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naplastic large cell lymphoma (ALCL) is a T-cell or null-phenotype lymphoma. Most cases have t(2;5)(p23;q25), which results in a fusion between the anaplastic lymphoma kinase (ALK) gene at chromosome band 2 and the nucleophosmin (NPM) gene at chromosome band 5. This fusion increases the activity of the ALK protein, which results in the proliferation of ALCL cells.

Childhood ALCL accounts for 10–20% of childhood non-Hodgkin lymphoma cases, and the advanced stages (stage III and IV) of childhood ALCL were reported to have a 2-year event-free survival (EFS) rate of 70–80%. Standard therapy for patients with stage I or II ALCL who have undergone a total resection of the tumor is only three courses of intensive combined chemotherapy, whereas patients at other advanced stages of cancer often receive 6 courses of chemotherapy. Hematopoietic stem cell transplantation (HSCT) is not recommended as a first-line therapy for ALCL [1–3]. The BFM, SIOP, and UKCCSG retrospective studies demonstrated that invasion to the mediastinum, skin, liver, and lung were risk factors for a poor prognosis of ALCL [4].

Here, we report 2 patients who relapsed 6 to 18 months after the end of ALCL99 therapy [5]. Our analysis of stored samples taken at the initial diagnosis identified the NPM-ALK fusion gene in both patients.
The prognostic impact of monitoring the NPM-ALK chimera will also be discussed.

**Case Report**

**Patient 1.** In September 2010, a 15-year-old Japanese female presented with fever and swelling of the cervical lymph nodes, cervix, axillae, supraclavicular fissure, mediastinum, hilus, paravertebrae, and the upper abdomen (Fig. 1A). Because she was in critical condition on admission, positron emission tomography (PET) images could not be obtained. The diagnosis of Stage III ALC was made by lymph node biopsy. Horseshoe-shaped large cells were positive for both CD30 and ALK in the nucleus and cytoplasm (Fig. 2A,B). Bone marrow (BM) aspirate was normal, and no malignancy was observed in the cerebrospinal fluid. She had achieved complete remission after undergoing the ALCL99 regimen; but 8 months after the end of chemotherapy, relapse was confirmed in the right inguinal lymph node. She was treated by with the Berlin-Frankfurt-Munster (BFM) regimen and achieved a second complete remission [1].

The patient eventually underwent an unrelated bone marrow transplantation (BMT) with a conditioning regimen of etoposide (VP16), endoxan (CY), and total body irradiation (TBI). Graft-versus-host disease (GVHD) prophylaxis of tacrolimus and short-term methotrexate was administered. She transiently suffered from pure red cell aplasia from an unknown cause, but she has maintained complete remission for 38 months since the BMT.

**Patient 2.** A 14-year and 5-month-old Japanese female presented with fever and lymph node swelling throughout her entire body in May of 2011 (Fig. 1B). Computed tomography (CT) and PET CT showed enlarged lymph nodes both above and below the diaphragm, and invasion into the left adrenal grand. The diagnosis of ALC was made based on the lymph node biopsy. Large cells positive for CD30 and ALK in both the nucleus and cytoplasm were observed (Fig. 2C,D). BM aspirate demonstrated normocellular marrow, and no malignant evidence found in the cerebrospinal fluid.

She achieved a first complete remission by ALCL99; however, relapse occurred in multiple lymph nodes, observed by CT 18 months after the end of the chemotherapy. She underwent a BMT with a conditioning regimen of VP16, CY, and TBI. GVHD prophylaxis was administered. She has maintained complete remission for 33 months since the BMT.

**The Histopathological Examinations.** Biopsy samples were stained with hematoxylin and eosin. The immunohistochemical analysis was performed using an automatic immunostainer (Ventana Medical Systems, Tucson, AZ, USA) with CD30 (Ber-H2) (Dako, Carpenteria, CA, USA), ALK, CD2, CD3 (polyclonal), CD5, CD43, CD15, CD20, CD79a, CD45RO(UCHL-1), CD45(LCA), S-100, TIA-1, Granzyme B, Ki-67 and epithelial membrane antigen (EMA; Dako) antibodies.

