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동시에 채취한 림프절과 골수에서 상이한 면역표현형을 보인 급성백혈병 2예

송민정[1] · 김희진[1] · 고영혜[2] · 김선희[1]

성균관대학교 의과대학 삼성서울병원 진단검사의학과[1] · 병리과[2]

Discrepant Immunophenotypic Characteristics between the Lymph Node and Bone Marrow in Two Mixed-Phenotype Acute Leukemia Patients

Min-Jung Song, M.D.[1], Hee-Jin Kim, M.D.[1], Young-Hyeh Ko, M.D.[2], and Sun-Hee Kim, M.D.[1]

Departments of Laboratory Medicine & Genetics[1] and Diagnostic Pathology[2], Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

The immunophenotypic profile of hematological malignancies is usually consistent among different sites of involvement; this consistency allows reliable diagnosis from peripheral blood, bone marrow, or lymph node, especially in cases of acute leukemia. Although in a minority of lymphoma patients, two or more different populations with discordant immunophenotypes have been described, either at the same or distinct sites. Here, we report two Korean patients with acute leukemia where the results of immunophenotypic analysis of the bone marrow specimen were different from those of immunohistochemical studies of a biopsy sample of a cervical lymph node, particularly with respect to myeloperoxidase and CD3. The clinical significance of the immunophenotypic disparity found in the patients still remains unknown; however, discrepancies between the different anatomic sites that are simultaneously involved can occur in a subset of leukemia patients. Therefore, integration of all the relevant results, including those of the bone marrow studies, may be helpful for accurate diagnosis and selecting appropriate treatment modalities. *(Korean J Lab Med 2009;29:396-401)*.

Key Words : *Mixed-Phenotype Acute Leukemia, Lymph node, Bone marrow, Immunophenotype*

INTRODUCTION

Most acute leukemias can be assigned a specific lineage as either ALL or AML according to the morphologic, cytochemical, and immunophenotypic characteristics of the blast cells. With the widespread availability of flow cytometry, acute leukemias that express cross-lineage antigens are increasingly being recognized [1]. These can either be bilineal acute leukemias with two separate populations of blasts, each from a different lineage, or biphenotypic acute leukemia (BAL) with a single blast population expressing multiple lineage markers. Both the above groups of leukemias are categorized as "acute leukemias of ambiguous lineage" in the new WHO classification system [2] and are believed to have arisen from the multipotent hematopoietic stem cells (HSC) [3]. In an effort to unify the definition of BAL, the European Group for the Immunological Classification of Leukaemias (EGIL) proposed guidelines for the scoring of

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Corresponding author : Sun-Hee Kim, M.D.
Department of Laboratory Medicine, Samsung Medical Center,
50 Irwon-dong, Gangnam-gu, Seoul 135-710, Korea
Tel : +82-2-3410-2704, Fax : +82-2-3410-2719
E-mail : sunnyhk@skku.edu

lineage-specific antigens commonly used in the assignment of lineage to acute leukemias [4].

In leukemia, the immunological profile is generally considered to be conserved, irrespective of the involved site [5]. Therefore, in most cases the definitive diagnosis in acute leukemia is based mainly on the results of bone marrow studies. Here, we present two cases of acute leukemia, which are interesting because the expression of some antigens was observed in the lymph node but not in the bone marrow.

CASE REPORTS

1. Patient 1

A 36-yr-old male patient was admitted with palpable neck masses that had developed 10 days prior to admission. His previous medical history was unremarkable, and his family history was negative for hematologic disorders. A computed tomographic scan of the larynx revealed multiple lymphadenopathy involving both cervical, mediastinal and left axillary lymph nodes. The laboratory findings included a hemoglobin level of 12.1 g/dL, a platelet count of 159,000/μL, and a leukocyte count of 1,910/μL. A peripheral blood smear showed left-shifted neutrophils, and 1% blasts. Under the presumptive diagnosis of malignant lymphoma, an excisional biopsy of the cervical lymph node and a bone marrow study were performed. The patient was diagnosed as having acute myeloid leukemia without maturation according to the French-American-British (FAB) classification and was treated with high-dose cytarabine, idarubicin, vincristine, and prednisone. He was documented to have attained complete remission of disease at day 28. He is now in the allogenic peripheral blood stem cell transplantation state without relapse.

