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Received on Mar. 13, 2018; Revised on Apr. 17, 2018; Accepted on May 10, 2018
https://doi.org/10.5045/br.2018.53.3.254

Authors' Disclosures of Potential Conflicts of Interest

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

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Philadelphia-positive mixed phenotype acute leukemia presenting with *PML-RARα* fusion transcript without t(15;17) on cytogenetic studies

TO THE EDITOR: About 1–5% of the acute leukemias are not possible to assign a single lineage, which is designated as mixed phenotype acute leukemia (MPAL) [1, 2]. Promyelocytic leukemia (*PML*) and retinoid acid receptor α (*RARα*) fusion transcript is a product of translocation of chromosome 15 and 17, which is a hallmark of acute promyelocytic leukemia (APL). Cryptic or masked t(15;17) (q24;q21) in APL, which has morphological APL and *PML-RARα* fusion transcript but no t(15;17)(q24;q21) on routine cytogenetic analysis, has been noted [3]. The detection of t(15;17) in biphenotypic acute leukemia (BAL) with French-American-British (FAB) L2 morphology has been rarely reported, including one with *PML-RARα* fusion transcript [4] and the other without *PML-RARα* fusion transcript. However, by far, there is no report about the expression of *PML-RARα* fusion transcript in Philadelphia chromosome-positive (Ph+) MPAL patients.

A 37-year old female presented with a two-week history of neck and pelvic area pain, fever, and chills. On physical examination, the spleen was palpable 10 cm below the lower costal margin on the left mid-clavicular line. The liver and lymph nodes were not palpable.

Initial blood test revealed elevated white blood cell (WBC) count with blasts (WBC 287.520×10^9/L; segmented neutrophil 32%; metamyelocyte 2%; myelocyte 13%; eosinophil 2%; basophil 1%; blast 13%; Hb 9.6 g/dL; platelets 187×10^9/L). Prothrombin time (PT) and activated partial prothrombin time (aPTT) were in the normal range (PT 13.5 sec; aPTT 25.5 sec), and fibrinogen was slightly increased (467.5 mg/dL).

A bone marrow (BM) aspiration and biopsy showed markedly hypercellular marrow and increased blasts (about 23.8% of all nucleated cells) with predominantly lymphoblast morphology with scant cytoplasm without Auer rods (Fig. 1A). Special staining showed negativity to all of MPO, Sudan black B, and specific and nonspecific esterases and periodic acid-Schiff staining. On immunophenotyping, blast cells expressed myeloid (cytoplasmic MPO 54.58%, CD13 77.94%, CD33 75.4%), B-lymphoid (CD10 26.87%, CD19 83.94%), and stem cell markers (CD34 3.11%, CD71 53.7%). Chromosomal analysis by G-banding showed 46,XX,t(9;22)(q34;q11) in 23 cells among the 25 metaphase cells analyzed (Fig. 1B). The FISH signals from Vysis indicated abnormal *BCR/ABL1* fusions in 304 of 312 (97.4%) interphase nuclei examined (Fig. 1C), and the *PML-RARα* probe reported no fusion in 325 interphase nuclei examined (Fig. 1D). RT-PCR with a Hemavision kit showed a single *PML-RARα*

