INTERNAL MEDICINE

□ CASE REPORT □

Primitive Neuroectodermal Tumor as a Differential Diagnosis of CD56-Positive Tumors in Adults

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Abstract

A 33-year-old Japanese man was referred to our hospital after a huge intrapelvic tumor with bilateral hydronephrosis was found following persistent lumbago. Natural killer/T-cell lymphoma was suspected due to positive immunostaining for CD56, but CHOP therapy was ineffective. Re-evaluation of the tumor cells showed that they were positive for CD99, neuron-specific enolase, and synaptophysin and had a t(11 ; 22) (q 24 ; q12) translocation, leading to the revised diagnosis of primitive neuroectodermal tumor (PNET). Systemic chemotherapies and radiation therapy were added to surgical resection, and no recurrence has been detected for 3 years. Taken together, PNET may be considered in adult patients with CD56-positive tumors.

Key words: primitive neuroectodermal tumor, CD56, CD99, ICE-CAV therapy

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Introduction

Primitive neuroectodermal tumor (PNET) belongs to the Ewing sarcoma family and makes up approximately 1% of all sarcomas in Japanese children (1). Recently, it has been shown that a t(11 ; 22) (q24 ; q12) translocation (EWS/FLI-1 fusion) is essential for the development of Ewing sarcoma family tumors (ESFT) (2, 3). ESFT is relatively common as a cause of bone tumors in children, but is quite rare in adults (4, 5). Here, we report an adult case of PNET initially diagnosed as natural killer (NK)/T-cell lymphoma based on a positive result for CD56.

Case Report

A 33-year-old Japanese man was referred to our hospital for treatment of an intrapelvic tumor. He had suffered from multiple liver and gastric tumors at the age of 27. The tumor cells obtained from gastric mucosa had large round-shaped nuclei and were positive for CD20 (Fig. 1), but not for CD3. Since Epstein-Barr virus (EBV)-encoded small RNA (EBER) was detected in the nuclei of tumor cells by *in situ* hybridization (Fig. 1), he was diagnosed with EBV-related B-cell lymphoproliferative disorder. He was successfully treated with 6-time cyclophosphamide, adriamycin, vincristine, and prednisolone (CHOP) therapy and was in good health with no recurrence for 6 years after the treatment. However, he became worried about lumbago that persisted for two months and first visited another hospital, where he showed no signs of weight loss, fever, night sweats, or dysuria. Physical examination showed tenderness in bilateral costovertebral angles. Hepatosplenomegaly, swelling of superficial lymph nodes or tonsils, or an abnormal mass were not detected. Laboratory tests revealed increases in serum concentrations of urea nitrogen, creatinine, lactate dehydrogenase, β2-microglobulin, and soluble interleukin-2 receptor (Table 1), and an abdominal computed tomography (CT) scan revealed a huge tumor (10×10×6 cm) in the pelvis with bilateral hydronephrosis and swelling of the para-

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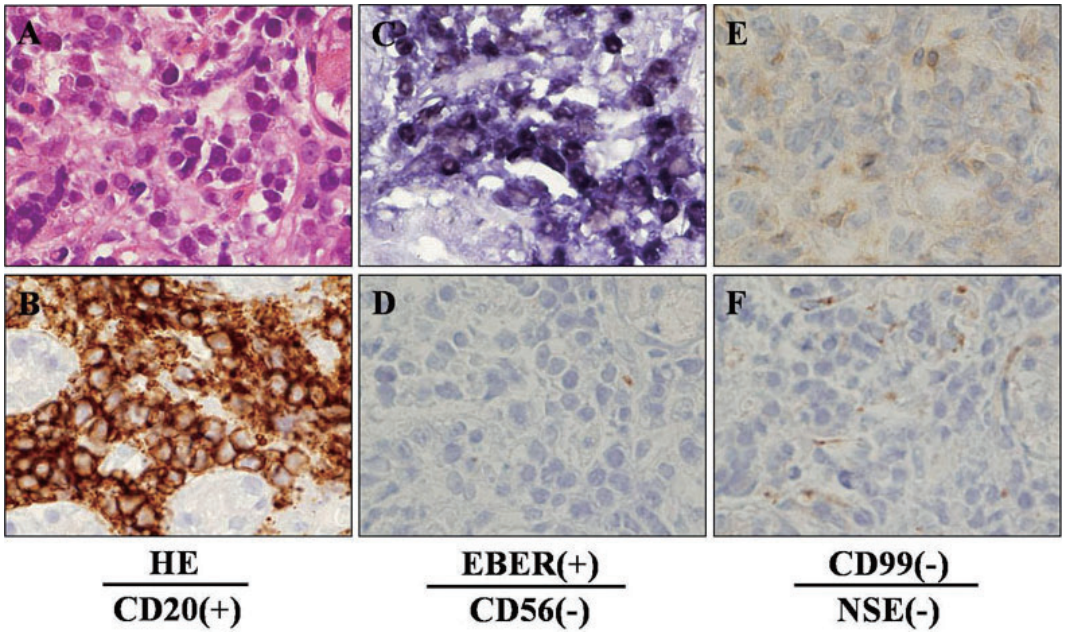

