Case Report

Diffuse Large B-cell Lymphoma Arising in a Patient with Neurofibromatosis Type I and in a Patient with Neurofibromatosis Type II

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We experienced two unusual cases of diffuse large B-cell lymphoma (DLBCL), which occurred in a patient with neurofibromatosis type I and a patient with neurofibromatosis type II. B-cell lymphoma is one of the most common phenotypic subgroups of malignant lymphoma. Neurofibromatosis I is characterized by café-au-lait spots and neurofibromas. Neurofibromatosis II typically consists of bilateral schwannomas of the acoustic nerve. Malignant lymphomas rarely coexist with neurofibromatosis I, and no coexistence with neurofibromatosis II has been reported. The patient with neurofibromatosis I was a 50-year-old Japanese woman, clinically manifesting von Recklinghausen’s disease since infancy, who noticed an egg-sized tumor in her shoulder. The patient with neurofibromatosis II was a 39-year-old Japanese man who noticed multiple soft tissue tumors in his neck, buttoc, and elbow. Biopsied materials from both cases were examined by multiparameter methods, including flow cytometry. Flow cytometry revealed large-scale cells to be tumor cells, and they were positive for CD19, CD20, and CD22. Both patients received chemotherapy, and the tumors disappeared. The patient with neurofibromatosis I was alive without recurrence at 4 years after treatment, while the patient with neurofibromatosis II died of recurrence. To the best of our knowledge, this is the first case of malignant lymphoma arising in a neurofibromatosis II patient. As for neurofibromatosis I, there were some reports about occurrence of malignant lymphoma. It is important to be aware of possibility of association of malignant tumors not only of the nervous system but also of unrelated to the nervous system when tumors appear in neurofibromatosis patients.

malignant lymphoma; neurofibromatosis; flow cytometry; immunohistochemistry

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Neurofibromatosis type I, or von Recklinghausen’s disease, is a genetic disease characterized by café-au-lait spots and neurofibromas (Enzinger and Weiss 1995). Neurofibromatosis type II is also a genetic disease, the main lesions of which are bilateral schwannomas of the acoustic nerve (Enzinger and Weiss 1995).

It is well known that malignant tumors of the nervous system, such as malignant schwannomas, gliomas, or astrocytomas, often coexist with neurofibromatosis I or II (Mulvihill et al. 1990). Malignant tumors unrelated to the nervous system rarely coexist with neurofibromatosis I or II, and most of such reported cases were cases with coexisting malignant lymphomas (Berman et al. 1977; Li et al. 1977; Kaplan et al. 1982; Tamaro et al. 1982; Kremen et al. 1985; Wertelecki et al. 1985; Sorensen et al. 1986; Matsuzaki et al. 1989; Itoh et al. 1998). However, the subclassification of neurofibromatosis, type I or type II, was not clear in those previous studies since the concept of typing was proposed recently.

B-cell lymphoma is the most common phenotypic subgroup of malignant lymphoma and consists of many subtypes defined by their histological features as well as by phenotypic, cytogenetic and molecular features according to the WHO classification (Jaffe et al. 2001). Since genetic analysis has become significant and is emphasized in the WHO classification, more detailed examinations such as polymerase chain reaction or DNA microarray analysis are applied for the evaluation of minimal residual disease and patients’ prognoses, or for choosing therapeutic modalities (Morgensztern and Lossos 2005). Extranodal invasion of malignant cells may be common in patients with non Hodgkin’s lymphoma (Hoshino et al. 2004). Detection at the molecular level of minimal residual disease in extranodal organs may be useful as a predictor of prognosis for non Hodgkin’s lymphoma (Hoshino et al. 2004).

Here, we report two cases of malignant lymphoma associated with neurofibromatosis, which were clinically subclassified as type I and type II. The lymphomas were diagnosed by multiparameter analyses.