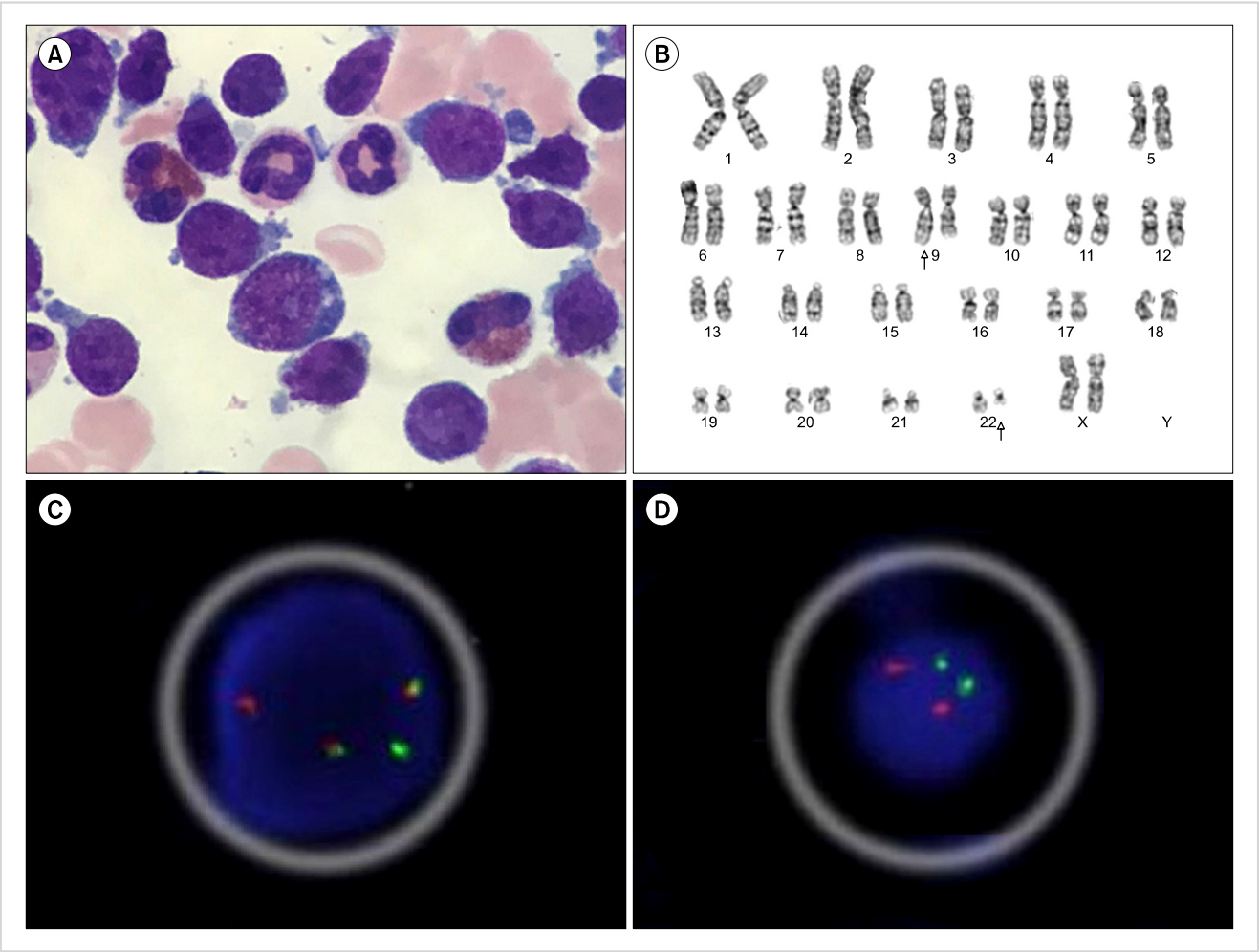

Fig. 1. Bone marrow aspiration smear shows lymphoblasts with scanty cytoplasms, lacking Auer rods and granules (Wright-Giemsa stain, ×1,000) **(A)**. Cytogenetic finding by Giemsa-banding indicates 46,XX,t(9;22)(q34;q11) in 23 cells among the 25 metaphase cells **(B)**. Fluorescence in situ hybridization (FISH) study showing *BCR/ABL1* dual-fusion translocation probe (Vysis, Downers Grove, IL, USA) at initial diagnosis. Interphase image showing an abnormal signal pattern, consisting of one BCR (green), one ABL1 (red), and two fused green/red (or yellow) signals representing the BCR/ABL1 fusion **(C)**. Interphase FISH image using *PML/RARα* dual-fusion translocation probe at initial diagnosis. Two separated PML (green) and RARα (red) signals, which indicate absence of the *PML/RARα* fusion **(D)**.

