CASE REPORT

Essential thrombocythaemia with aggressive megakaryocytosis after myelofibrotic transformation

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ABSTRACT

Background: Among myeloproliferative neoplasms, it is often difficult to distinguish essential thrombocythaemia (ET) from prefibrotic-stage primary myelofibrosis (PMF) with thrombocytosis given their overlapping clinicopathological phenotypes.

Case presentation: We encountered a 45-year-old male who was initially diagnosed with ET and eventually became transformed to secondary myelofibrosis 20 years later. Two distinct types of aberrant megakaryocytes were observed at diagnosis: one type characteristic of ET and the other type characteristic of PMF. With a proliferation in the bone marrow, aberrant megakaryocytes were infiltrated into the extramedullary organs and were even present in the thrombus were observed at autopsy. As a result of next-generation sequencing, the significant increase of variant allele frequency (VAF) of JAK2 V617F and U2AF1 S34Y mutations was observed in the bone marrow cells at the final stage.

Conclusions: This patient could be recognized as an atypical case of aggressive megakaryocytosis transformed from ET.

Introduction

Patients diagnosed with one of the myeloproliferative neoplasms (MPNs) show heterogeneous clinicopathological phenotypes. Essential thrombocythaemia (ET) and primary myelofibrosis (PMF) constitute two distinct entities of three BCR-ABL1-negative MPNs. It is often difficult to distinguish patients with ET from those with prefibrotic stage PMF with thrombocytosis. Here, we report a 45-year-old male who was initially diagnosed with ET and the condition transformed to secondary myelofibrosis 20 years later.

Case presentation:

A 45-year-old male experienced dizziness while driving and was admitted to a local hospital. An elevated hemoglobin level was noted on two recent consecutive annual medical check-ups. His blood analysis revealed pancytosis (WBC 23,100 /μl, RBC 577 × 10^6 /μl, Hb 18.1 g/dl, Plt 68.7 × 10^4 /μl), and he was referred to our institute. On physical examination, an enlarged spleen was palpable 2 fingerbreadths below the left costal margin. No other clinical symptom related to dysregulated proinflammatory cytokines or disturbed microvascular circulation or splenomegaly was noted. Laboratory findings in our institute also revealed pancytosis: WBC 19,600/μl (Stab 5.5%, Seg 74.5%, Eosinroblast: Ebl) accounted for 21.2% of total nucleated cells. The myeloid to erythroid ratio was slightly elevated (3.3) (Table 1). An initial bone marrow aspiration revealed hypercellularity (nucleated cell count (NCC): 51.5 × 10^4/μl) with an increased number of megakaryocytes (900/μl). Percentage of erythroid precursors (erythroblast: Ebl) accounted for 21.2% of total nucleated cells. The myeloid to erythroid (M:E) ratio was slightly elevated (3.3) (Table 1). A needle biopsy of the bone marrow performed after an aspiration revealed slightly hyperplastic bone marrow accompanied by an increased number of dysplastic megakaryocytes (Figure 1a, b). The
megakaryocytes exhibited two distinct morphological types. One type had abundant and mature cytoplasm and deeply lobulated or hyperlobulated nuclei often referred to as 'staghorn-like', whereas the other type had an altered nuclear:cytoplasmic ratio with abnormal chromatin clumping and plump (cloud-like) or bulbous lobulation of the nuclei (balloon-shaped) [1]. Both types of dysplastic megakaryocytes formed loose clusters. Silver staining of the bone marrow biopsy specimen revealed a very loose network of reticulin fibres that was categorized as grade 0 myelofibrosis (MF-0) according to the criteria of the European consensus on grading bone marrow fibrosis [3]. In addition, the presence of a V617F heterozygous mutation was confirmed by using quantitative PCR analysis at this time (Figure 1e). Coarse bundles of collagen fibres with dense reticulin fibres were observed in silver staining of the trephine biopsy specimen (Figure 1g), and grade 3 myelofibrosis (MF-3) was confirmed based on the European consensus on grading bone marrow fibrosis. With these consecutive data, we verified myelofibrotic transformation under the criteria for post-ET MF [3]. In addition, the presence of a JAK2 V617F heterozygous mutation was confirmed by using quantitative PCR analysis at this time (Figure 2).

