대한진단검사의학회지 제27권 제4호 2007
Korean J Lab Med 2007;27:253-6

비전형적인 형태를 보인 CD45-, CD19- B 전구세포림프모구성백혈병 1예

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A Case of CD45-, CD19- Precursor B Cell Acute Lymphoblastic Leukemia with an Atypical Morphology

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The differential diagnosis of acute lymphoblastic leukemia (ALL) from other small round blue cell tumors in children is very important for proper treatment, but sometimes difficult. CD45 is expressed on almost all-human leukocytes and not expressed on other small round blue cell tumors. Moreover, CD19 is expressed on all stages of B lineage cells and loss of this antigen is very rare in precursor B-cell ALL. We report a case of ALL with atypical morphology and immunophenotype. A 6-yr-old girl presented with fever and weight loss. Many abnormal cells with variable sized, high nuclear-cytoplasmic ratio and distinct nucleoli were counted 23% in bone marrow. The results of immunophenotyping were negative for CD45, CD19, CD10, CD20, CD3, CD5, CD7, CD56/16, CD13, and CD33 and positive for CD22, TdT, and CD34. The immunohistochemical staining of bone marrow biopsies was positive for CD79a, CD10, TdT and CD99. The cytogenetic study showed normal karyotype but amplification of *MLL* (myeloid/lymphoid or mixed lineage leukemia) gene was suggestive in the fluorescent in situ hybridization. The patient received the standard chemotherapy for acute lymphoblastic leukemia and reached complete remission. ***(Korean J Lab Med 2007;27:253-6)***

Key Words : *CD45, CD19, Acute Lymphoblastic Leukemia*

INTRODUCTION

The differential diagnosis of acute lymphoblastic leukemia (ALL) from other "small-round-cell tumors," such as neuroblastoma, rhabdomyosarcoma, and Ewing's sarcoma, is essential for proper treatment. The diagnosis based on morphologic findings may be difficult when clinical features are unclear. However, the immunophenotypic characteristics of these tumors may provide rapid and accurate diagnostic aids[1, 2]. In general, CD45 is expressed on almost all human leukocytes[3, 4], but is not expressed on neuroblastoma cells[1, 5, 6]. However, cases of CD45 negative ALL have been reported, although the clinical significance of these cases is unclear[3, 4]. In addition, CD19 is expressed on all stages of B lineage cells and loss of this antigen is rare in precursor B cell ALL[7]. Here, we present a case showing CD45-, CD19-, CD10-, CD22+, CD79a+, TdT+ and CD34+ with an atypical morphology undistinguishable from that of solid tumors like neuroblastoma.

접 수 : 2007년 3월 20일 **접수번호 :** KJLM2028
수정본접수 : 2007년 6월 26일
게재승인일 : 2007년 6월 26일
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CASE REPORT

A 6-yr-old girl presented with fever and weight loss. A physical examination showed splenomegaly, but no lymphadenopathy. Complete blood cell counts at admission were hemoglobin 6.2 g/dL, white blood cells 0.5×10^9/L, platelets 77×10^9/L, and blast was not noted in a peripheral blood smear. Bone marrow aspiration smear demonstrated many abnormal cells, which constituted 23%. Abnormal cells were of variable sizes, and had a high nuclear-cytoplasmic ratio, fragile cytoplasm, and loose chromatin (Fig. 1A). Dyserythropoiesis, such as karyorrhexis and multinuclearity (Fig. 1B), and myeloid cell hypogranulation were found. Megakaryocytes were markedly increased, which was an atypical finding in ALL. Moreover, many micromegakaryocytes and separate-nucleated forms were present (Fig. 1C). Bone marrow biopsies showed hypercellularity with increased megakaryocytes and many diffusely scattered abnormal cells (Fig. 1D). Immunophenotyping was performed as following combinations: CD10/CD19, CD45/CD19, CD20/CD5, CD3/CD22, CD7/CD33, HLA-DR/CD13, CD14/CD34, CD3/CD16+56 and TdT. Immunophenotyping results were negative for CD45, CD19, CD10, CD20, CD3, CD5, CD7, CD56/16, CD13, and CD33, and positive for CD22 (78%), TdT (67%), HLA-DR (57%), and CD34 (37%)(Fig. 2). The immunohistochemical staining of bone marrow biopsies was positive for CD79a, CD10, TdT, and CD99, and no neuroendocrine cells were present on synaptophysin and CD56 stain. The PAS stain was positive with a punctate pattern and peroxidase, SBB and nonspecific esterase stains were negative. The erythroid cells showed negative for PAS stain. The iron stain showed the presence of iron granules and no ringed sideroblast was noted. A cytogenetic study showed a normal karyotype but interphase fluorescent in

