J Clin Exp Hematopathol
Vol. 49, No. 1, May 2009

Case Study

Epstein-Barr Virus Associated Post-transplant Hodgkin Lymphoma in an Adult Patient after Cord Blood Stem Cell Transplantation for Acute Lymphoblastic Leukemia

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Post-transplant lymphoproliferative disorder (PTLD) is one of the most important complications of solid organ transplantation or hematopoietic stem cell transplantation. Most PTLDs are associated with Epstein-Barr virus (EBV) infection. Although post-transplant Hodgkin lymphoma (HL) is included in PTLD, there have been no studies in the literature on adult cases of post-transplant HL after cord blood stem cell transplantation (CBSCT). This is due to the fact that EBV infection of cord blood cells usually does not occur, and EBV-infected lymphocytes of the recipient should be eradicated by preconditioning therapy. We report a 26-year-old woman case of post-transplant HL, which occurred after CBSCT for relapsed acute lymphoblastic leukemia. Three years and eight months after CBSCT, the enlarged cervical lymph node was histologically diagnosed as EBV associated post-transplant HL, which showed immunophenotypes of classical HL and latency type II EBV infection. She underwent chemotherapy, and has survived 4 years and 6 months after CBSCT. Differential diagnosis of post-transplant HL with good prognosis and HL-like PTLD with aggressive behavior is important, and immunohistochemical methods were useful and essential for it. The source of EBV associated HL in this case will be discussed. 〔*J Clin Exp Hematopathol 49(1) : 45-51, 2009*〕

Keywords: Epstein-Barr virus, post-transplant lymphoproliferative disorders, Hodgkin lymphoma, cord blood stem cell transplantation

INTRODUCTION

Post-transplantation lymphoproliferative disorder (PTLD) is one of the complications after solid organ or hematopoietic stem cell transplantation (HSCT).[1,2] The PTLD classification of World Health Organization (WHO) includes major four categories ; (1) early lesions (reactive plasmacytic hyperplasia and infectious-mononucleosis-like PTLD), (2) polymorphic PTLD, (3) monomorphic PTLD (including B-cell neoplasms and T-cell neoplasms), and (4) Hodgkin lymphoma (HL) and HL-like PTLD.[3] The last category is very rare.[4] Its incidence is reported 1.8% or 3.4% of PTLD in childhood,[5,6] and post-transplant HL has not been known in adult cases. Most PTLDs are Epstein-Barr virus (EBV) associated B-cell lymphoproliferative disorders, and are caused by HSCT or administration of immunosuppressants. The cumulative incidence of PLTD is approximately 1% at 10 years in HSCT.[2] Here we report a very rare adult case of post-transplant HL in PTLDs after cord blood stem cell transplantation (CBSCT) for relapsed acute lymphoblastic leukemia (ALL) in the central nervous system (CNS).

Received : September 8, 2008
Revised : December 11, 2008
Accepted : December 18, 2008
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CASE REPORT

The patient was a Japanese woman who suffered from precursor B-cell ALL at 20 years of age. She received chemotherapy and obtained complete remission. This chemotherapy was finished 2 years and 4 months after the ALL onset. However, ALL relapsed in CNS 4 months after the end of the

therapy. She received radiation treatment for whole brain and spinal cord (total 24 Gy) with chemotherapy of high dose cytarabine and mitoxantron, and resulted in second complete remission. She underwent CBSCT from HLA 3-locus mismatch female. Preconditioning regimen was 120 mg/kg of cyclophosphamide and 12 Gy of total body irradiation. Prevention of acute graft-versus-host disease (GVHD) was performed by short-term methotrexate and tacrolims. Engraftment was achieved 24 days after the transplantation. Although grade II acute GVHD (skin stage 3) and cytomegalovirus infection (only positive reaction of antigenemia) occurred, which were improved by the administration of prednisolone and ganciclovir, respectively. She was discharged 122 days after CBSCT. The administration of tacrolimus was finished 6 months after CSBCT. She presented with enlarged cervical and inguinal lymph nodes 1 year and 3 months after CBSCT, and cervical lymph nodes were gradually enlarged. At the age of 26 (3 years and 8 months after CBSCT), she underwent biopsy of the cervical lymph node.

