Four Cases of Chronic Myelogenous Leukemia in Mixed Phenotype Blast Phase at Initial Presentation Mimicking Mixed Phenotype Acute Leukemia with t(9;22)

Woojin Choi, M.D.1, Myungshin Kim, M.D.1, Jihyang Lim, M.D.1, Kyungja Han, M.D.1, Seok Lee, M.D.2, Jae Wook Lee, M.D.3, Nack Gyun Chung, M.D.3, and Yonggoo Kim, M.D.1
Departments of Laboratory Medicine1, Internal Medicine2, and Pediatrics3, College of Medicine, The Catholic University of Korea, Seoul, Korea

The t(9;22)(q34;q11.2) translocation results in a BCR-ABL1 fusion gene located on the Philadelphia chromosome (Ph), causing a constitutively active BCR-ABL1 tyrosine kinase. This fusion gene is found in virtually all cases of CML, 5% of pediatric and 15-30% of adult cases of ALL, and 1-2% of cases of de novo AML [1-3]. While mixed phenotype acute leukemia (MPAL) with t(9;22)(q34;q11.2) is rare, this translocation is the most common recurrent genetic abnormality seen in MPAL [4].

Untreated CML typically follows a biphasic or triphasic clinical course. An initial indolent chronic phase (CP) is followed by an accelerated phase (AP) and/or a blast phase (BP) [5]. Only about 10% of patients initially present in BP [6]. Such cases can be easily misdiagnosed as acute leukemia with t(9;22) [4]. In about 70% of CML-BP cases, the blast lineage is myeloid, whereas the blasts are lymphoblasts in 20-30% of cases [5]. In addition, the blasts are of mixed phenotype only in rare cases of CML-BP [7]. Here, we report 4 cases of CML in mixed phenotype BP, diagnosed by FISH analysis performed on mature neutrophils, which were initially suspected to be acute leukemia with t(9;22). Splenomegaly, major BCR-ABL1, and peripheral granulocytic hyperplasia with all stages of granulocytes without dyspoiesis were observed in all cases. The clinical and laboratory findings at initial presentation are summarized in Table 1.

CASE 1: A 60-yr-old man presented with fatigue, myalgia, chills, dull pain in the left axillary region, and weight loss of 5 kg over 15 days. Ultrasonography revealed splenomegaly. Laboratory tests showed white blood cell (WBC) count of 41.9×10^9/L, Hb level of 11.2 g/dL, and platelet count of 74×10^9/L. Peripheral blood (PB) smear revealed granulocytic hyperplasia, 17% blasts of moderate size, and 2% basophils. Bone marrow (BM) aspirates revealed 100% cellularity with 68% blasts. Following the preliminary diagnosis of MPAL with t(9;22), the patient was started on ALL-type induction chemotherapy. A cytogenetic study showed 46,XY,t(9;22)(q34;q11.2) in all 30 cells analyzed with additional chromosomal aberrations. FISH analysis using BCR/ABL1 probe (Abbott Molecular/Vysis, Des Plaines, IL, USA) showed fusion signals in 95% of the cells analyzed. In addition to blasts, mature neutrophils also revealed BCR-ABL1 signals. Therefore, a final diagnosis of CML in mixed phenotype BP was made.
A BM study after the first induction cycle without tyrosine-kinase inhibitor (TKI) revealed 50% cellularity with less than 1% blasts; however, a cytogenetic study revealed 46,XY,t(9;22) (q34;q11.2) in 28 of 30 cells analyzed. After dasatinib and consolidation chemotherapy, a BM study showed 10% cellularity with less than 1% blasts, and 1 of 30 metaphase cells showed 46,XY,t(9;22)(q34;q11.2).

CASE 2: An 8-yr-old girl presented with headache and myalgia persisting for a week. Physical examination revealed cervical lymphadenopathy and hepatosplenomegaly. Laboratory tests showed WBC count of 513×10^9/L, Hb level of 5.3 g/dL, and platelet count of 48×10^9/L. PB smear showed granulocytic hyperplasia and 25% blasts of small size, but there was no increase in the number of either basophils or eosinophils. Her BM study revealed 100% cellularity and 68% blasts. Both blasts and mature neutrophils showed the BCR-ABL1 fusion signals (Fig. 1). A diagnosis of CML in mixed phenotype BP was made.

CASE 3: A 10-yr-old girl presented with emesis for 3 days and hepatosplenomegaly. Laboratory tests revealed WBC count of 376×10^9/L, Hb level of 6.7 g/dL, and platelet count of 33×10^9/L. PB smear showed granulocytic hyperplasia with 28% blasts of variable sizes and 4% eosinophils. Her BM study revealed 100% cellularity with 48% blasts. Following the preliminary diagnosis of AML with t(9;22), AML-type induction chemotherapy was started. However, FISH analysis revealed that both blasts and mature neutrophils were positive for BCR-ABL1. Therefore, she was initially diagnosed as having CML in myeloid BP.

