



Therapy-Related Acute Megakaryoblastic Leukemia in a Lung Cancer Patient

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Therapy-related AML (t-AML) is one of the newly expanded disease entities in the 2008 WHO classification, accounting for 10-20% of all cases of AML, and its incidence is increasing worldwide because of improved survival rates following treatment for other primary cancers [1, 2]. Acute megakaryoblastic leukemia (AMKL) (M7) is the least common of the t-AML French-American-British (FAB) subtypes, and only two such cases have been reported to date [3, 4], neither of which was in Korea. AMKL accounts for about 7-10% of childhood AML cases (frequently associated with Down syndrome), but only about 1% of adult AML cases [5]. Here, we describe a rare case of therapy-related acute megakaryoblastic leukemia (t-AMKL) with chromosome 5 and 7 abnormalities that presented ten years after chemoradiotherapy in an elderly lung cancer patient.

A 72-yr-old man with lung cancer (non-small cell lung cancer [NSCLC], right lower lobe [RLL], squamous cell cancer, stage T2N1M0) was treated with a combination of radiotherapy and a repeated chemotherapy regimen (4 trials, 13 cycles) consisting of docetaxel, cisplatin, gemcitabine, vinorelbine, gefitinib, irinotecan, and carboplatin, between March 2003 and February 2005 (Table 1). Subsequently, in April 2005, he underwent surgical resection (RLL lobectomy) of the cancer and thereafter achieved complete remission.

He was asymptomatic until being referred to our hospital for further evaluation of pancytopenia discovered at a local hospital,

to which he had been admitted for fever persisting for three days, upper respiratory infection symptoms, and aggravated dyspnea. He had signs of anemic conjunctiva, but no lymphadenopathy or organomegaly on physical examination. Abdominal sonography revealed hepatomegaly but no splenomegaly. A complete blood count (CBC) revealed Hb level of 5.2 g/dL; white blood cell counts of $1.7 \times 10^9/L$ (absolute neutrophil count $0.78 \times 10^9/L$); and platelet counts of $34 \times 10^9/L$. A peripheral blood smear showed macrocytic normochromic anemia with teardrop cells, a shift to the left in the neutrophilic series together with dysplastic features such as hypogranulation and the Pelger-Huët anomaly in segmented neutrophils, irregularly segmented monocyte nuclei, and giant platelets (Fig. 1A). The plasma Hb (19.5 mg/dL; reference interval [RI], 0.0-5.0 mg/dL) and D-dimer (4.59 $\mu\text{g/mL}$; RI, 0-0.5 $\mu\text{g/mL}$) levels were both elevated.

Most biochemical tests were normal, except for elevated levels of serum lactate dehydrogenase (513 IU/L; RI, 0-480 IU/L), beta-2 microglobulin (2.9 mg/L; RI, 0-2.4 mg/L), and C-reactive protein (22.24 mg/L; RI, 0-5.0 mg/L). Vitamin B12 was raised at 19,030 pg/mL (RI, 160-970 pg/mL), but the folate level was within the RI. Bone marrow (BM) aspirate smears and a touch print preparation of the BM biopsy revealed hypercellular marrow, dysplastic changes in all three hematopoietic cell lineages, and 23.5% blasts (Fig. 1B). The blasts were medium to large sized and had round, slightly irregular or indented nuclei, fine

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Table 1. Characteristics of previously reported therapy-related acute megakaryoblastic leukemia cases

Previous report	Age/ Sex	Primary malignancy	Previous treatment for primary malignancy	Secondary malignancy	Latency (yr)	Karyotype	AML immunophenotype
Yeasmin et al. [3]	73/F	Ovarian cancer	Pac, Carbo	t-MDS → t-AML	2*	der(5;9)(p10;q10), +19, etc. [†] der(5;19)(p10;q10), -6, etc. [†]	M7 (CD13+, glycophorin A+, CD41+; CD3-, CD79-)
Sakai et al. [4]	41/F	Peripheral T-cell lymphoma	CHOP, MEVP, Etop, Cis, Carbo, Cyt, BH-AC, MCNU, Proc, Bleo	t-AML → t-MDS → t-AML M7	2 6 2	t(8;21)(q22;q22), +1 → der(1;7)(q10;p10), +1 → der(1;7)(q10;p10)	M2 → M7 (CD34+, CD41+, CD3-, CD10-, CD13-, CD33-, CD56-, HLA-DR-)
Present case	72/M	Non-small cell lung cancer	RT, Taxol, Cis, Gemza, Vin, Ire, Irin, Carbo	t-AML	10	-5,-7,+2mar	M7II (CD13+, CD23+, cytoplasmic MPO+, CD61+, CD41+, factor VIIIa+)