In the physical examination, her left enlarged cervical lymph nodes were 2 to 3 cm in diameter. Axial or inguinal lymph nodes were not swollen, and hepatosplenomegaly was not found. There were no remarkable abnormalities in the blood test (complete blood count and biochemical tests) at the biopsy. Hematoxylin-eosin (H&E) stained sections showed the destruction of the normal lymph node structure (Fig. 1). There were Hodgkin and Reed-Sternberg (R-S) cells surrounded with the aggregation of reactive background cells including neutrophils, eosinophils, histiocytes, and plasma cells. Immunohistochemical stains demonstrated that

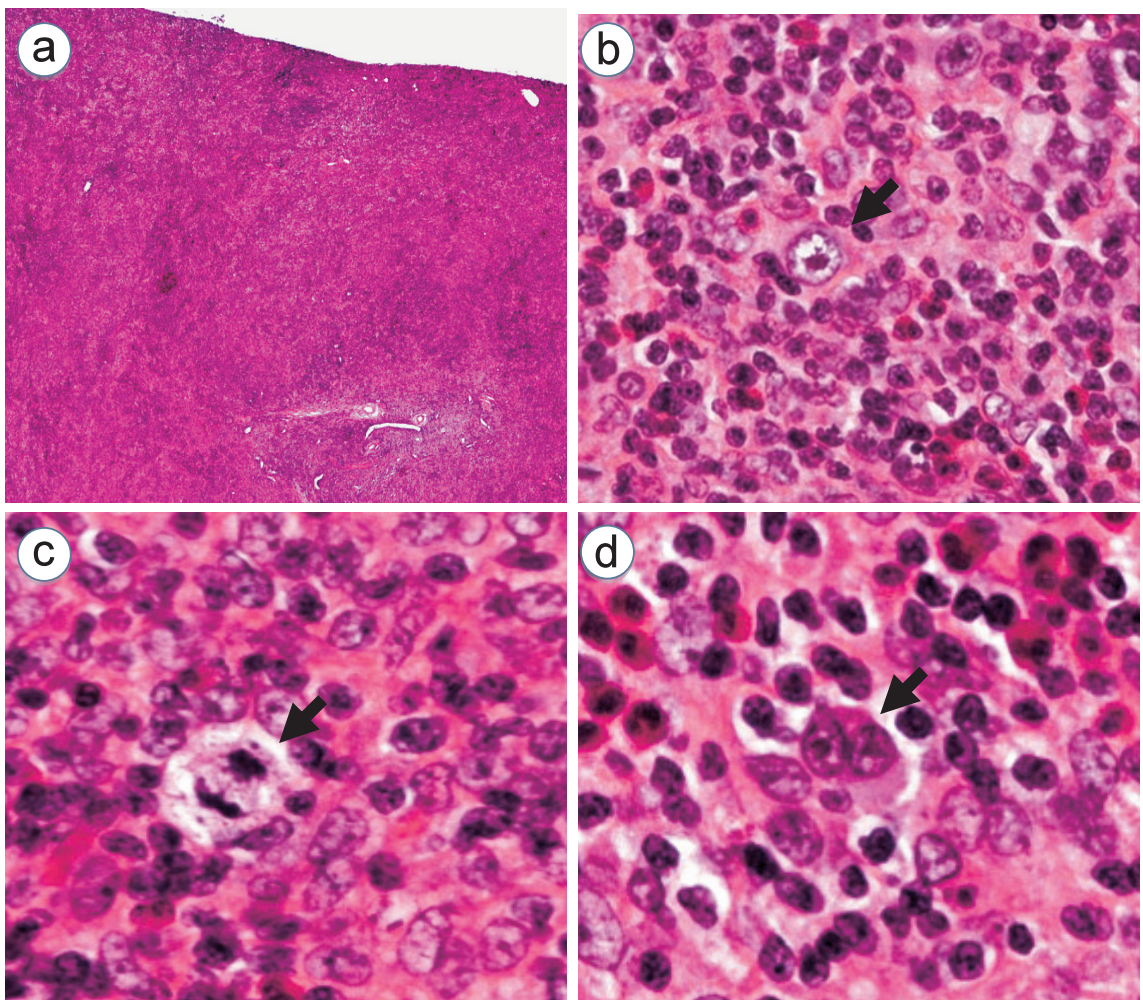

Fig. 1. Cervical lymph node biopsy. (***1a***) The normal structure of the swollen lymph node was destroyed, and lymphatic sinus and lymphoid follicles disappeared. (***1b- 1d***) Scattered small numbers of Hodgkin/Reed-Sternberg cells were surrounded by numerous reactive cells including small lymphocytes, eosinophils, plasma cells and histiocytes. (***1b***) Hodgkin cell, (***1c***) Mitotic large cell, (***1d***) Reed-Sternberg cell. They were indicated by *arrows*, respectively. (***1a- 1d***) Hematoxylin and eosin stain, (***1a***) x40, (***1b***) x200, (***1c***) x400, (***1d***) x400.

Hodgkin and R-S cells were positive for CD15 (partially), CD30, EBV latent membrane protein (LMP) 1, and negative for CD3, CD79a, ZEBRA (BamHI Z fragment Epstein-Barr replication activator), and EBV nuclear antigen (EBNA) 2 (Fig. 2 and Table 1). The background reactive cells included small