A BM study after the first induction cycle without TKI revealed 80% cellularity with 8% blasts; however, a cytogenetic study showed 46,XX,t(9;22)(q34;q11.2) in 28 of 30 cells analyzed. Real-time quantitative PCR (RQ-PCR) also showed a high level of BCR-ABL1 fusion transcript. Therefore, the first cycle of imatinib was initiated. At 3 months from diagnosis, the patient’s WBC count was 5.8×10^9/L with 43% blasts. A BM study showed 80% cellularity with 78% blasts, and BCR-ABL1 fusion signals were detected in 97.5% of the cells analyzed by FISH (390/400). Flow cytometry revealed that the blasts were positive for CD7, CD10, CD19, CD33, CD34, cCD79a, and HLA-DR. This switch in blast lineage from myeloid to B-lymphoid after only 3 months of therapy was surprising, but a very small population of B-lymphoblasts coexisting with a larger population of myeloblasts was noted after reviewing the initial flow cytometry results. A final diagnosis of CML in mixed phenotype BP was therefore made.

CASE 4: A 50-yr-old woman was transferred to our hospital with abdominal discomfort and splenomegaly with likely MPAL. Laboratory tests revealed WBC count of 366×10^9/L, Hb level of 8.0 g/dL, and platelet count of 208×10^9/L. PB smear showed granulocytic hyperplasia, 60% blasts of variable sizes, 1% baso-

Fig. 1. Interphase FISH results of case 2. (A) FISH analysis using a BCR/ABL1 Dual color, Dual fusion Translocation Probe, which showed a green signal (native BCR), a red signal (native ABL1), a fused signal, and a smaller green signal, indicating BCR-ABL1 and a loss of ABL1 in neutrophils as well as blasts: nuc ish(ABL1X2),(BCRX3),(ABL1 con BCRX1)[390/400]. (B) FISH analysis using BCR/ABL1 extra signal (ES) Dual color Translocation Probe, which revealed a green signal (native BCR), a red signal (native ABL1), and a fused signal without a smaller red signal (ES) in neutrophils as well as blasts. The absence of the smaller red signal indicates the loss of ABL1. While using BCR/ABL1 ES probe, if there is no loss of ABL1, a fusion signal and a smaller red extra signal indicate major BCR-ABL1, and 2 fusion signals indicate minor BCR-ABL1. The results of case 2 indicate major BCR-ABL1: nuc ish(ABL1X2),(BCRX2),(ABL1 con BCRX1)[390/400].
Choi W, et al.

CML in mixed phenotype BP

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phils, and 3% eosinophils. Her BM study revealed 100% cellularity with 77% blasts. Following the preliminary diagnosis of MPAL with t(9;22), ALL-type induction chemotherapy was started. Mature neutrophils as well as blasts revealed BCR-ABL1 signals by FISH analysis. Therefore, a final diagnosis of CML in mixed phenotype BP was made.

A BM study after the first induction cycle followed by imatinib revealed 10% cellularity with less than 1% blasts; however, a cytogenetic study still revealed 46,XX,t(9;22)(q34;q11.2)[20] due to resistance to imatinib despite good response to chemotherapy.

Acute leukemia with t(9;22) and CML-BP have very similar clinical presentations and morphologic features. Therefore, in Ph+ leukemias, CML-BP should be considered as a part of the differential diagnosis [8]. The 2008 WHO classification suggests caution when making the diagnosis of MPAL with t(9;22) in a case of myeloid leukemia with maturation that also shows expression of lymphoid markers, because CML-BP may show a similar pattern [4]. Splenomegaly, peripheral leukocytosis due to maturing and mature neutrophils, absolute basophilia, and a clinical history of CML may support the diagnosis of CML-BP [5]. Although our patients had splenomegaly and peripheral leukocytosis without dyspoiesis, 2 patients did not show absolute basophilia. In addition, CML patients did not show basophilia in the BP, whereas CML patients in mixed phenotype BP also showed a similar pattern [1]. In CML-BP, the diagnosis is made when the BP is present in more than 1% blasts [3]. Therefore, in CML patients, the diagnosis is made when the MPAL is confirmed by FISH analysis and the BP is present in less than 1% blasts.