2. Patient 2

A 14-yr-old female was referred to our institution in August 2007 because of a massive cervical lymphadenopathy and shortness of breath. A physical examination demonstrated multiple enlarged and tender lymph nodes in the retroauricular area. On admission, the patient's blood count showed the following: hemoglobin 13.5 g/dL, platelets 55,000/μL, and leukocytes 239,940/μL (with 7% blasts). A computed tomographic scan of the neck showed multiple enlarged lymph nodes with central low attenuation involving both the neck and the right paratracheal area; this suggested the presence of a hematologic malignancy such as a lymphoma or a metastatic cancer. Other work-ups, such as abdominal ultrasound and cerebrospinal fluid examination showed normal findings. Under the presumptive diagnosis of acute leukemia, an excisional biopsy of the cervical lymph node and a bone marrow study were performed. On the basis of the clinical features, morphology, and immunophenotype, the final diagnosis of mixed-phenotype acute leukemia, T/myeloid (not otherwise specified) was established. The patient was commenced on induction chemotherapy as for acute lymphoblastic leukemia (prednisolone, vincristine, daunorubicin, and L-asparaginase) and achieved a complete response after one cycle of treatment. She remains in continuous remission on routine follow-up, one yr after stem cell transplantation from a sibling donor.

3. Histomorphology and immunohistochemistry

Wright-Giemsa-stained peripheral blood and bone marrow smears were prepared for morphologic analysis. In addition, the aspirate was also stained with periodic acid-Schiff (PAS), peroxidase, and alpha-naphthyl butyrate esterase (ANBE). The histology of the cervical lymph node and bone marrow biopsy was analyzed in hematoxylin-eosin (HE) stained sections. The immunohistochemical stains that were applied on the paraffin-embedded tissue sections included CD2, CD3, CD5, CD7, CD56, CD79a, CD99, CD117, Ki-67, TIA-1, terminal deoxynucleotidyl transferase (TdT), and myeloperoxidase (MPO) for the lymph node and CD3, CD20, CD79a, TdT, and MPO for the bone marrow (all the stains were obtained from Dako Cytomation, Glostrup, Denmark, except for those of CD3, which were from Novocastra, Newcastle, UK).

The cervical lymph node biopsy of patient 1 showed almost complete replacement by malignant cells, which were medi-

