J Clin Exp Hematop
Vol. 53, No. 3, Dec. 2013

*Letters to the Editor*

# Refractory Case of ALK-Negative Anaplastic Large-Cell Lymphoma with PAX-5 Expression and T-Cell Receptor-$\gamma$ Gene Rearrangement

Keiki Kawakami,[1)] Akira Yazaki,[1)] Ryugo Ito,[1)] Yasutaka Tono,[1)] Tetsuya Murata,[2)] Yohichiro Baba,[2)] Tomoko Uchiyama,[2)] Hiroshi Imai,[3)] and Shigeo Nakamura[4)]

**Keywords:** ALCL, ALK-negative, PAX-5, TCR-$\gamma$, gene

## TO THE EDITOR

Anaplastic large-cell lymphoma (ALCL) is a rare T-cell lymphoma consisting of lymphoid cells that are usually bizarre and large CD30-positive cells with abundant cytoplasm and pleomorphic nuclei.[1] At present, two types of ALCL are recognized, that is, anaplastic lymphoma kinase (ALK)-positive ALCL (ALCL, ALK$^+$) and ALK-negative ALCL (ALCL, ALK$^-$), although they are not morphologically distinguishable. The former has been suggested to be a distinctive entity with ALK expression associated with a t(2;5) translocation, arising in children and young adults and showing favorable prognosis. On the other hand, the latter is more aggressive, occurs at an older median age, and is thought to be a heterogeneous group. Its peak incidence is reported to occur at 40-65 years old.[2,3]

Tumor cells of ALCL, ALK$^-$, usually express one or more T-cell lineage antigens such as CD2, CD3, CD5 or CD4, and rarely CD8. Many cases express the cytotoxic molecules TIA1, granzyme B or perforin A as ALCL, ALK$^+$ cases. Hence, most cases of ALCL, ALK$^-$, are supposed to be of T-cell origin, although a few cases lacking both T-cell and B-cell antigens have been reported, so-called null-ALCL. However, polymerase chain reaction (PCR) analysis revealed that most cases of null-ALCL showed clonal rearrangement of T-cell receptor (TCR) $\beta$-chain genes, suggesting its T-cell origin.[4] It has also been argued that ALCL, ALK$^-$, should not be excluded from the category of peripheral T-cell lymphoma, not otherwise specified.[3]

PAX-5 is thought to be expressed exclusively in B cells from the pro-B- to the mature B-cell stage and then silenced in plasma cells ; hence, it is considered to be B-cell lineage-specific marker.[5]

Here, we report a case of ALCL, ALK$^-$, with PAX-5 expression and TCR-$\gamma$ rearrangement, which was very aggressive and showed refractoriness to chemotherapy and radiotherapy.

A 64-year-old Japanese male was admitted to our hospital because of left inguinal mass lesion. B symptoms were not present. On admission, a mass lesion as large as 5 cm was palpable in the left inguinal lesion. He did not show another superficial lymphadenopathy, hepatosplenomegaly or skin lesion. Complete blood cell analysis revealed a white blood cell count of 7,700/μL, a platelet count of $26.7 \times 10^4$/μL and a hemoglobin concentration of 14.8 g/dL. An elevation of serum lactate dehydrogenase up to 311 IU/L was observed (normal range, 110-225 IU/L). Serum interleukin-2 receptor level was as high as 4,530 U/mL. Human chorionic gonadotropin-$\beta$ and $\alpha$-fetoprotein, germ cell tumor marker, were both negative. Computed tomographic scan revealed marked lymphadenopathy in the range from left inguinal to left iliac artery regions. Whole-body 2-[$^{18}$F]fluoro-2-deoxy-D-glucose positron emission tomography fused with computed tomography (PET/CT) demonstrated abnormal uptake in the inguinal and iliac artery regions (SUVmax = 15.4). Endoscopic examinations did not reveal any abnormal findings in the esophagus, stomach, duodenum and colon. Bone marrow biopsy did not show any pathological cells. The left inguinal lymph node was biopsied. Histological findings

Received : June 28, 2013
Revised : August 8, 2013
Accepted : August 18, 2013
[1)] Division of Hematology/Oncology and [2)] Division of Pathology and Clinical Laboratories, Suzuka General Hospital, Suzuka, Japan
[3)] Pathology Division, Mie University Hospital, Mie, Japan
[4)] Department of Pathology and Clinical Laboratories, Nagoya University Hospital, Nagoya, Japan
Corresponding author: Keiki Kawakami, M.D., Ph.D., Division of Hematology/Oncology, Suzuka General Hospital, 1275-53, Yasuzuka-cho, Suzuka-shi, Mie 513-8630, Japan
E-mail: kawakei@cocoa.ocn.ne.jp

