**BCR-ABL1** is a secondary event after JAK2V617F in a patient with essential thrombocythemia who develop chronic myeloid leukemia

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**Abstract**

Several cases such as myeloproliferative neoplasms (MPN) with the coexistence of JAK2 and BCR-ABL have been reported. However, cases of transformation of essential thrombocythemia (ET) into chronic myeloid leukemia (CML) during the disease progression were rarely reported. Here, we report the case of a patient with JAK2 V617F-positive ET who subsequently acquired BCR-ABL1, which transformed the disease into CML after 10 years from the initial diagnosis. In this study, we dynamically monitored JAK2 V617F and BCR-ABL and observed multiple gene mutations, including IDH2, IDH1, ASXL1, KRAS, and RUNX1. It is important to be aware of this potentially clone evolution in disease progression.

**Key words:** BCR-ABL1; Chronic myeloid leukemia; Clonal evolution; JAK2 V617F; Postessential thrombocythemia myelofibrosis

1. **INTRODUCTION**

Myeloproliferative neoplasms (MPN) are caused by hematopoietic stem cells (HSCs) with somatic mutations in the genes involved in the tyrosine kinase signaling. The main affected genes include the BCR-ABL1 in Philadelphia chromosome-positive chronic myeloid leukemia (CML) and JAK2/MPL/CALR mutations in MPNs. Although it was thought to be mutually exclusive, a number of cases with coexistence of JAK2 V617F and BCR-ABL in patient with MPN have been reported.1–4 However, the cases of transformation of essential thrombocythemia (ET) into CML during the disease progression were rarely reported. Here, we report the case of a patient with JAK2 V617F-positive ET who subsequently acquired BCR-ABL1 that transformed into CML after 10 years from the initial diagnosis. In this study, we dynamically monitored clinical variables, hematologic data, bone marrow (BM) histomorphologic features, karyotype, JAK2 V617F, and BCR-ABL and observed multiple gene mutations by next-generation sequencing (NGS), including IDH2, IDH1, ASXL1, KRAS, RUNX1 etc. Although the case was rare, it is important to be aware of this potential clone evolution during disease progression. These features can be misinterpreted to reflect resistance to therapy or disease progression.

2. **CASE PRESENTATION**

Clinical characteristics, laboratory results, BM biopsy results, response, and prognosis of a case of a 48-year-old male patient with ET who developed CML after 10 years from the initial diagnosis in The Second Affiliated Hospital of Harbin Medical University were retrospectively collected and analyzed.

A 48-year-old male patient was diagnosed with JAK2V617F-positive ET, normal cytogenetics, and absence of BCR-ABL 10 years ago (2010). He was treated with hydroxyurea or interferon-alpha (IFN-α) until March 2017. A routine blood monitoring showed that white blood cell (WBC) was between 5 and 10 × 10⁹/L, and the platelet (PLT) was between 400 and 600 × 10⁹/L. He had a history of coronary stent implantation and irregularly followed oral administration of antiplatelet aggregation drugs and statins in 2017. In the same year (2017), the patient suffered from right lower abdominal pain and his blood routine examination revealed high WBC: 26 × 10⁹/L. Thus, he was diagnosed with acute appendicitis and took anti-inflammatory medication and appendectomy. After surgery, the abdominal pain improved, but WBC was still higher than normal. At that time, the patient did not pay attention to it. Routine blood monitoring showed that the WBC was between 15 and 30 × 10⁹/L, and the PLT was between 100 and 300 × 10⁹/L. The patient continued to take hydroxyurea treatment.

However, in October 2020, the patient had splenomegaly and hepatosplenomegaly was clinically confirmed by ultrasound (spleen length: 16.5 mm). Full blood count revealed a WBC of 41.97 × 10⁹/L, HB of 128 g/L, PLT of 307 × 10⁹/L, and an absolute neutrophil count of 30.38 × 10⁹/L. BM aspirate was mildly hypercellular with increased megakaryopoiesis and a normal megakaryocyte:erythrocyte (M:E) ratio (Fig. 1A). A BM biopsy examination showed a hypercellular...