22 months to diagnosis of t-MDS, 3 months to diagnosis of t-AML; †Karyotype of t-MDS: 46,XX,der(5;9)(p10;q10), del(7)(q?), +19, add(3q21), der(12)add(12)(p11.2), t(3;12)(q21;q22), del(12)(p?) [17/20], Karyotype of t-AML: 46,XX,der(5;19)(p10;q10), -6, del(7)(q?), add(12p11.2), -13, -17, +19[20/20]. Abbreviations: Pac, paclitaxel; Carbo, carboplatin; CHOP, adriamycin, cyclophosphamide, vincristine, prednisolone; MEVP, mitoxantrone, etoposide, vindesine, prednisolone; Dox, doxorubicin; Etop, etoposide; Cis, cisplatin; Cyt, cytoxan; BH-AC, behenoylara-C; MCNU, ranimustine; Proc, procainamide; Bleo, bleomycin; RT, radiotherapy; Taxol, docetaxel; Gemza, gemcitabine; Vin, vinorelbine; Ire, iressa; Irin, irinotecan.

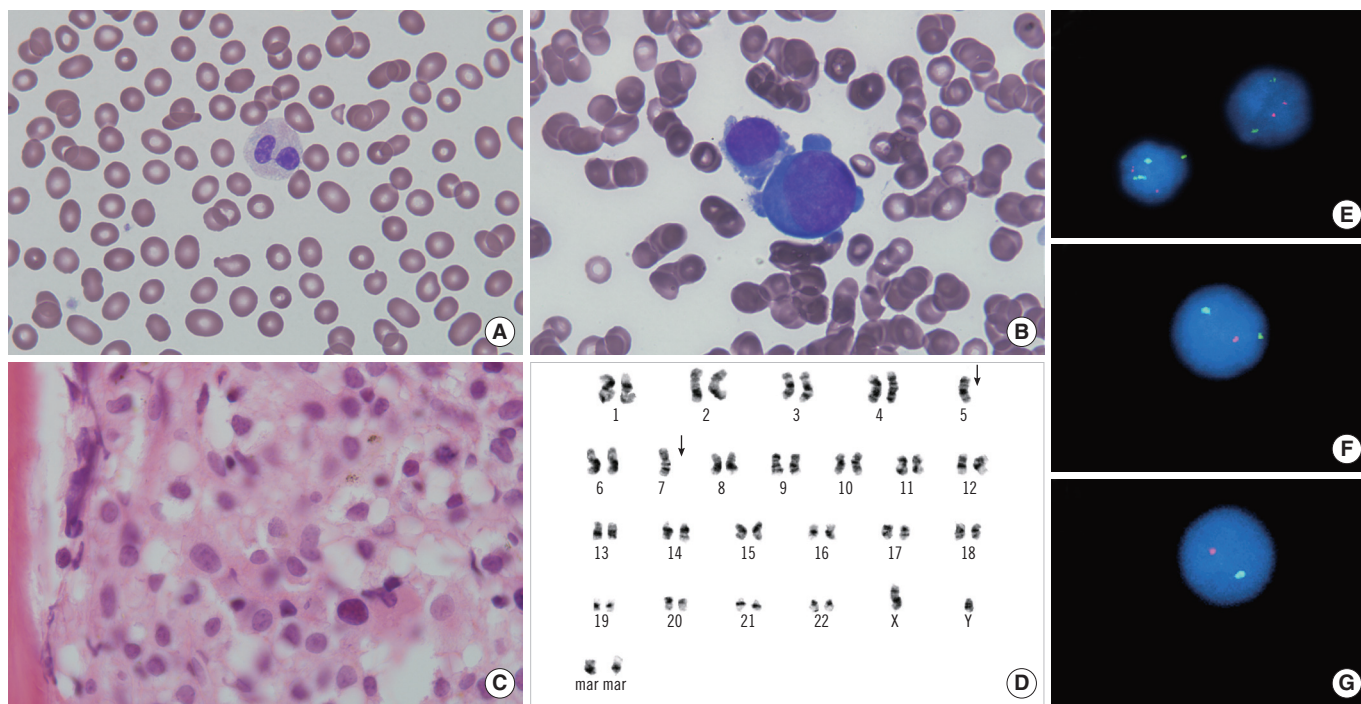


Fig. 1. (A) Peripheral blood (PB) smear findings (Wright-Giemsa stain, $\times 1,000$), (B) bone marrow (BM) aspirate smear (Wright-Giemsa stain, $\times 1,000$), and (C) BM biopsy section (H&E stain, $\times 1,000$). Cells in the PB smear (A) had dysplastic features, such as marked anisopoikilocytosis, and some red blood cells (RBCs) had a teardrop shape. Neutrophils showed the Pelger-Huët anomaly and cytoplasmic hypogranularity. Leukemic blasts (B), consistent with acute megakaryoblastic leukemia, were of medium to large size and had round, slightly irregular or indented nuclei, with fine reticular chromatin, one to three inconspicuous nucleoli, basophilic agranular cytoplasm, and frequent pseudopod formation. The biopsied marrow (C) showed hypercellularity relative to the patient's age (approximately 70%). (D) The karyotype of the abnormal clone in 2 out of 20 analyzed metaphase cells using G-banding. It was initially interpreted as 46,XY,-5,-7,+2mar. The arrows denote the abnormal chromosomes. (E-G) Interphase FISH for AML1-ETO (E), RELN/TES (F), and TAS2R1/EGR1 (G) at diagnosis. The three green (RUNX1 probe on 21q22) and two orange (RUNX1T1 probe on 8q22) signals (E) were consistent with an additional AML1 signal in 28.2% of the nuclei analyzed. The two green (D5S630 probe on 5p15.31) and one orange (EGR1 probe on 5q31) signal (F) and one green (RELN probe on 7q22) and one orange (TES probe on 7q31) signal (G) were consistent with 5q and 7q deletions, respectively. The two green (D5S630 probe on 5p15.31) signals (F) signified that there were two 5p loci. Abbreviation: H&E, hematoxylin and eosin.