Approximately 3 years after the discontinuation of hydroxyurea, leukocyte counts began to increase. The third bone marrow examination was performed one month after readministration of hydroxyurea and revealed more extensive myelofibrosis compared with the former examination (Figure 1f, h). Unfortunately, despite the administration of an escalated dose of hydroxyurea, several clinical symptoms possibly related to dysregulated proinflammatory cytokines, such as fever, general malaise and night sweats, emerged along with exaggerated leukocytosis. Even after being admitted, these lines of symptoms continued to progress, and the patient died 6 days after hospitalization. We did not have the opportunity to administer ruxolitinib. An autopsy revealed marked hepatosplenomegaly. A splenomegaly (SI: 55 x 180 mm)

### Table 1. Laboratory findings.

| Complete blood count | Biochemistry | Serology |
|----------------------|--------------|----------|
| WBC 19,600 /µL | AST 25 U/L | CRP < 0.4 mg/dL |
| Stab 5.5% | ALT 30 U/L | IgG 1350 mg/dL |
| Seg 74.5% | ALP 7.8 KAU | IgA 275 mg/dL |
| Eo 2.0% | LDH 489 U/L | IgM 113 mg/dL |
| Ba 1.0% | T-Bil 0.5 mg/dL | IgD <1.0 mg/dL |
| Mo 3.5% | TP 7.5 g/dL | IgE 35 mg/dL |
| Lym 13.5% | CHE 390 U/L | IEP (serum) |
| RBC 592 x 10⁴ /µL | UN 14 mg/dL | M-protein(-) |
| Hb 18.2g/dL | Creat 1.1 mg/dL |
| Ht 54.70% | Na 142 mEq/L |
| MCV 92.3 | K 5.7 mEq/L | Vitamin B12 760 pg/ml (230 - 800) |
| MCH 30.7pg | Cl 103 mEq/L | EPO 13.8 mlU/ml (8 - 36) |
| MCHC 33.30% | Ca 4.9 mEq/L |
| Pt 66.6 x 10⁴ /µL | IP 4.2 mg/dL |
| Reticulocyte 2.4% |

**Bone marrow**

| NCC 51.5 x 10⁹ /µl | Mgk 900 /µl |
| Blast 2.2% | M:E 3.3 |
| Ebl 21.2% |

Chromosome 46 XY, [10/10]

FISH analysis BCR-ABL: negative

**Abdominal ultrasonography**

- Mild hepatomegaly
- Splenomegaly (SI: 55 x 180 mm)

**Circulating red blood cell volume** 24.6 ml/kg

**Arterial blood gas analysis**

- pH 7.417
- paCO2 42.2 mmHg
- paO2 87.7 mmHg
- BE 2.9 mmol/L

**Hematology**

- RBC 592 x 10⁴ /µL
- UN 14 mg/dL
- CHE 390 U/L
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**Acute Leukemia**

- Blast 2.2%
- RBC 592 x 10⁴ /µL
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**Table 1.** Laboratory findings.
Figure 1. Bone marrow histology in low-power (a and c; x100) or high-power (b and d ~ h; x400) fields at diagnosis (a ~ d), transformation into fibrosis (e and g) and the exaggerated thrombocytosis phase (f and h). a, b: H-E staining revealed slightly hyperplastic bone marrow with an increased number of dysplastic megakaryocytes. Two distinct types of dysplastic megakaryocytes were observed. One type exhibited abundant and mature cytoplasm and deeply and hyperlobulated nuclei (staghorn-like), whereas the other type exhibited an altered nucleocytoplasmic ratio with abnormal chromatin clumping and plump (cloud-like) or bulbous (balloon-shaped) lobulation of the nuclei. These two types of abnormal megakaryocytes formed loose clusters. Arrows and arrowheads indicate abnormal megakaryocytes with staghorn-like and cloud-like nuclei, respectively. c, d: Silver staining of the initial bone marrow biopsy revealed a very loose network of reticulin fibre, and the condition was categorized as grade 0 myelofibrosis (MF-0) based on the criteria of the European consensus on grading bone marrow fibrosis. e, f: H-E staining revealed infiltration of dysplastic megakaryocytes in the secondary (e) and tertiary (f) analyses. g, h: Silver staining of the secondary (g) and tertiary (h) bone marrow biopsy revealed obvious fibrosis. Compared with the secondary biopsy (g), the tertiary sample (h) exhibited more prominent fibrosis.
were detected even in arterial and venous thrombi, and cell aggregate in pulmonary blood vessels (Figure 3g, i). These megakaryocytes were positive for CD42b (Figure 3b, d, f, h, j) but negative for CD34.

In order to clarify the molecular basis on distinct changes of clinical features in the terminal stage, the secondary (at the diagnosis of post-ET MF) and tertiary (at the further progression of myelofibrosis) bone marrow samples were subjected to next-generation sequencing analysis [4]. As a result, a significant increase of VAFs of both JAK2 V617F and U2AF1 S34Y mutations was observed in the bone marrow cells (0.424% to 0.789%, and 0.029% to 0.396%, respectively) (Figure 2). There was no mutation of CALR or MPL genes in either sample.

