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

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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.

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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-

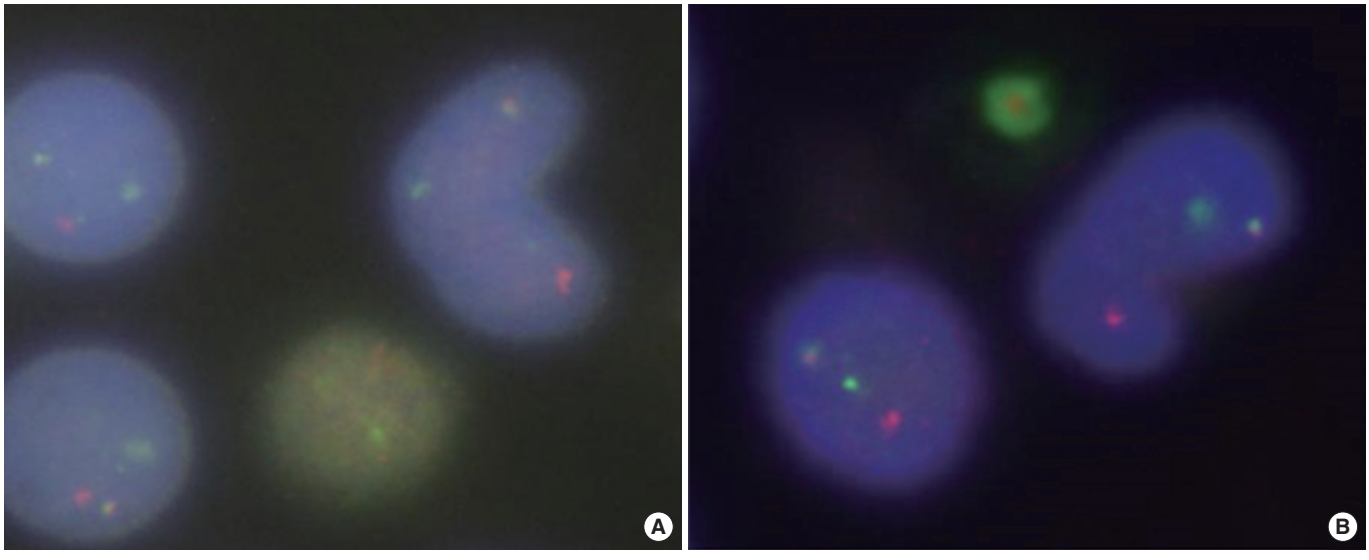

Fig. 1. Interphase FISH results of case 2. (A) FISH analysis using a *BCR/ABL1* Dual color, Dual fusion Translocation Probe, which showed a green signal (native *BCR*), a red signal (native *ABL1*), a fused signal, and a smaller green signal, indicating *BCR-ABL1* and a loss of *ABL1* in neutrophils 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].

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†
		PB	BM	MPO	PAS				
1/M/60	Present	17	68	+	-	CD10, CD19, CD20, cCD22, CD34, cCD79a, HLA-DR, cMPO	e14a2 (b3a2)	46,XY,t(9;22)(q34;q11.2)[23]/46,XY,-9,der(19)t(9;19)(q12;p13.3)t(9;22)(q34;q11.2),der(22)t(9;22)[9]/45,XY,-9,der(19)t(9;19)(q12;p13.3)t(9;22)(q34;q11.2),der(22)t(9;22)[6]/48,XY,-9,der(19)t(9;19)(q12;p13.3)t(9;22)(q34;q11.2),+21,der(22)t(9;22),+der(22)t(9;22),+mar[2]	nuc ish(ABL1x3~4),(BCRx3~4),(ABL1 con BCRx2~3)[380/400]
2/F/8	Present	25	68	+	-	CD10, CD19, cCD22, CD34, cCD79a, HLA-DR, cMPO	e13a2 (b2a2)	46,XX,t(9;22)(q34;q11.2)[29]/46,XX[1]	nuc ish(ABL1x2),(BCRx3),(ABL1 con BCRx1)[390/400]
3/F/10	Present	28	48	+	-	CD11c, CD19, CD33, CD34, CD64, CD117, HLA-DR, cMPO (1st) CD7, CD10, CD19, CD33, CD34, cCD79a, HLA-DR (2nd)	e14a2 (b3a2)	46,XX,t(9;22)(q34;q11.2)[20]	nuc ish(ABL1,BCR)x3 (ABL1 con BCRx2)[390/400]
4/F/50	Present	60	77	-	+	CD10, CD19, CD33, CD34, CD56, cCD79a, CD117, HLA-DR	e14a2 (b3a2)	46,XX,t(9;22)(q34;q11.2)[20]	nuc ish(ABL1,BCR)x3 (ABL1 con BCRx2)[380/400]

*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.

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 with all stages of granulocytes without dyspoiesis, 2 patients did not show absolute basophilia. In addition, CML presenting in the BP is very rare in Korea (1% of CML cases) [9]. CML in mixed phenotype BP is also rare, whereas t(9;22) is the most common recurrent genetic abnormality seen in MPAL [4]. Therefore, our patients were initially suspected of having MPAL with t(9;22) on the basis of preliminary BM morphology, molecular test results, and immunophenotype, but a few days later, they were later diagnosed with CML in mixed phenotype BP on the basis of the FISH results. The final diagnoses were also supported by follow-up cytogenetic studies and RQ-PCR.

CML is an abnormal pluripotent stem cell disease, and t(9;22) is found in all hematopoietic lineages [5]. In CML, leukemic stem cells can differentiate, and neutrophilic leukocytosis is a dominant morphologic feature. That is, BCR-ABL1 preferentially induces granulocytic differentiation as well as CML blast proliferation. This is partially because c-Jun, a monopoiesis-promoting transcription factor, is downregulated in both CML neutrophils and blasts by BCR-ABL1. In addition, the c-Jun downregulation can be reversed by imatinib [10]. Thus, the *BCR-ABL1* fusion gene is expected to be found even in mature neutrophils in CML. In addition, mature eosinophils and basophils carry the *BCR-ABL1* fusion gene [11].

FISH analysis can be used to determine cell lineages with the Ph chromosome in patients presenting with Ph+ lymphoblastic

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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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