T-cells ($CD3^+$, $CD4^+$ or $CD8^+$), B-cells ($CD79a^+$, $CD20^+$) and histiocytes ($CD68^+$). MIB-1 index of Hodgkin and R-S cells was about 70% (Table 1). *In situ* hybridization (ISH) of EBV-encoded small RNA (EBER) 1 was also performed. All Hodgkin and R-S cells expressed EBER1, and a few background reactive lymphocytes were also positive for EBER1. The computed tomography (CT) revealed that left deep cervical, supraclavicular, and pretracheal lymph nodes were swollen. In addition, positron emission tomography (PET) showed that right cervical, mediastinal, and para-aortic lymph nodes, bilateral tonsils and spleen were also involved. No malignant cells were found in the bone marrow aspiration smear. We diagnosed her as HL stage IIIA, and her international prognostic score was 0. Initially, we suggested a chemotherapy of ABVD (adriamycin, bleomycin, vincristine and dacarbazine) to her. However, she rejected it, because she was concerned about possible cardiac toxicity of adriamycin. Therefore, she received 6 courses of C-MOPP (cyclophosphamide, vincristine, procarbazine and prednisone), and she has been surviving 4 years and 6 months after CBSCT. The clinical course mentioned above was summarized in Fig. 3.

We reviewed her EBV-associated antibody titers, anti-viral capsid antigen (VCA)-IgG and EBNA-IgG, both of which were positive and consistent with persistent EBV infection (Fig. 4). She had already been infected EBV before CBSCT, because these antibodies were positive since her ALL onset. EBV DNA (*EBNA1* DNA) from the blasts of