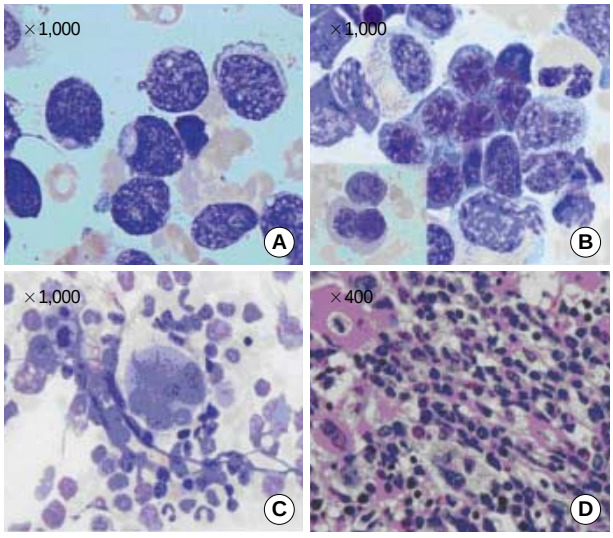

Fig. 1. Bone marrow findings showed many abnormal cells with highly variable sizes, a high nuclear-cytoplasmic ratio, fragile cytoplasm, and loose chromatin (A). Dysplastic changes were present in myeloid and erythroid series (B). Megakaryocytes showed dysplasia, e.g., separated nucleation (C). A biopsy showed scattered immature cells and an increased megakaryocyte count (D).

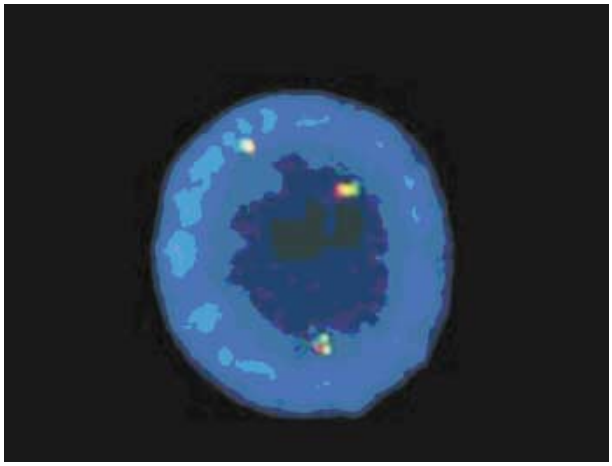

Fig. 3. Fluorescent in situ hybridization study using an MLL probe showed three fusion signals.

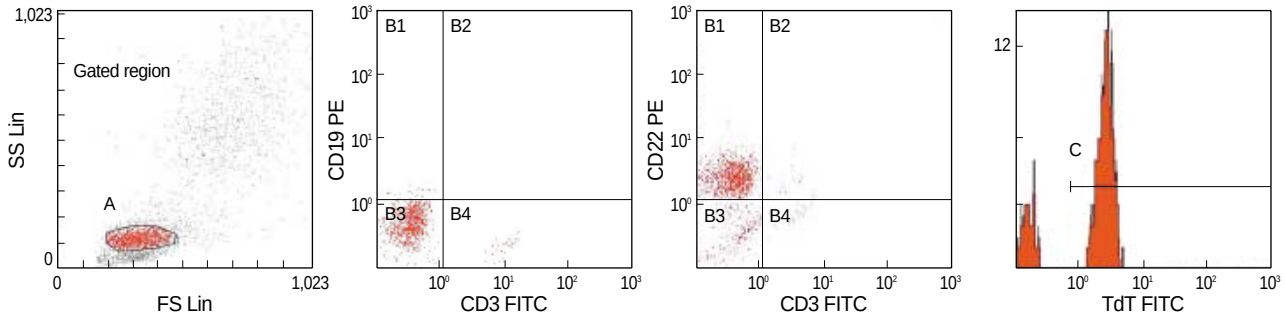

Fig. 2. The results of immunophenotyping. Leukemic cells were negative for CD19 and CD45 and positive for TdT, CD22, HLA-DR and CD34.