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marrow and predominant megakaryocytic proliferation, with very large and polyploid megakaryocytes arranged in tight clusters. Megakaryocytic proliferation was associated with a marked myeloid hyperplasia and the reticulin stain showed increased fibrosis (grade 3) (Fig. 1B and C). Cytogenetic analysis of the BM was abnormal (46, XY, del(13)(q13q21)[10]/46,XY[10]) when assessing twenty metaphases (Fig. 1D). In addition, we detected several mutations by next-generation sequencing (NGS). Nucleated cells (1.0 × 10⁷) were used for genomic DNA extraction after the lysis of red blood cells. NGS of genomic DNA was performed on the coding sequence (CDS) of the 175 hematological disease genes through Illumina NextSeq 550 with a mean sequencing depth of 2000 ◊. Data were analyzed using the bioinformatics pipeline in house (Data S1). The results found gene mutations, including JAK2 c.1849G>T/p.V617F (90.20%), IDH2 c.419G>A/p.R140Q (44.90%), ASXL1 c.1772dupA/p.Y519fs*1(12.10%), KRAS c.190T>G/p.Y64D (5.80%), RUNX1 c.508C>G/p.F203R.

Figure 1. Morphology and cytogenetic analysis. (A) Bone marrow (BM) morphology at post-ET MF diagnosis, Wright stained, ×100 magnification. (B and C) BM biopsy analysis at post-ET MF diagnosis, hematoxylin and eosin stained, ×40 magnification. (D) Karyotype at post-ET MF diagnosis: 46, XY, del(13)(q13q21). (E) BM morphology at CML diagnosis, Wright stained, ×100 magnification. (F) BM biopsy analysis at CML diagnosis, hematoxylin and eosin stained, ×40 magnification. (G and H) Karyotype at CML diagnosis: 46, XY, t(9;22)(q34;q11) and 46, XY, del(13)(q13q21). (I and J) Interphase fluorescence in situ hybridization (FISH) (Vysis dual color, dual-fusion translocation probe) analysis of BCR::ABL1 (ABL1 red, BCR green). Abbreviation: CML = chronic myeloid leukemia.
JAK2 V617F (PCR) Positive Positive 96.2
FISH BCR-ABL 0 0.50% 92.50%

Clinical characteristics of the patient in three phases.

| Characteristics | ET phase | Post-ET MF phase | CML phase |
|-----------------|----------|------------------|------------|
| Size of spleen (below costal margin, cm) | Normal | 15 | 15 |
| Hemoglobin, g/dL | 132 | 128 | 92 |
| Platelet count, x10^9/L | 658 | 307 | 135 |
| WBC count, x10^9/L | 7.2 | 41.97 | 128.6 |
| Neutrophils absolute count, x10^9/L (%) | 3.8 | 37.35 | 36 |
| Blasts (%) | 0 | 2 | 5 |
| Promyelocytes (%) | 0 | 7 | 8 |
| Myelocytes (%) | 0 | 2 | 3 |
| Metamyelocytes (%) | 0 | 1 | 11 |
| Bands (%) | 0 | 2 | 10 |
| Neutrophils (%) | 75 | 83.1 | 28 |
| Lymphocytes (%) | 20 | 2.9 | 7 |
| Monocytes (%) | 4 | 3.1 | 3 |
| Eosinophils (%) | 0 | 1.4 | 3 |
| Basophils (%) | 1 | 2.5 | 9 |
| LDH, IU/L | 189 | 661 | 1233 |
| Karyotype (ISCN) | 46, XY,del(13)(q14q14) | 46, XY,del(13)(q14q14) | 46, XY,del(13)(q14q14) |
| FISH BCR-ABL | 0.50% | 92.50% |
| JAK2 V617F (PCR) | Positive | Positive | 96.2 |

