CASE REPORT

Acute Megakaryoblastic Leukemia Harboring a Subclone Expressing BCR-ABL1 Fusion Gene Product

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Abstract:
Acute myeloid leukemia (AML) with BCR-ABL1, also termed Philadelphia chromosome-positive AML (Ph+ AML), is a rare subtype classified by the World Health Organization in 2016. The characteristics of Ph+ AML have not been fully identified yet. We herein report a patient with Ph+ AML who phenotypically exhibited megakaryoblastic characteristics, FAB:M7 and harbored a subclone expressing BCR-ABL1 gene fusion products. This case suggests that BCR-ABL1 was acquired as a subclone due to a secondary event that might have occurred late during leukemia evolution. Our findings may aid in deciphering the mechanism underlying Ph+ AML development in future studies.

Key words: Ph+ AML, acute megakaryoblastic leukemia, BCR-ABL1 fusion gene

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Introduction

Acute myeloid leukemia (AML) with BCR-ABL1, also termed Philadelphia chromosome-positive AML (Ph+ AML), is a rare subtype of leukemia. It contributes to approximately 0.48%-3% of newly diagnosed cases of AML (1, 2). Ph+ AML should be distinguished from chronic myeloid leukemia in myeloid blast crisis (CML-MBC) (1) or mixed phenotype acute leukemia (MPAL) with t(9;22)(q34;q11); BCR-ABL1 (3). However, the disease characteristics of Ph+ AML have not yet been fully identified (4).

Ph+ AML was classified as a high-risk disease in the recently updated guidelines of European Leukemia Net (ELN) (5) and current National Comprehensive Cancer Network (NCCN) (6). Its treatment has not been established yet and remains controversial due to the absence of systematic clinical data (1). Furthermore, although the BCR-ABL1 oncoprotein has been shown to be a selective target in the treatment of CML, positioning of a targeted therapy using tyrosine kinase inhibitors (TKIs) in Ph+ AML is controversial (2). Neuendorff et al. suggested that the co-incidence of spontaneously occurring BCR-ABL1 within clonal hematopoiesis may explain the development of Ph+ AML and that BCR-ABL1 is an additional modification that emerges as a BCR-ABL1-positive subclone (1). We experienced a patient presenting acute megakaryoblastic leukemia with a BCR-ABL1-expressing subclone who was diagnosed with Ph+ AML based on the morphological, phenotypical, and cytogenetic properties according to the World Health Organization (WHO) classification (7).

We herein report a patient with Ph+ AML who might have acquired BCR-ABL1 as a subclone due to a secondary event during leukemia evolution, as proposed previously (1).

Case Report

A 69-year-old woman presented at Suzuka Kaisei Hospital for general malaise that had persisted for 1 month. She did not have any remarkable history or mention any antecedent hematological abnormality.

A physical examination revealed anemia in the conjunctiva. No other abnormalities, such as splenomegaly, were observed. The laboratory findings were as follows: white blood cell count, 2.2x10⁹/L; blasts, 4%; myelocytes, 2%; neutrophils, 41%; lymphocytes, 46%; monocytes, 4%; basophils, 2%; eosinophils, 1%; hemoglobin, 61 g/L; and platelet count, 20x10⁹/L. The blasts were lymphoblast-like with a
Figure 1. (1) Peripheral blood smear findings using May-Giemsa stain at (A) 400, (B) 1,000 magnification, and (C) MPO at 1,000 magnification. (2) Histological findings of bone marrow biopsy using (A) HE, (B) CD34, (C) CD42b, (D) c-kit, and (E) silver impregnation staining at 400× magnification. HE: Hematoxylin and Eosin staining, MG: May-Giemsa, MPO: myeloperoxidase, SIM: silver impregnation method

high nuclear-cytoplasmic ratio and negative for myeloperoxidase (MPO) staining, and some exhibited cytoplasmic blebs (Fig. 1-(1)).

Bone marrow aspiration could not be performed due to ‘dry tap.’ Instead, a bone marrow biopsy was performed, which revealed that the megakaryoblasts were immunophenotypically positive for CD34, CD42b, and c-kit and negative for MPO (Fig. 1-(2) A to D). The immunophenotypes by flow cytometry of the peripheral blood revealed that the blasts were positive for CD13, CD34, CD117(c-kit), and HLA-DR (Fig. 2). Bone marrow fibrosis was categorized as grade 2 according to the European consensus (8) (Fig. 1-(2) E).

