Letter to the Editor
Diagnostic Hematology
Ann Lab Med 2013;33:371-374
http://dx.doi.org/10.3343/alm.2013.33.5.371
ISSN 2234-3806 • eISSN 2234-3814
ANNALS OF LABORATORY MEDICINE

# Two Cases of Near-Tetraploidy in Acute Leukemias of Ambiguous Lineage

Bo Hyun Kim, M.D.[1], Hye Ryoun Kim, M.D.[1], Mi-Kyung Lee, M.D.[1], and HyunYoung Chi, M.D.[2]
Department of Laboratory Medicine, Chung-Ang University College of Medicine[1]; Samkwang Medical Laboratories[2], Seoul, Korea

Acute leukemia of ambiguous lineage (ALAL) is a rare subtype of acute leukemias that does not show any clear evidence of differentiation along a single lineage [1].

Although both numerical and structural chromosomal abnormalities are commonly associated with acute and chronic hematologic malignancies, near-tetraploidy has been reported rarely (1.2% of AML cases) [2, 3]. Adult AML patients with tetraploidy are characterized by insensitivity to chemotherapy, low remission rates, and short survival periods (median, 4 months; range, from 0.1 to 32 months) [4].

There are very few cases described in the literature to determine whether there are any chromosomal abnormalities associated with ALAL [1, 2]. Here, we report 2 cases of adult ALAL with near-tetraploidy, representing the first such case reports in Korea.

Case 1 involved a 63-yr-old Korean man. A complete blood cell (CBC) count at admission showed pancytopenia (hemoglobin level: 4.6 g/dL; white blood cell count: $3.17\times10^9$/L; absolute neutrophil count: $1.26\times10^9$/L; platelet count: $101\times10^9$/L). Immature cells were rare on the peripheral blood (PB) smear. The 500-cell differential count from the BM aspirate revealed that 70% of all nucleated cells were blasts with large sizes, high nuclear/cytoplasm (N/C) ratios, irregularly-shape nuclei, dispersed chromatin, distinct nucleoli, and basophilic cytoplasm that occasionally formed pseudopods (Fig. 1A). Cytochemical staining was negative for myeloperoxidase (MPO), periodic acid-Schiff (PAS), and nonspecific esterase (NSE). The BM biopsy specimen showed hypercellular marrow infiltrated by large immature cells. CD34-specific immunohistochemical staining showed strong positive staining for leukemic blasts.

Flow cytometric analysis of the BM aspirate was performed with a 2-color Beckman Coulter Cytomics FC 500 flow cytometer (Beckman-Coulter, Fullerton, CA, USA). The result revealed that approximately 66% of the large cells were strongly positive for CD19 (99.0% of the gated blasts) and HLA-DR (98.7% of the gated blasts), and negative for all other myeloid (CD13, CD33, CD117, and MPO), B-lymphoid (CD20, CD22, and CD79a), T-lymphoid (CD2, CD3, CD5, CD7, and CD10), and terminal deoxynucleotidyl transferase (TdT) antigens.

On the basis of morphologic features and immunophenotyping results of the BM aspirate, the patient was diagnosed with ALAL according to the 2008 WHO classification [1]. The result of conventional G-banding chromosomal analysis of the bone marrow cells was 89-93,XXYY,+X,-Y,-6,+7,-9,-14,-16,-18,-19,-20[cp6]/46,XY [14] (Fig. 1B). Interphase FISH using a DNA probe set specific for the *AML1/ETO, BCR/ABL, MLL, PML/RARA,* and *CBFB* genes (Vysis, Downers Grove, IL, USA) was performed according to the manufacturer's protocols, and the result was nuc ish (RUNX1T1, RUNX1) ×4 [23/200]/(RUNX1T1, RUNX1) ×2 [173/200], (ABL ×3, BCR ×4) [20/200]/(ABL, BCR) ×2 [178/200] (MLL ×4) [21/200]/(MLL ×2) [175/200], (PML, RARA) ×4 [31/200]/(PML, RARA) ×2 [167/200], (CBFB×4) [37/200]/(CBFB