Abbreviations: CML = chronic myeloid leukemia, ET = essential thrombocythemia, FISH = fluorescence in situ hybridization, LDH = lactate dehydrogenase, MF = myelofibrosis, WBC = white blood cell.
| References, year | No. patients | Sex | Age at 1st D | Diagnosis* | Time to second Dx, years | Splenomegaly (cm) | WBC (×10^9/L) | PLT (×10^9/L) | HB (g/L) | BCR-ABL quantitation (PCR) | FISH | JAK2 (% Allele frequency) | Bone marrow reticulin | Treatment | Alive/Dead |
|------------------|-------------|-----|-------------|------------|--------------------------|------------------|----------------|---------------|---------|---------------------------|-------|------------------------|-----------------|-----------|------------|
| Soderquist et al, 2018 | 1            | F   | 66          | ET         | 3                        | ND               | ND             | ND            | ND      | Positive, e/3a2            | ND    | Positive, 24%           | Moderate        | Hy and Rux | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Nil, Hy and Rux | A          |
| Kandarpa et al, 2017 | 2            | M   | 63          | ET         | 2                        | ND               | ND             | 42            | 38      | Negative, 4.40%            | ND    | Negative, 50%           | Moderate        | Das and Rux | Im A      |
| Kandarpa et al, 2017 | 3            | M   | 70          | ET         | 4                        | ND               | ND             | 3.8           | 589     | No detected               | ND    | 93.3%                  | Moderate        | Im         | Im A      |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Das and Rux | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Calr       | A          |
| Kandarpa et al, 2017 | 4            | F   | 59          | ET         | 13                       | ND               | ND             | 48.2          | 380     | Negative                  | ND    | Positive, 24%           | Mild to none    | Das and Rux | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Anagrelide  | A          |
| Grisouard et al, 2013 | 5           | F   | 51          | ET         | 5                        | ND               | ND             | ND            | ND      | Negative                  | ND    | Negative, 24%           | Moderate        | Im         | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Nil, Hy and Rux | A          |
| Jallades et al, 2008 | 6           | F   | 56          | ET         | 4                        | ND               | ND             | ND            | ND      | Positive, b/3a2            | ND    | t(9;22)(q34;q11)         | Moderate        | Hy and Rux | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Das and Rux | A          |
| Curtin et al, 2005 | 7            | M   | 73          | ET         | 12                       | 16.7             | 1000           | 1000          | 13.5    | Negative                  | 81%   | t(9;22)(q34;q11)         | Positive        | Aspirin    | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       | Positive, 24%           | Moderate        | Hy or Im   | A          |
| Wahlin and Golovleva, 2003 | 8      | M   | 41          | ET         | 18                       | 60.1             | Normal         | 9180          | 93.5%   | Positive, 24%            | ND    | Positive, 24%           | Moderate        | Hy and IFN  | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Peripheral SCT | A          |
| Soderquist et al, 2018 | 9           | F   | 48          | PV         | 5                        | 26.1             | 66             | 13.4          | e/3a2   | ND                         | 46,XX:t(9;22)(q34;q11) | 24.4%|                       |                 | Phleb, Hy, IFN | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | IFN and Rux | D          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Hy          | D          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Th or Rux    | D          |
| Kandarpa et al, 2017 | 11          | M   | 76          | PV         | 6                        | 60.2             | 77             | 8.8           | e/1a2   | 63%                       | 46,XX:t(9;22)(q34;q11)| >50%|                       |                 | Im, Th and Rux | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Hy or Im    | D          |
|                       |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Das and Rux | A          |
| Zhou et al, 2015 | 13           | F   | 45          | PV         | 11                       | Yes              | 45             | 799          | 95      | 46,XX:t(9;22)(q34;q11)  | 6%   |                       |                 | Das, Hy and Rux | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Hy          | A          |
| Wang et al, 2013 | 14           | M   | 45          | CML        | 12                       | 45               | ND             | 95            | 95      | Positive, 24%            | ND    | Positive, 24%           | Moderate        | Hy and Rux | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Im and Das   | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Phleb       | A          |
| Pieri et al, 2011 | 16           | M   | 72          | PV         | 10                       | 46               | 462            | 118           | b/3a2   | 61%                       | 46,XY:t(9;22)(q34;q11)| 61%|                       |                 | Hy          | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       |                       |                 | Im and Das   | A          |
| Pingali et al, 2009 | 17          | M   | 39          | PV         | 15                       | 8                | 662            | 342           | 129     | Negative                  | ND    | t(9;22)(q34;q11)        | Positive        | Im, Nil and Das | A          |
|                   |              |     |             |            |                          |                  |                |               |         |                           |       | 62%                     | Moderate        | Im and IFN   | D          |
| Hussein et al, 2008 | 18          | M   | 48          | PV         | 15                       | 16.7             | 1000           | 1000         | 135     | ND                        | b/3a2 | 81%                     | Positive        | Im and IFN   | D          |