**Discussion**

Up to 30% of PMF patients are asymptomatic but are consistently associated with thrombocytosis [1]. It is difficult to make an accurate differential diagnosis between ET and early PMF with thrombocytosis. In addition, 30 - 40% of PMF patients exhibit prefibrotic or early fibrotic stage disease [1]. In both stages, an increased number of megakaryocytes is the exclusive positive finding without obvious fibrosis in the bone marrow [1]. To address this issue, PMF is further subclassified into two stages in the World Health Organization (WHO) classification of myeloid neoplasms revised in 2017 (WHO 2017 classification): prefibrotic/early and overt fibrotic stages [5]. In this revision, the significance of morphological findings is emphasized to differentiate ET patients from PMF patients in the prefibrotic stage (prePMF). The most striking abnormalities of megakaryocytes in ET patients include abundant and mature cytoplasm and deeply lobulated and hypersegmented (staghorn-like) nuclei. In contrast, the important morphological findings in PMF patients include deviations from the normal nuclear:cytoplasmic ratio suggestive of defective maturation; abnormal patterns of chromatin clumping exhibiting hyperchromatic, cloud-like, or balloon-shaped nuclei; and bare nuclei [5]. Based on the WHO 2017 classification, the patient’s clinical manifestation and laboratory data did not fulfill the criteria for ET or prePMF at the time of administration. Histopathological analysis of the bone marrow samples, especially megakaryocytes, should have been critical to distinguish these two types of diseases. This patient’s bone marrow exhibited increased cellularity compared with the typical bone marrow of ET patients. No obvious dominance of erythroid precursor cells was noted. Intriguingly, two distinct types of dysplastic megakaryocytes were concurrently observed: one with ‘staghorn-like’ nuclei typically observed in ET and the other with ‘cloud-like’ or ‘balloon-shaped’ nuclei in PMF. Both types of dysplastic megakaryocytes were increased in number and formed loose clusters.

The discriminatory power of the WHO classification criteria remains controversial, especially between ET and prefibrotic/early PMF [6]. Various factors, such as disease diversity and interobserver discordance, may affect this problem [6, 7]. To address this issue, a multicentre study with a total of 295 patients with MPNs was performed, focusing on validation of the discriminatory power of the WHO classification system [8]. As a result, acceptable consensus concerning the discrimination between ET and PMF was achieved in 88% of cases. However, among the MPN patients analysed in this study, a few cases could not be categorized into
Figure 3. H-E staining of the bone marrow (a), liver (c), spleen (e), thrombus (g) and lung (i) at autopsy. Infiltration of dysplastic megakaryocytes was confirmed with anti-CD42b immunostaining in the bone marrow (b), liver (d), spleen (f), thrombus (h) and lung (j). Arrows and arrowheads indicate abnormal megakaryocytes with staghorn-like and cloud-like nuclei, respectively (a).
any typical disease entities. Some typical patients exhibited mixed characters of polycythaemia vera (PV) and ET and were categorized as ET/PV. The other undistinguishable patients were categorized as MPN-U. It is quite interesting that no patient exhibited a mixed appearance of ET and PMF similar to that noted in this patient even in this large study. Therefore, this patient is rare and valuable for solving the discriminatory issue between these two difficult-to-distinguish diseases. It was difficult to obtain an accurate diagnosis in this patient at the time of initial administration even under the newly revised WHO 2017 classification.

Patients with PMF generally exhibit significantly worse clinical outcomes compared with ET patients. The median overall survival of patients with ET and prefibrotic PMF from initial diagnosis is 21 and 14 years, respectively [8]. The ten-year and 15-year survival rates of ET and early/prefibrotic PMF are 89% and 76% and are 80% and 59%, respectively [9]. Myelofibrotic transformation along with the development of acute myeloid leukaemia in MPN patients is a potentially fatal complication, and the condition is evident earlier in early/prefibrotic MF compared with ET. The frequency of myelofibrotic transformation in ET patients is 0.8-4.9% and 4-11% at 10 and 15 years after diagnosis, respectively [10], whereas the frequency in early/prefibrotic MF patients is 2.3%, 12.3% and 16.9% at 5, 10 and 15 years, respectively [9]. In this patient, apparent bone marrow fibrosis was detected more than 20 years after diagnosis. Although the initial findings were inconsistent with the typical pathologic manifestations of ET, our patient collectively exhibited an ET clinical course rather than PMF. Moreover, given that the median time to fibrotic transformation in ET patients is approximately 7–16 years from the time of diagnosis [10], transformation was delayed in this patient compared with the average duration. Seven risk factors, including advanced age (≥60), leukocytosis (≥11,000/μl), anaemia, reticulin fibrosis and increased cellularity of the bone marrow, absence of the JAK2 V617F mutation, use of anagrelide and the presence of an ASXL1 mutation, affect fibrotic transformation in ET patients [10]. Although the mutational status of JAK2 or ASXL1 was not tested, the clinical findings of our patient met only two of the seven factors, namely, leukocytosis (19,600/μl) and hypercellularity of the bone marrow at the time of diagnosis, which could have prolonged the period before the transformation.