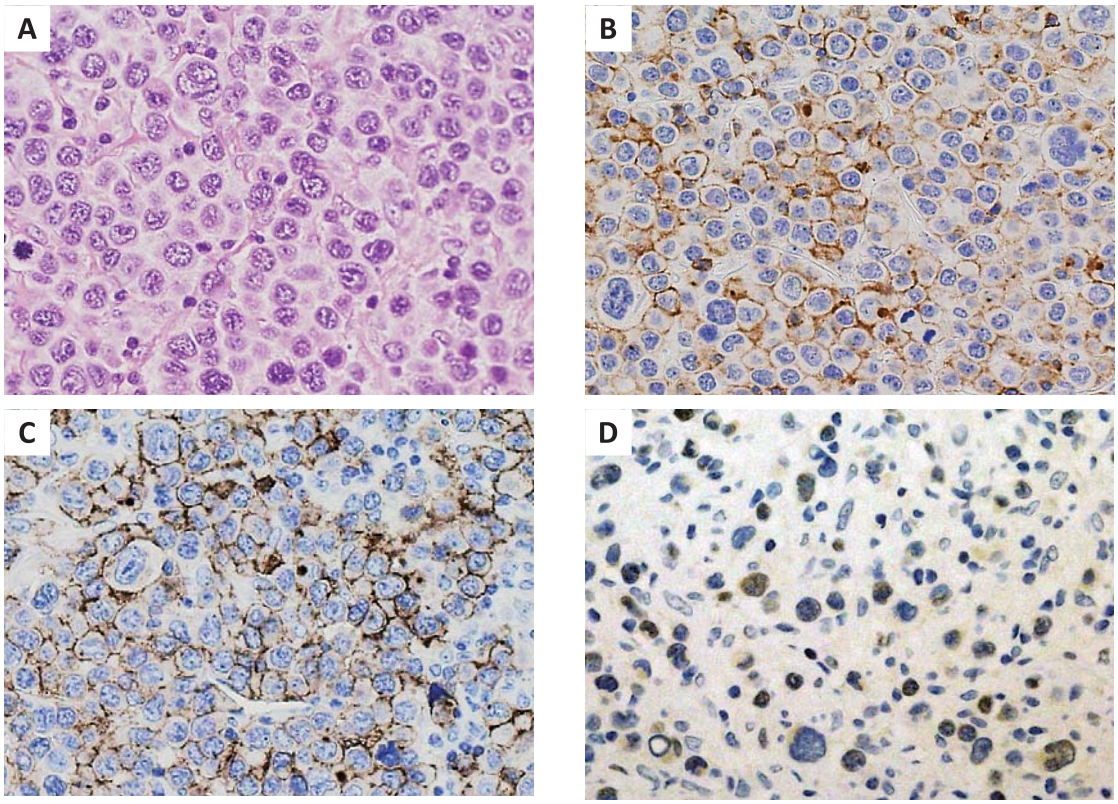


**Fig. 1.** Histopathological findings of the left inguinal lymph node. (***1A***) Lymph node biopsy sections stained with hematoxylin and eosin revealed disruption of the lymph node architecture and diffuse proliferation of large neoplastic cells that had clear cytoplasma and eccentric nucleus showing horseshoe shaped or reniform with prominent nucleoli. H&E stain. (***1B***) Large neoplastic cells showed positive staining for CD5. (***1C***) Large neoplastic cells showed positive staining for CD30. (***1D***) Expression of the PAX-5 was detected weakly in the nuclei of the large neoplastic cells and strongly in those of the non-neoplastic bystander B-cells.

were as follows : The normal nodal structure was effaced by solid, cohesive sheets of large pleomorphic neoplastic cells with variably shaped nuclei such as kidney-shaped, round or oval, prominent nucleoli, and abundant pale cytoplasm (Fig. 1A). Flow cytometric analysis using suspension cells revealed that tumor cells were positive for CD5 and CD30, and negative for CD2, CD3, CD4, CD8, CD7, CD10, CD19, CD20, CD56 and surface immunoglobulins. Terminal deoxynucleotidyl transferase activity was not detected. Immunohistochemically, tumor cells were positive for CD5 (Fig. 1B), CD30 (Fig. 1C), bcl-2, vimentin, fascin and MUM1. PAX-5 positivity was also seen with weak staining intensity similar to that observed in classical Hodgkin lymphoma (Fig. 1D).[6] The tumor cells were negative for ALK, CD45 (LCA), CD45RO (UCHL-1), CD45RA, CD1a, CD3, CD2, CD7, CD4, CD8, TIA1, granzyme B, CD56, L-26, CD10, CD15, c-kit, placental alkaline phosphatase, MNF116, CAM5.2, EMA and *in situ* hybridization for EBER (Epstein-Barr virus-encoded RNA). Approximately 50% of the tumor cells were positive for MIB1/Ki-67. On the basis of these findings, a diagnosis of ALCL, $ALK^-$, null cell type, was made. The patient was treated with systemic administration of a standard CHOP regimen (cyclophosphamide, doxorubicin, vincristine and prednisolone). Even after the completion of eight courses of CHOP therapy, CT scan detected a small residual mass in the left inguinal lesion and PET-CT showed abnormal uptake there, suggesting an active residual disease. Therefore, involved field irradiation of 50 Gy was performed from the left iliac artery to the left inguinal regions. However, even after the radiotherapy, the left inguinal lesion remained. Biopsy confirmed the presence of active tumor cells there. Thereafter, the tumor grew rapidly and spread systemically, being accompanied by a fulminant hemophagocytic syndrome. Several systemic chemotherapies such as DeVIC (dexamethasone, VP16, iphosphamide and carboplatin) regimen were applied, but in vain. The patient died of multiorgan failure due to systemic tumor invasion. An autopsy was not allowed.