**Received:** March 13, 2013
**Revision received:** May 2, 2013
**Accepted:** July 1, 2013

**Corresponding author:** Hye Ryoun Kim, M.D.
Department of Laboratory Medicine, Chung-Ang University College of Medicine, 102 Heukseok-ro, Dongjak-gu, Seoul 156 755, Korea
Tel: +82-2-6299-2718, Fax: +82-2-6298-8630, E-mail: hyekim@cau.ac.kr

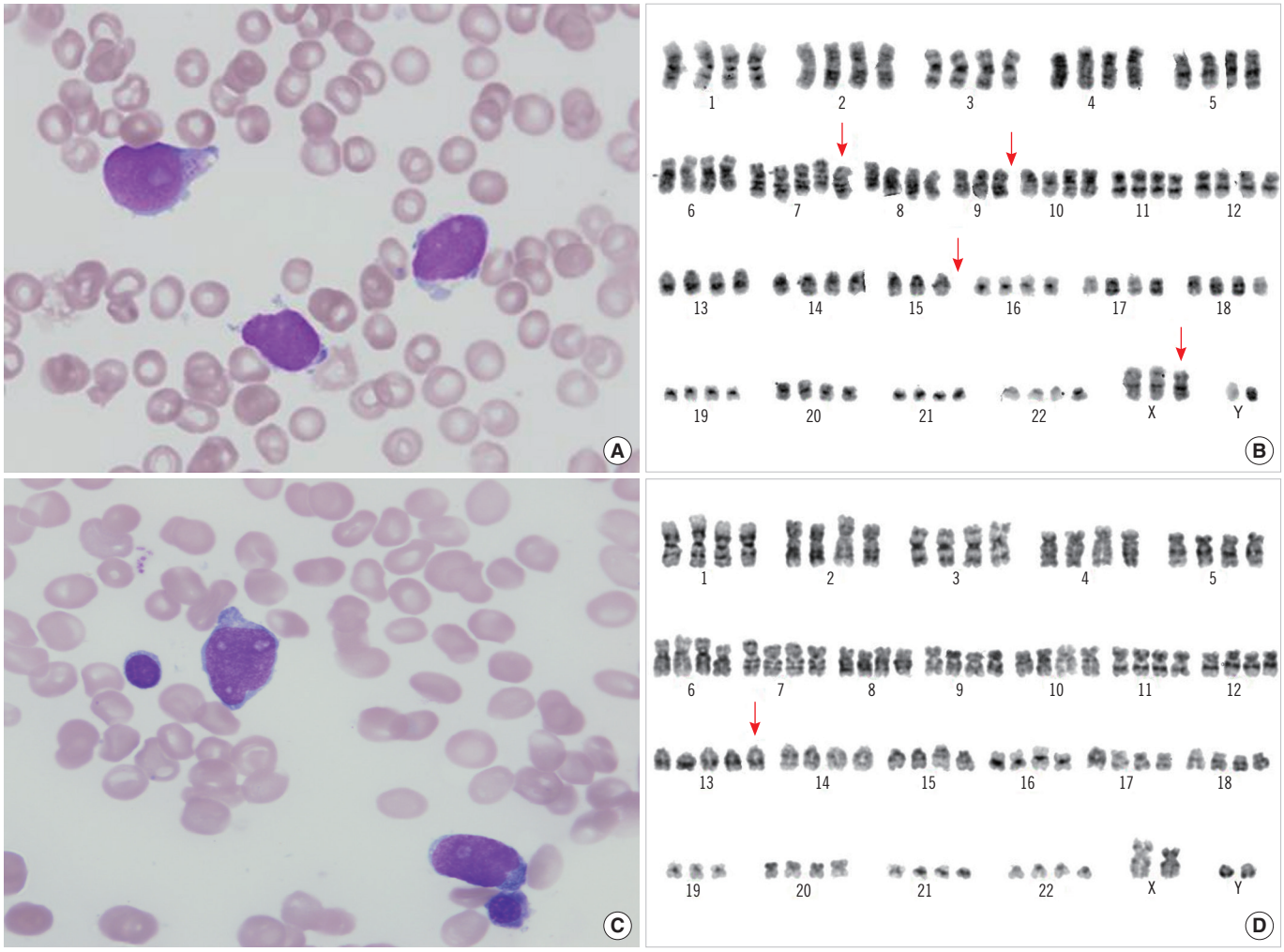


**Fig. 1.** Histomorphologic features of leukemic blasts from bone marrow aspirates and evidence of near-tetraploidy in 2 cases. (A) Leukemic blasts in case 1. Blasts were large and had high nuclear/cytoplasm ratios, irregular nuclei, a dispersed chromatin pattern, distinct nucleoli, and basophilic cytoplasm with pseudopods (Wright-Giemsa stain, ×1,000). (B) Karyotype of the abnormal clone in case 1. The arrows indicate additional or missing chromosomes from tetraploidy. The final karyotype was 89-93,XXYY,+X,-Y,-6,+7,-9,-14,-16,-18,-19,-20[cp6]/46,XY[14]. (C) Leukemic blasts in case 2. Blasts were large and had high nuclear/cytoplasm ratios, irregular nuclei, a dispersed chromatin pattern, and distinct nucleoli (Wright-Giemsa stain, ×1,000). (D) Karyotype of the abnormal clone in case 2. The arrows indicate additional or missing chromosomes from tetraploidy. The final karyotype was 93,XXYY,+13[8]/45,X,-Y[7]/46,XY[15].

×3) [160/200]. Genetic mutation analyses for internal tandem duplication of fms-related tyrosine kinase 3 (*FLT3*/ITD) and nucleophosmin 1 (*NPM1*) were performed with bidirectional Sanger sequencing using an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The *FLT3*/ITD results were negative, and the mutation status of *NPM1* was wild type.

After the patient received induction chemotherapy, first follow-up BM examination and cytogenetic analyses were performed. Follow-up BM aspiration showed blasts within the normal range (1.3% of total nucleated cells); however, diffuse infiltration and clusters of immature cells were detected on BM biopsy. Follow-up cytogenetic analysis showed the presence of near-tetraploidy (88-92,XXYY,+X,-Y,-4,-5,+7,-9,-21,-22[cp2]/46,XY[18]). Subsequently, the patient received re-induction chemotherapy, and second follow-up BM examination and cytogenetic analyses were performed. The blast counts in the BM aspirate were normal (3.6% of all nucleated cells). However, diffuse infiltration and clusters of immature cells were still present in the BM biopsy, and cytogenetic analysis revealed the presence of near-tetraploidy (92,XXYY[2]/46,XY[18]). The patient died after 82 days of hospitalization.