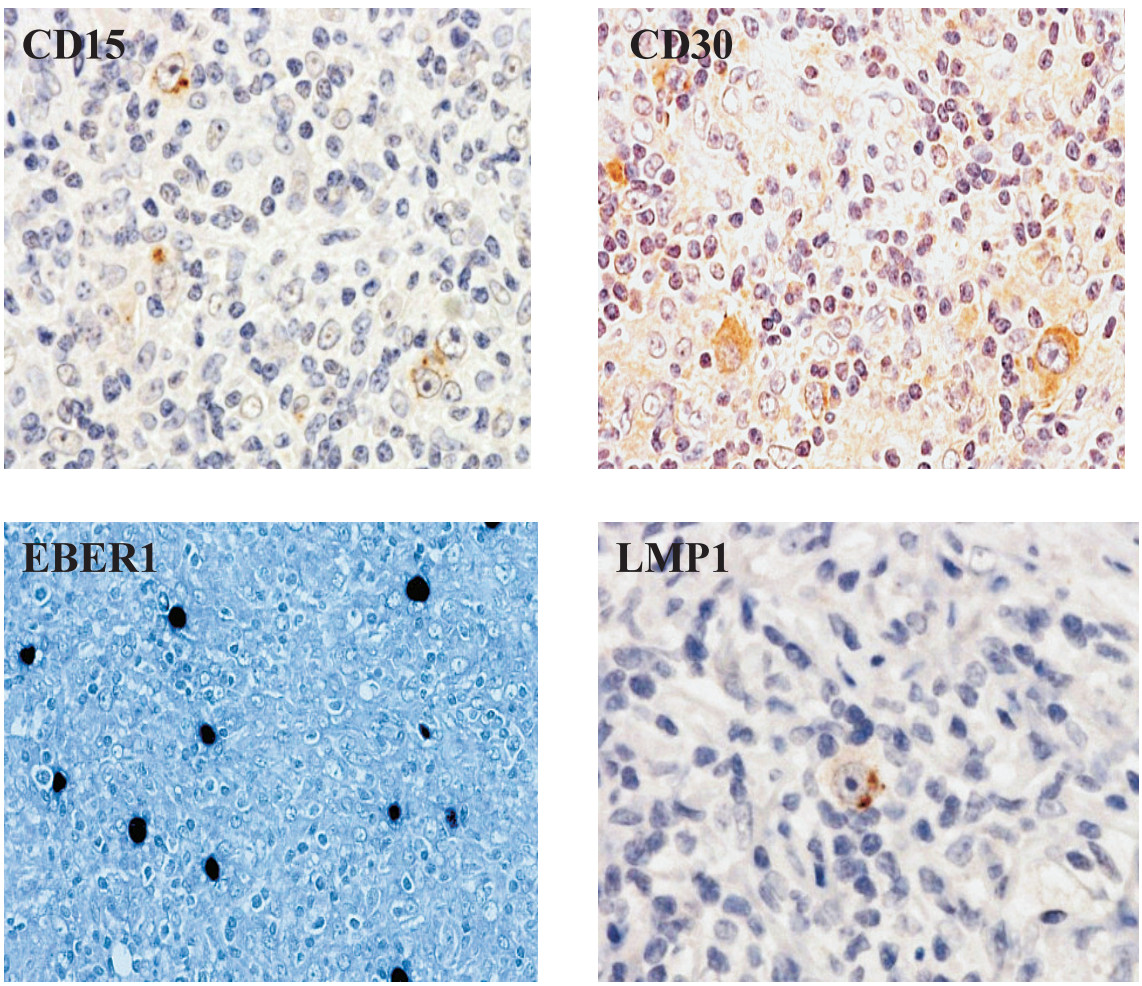

Fig. 2. Immunohistological examination and *in situ* hybridization of *Epstein-Barr virus-encoded small RNA* (*EBER*) *1*. Hodgkin/Reed-Sternberg cells were positive for CD15, CD30, *EBER1*, and latent membrane protein (LMP) 1, compatible with phenotype of Hodgkin lymphoma. Counterstained with hematoxylin, (CD15) x200, (CD30) x200, (*EBER1*) x200, (LMP1) x200.

Table 1. Summary of histological examination

Antibodies	Clone, Manufacturers	Hodgkin/ R-S cells	Reactive background cells (lymphocytes, granulocytes, histiocytes)
CD3	PS1, Nichirei-Zymed	−	+
CD4	1F6, Nichirei-Zymed	−	+
CD8	C8/144B, DAKO	−	+
CD79a	JCB117, DAKO	−	+
CD20	L26, DAKO	−	+
CD68	PG-M1, DAKO	−	+
CD15	CD3- 1, DAKO	+ (partially)	+ (partially)
CD30	Ber-H2, DAKO	+	+ (partially)
EBER1 (ISH)	Chang *et al*. (1992)[17]	+	+ (a few)
LMP1	CS1- 4, DAKO	+	−
EBNA2	PE2, DAKO	−	−
ZEBRA	BZ. 1, DAKO	−	−
Ki-67 (index)	MIB-1, DAKO	+ (70%)	+

R-S cells ; Reed-Sternberg cells, +, positive ; −, negative ; POLY, polyclonal antibody ; ISH, *in situ* hybridization