reticular chromatin, one to three inconspicuous nucleoli, basophilic agranular cytoplasm, and frequently formed pseudopod.

There were relatively fewer erythroid cells, and these had dysplastic features such as nuclear-cytoplasmic asynchrony. Con-

versely, there were relatively more granulocytes, but these also showed dysplastic features such as megaloblastoid change, hyposegmentation, and cytoplasmic hypogranularity. Markedly, more megakaryocytes that varied considerably in size were present, and they were multi-nucleated with hypolobated nuclei. Micromegakaryocytes, megakaryocytic fragments, and bare megakaryocytic nuclei were frequently observed. It was also noteworthy that basophils accounted for up to 6.7% of the nucleated marrow cells.

Cytochemical staining of the BM aspirate revealed that blasts were positive for periodic acid-Schiff, acid phosphatase (punctuate), and non-specific esterase, and negative for Sudan black B, myeloperoxidase (MPO), and specific esterase.

The biopsied marrow was hypercellular relative to the patient's age (approximately 70%) (Fig. 1C), consisting predominantly of dysplastic megakaryocytes of variable size and leukemic blasts. Immunohistochemical staining of the paraffin-embedded BM biopsy specimen showed that the blasts and megakaryocytes were positive for Factor-VIII, CD61, MPO, and c-Kit. There were a reduced number of glycophorin-positive erythroid cells, and reticulin staining showed mild myelofibrosis (grade MF-1) [1].

Immunophenotyping of leukemic blasts in the BM using flow cytometry revealed a weakly positive result for CD45, CD13, CD23, and cytoplasmic MPO and strong positivity for CD61 and CD41, consistent with a megakaryocytic lineage. The same blasts were negative for CD2, CD5, CD7, CD10, CD11a, CD14, CD20, CD22, CD33, CD34, CD64, CD79a, CD34, CD117, HLA-DR, cytoplasmic CD3, cytoplasmic CD19, and terminal deoxynucleotidyltransferase (TdT).