Case 2 involved an 80-yr-old Korean man. Results of a CBC count were as follows: hemoglobin level, 8.3 g/dL; white blood cell count, $1.55\times10^9$/L; absolute neutrophil count, $0.22\times10^9$/L;


http://dx.doi.org/10.3343/alm.2013.33.5.371

and platelet count, $92\times10^9$/L. No immature cells were found on the PB smear. In the BM aspirate, 27.4% of all nucleated cells were large blasts with high N/C ratios, irregularly-shape nuclei, dispersed chromatin, distinct nucleoli, and basophilic cytoplasm (Fig. 1C). Cytochemical staining was negative for MPO, PAS, and NSE. The BM biopsy specimen showed normocellular marrow infiltrated by large immature cells. CD34-specific immunohistochemical analysis showed strong positive staining for leukemic blasts. The result of flow cytometric analysis of the BM aspirate revealed that approximately 21% of the large cells were strongly positive for CD13 (60.2% of the gated blasts), cCD79a (39.5% of the gated blasts), CD61 (25.9% of the gated blasts), TdT (56.6% of the gated blasts), and HLA-DR (62.7% of the gated blasts), and negative for all other myeloid, B-lymphoid, and T-lymphoid antigens.

On the basis of the morphologic features and immunophenotyping results of the BM aspirate, the patient was diagnosed with ALAL according to the 2008 WHO classification [1]. The result of conventional G-banding chromosomal analysis was 93,XXYY,+13 [8]/45,X,-Y[7]/46,XY[15] (Fig. 1D). The result of interphase FISH was nuc ish (RUNX1T1, RUNX1) ×4 [20/200]/(RUNX1T1, RUNX1) ×2 [178/200], (ABL, BCR) ×4 [26/200]/(ABL, BCR) ×2 [170/200], (MLL ×4) [26/200]/(MLL ×2) [171/200], (PML, RARA) ×4 [24/200]/(PML, RARA) ×2 [171/200], (CBFB ×4) [22/200]/(CBFB ×2) [173/200]. Neither FLT3/ITD nor NPM1 mutations were detected during genetic analyses. The patient refused chemotherapy and underwent only outpatient care. The last visit was 6 months after the initial BM diagnosis, and he discontinued follow-up thereafter. Follow-up PB and BM analyses were not performed.