situ hybridization (FISH) study using a myeloid/lymphoid or mixed lineage leukemia (MLL) probe (LSI MLL Dual color, break apart rearrangement probe, Vysis, Downer Grove, IL, USA) showed three fusion signals (Fig. 3). The interphase FISH finding was suggestive of *MLL* gene amplification but metaphase FISH was needed to exclude trisomy 11. The patient received standard chemotherapy for ALL. One month after induction therapy, an immunophenotypic study was performed to detect residual cells. The initial phenotypes of leukemic cells (CD45-/CD34+, CD45-/CD22+, CD34+/TdT+), were used to detect leukemic cells, but none of these cells were detected. She was regarded as being in a complete remission and has been under maintenance chemotherapy.

DISCUSSION

CD45, also referred to as leukocyte common antigen (LCA), consists of a group of high-molecular-weight proteins, which are selectively expressed on the surfaces of all hematopoietic cells except erythrocytes, platelets, and plasma cells[3, 8]. In contrast, "small-round-cell tumors", such as neuroblastoma, rhabdomyosarcoma, and Ewing's sarcoma, etc, which are sometimes similar to ALL, do not express CD45 antigen[1, 2, 5]. In the present case, immature cells were highly variable in size and had fragile cytoplasm and loose chromatin, which is not a typical ALL morphology. Myeloid cells and erythroid cells showed dysplastic changes, and megakaryocytes were also dysplastic and markedly increased in number, which led us to consider them as solid tumors like neuroblastoma rather than ALL. Moreover, CD45 and CD19 were not expressed, which is a rare finding in ALL. A literature review revealed that the frequency of CD45 negative ALL is variable (20 to 58%)[3, 4, 9]. One author showed that the lack of CD45 expression is restricted almost exclusively to precursor B cell ALL[3]. However, the clinical significance and morphologic finding of lack of CD45 expression remain unclear[3, 4, 10]. CD19 is expressed in the majority of precursor B cell ALL cases, and in one study, it was found that 99% of these cases expressed CD19 antigen[7]. Because the present case expressed other hematopoietic markers, e.g., TdT, CD34, CD22 and CD79a, showed a typical punctate PAS staining pattern, and there was no radiological or laboratory evidence of lymphoma or another solid tumor, we were able to diagnose precursor ALL. Moreover, final diagnosis of ALL was made based on a careful morphologic examination by two independent experts. However, immunoglobulin gene rearrangement or cytoplasmic antigen expression was not performed to confirm B cell precursor. These aberrant expressions in this case will be applied at follow-up immunophenotypic studies to detect minimal residual disease and confirm a complete remission. In conclusion, as it has been reported that a number of B cell ALL cases lack CD45 expression, CD45 negative cases should not be considered small round cell tumors when morphologic findings are undistinguishable. Proper diagnosis should be based on immunophenotype profiles, cytochemistry findings, molecular results, and clinical features.

요 약

소아에서 급성림프모구백혈병과 다른 작은 원형세포 종양(small round blue cell tumors)의 감별은 치료결정에 중요하지만 어려운 경우가 있다. CD45는 거의 모든 백혈구계 세포에서 발현되며 작은 원형세포 종양에서는 발현되지 않는다. 또한 CD19는 B 세포 분화의 모든 단계에서 발현되며 B 전구세포 림프모구백혈병에서 발현되지 않는 경우는 매우 드물다. 저자들은 비전형적인 형태와 면역표현형을 보인 급성림프모구성백혈병 1예를 경험하였다. 6세 여자 환자로 발열과 체중감소로 내원하였다. 골수검사에서 23%로 관찰된 비정상 세포는 크기가 다양하고, 핵소체는 비교적 뚜렷하며, 높은 핵-세포질 비를 보였다. 면역표현형은 CD45, CD19, CD10, CD20, CD3, CD5, CD7, CD56/16, CD13, CD33이 음성이며, CD22, TdT, CD34가 양성이었다. 면역조직화학검사는 CD79a, CD10, TdT, CD99가 양성이었다. 염색체 검사에서는 정상핵형이었으나, 형광제자리부합법 검사에서 *MLL* (myeloid/lymphoid or mixed lineage leukemia) 유전자의 증폭을 시사하는 소견이 관찰되었다. 환자는 급성림프모구백혈병의 표준 항암 치료를 받았으며 관해에 도달하였다.

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