In ET patients, a homozygous JAK2 V617F mutation is rare (2.2%) and is associated with stimulated erythropoiesis, myelopoiesis, reduced platelet count, increased incidence of splenomegaly, increased spleen size, an increased necessity of cytoreductive therapy, an increased risk of thrombotic events and fibrotic transformation [11]. Approximately 3 years after confirming JAK2 V617F heterozygosity (VAF, 0.424), the VAF was increased to 0.789, indicating that the mutation became homozygous at least in some part of the cell population. In parallel, fibrotic transformation became more prominent, and the patient died of thrombosis in the pulmonary arteries. This clinical course is essentially consistent with previous reports regarding clinical correlates of the JAK2 V617F allele burden in MPNs [12].

The most outstanding pathological feature of this patient at autopsy was extensive infiltration of dysplastic megakaryocytes in the extramedullary lesions. In ET patients, leukaemic transformation is quite rare [13]. Dysplastic megakaryocytes, but not megakaryoblasts, proliferated in the bone marrow of this patient. Dysplastic megakaryocytes were also observed not only in the representative organs for pathological extramedullary haematopoiesis, i.e. the spleen and liver, but also in thrombi and in cell aggregate in blood vessels of the lung. This finding might represent evidence that pathological megakaryocytes circulated in the peripheral blood. Recently it is reported that megakaryocytes migrate out of the bone marrow to the lungs, where they produce platelets accounting for 50% of total platelet production [14]. In this patient, however, there was no evidence that thrombopoiesis in the lung was established. Actually, the number of platelets was below the level of normal range. CD34 is typically expressed on leukaemic blasts transformed from MPNs. An anti-CD34 antibody failed to detect megakaryocytes in the bone marrow of this patient. This patient could be recognized as exhibiting an aberrant type of megakaryocytosis transformed from ET.

As a result of next-generation sequencing analysis, a significant increase of VAFs of both JAK2 V617F and U2AF1 S34Y mutations was observed when compared between secondary (at the diagnosis of post-ET MF) and tertiary (at the further progression of myelofibrosis) bone marrow samples (0.424% to 0.789%, and 0.029% to 0.396%, respectively) (Figure 2). U2AF1 gene encodes RNA splicing factor, and its mutations (S34Y and S34F) were firstly identified in the bone marrow cells derived from patients of myelodysplastic syndromes (MDS) [15], and play a significant role in leukemogenesis in myeloid malignancies [16]. U2AF1 mutations are also observed in patients with PMF at a frequency of 16%. [17, 18]. Although the underlying molecular mechanism is not fully elucidated, U2AF1 mutations are significantly correlated with anemia and thrombocytopenia, and are recognized as adverse factors associated with inferior overall or leukemia-free survival [17]. In addition, co-occurrence of mutation of MPN-associated driver genes (JAK2, CALR or MPL) and spliceosome genes is observed in certain myeloid malignancies [19], such as JAK2 V617F and SF3B1 mutation in MDS/MPN with ring sideroblasts.
and thrombosis (MDS/MPN-RS-T) [20, 21]. These lines of evidence imply that the U2AF1 mutation also may have contributed to an aggressive proliferation of mature megakaryocytes at the final stage in this case.

In summary, considering the clinicopathological features of this patient that megakaryocytes of ET and prefibrotic MF types were mixed up in the bone marrow, it might have been impossible to clearly discriminate ET and prefibrotic MF at diagnosis and predict the clinical course. The occurrence of U2AF1 S34Y mutation at least partly could have modified the clinical course and accelerated fibrosis in the bone marrow. However, the molecular basis on the rapid production of mature infiltrative megakaryocytes at the final stage remains unknown. It is interesting to analyse how mutations of MPM drivers and spliceosome genes collaborate molecularly. We need further examination on clinical information from a large number of patients to clarify whether their co-existence determines some specific entities in MPN.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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