**CASE REPORT**

**Case 1**

A 50-year-old Japanese woman noticed an egg-sized tumor in her shoulder. Her family history was unremarkable. She had café-au-lait spots and tubercles of the skin over her entire body and had been diagnosed as having von Recklinghausen’s disease during infancy. At the age of 42 years, she suffered from schwannomas in her right brachial plexus, cubital tunnel and cervical epidural space, all of which were excised. She also suffered from paraplegia and urinary disturbance due to a thoracolumbar epidural tumor. The tumor was excised and diagnosed as malignant lymphoma. She received chemotherapy and the symptoms disappeared.

Her shoulder tumor grew rapidly. The mobility of the shoulder tumor relative to the skin was good, and the mobility relative to deep tissue was poor. There was no tenderness or redness. A magnetic resonance image (MRI) scan revealed a tumor of 3 × 6 × 10 cm in the subcutis of the left shoulder. The tumor showed low signal intensities on T1 weighted images, slightly enhanced after gadolinium injection and some extent of high signal intensities on T2-weighted images as compared with muscles (Fig. 1). No other tumors were detected by whole body inspection.

Open biopsy was performed, and biopsy

![Fig. 1. MRI of the left shoulder tumor (arrows) in Case 1. Sagittal view showing mild high signal intensity in contrast to muscles on T2-weighted image.](image)
tissue specimens were sent to a laboratory for the registration-examination-analysis-description of lymphoproliferative disorders, which consists of the registration of hospitals, examination analyses of the specimen, and description of the diagnosis (READ system: Special Reference Laboratories, Tokyo) (Harigae et al. 2002).

Sections of the formalin-fixed, paraffin-embedded biopsy specimens of the tumors were stained with hematoxylin and eosin for histological diagnosis. For immunohistochemistry, thin tissue sections were fixed in 1% paraformaldehyde at 4°C on a shaker for 1.5 hours, washed in phosphate buffered saline (PBS) with 20% sucrose at room temperature overnight, and then snap-frozen in acetone at −70°C in a freezing machine. After incubation with each non-labeled antibody at 4°C overnight, the avidin-biotin peroxidase method with second antibodies was used for visualization of the reactivity (Ichinohasama et al. 1996) followed by methyl green counter-staining. Non-immune mouse immunoglobulin was used as a negative control (Hatori et al. 1997).

For flow cytometry, a single-cell suspension of tissues was prepared by cutting and teasing unfixed materials in phosphate-buffered saline. The washed cells were applied to a Cytorun Absolute flow cytometer (Ortho Diagnostic Systems, Raritan, NJ, USA) after incubating with monoclonal antibodies, as described previously (Ichinohasama et al. 1998).

In hematoxylin and eosin staining (Fig. 2), cells of large to moderate size had proliferated in a diffuse pattern. There were also scattered small lymphocytes. CD20 and CD79α as B cell markers were positive, and CD3 as a T cell marker was negative (Fig. 3). Cytokeratin, epithelial mem-

Fig. 2. Morphologic demonstration of the skin tumor (a, b) and lymphoma (c) of case 1 (hematoxylin and eosin stain: × 40, × 500, × 400, respectively).

a. The tumor was located in the deep portion of the dermis (arrows) with much denser cellularity than the dermis of loose fibrous connective tissue.

b. The skin tumor consisted of eosinophilic, thin, wavy fibers with long, spindle-shaped nuclei (some of them are indicated by arrows) and was diagnosed as neurofibroma.

c. Generally large lymphoma cells (some of them are indicated by arrows) had proliferated diffusely, and the diagnosis was diffuse large B-cell lymphoma based on the immunophenotypic findings shown in Fig. 3.
brane antigen (EMA), CD5, and CD30 were negative. The MIB-1 index was about 70%, and p53 was positive in many of the tumor cells. In flow cytometry, large-scale cells revealed the plain light chain reaction of a dominant lambda and were indicated to be tumor cells (Fig. 4). Therefore, the tumor was diagnosed as malignant lymphoma, diffuse B cell type (DLBCL).

Fig. 3. Immunohistochemical examination of the shoulder tumor in Case 1.
Large lymphoma cells (some of them are indicated by arrows) were positive for CD20 (a: × 400), while CD3-positive reactive lymphocytes (some of them are indicated by arrows) were smaller than the lymphoma cells (b: × 400).