**Quantification of NPM-ALK Chimera by Real-time PCR and ALK antibody.** We investigated the presence of the NPM-ALK chimera by conducting a real-time polymerase chain reaction (PCR), and we measured the antibody response to ALK as described [6,7]. The copy number of the NPM-ALK fusion transcript was normalized by 10^4 copies of ABL, and the antibody dilutions used were 1/50, 1/100, 1/250, 1/750, 1/6,750, 1/20,250, and 1/60,750. Informed consent was obtained from the...
patients’ parents, and approval was obtained for this study from the Ethics Committee of our institution.

In Patient 1, the copy number of NPM-ALK was 90 copies/10,000 copies of ABL in the BM, and 1,278 copies/10,000 copies of ABL in the peripheral blood (PB) at the initial diagnosis. On the other hand, the antibody titer of ALK was ×100 times at diagnosis. NPM-ALK in the BM was shown to be positive by reverse transcribed (RT)-PCR (the titer was too low to measure by real-time PCR) at relapse, and NPM-ALK in the BM was negative before BMT.

In Patient 2, the copy number of NPM-ALK was 92 copies/10,000 copies of ABL in the BM; however, NPM-ALK was not detected in the PB at initial diagnosis. The antibody titer of ALK was ×750 times at diagnosis, and ×2,250 times at relapse. Due to the lack of a sample, NPM-ALK could not be examined in the relapsed BM of Patient 2.

**Discussion**

We report 2 cases of relapsed stage III ALCL. Although both patients achieved complete remission by the ALCL99 regimen, they relapsed after the end of chemotherapy. Both patients were successfully treated with HSCT, and they survived without disease. As described above, we consider allogeneic HSCT for relapsed ALCL patients based on the results of a retrospective study of Japanese childhood ALCL [8]. The possibility of performing autologous HSCT remained when the minimal residual disease (MRD) was negative, but it was reported that a relatively high relapse rate was observed in ALCL patients with autologous HSCT compared to those of allogeneic HSCT, and we therefore chose allogeneic HSCT in the present 2 cases.

The NPM-ALK chimera was identified in BM samples from the initial diagnosis in both of our patients, although BM invasion was negative by usual examination. In addition, ALK antibodies were relatively low in samples from the initial diagnosis in both patients.

Mussolin et al. reported that in ALCL patients with NPM-ALK chimera and minimal disseminated disease (MDD) shown by RT-PCR, the 5-year progression-free survival was 41 ±11% for patients with positive BM versus 100% for patients with negative BM [9]. Damm Welk et al. reported that ALCL patients whose BM was NPM-ALK-positive by RT-PCR showed a higher cumulative incidence of relapse than those who were NPM-ALK negative [6,10]. Mussolin et al. proposed that a low ALK antibody titer was correlated with relapse, and patients were classified in a high-risk group when they were MDD-positive and had an antibody titer ≤1/750 [11]. The autologous
anti-ALK antibodies were produced by NPM-ALK oncogenic fusion protein, and the strength of the ALK autoantibody response was inversely correlated with stage and tumor dissemination, as well as with the risk of relapse [7].

MDD in childhood ALCL detected by qualitative RT-PCR was reported to be a strong risk factor for a poor prognosis, and it was also observed to be correlated with minimal residual disease (MRD) and the ALK antibody titer [12-14]. In our Patient 1, NPM-ALK was negative in the MRD, prior to BMT.

Although it remains to be clarified what percentage of patients with Stage III ALCL have MDD-positive BM by real-time PCR, the EFS rate in advanced ALCL patients was reported to be approx. 25% [15]. Although we did not monitor MDD in the present two cases, the survival rate for MDD-positive patients might improve in the future with the use of combined treatment, such as TKI targeting ALK and/or HSCT.

In conclusion, we report the cases of 2 patients with relapsed stage III ALCL who were successfully treated by HSCT. Both showed the presence of the NPM-ALK chimera and low ALK antibody at diagnosis.

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References