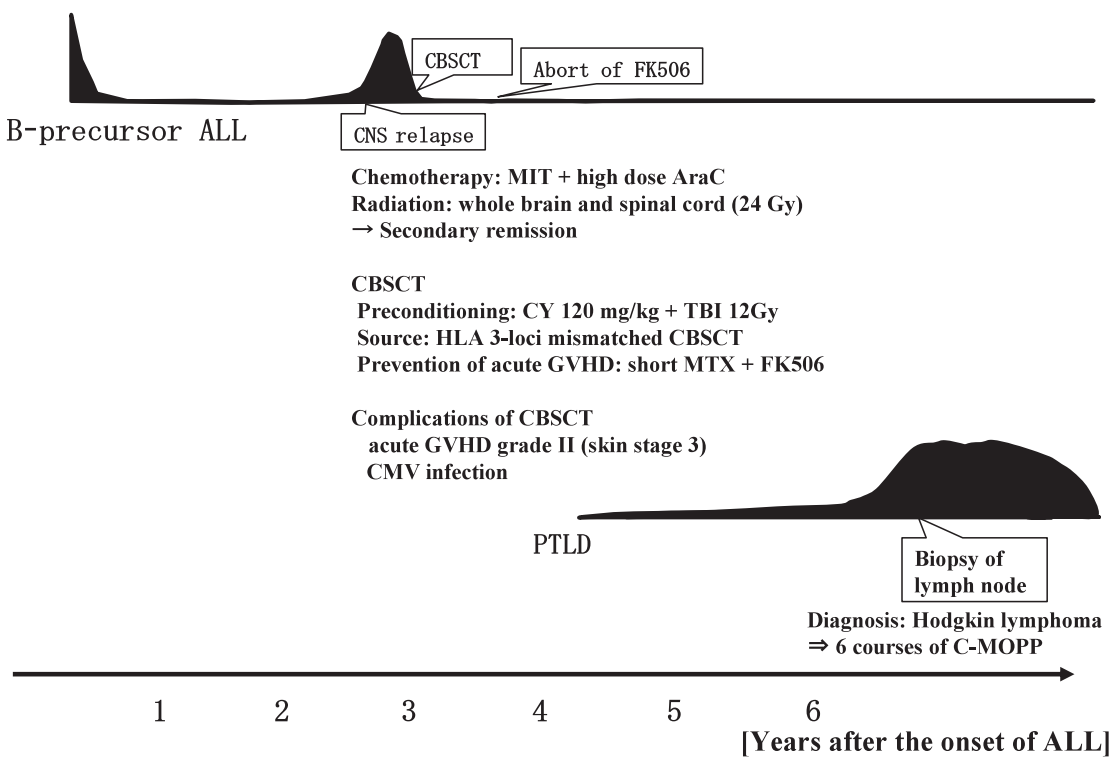

Fig. 3. Clinical course of the patient. The patient received cord blood stem cell transplantation (CBSCT) for relapsed acute lymphoblastic leukemia (ALL) in the central nervous system (CNS). Immunosuppressants were finished 6 months after CSBCT. She was pointed out enlarged cervical and inguinal lymph nodes 1 year and 3 months after CBSCT. Three years and eight months after CBSCT, she underwent the biopsy of the enlarged cervical lymph nodes. Then, she was diagnosed as Hodgkin lymphoma, and received chemotherapy by cyclophosphamide, vincristine, procarbazine, and prednisone (C-MOPP). MIT, mitoxantrone ; AraC, cytarabine ; CY, cyclophosphamide ; TBI, total body irradiation ; GVHD, graft versus host disease ; FK506, tacrolimus ; CMV, cytomegalovirus, MTX, methotrexate ; PTLD, post-transplant lymphoproliferative disorders