Table 1. The clinical and laboratory findings of each case at initial presentation

| Case No./ Sex/Age | Splenomegaly | % Blasts | Cytochemistry | Immunophenotype* | RT-PCR | Karyotype | FISH† |
|------------------|--------------|----------|---------------|-----------------|---------|-----------|-------|
| 1/M/60           | Present      | 17       | 68 +         | CD10, CD19, CD20, CD34, CD79a, HLA-DR, MPO | 14a2    | 46,XY,t(9;22)(q34;q11.2)(23)/46,XY,der(19)t(9;19)(p13.3)(9;22)(q12) | nuc ish(ABL1x3~4),(BCR3~4), (ABL1 con BCRx2~3)[380/400] |
|                  |              |          |              |                 | (b3a2) | 46,XY,t(9;22)(q34;q11.2)(23)/46,XY,der(19)t(9;19)(p13.3)(9;22)(q12) | (ABL1 con BCRx3)[390/400] |
| 2/F/8            | Present      | 25       | 68 +         | CD10, CD19, CD22, CD34, CD79a, HLA-DR, MPO | 13a2    | 46,XX,t(9;22)(q34;q11.2)(23)/46,XX[1] | nuc ish(ABL1x2),(BCR3), (ABL1 con BCRx1)[390/400] |
|                  |              |          |              |                 | (b3a2) | 46,XX,t(9;22)(q34;q11.2)(23)/46,XX[1] | (ABL1 con BCRx2)[390/400] |
| 3/F/10           | Present      | 28       | 48 +         | CD11, CD19, CD33, CD34, CD64, CD7, CD10, CD19, CD33, CD34, CD79a, HLA-DR (1st) | 14a2    | 46,XX,t(9;22)(q34;q11.2)(23)/46,XX[1] | nuc ish(ABL1, BCR)x3 |
|                  |              |          |              |                 | (b3a2) | 46,XX,t(9;22)(q34;q11.2)(23)/46,XX[1] | (ABL1 con BCRx2)[390/400] |
| 4/F/50           | Present      | 60       | 77 +         | CD10, CD19, CD33, CD34, CD56, CD79a, HLA-DR, MPO | 14a2    | 46,XX,t(9;22)(q34;q11.2)(23)/46,XX[1] | nuc ish(ABL1, BCR)x3 |
|                  |              |          |              |                 | (b3a2) | 46,XX,t(9;22)(q34;q11.2)(23)/46,XX[1] | (ABL1 con BCRx2)[390/400] |

*Data obtained by flow cytometry; †Data obtained by interphase FISH using BCR/ABL1 Dual color, Dual fusion Translocation Probe.

Abbreviations: PB, peripheral blood; BM, bone marrow; MPO, myeloperoxidase; PAS, periodic acid-Schiff; RT-PCR, reverse transcription PCR.
malignancies to distinguish CML presenting in lymphoblastic BP from lymphoblast-restricted Ph+ ALL [12]. The cytoplasmic membranes of cultured BM cells are lysed and the nuclei are swollen during preparation for the typical FISH analysis performed in our laboratory. Nevertheless, the lobulated nuclei of mature neutrophils are relatively easy to identify. Although these nuclei may occasionally be those of mature basophils or eosinophils, they stem from mature myeloid lineage cells with the Ph chromosome in CML. The 

\( BCR-ABL1 \) fusion signals in both the blasts and lobulated nuclei indicate the involvement of the Ph chromosome in multilineage and mature cells in our patients. A routine FISH report describes only the number of cells expressing the fusion signal from the interphase cells analyzed. Therefore, while interpreting FISH results for leukemia with t(9;22) that also shows granulocytic hyperplasia in PB, care should be taken to identify 

\( BCR-ABL1 \) fusion signals in mature neutrophils.

The follow-up cytogenetic analysis after initial induction chemotherapy for case 1 still showed t(9;22) in 28 of 30 cells analyzed with less than 1% blasts in the BM. This data led us to reconfirm the diagnosis of CML presenting in BP. Similar results were found in cases 3 and 4. RQ-PCR can be used at follow-up in a similar way. After an initial diagnosis of acute leukemia with t(9;22) and induction chemotherapy alone without TKI, if a cytogenetic study still shows a high proportion of Ph+ cells with a low proportion of blasts in the BM, the final diagnosis should be changed to CML.

CML-BP has a poor prognosis and should be treated with a TKI, with or without induction chemotherapy based on the blast phenotype, with the goal of reverting the disease to the chronic phase and proceeding to allogeneic stem cell transplantation as quickly as possible [13].

In summary, we reported 4 CML cases that initially presented in mixed phenotype BP, which was confirmed by FISH analysis. All patients were treated with chemotherapy combined with TKI. Differentiating CML that presents in BP from acute leukemia with t(9;22) may be difficult, especially in the case of CML in mixed phenotype BP; however, if 

\( BCR-ABL1 \) fusion signals are detected by FISH analysis in mature neutrophils as well as blasts, CML-BP is the most likely diagnosis. Laboratory hematologists should make an effort to identify 

\( BCR-ABL1 \) fusion signals in mature neutrophils while interpreting FISH results for leukemias with t(9;22) in order to distinguish between CML-BP and acute leukemia with t(9;22). In future studies, this type of thorough analysis may help establish the diagnostic guidelines and guide the therapeutic protocol.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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