TCR-$\gamma$- and $\beta$- and IgH-gene rearrangement analysis; Total DNA from the specimens of the biopsied lymph node in

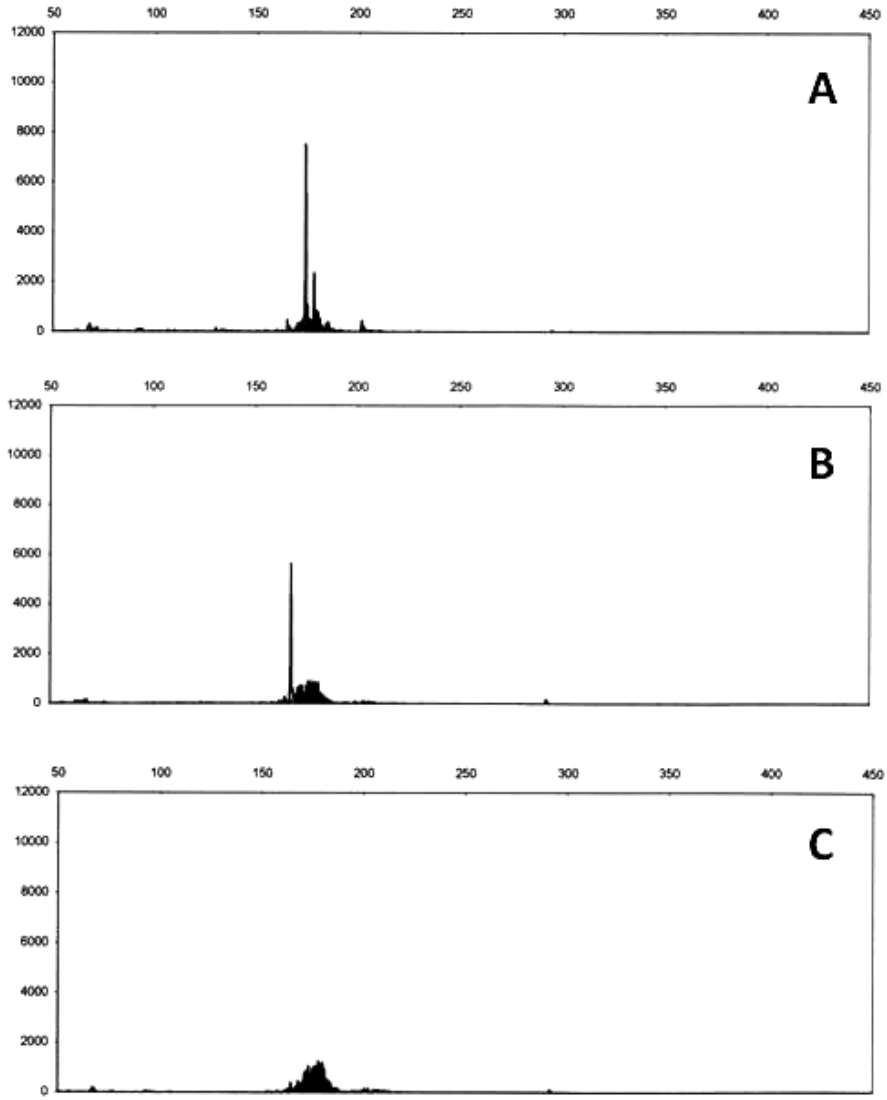


**Fig. 2.** GeneScan analysis of amplificates obtained by polymerase chain reaction for detection of T-cell receptor $\gamma$-chain gene rearrangements (V$\gamma$9, V$\gamma$11/J$\gamma$ gene) (***2A***) The case showed a solitary peak at 172 bp demonstrating the presence of clonally rearranged T-cell populations. (***2B***) Positive control (clonal T-cell). (***2C***) Negative control (polyclonal T-cells).

the present case was extracted and multiplex PCR analysis of IgH- and TCR-$\gamma$- and $\beta$-gene rearrangements was performed as described previously.[4,7] Rearrangements of clonal TCR-$\gamma$ gene, but not TCR-$\beta$- or IgH-gene, were detected by PCR.