Here, we report 2 cases of rare ALAL with near-tetraploidy. Immunophenotyping of the first patient showed strong positivity only for CD19 and negativity for CD79a, cCD22, and CD10. Therefore, we could not assign a B-lineage. Overall, no clear evidence of differentiation along a single lineage was found, but due to strong positivity for CD19, we could not diagnose acute undifferentiated leukemia. Finally, we were able to diagnose the first case as ALAL. In the second case, since the patient's immunophenotype showed no activity for MPO, monocytic markers, CD3, or CD19, we also could not assign any specific lineage. Although cCD79a staining was positive, the staining intensity was dim and there was no positivity for other B-cell lineage markers; hence, we excluded the possibility of B-cell ALL. CD61 staining also showed weak positivity, but the morphologic characteristics of the blasts were not similar to those of megakaryoblasts. In addition, it is well known that false-positive flow cytometric detection of platelet glycoprotein expression in myeloid leukemia secondary to platelet adherence to leukemic blasts occurs frequently (up to 85% of AML cases) [5]. The patient's flow cytometry dot plot for CD45 and side scatter (SSC) showed a diffuse platelet adhesion pattern in other cell populations, and therefore, he could not be diagnosed with acute megakaryoblastic leukemia. The WHO suggests that the lack of an assignable lineage in combination with the expression of no more than 1 nondefinitive antigen for any given lineage could be called acute undifferentiated leukemia, while similar neoplasms with more than 1 suggestive antigen in each of more than 1 lineage should be considered acute unclassifiable leukemia [6]. By definition, acute undifferentiated leukemia lacks the T or myeloid lineage-specific markers and does not express B-cell specific markers [1]. In case 2, the immunophenotype showed positivity for 1 myeloid nondefinitive antigen (CD33), but also for 1 B-cell lineage specific antigen (cCD79a); therefore, a diagnosis of acute undifferentiated leukemia and acute unclassifiable leukemia could not be made. There was no evidence of nonhematopoietic neoplasm, NK-cell leukemia, or other myeloid leukemia such as acute erythroid leukemia. For these reasons, the second patient was diagnosed with ALAL.

CD34 immunohistochemical staining showed strong positivity in both our patients, and this is similar to findings from other reports [7]. This finding revealed that the blasts of the 2 patients in this study had immature characteristics.

Previous study showed that increased DNA content is directly related to blast size [8]. In the 2 cases presented here, the blasts were large in size with high N/C ratios, irregular nuclear configuration, and distinct multiple nucleoli, which were consistent with those by Zhang et al. [8]. These results indicate that if giant and bizarre blasts are found in BM aspirate smears from patients with leukemia, tetraploid or near-tetraploid karyotypes should also be considered.

In general, tumors with tetraploidy have a high degree of malignancy and patient survival is relatively short [8]. Some authors have suggested that the great variety of chromosome numbers suggests a global defect in cell cycle control, leading to poor responses to chemotherapy [9]. The first patient did not achieve complete remission and had short survival duration of 82 days. We believe that abnormal tetraploid karyotypes may contribute to the observed laboratory and clinical features and lead to poor responses to therapy and poor prognoses.

In the second case, the patient refused chemotherapy for ALAL. If we assume that this patient survived for 6 months after the diagnosis of ALAL without chemotherapy, then the second

patient's outcome was better than that of the first. The following reasons may explain the difference in prognosis. First, the ages of the 2 patients differed. Human cell proliferation slows with increasing age. Thus, the more advanced age of the second patient may have led to slower disease progression. Second, the marrow blast count of the second patient was lower than that of the first patient. Third, according to the literature, tetraploidy or near-tetraploidy together with additional cytogenetic changes could be considered a prognostic marker of resistance to chemotherapy and poor outcomes [10]. The first patient in our study had more chromosome aberrations than the second patient, which may have contributed to the difference in outcomes. In addition, some studies have shown that loss of chromosomal material is associated with poor prognosis in cancer patients [11]. The first case showed more chromosomal loss than the second case; hence, we suggest that loss of chromosome material may have been associated with his poorer prognosis. Further studies, such as a comparative genomic hybridization study with more cases of near-tetraploidy, will be needed to confirm these hypotheses.