fusion transcript (325 bp) (Fig. 2A) and *BCR-ABL1* fusion transcript (b2a2, 397 bp) (Fig. 2B). RQ-PCR with an IPSOGEN BCR-ABL1 IS-MMR RQ kit showed 73.46% IS (ABL copy number 167,139; BCR-ABL copy number 139,300). Gene sequencing with electrophoresis showed a breakpoint between the exon 3 of *PML* and exon 3 of *RARα*, corresponding to the variant form (bcr3) fusion transcript in the examined breakpoint sites on chromosomes 15 and 17, respectively (Fig. 2C), and a breakpoint between the middle of exon 13 of BCR and exon 2 of ABL, representing the major type of bcr/abl (b2a2) fusion transcript in the studied breakpoint sites on chromosomes 22 and 9, respectively (Fig. 2D).

The patient had leptomeningeal seeding metastasis at diagnosis by cerebrospinal fluid (CSF) analysis. Meanwhile, the patient received leukocyte reduction by leukapheresis from the day of admission. Treatment was started on admission day 8 with induction chemotherapy of hyperCVAD [cyclophosphamide 300 mg/m^2 days 1–3; vincristine 1.4 mg/m^2 (max. 2 mg) days 4,11; adriamycin 50 mg/m^2 day 4; dexamethasone 40 mg days 1–4, 11–14] in combination with Imatinib 600 mg. On induction chemotherapy day 3, the patient showed profuse epistaxis, due to hypofibrinogenemia (platelet 90×10^9/L, fibrinogen 17 mg/dL). All-trans retinoic acid (ATRA) 45 mg/m^2 was added on induction day 7 to the treatment until confirmation of negative *PML-RARα* fusion transcript on PCR. On induction chemotherapy day 8, intrathecal triple chemotherapy (cytarabine 40 mg; methotrexate 15 mg; hydrocortisone 15 mg/m^2) three times per week was commenced after recovery from hypofibrinogenemia and bleeding tendency. On induction day 41, she had reached complete remission (CR) with incomplete blood count recovery (peripheral blood WBC 1.94×10^9/L; segmented neutrophil 70%; blast 0%; pla-