(Continued)
### Table 2

(Continued)

| Reference | No. of patients | Age at 1st diagnosis | Gender | Sex | Diagnosis* | Time to 2nd diagnosis | Bone marrow studies | JAK2| Bone marrow reticulin | FISH | BCR-ABL quantitation (P2R) | WBC | PLT | HB | Alive/Dead | Treatment |
|-----------|----------------|---------------------|--------|-----|------------|----------------------|--------------------|-----|----------------------|-------|------------------------|------|------|-----|------------|----------|
| Soderquist et al, 2007 | 22 | 45 | PMF | M | CML | 16 | Yes | F | 66 PMF | CML | Philadelphia | 102 | 142 | 557 | 83 | Positive | Im | + |
| Mirza et al, 2007 | 23 | 48 | PMF | M | CML | 3 | Yes | F | 67 | CML | Philadelphia | 65 | 625 | 90 | -50 | Positive | Im | - |
| Mirza et al, 2014 | 23 | 48 | PMF | M | CML | 1 | Yes | F | 67 | CML | Philadelphia | 65 | 625 | 90 | -50 | Positive | Im | - |

| Reference | No. of patients | Age at 1st diagnosis | Gender | Sex | Diagnosis* | Time to 2nd diagnosis | Bone marrow studies | JAK2| Bone marrow reticulin | FISH | BCR-ABL quantitation (P2R) | WBC | PLT | HB | Alive/Dead | Treatment |
|-----------|----------------|---------------------|--------|-----|------------|----------------------|--------------------|-----|----------------------|-------|------------------------|------|------|-----|------------|----------|
| Soderquist et al, 2014 | 23 | 48 | PMF | M | CML | 1 | Yes | F | 67 | CML | Philadelphia | 65 | 625 | 90 | -50 | Positive | Im | - |

Abbr: No. = number of patients, PMF = Philadelphia positive CML, CML = chronic myelogenous leukemia, EA = essential myeloid neoplasms, BCR::ABL = BCR-ABL1 translocation and JAK2 V617F mutation. Other abbreviations are defined in the table.

In conclusion, we describe a rare case of a patient who was diagnosed with ET followed by a diagnosis of CML and demonstrated that a preexisting JAK2 V617F positive clone acquired BCR::ABL1 translocation. This might suggest the necessity of screening for BCR::ABL1 translocation in patients with MPN with poor treatment responses and a rapid megalosplenia.

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This can explain the presence of 2 diseases in the same patient. In addition, the presence of these mutations indicates poor prognosis in MF and suggest short overall survival. How to explain the transformation of MPN to CML? Is it clonal evolution or therapy-related CML? Therapy-related CML has been reported in other cancers, including breast, lung, and gastric cancers, but very little is known about its clinical presentation and pathologic features. Although treatment strategies would be exactly the same as those for de novo CML, little is known about the responses and outcomes of therapy-related CML patients treated with TKIs. Iriyama et al[15] reported 11 patients with therapy-related CML treated with TKIs. The responses, prognoses, treatment responses, and outcomes were favorable as those of patients with de novo CML.[15] Although therapy-related CML have been reported to be potentially related to chemotherapy, radiotherapy, and immunosuppressive therapy, the pathogenesis of therapy-related CML is unclear. Either chemotherapy or radiotherapy could have had their immunity aggrieved or BM microenvironment injured.

Interestingly, low levels of BCR::ABL1 transcripts have been detected in some MPN at the cytogenetic level.[6,7] This and the chronology of reported transformations of PV or ET to CML suggest that the emergence of CML is likely a secondary event that results in the expansion of a clone with a greater proliferative advantage. It also appears to be consistent with the epidemiologic data that have shown that the pathogenesis of chronic phase CML is a result of 2 or more genetic events rather than a single hit.[18] However, others have suggested the mutations may arise in two independent clones. Nevertheless, both these scenarios presuppose an unstable genome that induces multiple changes in a stem cell or favors emergence of other competing clones. However, another hypothesis suggests that JAK2 V617F mutation increases genetic instability, resulting in the acquisition of a translocation or loss of heterozygosity. Alternatively, the patient may have a germline predisposition to leukemia, or a JAK2 V617F mutation that may have occurred in an HSC together with other somatic mutations that induce genetic instability.[10]

In conclusion, we describe a rare case of a patient who was diagnosed with ET followed by a diagnosis of CML and demonstrated that a preexisting JAK2 V617F positive clone acquired BCR::ABL1 translocation. This might suggest the necessity of screening for BCR::ABL1 translocation in patients with MPN with poor treatment responses and a rapid megalosplenia.
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