Real-time polymerase chain reaction (RT-PCR) screening of the peripheral blood detected 4,000 copies of the minor BCR-ABL1 gene and 40,000 copies of the WT1 gene. Interphase fluorescence in situ hybridization (FISH) of BCR-ABL1 revealed the presence of a BCR-ABL1 fusion signal. Interphase FISH for BCR-ABL1 of peripheral blood neutrophils showed findings of 0% in segmented cells and 4% in mononuclear cells. A G-banding karyotype analysis did not exhibit t(9;22), and complex karyotype abnormalities were observed (Fig. 3-(1)). In addition, a gene-mutation analysis was negative for NPM1, FLT3-ITD, JAK2V617F, MPLW515L/K, and CALR types 1 and 2.

Based on these findings, she was diagnosed with acute megakaryoblastic leukemia expressing the BCR-ABL1 gene (AML with BCR-ABL1 based on the WHO 2016 classification (7)). FISH of the bone marrow biopsy sample revealed the presence of one BCR-ABL1-positive cell among 200 blasts (Fig. 4). We considered the BCR-ABL1-positive clone to be a subclone.

The patient was hospitalized and treated with cytarabine/idarubicin as induction therapy without administering a tyrosine kinase inhibitor (TKI). She successfully achieved hematological complete remission. The number of minor BCR-ABL1 gene copies decreased to 260, and interphase FISH was not detected in the bone marrow.

She was subjected to two additional courses of consolidation therapy. Four months later, the disease recurred with regrowth of blasts in both peripheral blood and bone marrow. At the relapse, 39,000 copies of WT1 mRNA were detected using RT-PCR in the bone marrow; however, minor BCR-ABL1 was undetectable. A G-banding karyotype analysis did not exhibit t(9;22), and complex karyotype abnormalities were observed at the relapse (Fig. 3-(2)). FISH of the bone marrow biopsy sample revealed no fusion signal corresponding to BCR-ABL1. The disease was uncontrollable even through the treatment with gemtuzumab/ozogamicin, CAG therapy with cytarabine, aclarubicin, and granulocyte colony-stimulating factor (Fig. 5).

She refused further treatment and switched to best supportive care.

Discussion

AML with BCR-ABL1 is a rare subtype of leukemia and was considered a provisional entity of myeloid neoplasm
and acute leukemia (1). It was newly included in the revised WHO classification in 2016 (3). However, the disease characteristics of Ph+ AML have not been fully identified yet (4). It is important to distinguish Ph+ AML from CML-MBC (1). It was reported that the following characteristics supported the diagnosis of AML rather than CML: no antecedent hematological anomaly, no basophilia or significant splenomegaly at the diagnosis, the detection of p190-transcript, the occurrence of BCR-ABL1 transcripts in <100% of metaphases, and a persistent complete cytogenetic response after conventional chemotherapy (1).

We encountered a case of Ph+ AML expressing a subclone of BCR-ABL1 fusion gene product and a majority of non-BCR-ABL1 clones. The leukemic blasts exhibited morphologically homogeneous megakaryoleukemic characteristics. These findings suggest that the Ph+ AML subclone expressing BCR-ABL1 might have developed as a secondary clone during leukemogenesis.

Ph+ AML cells often aberrantly express lymphoid markers (9). These should be distinguished from MPAL with t(9;22)(q34;q11); BCR-ABL1, which express two types of key lineage-defining markers according to the 2016 WHO classi-
Figure 4. A FISH analysis of the bone marrow biopsy sample. The white arrow indicates a native BCR gene and a native ABL1 gene in a nucleus. The green signal shows a native BCR gene, and the red one shows a native ABL1 gene. The yellow arrow indicates a fusion signal corresponding to a BCR-ABL1 gene. FISH fluorescence in situ hybridization.

Figure 5. Temporal changes in the expression of BCR-ABL1 and WT1 genes assessed through an RT-PCR analysis of bone marrow or peripheral blood. At the relapse, 39,000 copies of WT1 mRNA were detected, and the copy number of BCR-ABL1 gene was below the detection level. HCR: hematological complete remission, RT-PCR: real-time polymerase chain reaction.