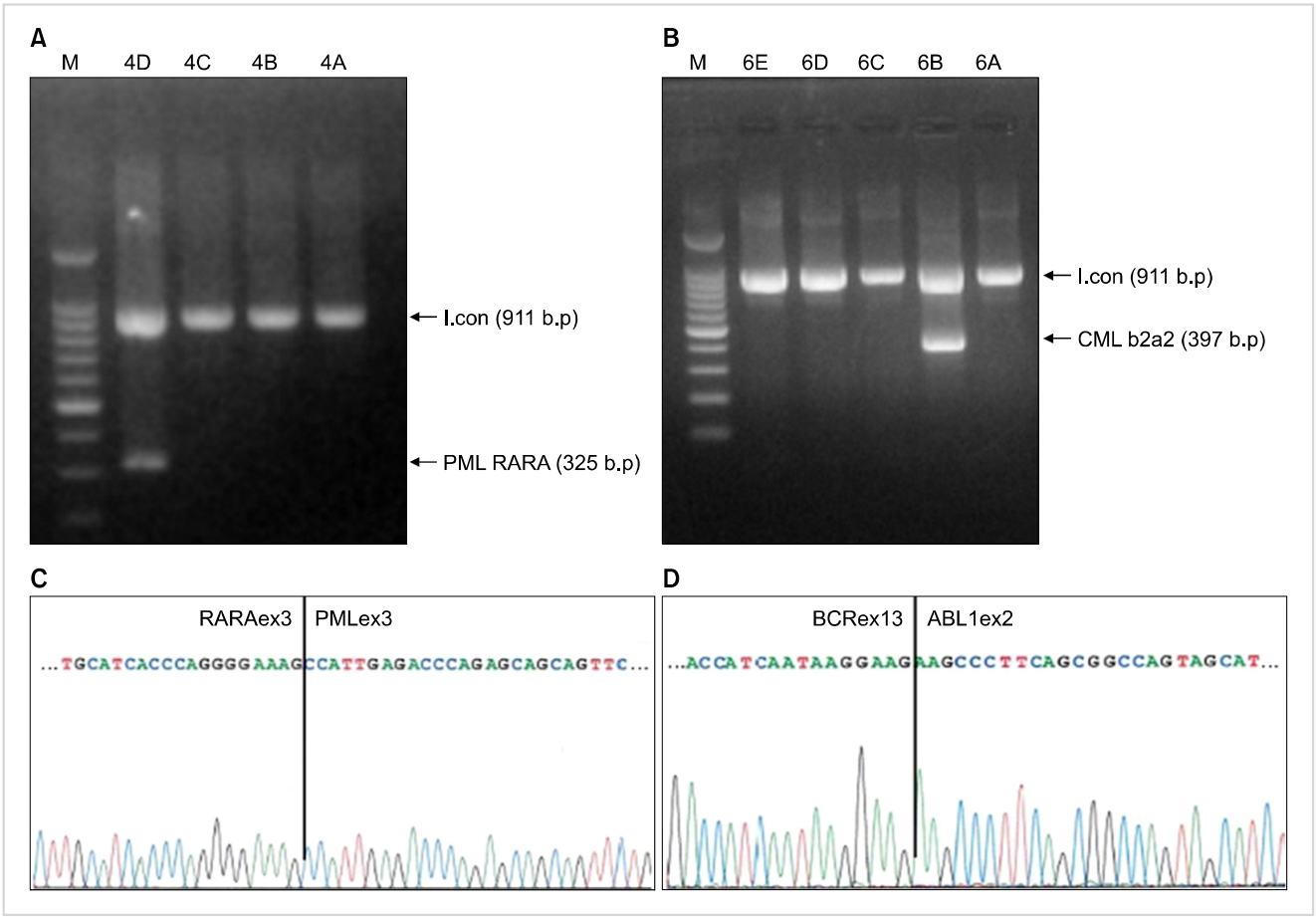

Fig. 2. **(A)** Agarose gel electrophoresis of the RT-PCR products showing *PML-RARα* fusion transcript (size: 325 bp) in lane 4D and **(B)** *BCR-ABL1* fusion transcript (size: 397 bp) in lane 6B. Sanger sequencing showing *PML-RARA* **(C)** and *BCR-ABL1*, b2a2 **(D)**.
Abbreviations: M, nucleic acid marked ladder; IC, internal control.

telets 70×10^9/L; BM blast 0.4%), and still Philadelphia chromosome was detected in 6.7% and 0.65% of the cells by conventional G-banding analysis and FISH, respectively; *BCR-ABL1* fusion transcript was positive by RT-PCR but no *PML-RARα* fusion gene or fusion transcript were detected on FISH and RT-PCR, respectively; and RQ-PCR showed 0.2007% IS (ABL copy number 68,581; BCR-ABL copy number 136.7). During the induction chemotherapy, the patient complained of grade 3 nausea and vomiting due to imatinib. She proceeded to 1 cycle of consolidation chemotherapy (which consisted of dasatinib 140 mg, high-dose methotrexate 1 g/m^2 day 1; cytarabine 3 g/m^2 days 2–3) with intrathecal triple chemotherapy for central nervous system (CNS) prophylaxis and reached cytogenetic complete remission confirmed by *BCR-ABL1* FISH. The patient received allogeneic hematopoietic stem cell transplantation (Allo-HSCT) from a full-matched unrelated donor with myeloablative conditioning chemotherapy (busulfan-cyclophosphamide). The patient suffered from grade 2 acute graft-versus-host disease but soon recovered by steroids and is still in CR state with complete donor chimerism after 8 months after HSCT.