*FLT3*/ITD and *NPM1* mutations are associated with outcomes of AML [1]. *FLT3* analysis in patients with AML with near-tetraploidy has been recommended to better understand the role of this mutation [12]. On the basis of these considerations, we analyzed *FLT3*/ITD and *NPM1* mutation status in our cases, but the results were all negative. Further studies will be needed to evaluate the relationship between gene mutations and near-tetraploidy.

To our knowledge, these are the first reported cases of near-tetraploidy in patients with ALAL in Korea. Cases of acute leukemias with tetraploid karyotypes are rare. Consequently, the number of studies on the laboratory and clinical features of these patients are also limited. Further studies, such as DNA content determination, will provide more convincing evidence to make a tentative diagnosis of leukemia based on morphologic observations. The findings of these case reports will be helpful for evaluating tetraploidy or near-tetraploidy associated with ALAL.

## Authors' Disclosures of Potential Conflicts of Interest

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

## Acknowledgements

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012-0007974).

## REFERENCES

1. Swerdlow SH, Campo E, et al. eds. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC, 2008.
2. Béné MC, Castoldi G, Derolf A, Garand R, Haas T, Haferlach T, et al. Near-tetraploid acute myeloid leukemias: an EGIL retrospective study of 25 cases. Leukemia 2006;20:725-8.
3. Berger R, Flandrin G, Bernheim A, Le Coniat M, Vecchione D, Pacot A, et al. Cytogenetic studies on 519 consecutive de novo acute nonlymphocytic leukemias. Cancer Genet Cytogenet 1987;29:9-21.
4. Lemez P, Michalová K, Zemanová Z, Marinov I, Trpaková A, Moravcová J, et al. Three cases of near-tetraploid acute myeloid leukemias originating in pluripotent myeloid progenitors. Leuk Res 1998;22:581-8.
5. Betz SA, Foucar K, Head DR, Chen IM, Willman CL. False-positive flow cytometric platelet glycoprotein IIb/IIIa expression in myeloid leukemias secondary to platelet adherence to blasts. Blood 1992;79:2399-403.
6. Czuchlewski D. Acute leukemias of ambiguous lineage. In: Foucar K, Reichard K, Czuchlewski D, eds. Bone marrow pathology. 3rd ed. Chicago: ASCP Press, 2010:441-7.
7. Xiao Z, Liu S, Liu X, Yu M, Hao Y. Tetraploidy or near-tetraploidy clones with double 8;21 translocation: a non-random additional anomaly of acute myeloid leukemia with t(8;21)(q22;q22). Haematologica 2005;90:413-4.
8. Zhang JH, Zheng YC, Wang YX, Zhang JY, Liu ZG. Enlarged and prominent nucleus may be indicative of tetraploidy: a laboratory study of a rare near-tetraploidy in a child patient with acute myelogenous leukemia AML-M4. J Pediatr Hematol Oncol 2010;32:19-21.
9. Yeh SP, Wang Y, Su J, Hsueh E, Yu M, Wu H. Near-tetraploid minimally differentiated acute myeloid leukemia with extensive erythrophagocytosis by leukemic blasts. Ann Hematol 2000;79:36-9.
10. Jarosova M, Nedomova R, Hubacek J, Holzerova M, Mickova P, Katrincsakova B, et al. Rare tetraploidy with large 5q deletion in acute myeloid leukemia with myelodysplasia-related changes (AML-MRC). Leuk Res 2012;36:e68-70.
11. Gutiérrez NC, García JL, Hernández JM, Lumbreras E, Castellanos M, Rasillo A, et al. Prognostic and biologic significance of chromosomal imbalances assessed by comparative genomic hybridization in multiple myeloma. Blood 2004;104:2661-6.
12. Jurisić V, Pavlović S, Colović N, Djordjevic V, Bunjevacki V, Janković G, et al. Single institute study of FLT3 mutation in acute myeloid leukemia with near tetraploidy in Serbia. J Genet 2009;88:149-52.


http://dx.doi.org/10.3343/alm.2013.33.5.371