Here, we report a case of PAX-5-positive ALK-ALCL with monoclonal TCR-$\gamma$ gene rearrangement but without TCR-$\beta$ gene rearrangement.

PAX-5 is considered to be a B-cell lineage-specific marker.[5] However, Feldman *et al*. reported that aberrant expression of PAX-5 occurs rarely in T-cell anaplastic lymphomas and may be associated with extra copies of the PAX-5 gene.[6] Furthermore, Alexander *et al*. reported a case of PAX-5-positive peripheral T-cell lymphoma-unspecified with monoclonal TCR-$\gamma$ gene rearrangement.[5] They described that PAX-5 may contribute to T-cell lymphomagenesis, showing that PAX-5 completely blocks cellular development by repressing the T-cell-specific gene Notch. 1.7 in the T-cell lineage. In addition to hematological malignancies, PAX-5 is reported to be expressed and to display oncogenic potential in bladder carcinomas, neuroendocrine tumors and astrocytomas, in all of which its presence is associated with morphological and/or clinical aggressiveness.[5] Thus, the detection of PAX-5 in lymphoid malignancies cannot be considered to be definitively B-cell lineage-specific.

On the other hand, different from the TCR-$\beta$ gene, rearrangement of the TCR-$\gamma$ gene is thought not to be lineage-specific. Many cases of B-cell non-Hodgkin lymphoma with clonal TCR-$\gamma$ gene rearrangement have been reported. Hence, the results for TCR gene rearrangement should always be interpreted in conjunction with histology and immunophenotyping.[8] These findings show that tumor cells in the present case did not have any lineage-specific marker.

In this way, the present case may be called "real" null-ALCL. The cell origin of the tumor cells in the present case is of interest. They might have been immature lymphoid stem cells or "defective" T-cells, that is, T-cells with defective expression of TCRs analogous to defective immunoglobulin expression seen in HL.[9] Although we do not know the exact significance of PAX-5 expression in the present case, there is a possibility that this aberrant expression of PAX-5 is associated with the refractoriness and aggressiveness seen in the present case.[5,10] To confirm this speculation, further cases need to be accumulated.

## REFERENCES


1 Stein H, Mason DY, Gerdes J, O'Connor N, Wainscoat J, *et al*.: The expression of the Hodgkin's disease-associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: Evidence that Reed-Steinberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 66:848-858, 1985

2 Nakamura S, Shiota M, Nakagawa A, Yatabe Y, Kojima M, *et al*.: Anaplastic large cell lymphoma : a distinct molecular pathologic entity: a reappraisal with special reference to p80 (NPM/ALK) expression. Am J Surg Pathol 21:1420-1432, 1997

3 Falini B: Anaplastic large cell lymphoma: pathological, molecular and clinical features. Br J Haematol 114:741-760, 2001

4 Foss HD, Anagnostopoulos I, Araujo I, Assaf C, Demel G, *et al*.: Anaplastic large-cell lymphomas of T-cell and null-cell phenotype express cytotoxic molecules. Blood 88:4005-4011, 1996

5 Tzankov AS, Went PT, Münst S, Papadopoulos T, Jundt G, *et al*.: Rare expression of BSAP (PAX-5) in mature T-cell lymphomas. Mod Pathol 20:632-637, 2007

6 Feldman AL, Law ME, Inwards DJ, Dogan A, McClure RF, *et al*.: PAX5-positive T-cell anaplastic large cell lymphomas associated with extra copies of the PAX5 gene locus. Mod Pathol 23:593-602, 2010

7 van Dongen JJ, Langerak AW, Brüggemann M, Evans PAS, Hummel M, *et al*.: Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 17:2257-2317, 2003

8 Tai YC, Peh SC: Feasibility of T-cell receptor $\gamma$ (TCR $\gamma$) gene rearrangement on formalin-fixed, paraffin-embedded tissues by PCR assays. Singapore Med J 44:250-255, 2003

9 Bonzheim I, Geissinger E, Roth S, Zettl A, Marx A, *et al*.: Anaplastic large cell lymphomas lack the expression of T-cell receptor molecules or molecules of proximal T-cell receptor signaling. Blood 104:3358-3360, 2004

10 Tamaru J, Tokuhira M, Nittsu N, Nakamura S, Ichinohasama R, *et al*.: Hodgkin-like anaplastic large cell lymphoma (previously designated in the REAL classification) has same immunophenotypic features to classical Hodgkin lymphoma. Leuk Lymphoma 48:1127-1138, 2007