Discussion

To date, cytogenetic abnormality of t(15;17) has been noted in two BAL cases. One BAL case had FAB ALL-L2 morphology with immunophenotypic pattern showing MPO 53%, CD10 83%, and CD19 87%, a cytogenetic result of t(15;17), +8, and a *PML-RARα* transcript [4]. Induction chemotherapy in this patient was done with ALL-type of chemotherapy, and at the end of the induction phase, as there was minimal residual disease by flow cytometry, FISH, and RT-PCR, the protocol was changed to AML-type of chemotherapy with ATRA, with intensification with a high dose of cytarabine and maintenance protocol for APL. The patient sustained in hematological CR for a three-year follow-up. This case should have been classified to APL in current WHO 2008 criteria, which has a different treatment approach and survival from typical MPAL. Another BAL case had also FAB ALL-L2 morphology with immunophenotypic pattern showing MPO 2%, CD13 40.9%, CD33 94.6%, CD19 90.8%, and CD22 45.4%, a karyotype result of 46,XX,t(4;12)(q21;p11), t(15;17)(q22;q12), and no *PML-RARα* fusion signal on FISH [5]. The patient received ALL-type of chemotherapy without ATRA. Although the

Table 1. Summary of published cases of co-expressing BCR-ABL1 and *PML-RARα*, biphenotypic acute leukemias with t(15;17), and this case.

No.	Age	Sex	Complete blood count			Diagnosis	Morphology	Cytogenetics	PCR	Chemotherapy	CR	Survival, mo	Reference
			WBC, 10^9/L	Hb, g/dL	PLT, 10^9/L								
1	69	F	1.17	118	79	APL	APL	46,XX,t(1517)(q24.1;q21.1)[8]/46,XX[16]	BCR-ABL1(+) PML-RARA(+)	ATRA+Ida	Yes	6 (died)	[9]
2	39	F	242.2	8.8	20	APL	APL	46,XX,t(9;22)	PML-RARA	Ida+Ara-C	Yes	5 (died)	[10]
3	38	F	1.8	6.1	12	APL	APL	46,XX,t(9;22)(q34;q11),t(15;17)(q24;q21)[4]/46,XX[16]	BCR-ABL1(+) PML-RARA(+)	ATRA+Arsemoc trioxide	No	<1 (died)	[11]
4	48	M	1.15	8.6	15	APL	APL	46,XY,t(9;22)(q34;q11),t(15;17)(q24;q21)[10]/47,idem,+8[4]/46,idem,der(14)t(9;14)(q10;q10)[6]	BCR-ABL1(+) PML-RARA(+)	ATRA+Arsemoc trioxide	Yes	>18	[12]
5	50	M	0.45	7.3	3	APL	APL	46,XY,t(15;17)(q22;q12)[9]/46,XY,del(6)(q?),t(9;22)(q34;q11.2)[1]/46,XY[10]	BCR-ABL1(+) PML-RARA(+)	ATRA	Yes	>2	[13]
6	51	F	287.83	7.4	116	APL	APL	46,XX,t(9;22)(q34;q11),t(15;17)(q24;q21)	BCR-ABL1(+) PML-RARA(+)	ATRA+Dauno+Ara-C	Yes	>11	[14]
7	7	F	2.8	7	138	BAL	FAB-L2	t(15;17), +8	PML-RARA(+)	ALL-type regimen→AML-type regimen+ATRA	Yes	>36	[15]
8	55	F	13.4	9.3	110	BAL	FAB-L2	46,XX,t(4;12)(q21;p11),t(15;17)(q22;q12)[24]	PML-RARA(+)	ALL-type regimen	Yes	>43	[16]
9	37	F	287.5	9.6	187	MPAL	Lymphoblast	46,XX,t(9;22)(q34;q11.2)[23]/46,XX[2]	BCR-ABL1(+) PML-RARA(+)	Imatinib+ALL-type regimen+ATRA	Yes	>6	Present case

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; ATRA, all trans-retinoid acid; Ara-C, cytosine arabinoside; BCR-ABL1, breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1; CR, complete remission; Dauno, daunorubicin; F, female; FAB, French-American-British; Hb, haemoglobin; Ida, idarubicin; M, male; PCR, polymerase chain reaction; PLT, platelet; PML-RARα, promyelocytic leukemia-retinoid acid receptor; WBC, white blood cell.

patient suffered from septic shock during consolidation phase, the patient is still in hematologic CR over 3.7-year follow-up. The presence of t(15;17) is now categorized as APL; however, this case had lymphoid morphology and immunophenotype according to 2008 WHO classification, which might have led to a favorable response to ALL-type of chemotherapy without ATRA.