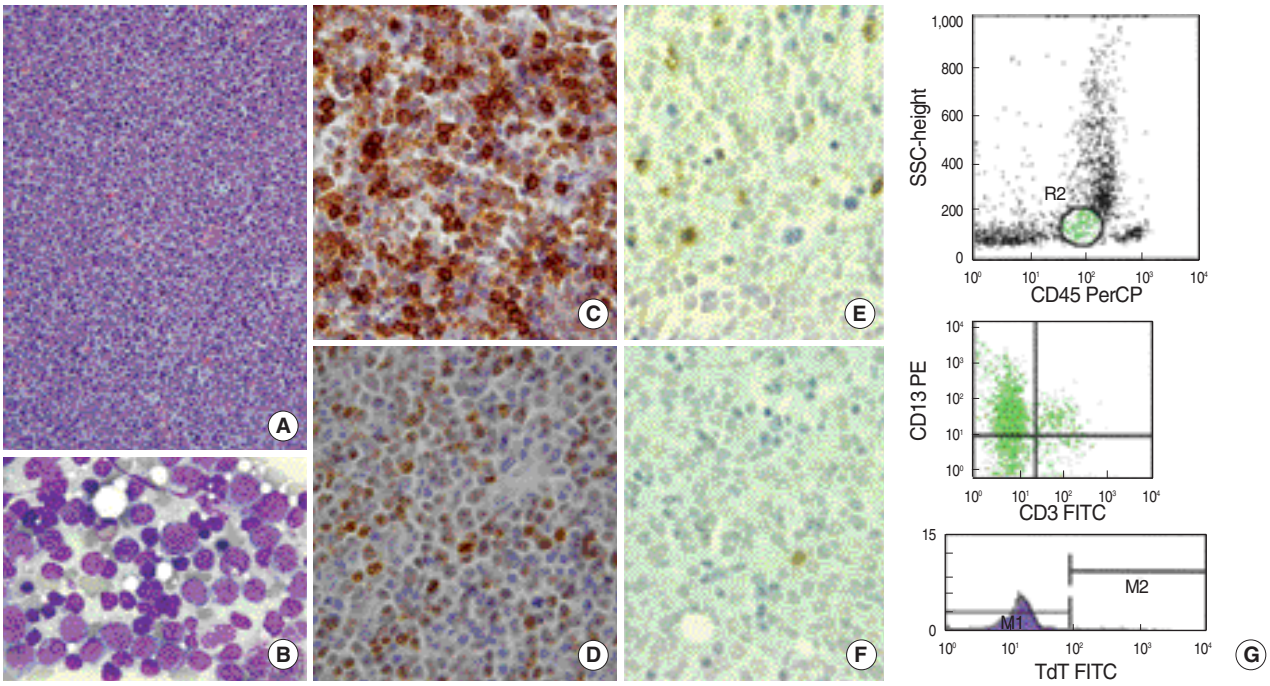

Fig. 1. Patient 1. (A) Cervical lymph node biopsy showing almost complete replacement by malignant cells (H&E stain, ×400). (B) Bone marrow aspirate smear showing blast cells with rather abundant cytoplasm with granules (Wright-Giemsa stain, ×1,000). (C, D) Immunohistochemical stains on lymph node biopsy. The majority of neoplastic cells are strongly positive for CD3 (C) and TdT (D) (×1,000). (E, F) Immunohistochemical stains on bone marrow biopsy. The majority of neoplastic cells are negative for CD3 (E) and TdT (F) (×1,000). (G) Flow cytometric immunophenotyping of the marrow blasts showing negative expression of CD3 and TdT.
Abbreviation: Tdt, terminal deoxynucleotidyl transferase.

Table 1. Immunohistochemistry results in the cervical lymph node (LN) and immunophenotypic results of the marrow (BM) aspirate

Antigens	Patient 1		Patient 2	
	LN	BM	LN	BM
CD2	–	–	–	–
CD3	**+**	**–**	+	+
CD5	–	–	ND	+
CD7	+	+	ND	+
CD10	ND	–	ND	+
CD13	ND	+	ND	+
CD14	ND	–	ND	–
CD15	ND	–	ND	–
CD19	ND	–	ND	–
CD20	ND	–	ND	–
CD33	ND	+	ND	–
CD34	ND	+	ND	–
CD56	–	ND	+	ND
CD64	ND	–	ND	–
CD79a	–	ND	ND	ND
CD117	+	+	ND	+
MPO	+	+	**+**	**–**
TdT	**+**	**–**	+	W+

Abbreviations: ND, Not done; MPO, myeloperoxidase; Tdt, terminal deoxynucleotidyl transferase; W+, Weakly positive.

um-sized with one or two prominent nucleoli (Fig. 1). Immunohistochemical staining on the paraffin-embedded tissue sections demonstrated that the tumor cells were positive for polyclonal CD3, CD7, CD99, CD117, Ki-67, MPO, and TdT (Table 1). The bone marrow studies showed normocellular marrow (cellularity 40%) for the patient's age; however, most nucleated cells were leukemic blasts having rather abundant cytoplasm with granules, and exhibited up to 80.1% of all nucleated cells. The blasts were negative for PAS, ANBE, TdT, and polyclonal CD3 and stained positively for Sudan black B (SBB), MPO, and peroxidase.