G-banded metaphase analysis and FISH were performed on the BM aspirate using standard cytogenetic techniques. The karyotype was 46,XY,-5,-7,+2mar in 2 of the 20 analyzed BM cells (Fig. 1D), and 46,XY in the remaining 18 cells. BCR/ABL, AML1/ETO, PML/RARA, CBFβ, MLL, D5S630/EGR1, and RELN/TESEFISH probes (Abbott Molecular/Vysis, Des Plaines, IL, USA) and the D5S630/EGR1 probe from Cytocell (Cambridge, UK) showed an additional *AML1* signal, a 5q deletion, and a 7q deletion (Fig. 1E-G). The FISH findings suggested that at least part of the marker chromosomes were derived from chromosome 21 and the short arm of chromosome 5, and not from 5q or 7q. Multiplex reverse transcriptase-PCR (RT-PCR) with the Hemavision kit (DNA Technology, Aarhus, Denmark) did not detect any fusion transcripts.

The patient was diagnosed with t-AMKL and was treated for neutropenic fever and pneumonia. No further therapy was administered for the leukemia because of his poor general condi-

tion, and he is currently being followed up in our hospital with routine CBC check-ups.

The previous 2008 WHO classification described two subsets of therapy related myeloid neoplasm, consisting of t-AML/therapy-related myelodysplasia (t-MDS) and t-AML/t-MDS/therapy-related myeloproliferative neoplasm (t-MPN). The former subset commonly caused by exposure to alkylating agents or radiotherapy has the following characteristics: 1) It typically has a long latent period; 2) Its incidence increases with age; 3) It is frequently present with t-MDS, t-AML after a myelodysplastic phase or t-AML with dysplastic features; 4) It has a poorer prognosis; 5) It is associated with an unbalanced loss of genetic material; 6) It commonly affects chromosomes 5 or 7. The other subset is characterized by 1) exposure to topoisomerase II inhibitors; 2) a short latency period; 3) a similar incidence across all ages; 4) the presence of overt t-AML without a preceding myelodysplastic phase or dysplastic features; 5) a more indolent prognosis; and 6) balanced translocations involving 11q23 or 21q22 [1, 2]. The patient in this case was elderly, presented with overt t-AML with prominent dysplastic features after ten years of remission and unbalanced chromosomal abnormalities in chromosomes 5 and 7. Alkylating agents, anti-tubulin agents, topoisomerase I inhibitors and ionizing radiation were all used in his treatment, but not topoisomerase II inhibitor. This case therefore showed clinical features typical of the former subset of t-AML/t-MDS. The two previously reported t-AMKL cases also involved dysplastic features, unbalanced chromosomal rearrangements, and a history of alkylating agent or radiation therapy (Table 1) [3, 4].

AMKL in non-Down syndrome patients is rare, accounting for less than 1% of all adult AML cases [6]. A previous study reported t-AMKL in 4 of 37 (10.8%) adult AMKL cases [5], and another study found t-AMKL in 2 of 23 (8.7%) adult AMKL cases [6], and on this basis the incidence of t-AMKL is about 0.1% of all adult AML cases.

Adherence of platelets to leukocytes during flow cytometry and the procedures used for fixation and decalcification in immunohistochemistry can lead to difficulties in identifying megakaryocytic lineages [7]. Recent studies have stated that accurate diagnosis of AMKL additionally requires that cells are negative for CD11a and HLA-DR, as well as being positive for previously used markers such as CD41, CD42, CD61, or Factor VIII [8]. Likewise, in this case, the patient also showed a CD61+, Factor VIII+, CD11a-, and HLA-DR immunophenotype.

In summary, to the best of our knowledge, this is the third case report of t-AMKL worldwide and the first report of t-AMKL in Korea. Furthermore, it is the first reported case of overt t-

AMKL without any evidence of preceding myelodysplasia.

Authors' Disclosures of Potential Conflicts of Interest

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

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