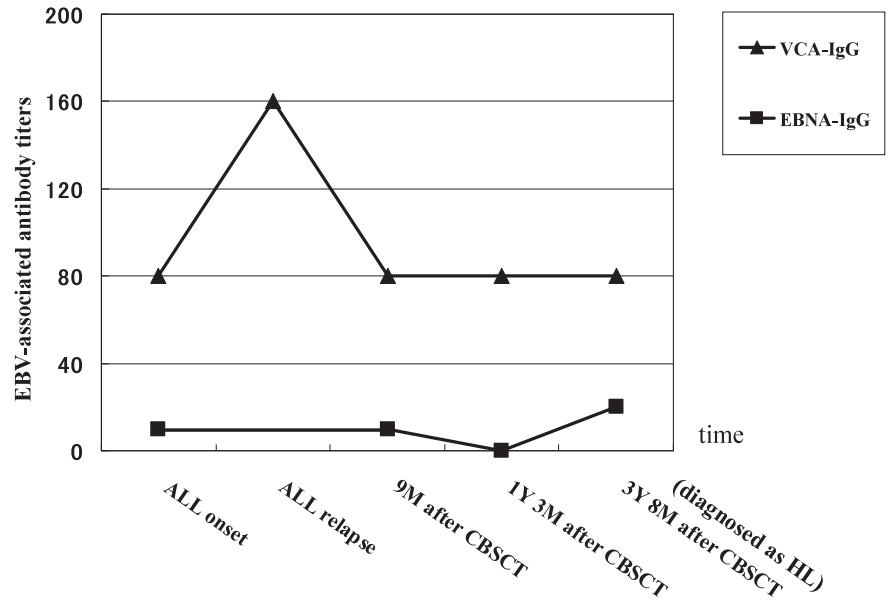

Fig. 4. Temporal change of Epstein-Barr virus (EBV) associated antibody titers. These EBV-associated antibody titers showing both anti-viral capsid antigen (VCA)-IgG and anti-EBV nuclear antigen (EBNA)-IgG positive were consistent with persistent EBV infection pattern since the onset of acute lymphoblastic leukemia (ALL). Y, year (s) ; M, month (s) ; CBSCT, cord blood stem cell transplantation

primary ALL, was not detected by polymerase chain reaction (PCR) (Fig. 5) and EBER1 ISH (Data not shown).

DISCUSSION

The risk factors of PTLD are HSCT from HLA mismatched related or unrelated donor, administration of antithymocyte globulin or anti-CD3 monoclonal antibody for prophylaxis or treatment of acute GVHD (graft versus host disease), GVHD grade II to IV, conditioning regimens that included radiation and HSCT of T-cell-depleted graft.[1,2] The donor of this case was HLA mismatched unrelated. Therefore, this was a high risk case of PTLD. We diagnosed her as PTLD by the biopsy of her cervical lymph node because it had gradually enlarged for 2 years and 5 months. It is very difficult to diagnose early stage PTLD. Baker *et al*. reported that 35% of PTLD cases were diagnosed on postmortem examination.[1] These were often present with a disseminated or fulminant form of the disease that lacks a well defined tumor mass or lymph node swelling. Monitoring of EBV DNA in peripheral blood mononuclear cells by a real-time quantitative PCR is useful for the early detection and intervention in PTLD.[7,8] However, it is expensive, and is not covered by public health insurance in Japan. For this reason, it was not done in our case.

Post-transplant HL and HL-like PTLD are included in the same category in WHO classification of PTLD.[3] They show similar histology with Hodgkin/R-S cells or Hodgkin/R-S like cells in H&E stained specimens, but background cells in post-transplant HL are reactive, whereas those in HL-like PTLD are atypical and neoplastic. Their clinical courses are quite different.[4] HL-like PTLD is more aggressive than post-transplant HL, and therapeutic approaches for HL-like PTLD include a reduction in immunosuppression as well as anti-CD20 antibody (rituximab), whereas treatment for HL consists of a chemotherapy and/or radiation.[4,5] Immunohistochemically, large atypical cells (R-S like cells) in HL-like PTLD express CD45 and B-cell markers (CD20 and/or CD79a) with variable expression or without expression of CD30, but do not express CD15.[5,9,10] Meanwhile, the R-S cells of post-transplant HL express CD30, CD15, and occasionally CD20, but not CD45.[5,9,10] In HL-like PTLD cases, EBER1 is positive not only in R-S like cells but also in background atypical lymphocytes.[5,11] In contrast, it is positive only in R-S cells in post-transplant HL.[5,11] Most of HL-like PTLD cases have demonstrable immunoglobulin gene rearrangements in whole-tissue DNA. In post-transplant HL, these rearrangements are not easily detected in whole-tissue DNA, but often detected in the DNA of isolated single Hodgkin/R-S cells.[9] The expression pattern of EBV proteins may aid in the differentiation between post-transplant HL and HL-like PTLD. HL is usually latency type II ; EBER1 and LMP1 are positive, but EBNA2 is negative, while HL-like PTLD is usually latency type III ; EBER1, LMP1 and EBNA2 are all positive.[12] According to the above-mentioned reasons, our case is compatible with post-transplant HL with