The cervical lymph node biopsy of patient 2 showed effacement of nodal architecture by diffuse infiltration of tumor cells that were medium to large-sized with a high nuclear cytoplasmic ratio, dispersed nuclear chromatin, and prominent nucleoli (Fig. 2). CD3, CD56, CD99, TIA-1, and MPO were expressed by the majority of cells and scattered TdT positivity was detected among the cells (Table 1). The bone marrow aspiration smear showed medium to large-sized

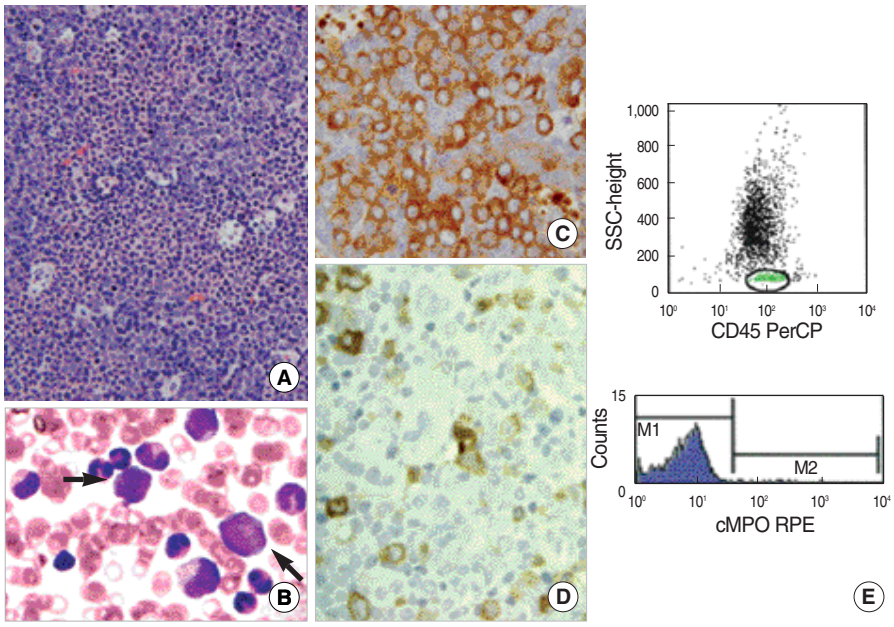

Fig. 2. Patient 2. (A) Cervical lymph node biopsy showing diffuse infiltration by neoplastic cells (H&E stain, ×400). (B) Bone marrow aspirate smear showing blast cells (arrows) with high nucleus/cytoplasm ratio and prominent nucleoli (Wright-Giemsa stain, ×1,000). (C) Immunohistochemical stain on lymph node biopsy. The large neoplastic cells showed positivity to MPO (×1,000). (D) Immunohistochemical stain on bone marrow biopsy. The neoplastic cells showed negativity to MPO (×1,000). (E) Flow cytometric immunophenotyping of the marrow blasts showing negative expression of MPO.
Abbreviation: MPO, myeloperoxidase.

tumor cells accounting for 12.4% of all nucleated cells. These cells were large and heterogenous with irregular nuclei, lacy chromatin, and prominent nucleoli. They were negative for all cytochemical stains except ANBE. The bone marrow biopsy showed hypercellular marrow (cellularity 90%) filled with leukemic blasts and markedly increased granulocytic precursors (Myeloid/erythroid ratio, 18.6:1). The blasts stained positively for CD3 and TdT. However, the majority of the blasts were negative for MPO stain.

4. Immunophenotyping by flow cytometry and cytogenetic analysis

The bone marrow aspirate was stained using two combinations of monoclonal antibodies (MoAbs) directly conjugated to fluorescein isothiocyanate (FITC) and phycoerythrin (PE). The analysis was performed on the FACSort flow cytometer (Becton-Dickinson, San Jose, CA, USA), gating the blast population on CD45/side-scatter. A marker was considered positive if expressed in more than 20% of the gated cells in excess of the negative control.