Coexistence of *BCR-ABL1* and *PML-RARα* transcript has been noted in several typical or cryptic cases of APL [6-11] (summarized in Table 1). Their treatment strategies were mostly ATRA with or without chemotherapy, and although the cases were small and had short-term follow-up, APL patients with Philadelphia chromosome showed worse outcome compared with the general APL group. The reported cases were mostly from South Eastern Asia, and whether there is selection bias and the causal relationship is unknown, including this case, there might be an ethnic preponderance of co-expression of the *BCR-ABL1* and *PML-RARα* genes in acute leukemias. No other case report was available aberrantly expressing *PML-RARα* transcript in typical Philadelphia chromosome-positive acute leukemia or CML. The pathogenesis of MPAL is explained by several hypothesis, but recent studies suggest that the timing and level of expression of a specific transcription factor may affect lineage determination to the mixed phenotype, such as the low expression of PAX5 and its suppression by C/EBPα in B/myeloid MPAL and the aberrantly activated NOTCH1 signaling, leading to C/EBPα promoter hypermethylation and gene silencing in lineage switch from AML to T-ALL [12, 13]. The simultaneous presence of multiple driver genes of both AML and ALL might also be able to function for biphenotypic expression of acute leukemia.

Among the MPAL patients, t(9;22)/*BCR-ABL1* was observed in 15–32% of patients [14]. Before the advent of tyrosine kinase inhibitors (TKI), the survival of Philadelphia-positive MPAL patients was dismal, but after the introduction of TKIs to the treatment, the prognosis of this group of patients has markedly improved showing similar survival rate with Philadelphia-positive ALL patients [15]. Although no prospective, randomized controlled trials are

available to guide proper treatment, limited retrospective studies suggest ALL-like regimen followed by Allo-HSCT, and the addition of TKI in patients with t(9;22) translocation is advisable. Moreover, as MPAL patients show high frequency (about 20%) of CNS involvement at diagnosis, CNS prophylaxis is needed as a part of the treatment. The case in this report initiated induction chemotherapy with ALL-type chemotherapy, Imatinib 600 mg with the addition of ATRA, and CNS-directed therapy. Although she had reached hematologic incomplete CR without cytogenetic or molecular CR after first induction chemotherapy, the patient finally reached cytogenetic CR after consolidation chemotherapy and proceeded to Allo-HSCT. The addition of ATRA was done on induction chemotherapy day 7 after the recognition of *PML-RARα* transcript and FISH positivity, and the resolution of bleeding tendency was at induction day 8, which suggests a minimal role of ATRA on the cessation of bleeding diathesis in this patient. The contribution of ATRA to achieving *PML-RARα* fusion transcript negativity after induction chemotherapy is not certain, but the role of ATRA in these rare cases of patient needs further studies.

To the best of our knowledge, this is the first case of MPAL with t(9;22) with cryptic expression of *PML-RARα* transcript confirmed by gene sequencing. In MPAL patients, the presence of uncommon karyotype or gene transcript should not be neglected, and also the effort to reveal such cases with multiple diagnostic modalities must be done to guide best management option. Further study is needed to evaluate the incidence and prognosis of MPAL with t(9;22) and aberrant expression of *PML-RARα* transcript without detection of t(15;17) on cytogenetic analyses, and furthermore, to suggest proper treatment approaches in this rare type of disease.

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Received on Apr. 23, 2018; Revised on May 19, 2018; Accepted on May 23, 2018

https://doi.org/10.5045/br.2018.53.3.256

Authors' Disclosures of Potential Conflicts of Interest

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

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