Ph+ AML consists of a morphologically single blast population, whereas most MPAL cases exhibit a dimorphic blast population with some blasts resembling lymphoblasts and others myeloblasts (7). In our case, the morphological analysis indicated the presence of a single blast population, which represented a typical immunophenotype of acute megakaryoblastic leukemia (M7) according to the FAB classification (7). Considering these morphological, phenotypical, and cytogenetic properties, our patient was diagnosed with Ph+ AML.

BCR-ABL1 transcripts may temporarily be expressed at low levels in the blood of healthy individuals. However, the consequences associated with leukemogenesis are unknown (10). The higher frequency of BCR-ABL1-positive carriers in healthy populations than the incidence of Ph+ leukemia indicates that the BCR-ABL1 aberration is not sufficient for the malignant transformation of hematopoietic cells (11). Neuendorff et al. showed that Ph+ AML develops with the co-occurrence of spontaneously occurring BCR-ABL1 during clonal hematopoiesis on an underlying ‘mutational background’ and displays a small and/or temporary fraction as a BCR-ABL1-positive subclone (1). Bacher et al. reported that several AML cases harbored BCR-ABL1 as subclones, with other chromosomal abnormalities in most of
the cells in metaphase in addition to t(9;22) (12). These additional BCR-ABL1 alterations were supposedly acquired as secondary changes occurring in the late phase of leukemia evolution, so it is not the primary driver mutation (13).

Furthermore, the prognosis of Ph+ AML depends on the genetic background (concurrent aberrations) compared to BCR-ABL1 (13), whereas Ph+ AML is classified as a high-risk disease according to the recently updated guidelines of ELN (5) and current NCCN (6). In our case, the expression of a minor BCR-ABL1 gene in RT-PCR was low, and a BCR-ABL1 fusion signal was detected using FISH in only 1 out of 200 blasts in the bone marrow biopsy sample. Therefore, we suspected that BCR-ABL1-positive clone might be a subclone that emerged due to a secondary event during the disease development and not as the founding clone of AML. Considering the temporal changes in the expression of minor BCR-ABL1 and WT1 genes in the bone marrow or peripheral blood using RT-PCR, it is conceivable that the BCR-ABL1-positive subclone was present in a minor population at the early phase of the disease, and BCR-ABL1-negative founding clones survived, thereby leading to relapse after two additional courses of consolidation therapy. At the time of relapse, RT-PCR revealed 39,000 copies of WT1 mRNA with the minor BCR-ABL1 below the detection level. A FISH analysis of the bone marrow biopsy sample revealed the absence of positive cells among 200 blasts. The results of RT-PCR and FISH and the clinical course of our case support the proposed mechanism underlying the development of Ph+ AML (1).

Treatment of Ph+ AML has not been established yet; it remains controversial due to the absence of systematic clinical data (1). In particular, targeted therapy using TKIs has not been established (14). Thus far, several case reports have described implementing TKI treatments either alone or in combination with chemotherapy for Ph+ AML (1, 2, 4, 12-17). Some cases achieved hematologic responses to TKI treatment, but the response durations were limited (2). The reason for the limited response duration of TKIs against Ph+ AML is due to the clonal diversity of Ph+ AML, as opposed to CML in which the TKIs respond for a longer duration (1). A previous case report described how TKI treatment led to the eradication of BCR-ABL1-positive clones in AML, despite the overall growth of refractory AML cells (15). This suggests that TKI treatment carries a risk of clonal selection of BCR-ABL1-negative clone (1), leading to mutational evolution of clones other than BCR-ABL1-positive clones (15). Therefore, treatment with a single molecule-targeting agent, such as a TKI, may not be sufficient for achieving disease control of Ph+ AML, possibly because BCR-ABL1 is not a driver mutation present in the founding clone. However, such treatment might temporarily inhibit the proliferation of the BCR-ABL1-positive subclone (1). Therefore, while TKI treatment cannot be routinely recommended as part of first-line therapy (1), it might be a reasonable approach as a part of salvage therapy or may be able to be implemented in combination with allogeneic stem cell transplantation (4).

TKIs might be a safe and effective option for treating patients with Ph+ AML who are in poor general condition and cannot tolerate chemotherapy (18). Furthermore, combination therapy using venetoclax with TKI-based regimens showed encouraging activity in heavily treated patients, and the patients who achieved hematological responses showed higher number of Ph+ cells in metaphase or more BCR-ABL1 PCR copies at baseline than non-responders (18). Therefore, TKIs may be useful as an option for salvage therapies, especially in heavily treated patients with a poor performance status and high baseline Ph+ cells in metaphase (1, 4, 18). Our patient was newly diagnosed with AML, and her general appearance was good (performance status 0). At the time of the diagnosis, a low level of minor BCR-ABL1 expression was detectable in the peripheral blood, and AML cells exhibited complex karyotypes without t(9;22) in metaphase. Therefore, TKI treatment was not administered. At the relapse, TKIs were unsuitable as salvage therapy because the BCR-ABL1-positive clone had already disappeared.