Fig. 4. Results of flow cytometry of Case 1.
- a. Non-specific fluorescent signals were identified using non-labeled mouse immunoglobulin as a negative control.
- b. Lambda-positive cells were considerably greater in number than kappa-positive cells, indicating a clear light chain restriction as well as clonal proliferation of lambda-positive neoplastic cells.
- c. CD19-positive cells, considered to be neoplastic from the findings of (b), were negative for CD10.
Mitoxantrone, cyclophosphamide, vindesine, etoposide, and methotrexate were used for chemotherapy, to which the tumor responded. There was no recurrence at 4 years after the appearance of the tumor.

**Case 2**

A 39-year-old Japanese man noticed multiple soft tissue tumors in his neck, right buttock, and right elbow. He had suffered from facial nerve palsy in infancy, had undergone removal of soft tissue tumors in his back, and had experienced hypoglossal nerve palsy at the age of 20 years. Family history was unremarkable. Café-au-lait spots and tubercles of the skin were absent.

There was no tenderness or redness in his neck. The mobility of the tumor relative to the skin was preserved, and the mobility relative to deep tissue was poor. MRI demonstrated an acoustic tumor in his left cerebral nerve VIII. The pathological diagnosis of biopsy specimens of the tumors in his neck, right buttock, and right elbow was schwannoma.

One of the neck tumors became enlarged. Open biopsy was performed for this tumor, and biopsy specimens were examined by the same method as that for Case 1. In hematoxylin and eosin stainings (Fig. 5), cells of large to moderate size had proliferated in a diffuse pattern. In immunohistochemistry, CD19, CD20 and CD22 as B cell markers were positive. In flow cytometry atypical lymphoid cells were positive for CD19, CD20, and CD22. There was a slight shift to lambda chain. Therefore, the tumor was diagnosed as DLBCL.

The patient received chemotherapy with doxorubicin, cyclophosphamide, vincristine, and predonizolone, and the tumors disappeared. The

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**Fig. 5.** Morphologic demonstration of the skin tumor (a) and lymphoma (b) of case 2 (hematoxylin and eosin stain: × 150, × 400, respectively).

a. The tumor, diagnosed as schwannoma, consisted of Schwann-like cells with elongated nuclei and long processes showing both a palisading proliferation pattern and focal Verocay bodies (some of them are indicated by arrows).

b. Large centroblast-like lymphoma cells (some of them are indicated by arrows) biopsied from the neck tumor showed CD20-positivity and CD3-negativity defined by both flow cytometry and immunohistochemistry (figure not shown). These features are consistent with features of diffuse large B-cell lymphoma.
tumors in his neck recurred and he died of sudden respiratory arrest.

**DISCUSSION**

Sorensen et al. (1986) reported 57 malignant tumors in 212 patients with neurofibromatosis. There were 21 central nervous malignancies, 6 peripheral nervous sarcomas, and 57 other cancers or sarcomas. The relative frequencies of tumors differed markedly from those in the general population. In the general population, over half of all tumors are found in three sites: the lung, prostate and colorectum in men and the breast, colorectum and uterus in women.

As for the coexistence of blood diseases other than malignant lymphoma, multiple myeloma, acute myelocytic leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome have been reported (Reich and Wiernik 1976; Gregory and Hill 1990; Sanada et al. 1991).

Neurofibromatosis occurs in two varieties, neurofibromatosis I and II. Neurofibromatosis I, or Von Recklinghausen’s disease, is a genetic disease that affects one in every 2,500 to 3,000 live births (Lowman and LiVolsi 1980). It is an autosomal dominant inherited disorder with a high rate of penetrance (Enzinger and Weiss 1995). Café-au-lait spots and neurofibromas are the main symptoms (Enzinger and Weiss 1995). The clinical features in case 1 are compatible with those of neurofibromatosis I.