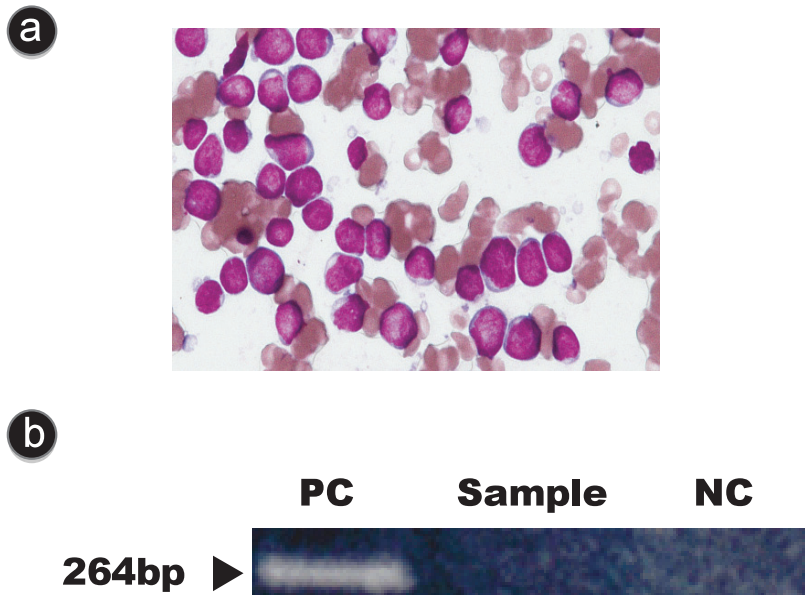

Fig. 5. The blasts of primary acute lymphoblastic leukemia (ALL) in bone marrow (***1a***) and polymerase chain reaction (PCR) analysis of Epstein-Barr virus (EBV) DNA (***1b***). (***1a***) Primary ALL blasts stained by Giemsas method. Blasts was 97.2% of all nucleated cells in the bone marrow smear. (***1b***) EBV DNA analysis by PCR using *EBV nuclear antigen* (*EBNA*) *1* DNA primers. *EBNA1* DNA was not detected in this sample. PC, the positive control (B95-8) ; NC, the negative control (distilled water)

latency type II EBV infection.

Although few studies about PTLD after CBSCT in childhood exist,[13-16] this is the first case of an adult EBV associated HL after CBSCT in the literature. In this case, the EBV genome was not detected in the blasts of primary ALL, while EBV markers (EBER1 and LMP1) were detected in post-transplant HL tissue. This means that the post-transplant HL was not derived from the former ALL. Instead, HL tumor cells were more likely to be derived from a donor lymphocyte, because lymphocytes of the patient were almost eradicated by preconditioning therapy. EBV is generally derived from donor lymphocytes in PTLD after HSCT. However, EBV was more likely to be derived from the patient in this case, because EBV associated antibodies of the patient indicated that EBV had already infected in her lymphocytes, and graft source was cord blood, which had little chance of EBV infection.

In summary, we report a case of post-transplant HL in a CBSCT patient of recurrent ALL. Monitoring EBV DNA load by a real time PCR is recommended in order to predict the occurrence of PTLD. Although the histological picture of post-transplant HL resembles that of HL-like PTLD in H&E staining, the clinical course and treatment strategy of them are different. Therefore, we should distinguish their difference by immunostaining, the pattern of immunoglobulin gene arrangement and the type of EBV latent infection.

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