS, SETBP1, BCOR, BRAF, KIT, or KRAS, is required. In addition, as leukocytosis may promote thrombosis in ET or PV, we tried to reduce the hyperleukocytosis using a higher dose of hydroxyurea knowing that it would adversely affect erythropoiesis in the present patient.

The hematological results for the present patient at presentation in 2011 reflected ET, not MDS-RS, in terms of marked thrombocytosis, mild leukocytosis, and the absence of anemia. Cazzola et al. described a multistep molecular
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pathogenesis of MDS/MPN with RS-T through the sequential acquisition of SF3B1 and JAK2 mutations. In the present patient, however, the hematological evidence for ET lasted for at least 5 years from presentation; therefore, the acquisition of the SF3B1 mutation may have been preceded by JAK2 mutation. Cazzola et al. mentioned this possibility in a small proportion of patients with MDS/MPN with RS-T. However, as bone marrow examination was not performed at presentation for the present patient, the precise pathogenesis remains unclear. Thus, molecular examinations at the early stage of this disorder may be highly important.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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