In conclusion, Ph+ AML cells are cytologically heterogeneous with clonal diversity (14), and the BCR-ABL1 fusion gene may emerge as a subclone due to a secondary event possibly occurring at the late phase of leukemia evolution (13). These findings support the concept of the previously proposed mechanism underlying the development of Ph+ AML (1).

The authors state that they have no Conflict of Interest (COI).

References

1. Neuendorff NR, Burmeister T, Dörken B, Westermann J. BCR-ABL-positive acute myeloid leukemia: a new entity? Analysis of clinical and molecular features. Ann Hematol 95: 1211-1221, 2016.
2. Soupp CP, Vergilio JA, Dal Cin P, et al. Philadelphia chromosome-positive acute myeloid leukemia: a rare aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. Am J Clin Pathol 127: 642-650, 2007.
3. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127: 2391-2405, 2016.
4. Shao X, Chen D, Xu P, et al. Primary Philadelphia chromosome positive acute myeloid leukemia: A case report. Medicine 97: e12949, 2018.
5. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129: 424-447, 2017.
6. National Comprehensive, Cancer Network. Clinical Practice Guidelines in Oncology on acute myeloid leukemia. Version 2 [Internet]. 2021 [cited 2020 Dec 22]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf
7. Borowitz MJ, Béné MC, Harris NL, Porwit A, Matutes E, Arber DA. Acute leukemia of ambiguous lineage. In: World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. (Revised 4th edition). Swerdlow SH, Campo E, Harris NL, et al., Eds. International Agency for Research on Can-
cancer, Lyon, 2017.
8. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica 90: 1128-1132, 2005.
9. Cuneo A, Ferrant A, Michaux JL, et al. Philadelphia chromosome-positive acute myeloid leukemia: cytoimmunologic and cytogenetic features. Haematologica 81: 423-427, 1996.
10. Ismail SI, Naffa RG, Yousef AM, Ghanim MT. Incidence of bcr-abl fusion transcripts in healthy individuals. Mol Med Rep 9: 1271-1276, 2014.
11. Song J, Mercer D, Hu X, Liu H, Li MM. Common leukemia- and lymphoma-associated genetic aberrations in healthy individuals. J Mol Diagn 13: 213-219, 2011.
12. Bacher U, Haferlach T, Alpermann T, et al. Subclones with the t(9;22)/BCR-ABL1 rearrangement occur in AML and seem to cooperate with distinct genetic alterations. Br J Haematol 152: 713-720, 2011.
13. Neuendorff NR, Hemmati P, Arnold R, et al. BCR-ABL+ acute myeloid leukemia: are we always dealing with a high-risk disease? Blood Adv 2: 1409-1411, 2018.
14. Rebourcier E, Chanterie S, Gac AC, Reman O. Rare but authentic Philadelphia-positive acute myeloblastic leukemia: two case reports and a literature review of characteristics, treatment and outcome. Hematol Oncol Stem Cell Ther 8: 28-33, 2015.
15. Neuendorff NR, Schwarz M, Hemmati P, et al. BCR-ABL1+ acute myeloid leukemia: clonal selection of a BCR-ABL1- subclone as a cause of refractory disease with nilotinib treatment. Acta Haematol 133: 237-241, 2015.
16. Konoplev S, Yin CC, Kornblau SM, et al. Molecular characterization of de novo Philadelphia chromosome-positive acute myeloid leukemia. Leuk Lymphoma 54: 138-144, 2013.
17. Piedimonte M, Ottone T, Alfonso V, et al. A rare BCR-ABL1 transcript in Philadelphia-positive acute myeloid leukemia: case report and literature review. BMC Cancer 19: 50, 2019.
18. Maiti A, Franquiz MJ, Ravandi F, et al. Venetoclax and BCR-ABL Tyrosine Kinase Inhibitor Combinations: Outcome in Patients with Philadelphia Chromosome-Positive Advanced Myeloid Leukemias. Acta Haematol 143: 567-573, 2020.

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