Sixteen cases of coexistence of neurofibromatosis I and malignant lymphoma have been reported (Berman et al. 1977; Li et al. 1977; Kaplan et al. 1982; Tamaro et al. 1982; Kremen et al. 1986; Matsuzaki et al. 1989; Itoh et al. 1998; Herbert et al. 2003; Kim et al. 2003; Zein et al. 2004). Three cases were B cell type (Matsuzaki et al. 1989; Kim et al. 2003; Zein et al. 2004), the majority being T cell type (Kaplan et al. 1982; Tamaro et al. 1982; Wertelecki et al. 1985; Itoh et al. 1998; Herbert et al. 2003). The ages of the patients ranged from 1 to 65 years, and the number of males was twice that of females. Our case was a 50-year-old female. Two of the previously reported cases were twins. Most cases had family histories of neurofibromatosis (Li et al. 1977; Kaplan et al. 1982; Tamaro et al. 1982; Wertelecki et al. 1985; Matsuzaki et al. 1989; Itoh et al. 1998). There was no remarkable family history in case 1. Other tumors arising in patients with neurofibromatosis I include pheochromocytomas (Kremen et al. 1954; Berman et al. 1977), glioblastoma multiforme (Li et al. 1977), Gardner syndrome (Kaplan et al. 1982), renal artery stenosis (Kremen et al. 1954), and acute lymphocytic lymphoma (Wertelecki et al. 1985).

Neurofibromatosis II affects one in 50,000 persons and is also autosomal dominant with a high rate of penetrance (95%) (Enzinger and Weiss 1995). Usually, bilateral schwannomas of the acoustic nerve are the main lesions (Enzinger and Weiss 1995).

According to the National Institutes of Health Consensus Development Conference Statement on acoustic neuroma in 1991, the presumptive diagnostic criteria of neurofibromatosis II are unilateral cerebral nerve VIII schwannomas of early onset detected by MRI or computed tomography (CT) before the age of 30 years and one of the bilateral acoustic nerve tumors detected by an appropriate imaging technique or a first-degree relative with neurofibromatosis II. Case 2 was diagnosed as presumptive neurofibromatosis II because of onset at the age of 20 years and the presence of a tumor in the left acoustic nerve. It is noteworthy that there have been no other reports of the coexistence of neurofibromatosis II and malignant lymphoma.

Malignant lymphoma is defined as a solid neoplasm of the immune system (Lukes 1979). There are two kinds of lymphomas: Hodgkin’s disease and non-Hodgkin’s lymphoma. All of the reported cases with neurofibromatosis, including our cases, were non-Hodgkin’s lymphomas. We performed immunohistochemical and genetic analyses of the present two cases in order to characterize the coexisting malignant lymphomas. Flow cytometry provides a rapid and objective means to confirm the immunological characteristics of lymphomas (Ichinohasama et al. 1996; Hatori et al. 1997). It can distinguish lymphoma cells from other cells and show more easily the
correlation of positivity and negativity of plural antigens in lymphoma cells. There have been no reports describing flow cytometrical analysis of malignant lymphomas in association with neurofibromatosis. Chemotherapy was performed in all of the reported cases of malignant lymphoma. The prognoses of the reported cases varied greatly and may have depended on the responsiveness to chemotherapy. Both of our cases responded to chemotherapy.

Based on previous reports on neurofibromatosis (Hope and Mulvihill 1981), the association of neurofibromatosis with Wilm’s tumor is considered to be weak, that with leukemia to be credible, and that with rhabdomyosarcoma to be firm. The mechanism of the association of neurofibromatosis and malignant lymphomas has not been clarified yet.

Neurofibromatosis I is genetically related to an alteration of chromosome 17 (Barker et al. 1987). The neurofibromatosis I (NF-1) gene is thought to be a kind of tumor suppressor gene. Neurofibromin, a product of the NF-1 gene, has an important role in the activation of ras protein and in controlling the proliferation and differentiation of cells. In neurofibromatosis I, the absence of NF-1 gene is assumed to cause the tumorigenesis of cells, though the relationship with malignant lymphomas has not been clarified yet (Izawa et al. 1996).

Neurofibromatosis II is related to a genetic defect of chromosome 22 (Campanacci 1999). The NF-2 gene is also thought to be a kind of tumor suppressor gene. In neurofibromatosis II, the absence of schwannomin, a product of the NF-2 gene, may have something to do with lymphomas arising from neurofibromatosis (Takeshima et al. 1998).

References