In patient 1, there was lymphoid light scatter with dim to moderate CD45-expression, which showed a single blast population with distinct positivity for numerous myeloid markers. In brief, the blasts were positive for CD7, CD13, CD33, CD34, CD117, and MPO. They were negative for CD3 and TdT (Table 1, Fig. 1). Cytogenetic analysis was performed using 24-hr unstimulated cultures with bone marrow specimen, and the karyotype was described according to the International System for Human Cytogenetics Nomenclature 2005. His karyotype was 46,XY,del(5)(q33)[5]/46,idem, del(6)(q21q23)[6]/46,XY[9].

Flow cytometric immunophenotyping of patient 2 revealed a single blast population with distinct positivity for numerous myeloid and T-cell markers. The blasts were positive for CD5, CD7, CD10, cytoplasmic CD3, CD13, CD117, and nuclear TdT. They were negative for MPO (Table 1, Fig. 2). At diagnosis, the cytogenetic study showed normal 46XX female karyotype in all the 20 metaphases that were analyzed.

DISCUSSION

It is well known that patients with non-Hodgkin lymphoma (NHL) may have histologic discordance between the different anatomic sites involved [6-8]. Earlier studies demonstrated differential expression of integrins (i.e., LFA-1) in tissue-based and leukemic NHL [9, 10]. Differences in the expression of lymphocyte homing receptors have also been postulated as a mechanism for specific tissue localization

of lymphocytes, as suggested by experimental studies. The authors indicated that antigen modulation in response to the tissue-specific microenvironment or preferential recirculation of cells with a certain immunophenotype may be associated with a disease mechanism [10]. Little has been published, however, about the immunophenotypic variation of acute leukemia involving different anatomic sites, especially at the time of diagnosis.

Here, we describe and characterize two cases of acute leukemia with discrepancy in the findings of immunophenotypic analysis of bone marrow specimen and those of immunohistochemical study of a biopsy sample of a cervical lymph node. The double-positive CD3/MPO leukemic cells described above were present in the lymph node but not in the bone marrow. Unfortunately, we were unable to perform immunophenotyping of the lymph node sample because it is not routinely used in our laboratory for the diagnosis and sub-classification of an acute leukemia. Therefore, it remains unclear whether the discrepancy of immunophenotyping is due to two different clones in the same patient, or an aberrant marker expression as a result of the neoplastic state and/or modulation of antigenic expression in relation to the host environment. However, considering the clinical manifestations of the patients and other laboratory findings, the latter is the more likely explanation.

A discrepancy was noted between the clinical manifestations and the findings of the bone marrow study in patient 2. The rapid clinical progression observed in patient 2 is a usual finding in acute leukemia. Further, she received an ALL-designed chemotherapy and achieved complete remission. However, the blast count in the bone marrow was less than 20% at initial diagnosis, which was insufficient to render the diagnosis of an acute leukemia. It is unclear whether the final diagnosis of patient 2 is the bone narrow involvement of malignant lymphoma or an atypical acute leukemia. Taking into effect the clinical manifestations, the treatment outcome, and findings of the lymph node biopsy, the final diagnosis was mixed-phenotype acute leukemia, T/myeloid (not otherwise specified).

The clinical significance of the immunophenotypic disparity found in the patients still remains unknown; however, the discrepancies between different anatomic sites that are simultaneously involved can occur in a subset of leukemia patients. Therefore, it is important that a definitive diagnosis should be assigned only after careful integration of all relevant results, including those of bone marrow studies.

To the best of our knowledge, this is the first report identifying an acute leukemia case with a different immunophenotype of leukemic cells in the bone marrow and in the lymph node. It is likely that other similar cases exist and go undetected because immunophenotypic studies of the lymph node are not routinely performed in all acute leukemia patients. Therefore, larger studies are warranted to determine the precise incidence of immunophenotypic discordance between different anatomic sites involved simultaneously in patients with acute leukemia, as well as the clinical behavior and response to treatment in this group of patients.

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