Figure 1. **Histological findings of the gastric tumor that appeared 6 years ago. The tumor cells were relatively large, round-shaped [A: Hematoxylin and Eosin staining], and positive for immunostaining against CD20 (B) and *in situ* hybridization against EBV-encoded small RNA (EBER) (C). However, CD56 (D), CD99 (E) and NSE (F) were all negative.**

Table 1. **Laboratory Data on Admission**

	Normal Range			Normal Range	
WBC (/μL)	3500-9000	6580	CRP (mg/dL)	<0.3	0.6
Hb (g/dL)	13-18	13.9	PT (%)	70-130	81.9
Platelet counts ($\times 10^3$/μL)	143-333	220	APTT (sec)	25-40	31.9
Albumin (g/dL)	3.8-5.3	3.6	Fibrinogen (mg/dL)	150-450	589.2
Bilirubin (mg/dL)	0.3-1.2	0.4	FDP (μg/dL)	<5	10.1
AST (IU/L)	13-33	12	FDP-D dimer (μg/dL)	<1	4.2
ALT (IU/L)	8-42	10	CEA (ng/mL)	<5	1.1
ALP (IU/L)	115-359	143	CA19-9 (U/mL)	<37	15.9
γGT (IU/L)	10-47	14	β2-MG (U/mL)	0.7-2.0	2.11
LDH (IU/L)	119-229	284	sIL2-R (mg/dL)	220-530	664
BUN (mg/dL)	8-22	48.1	anti EBV VCA IgM	< ×10	< ×10
Creatinine (mg/dL)	0.6-1.1	4.4	anti EBV VCA IgG	< ×10	×320
UA (mg/dL)	3.6-7.0	6.3	anti EBV EBNA	< ×10	×10
Na (mEq/L)	138-146	141	TPHA	(-)	(-)
K (mEq/L)	3.6-4.9	4.0	HBs antigen	(-)	(-)
Cl (mEq/L)	99-109	104	anti HCV antibody	(-)	(-)

Abnormal values were underlined. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γGT, γ- glutamyltransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; UA, uric acid; CRP, C-reactive protein; PT, protoronbin time; APTT, activated partial thromboplastin time; FDP, fibrin and fibrinogen degradation product; β2-MG, beta2 microglobulin sIL2-R, soluble IL2 receptor; CEA, carcinoembryonic antigen; TPHA, *Treponema pallidum* latex agglutination; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; (-), negative.

aortic lymph nodes (Fig. 2). Intra-ureteral stents were inserted and a needle biopsy of the tumor was performed. The tumor cells were round-shaped and resembled the abnormal lymphoid cells obtained from his multiple gastric tumors 6 years earlier. These cells were also positive for CD56, but not for CD3 or CD20 (Fig. 2). Based on these results, a diagnosis of NK/T-cell lymphoma was made and the patient was transferred to our hospital for immediate chemotherapy.

According to the diagnosis of NK/T-cell lymphoma, CHOP therapy was commenced but did not improve the patient's lumbago or tumor. Re-examination of the needle biopsy specimen obtained at the previous hospital showed that the tumor cells were also positive for CD99, a hallmark of ESFT. In addition, a t(11 ; 22) (q24 ; q12) translocation (EWS/FLI-1 fusion) was detected by fluorescence *in situ* hybridization (FISH) (Fig. 3A) and reverse transcriptase-

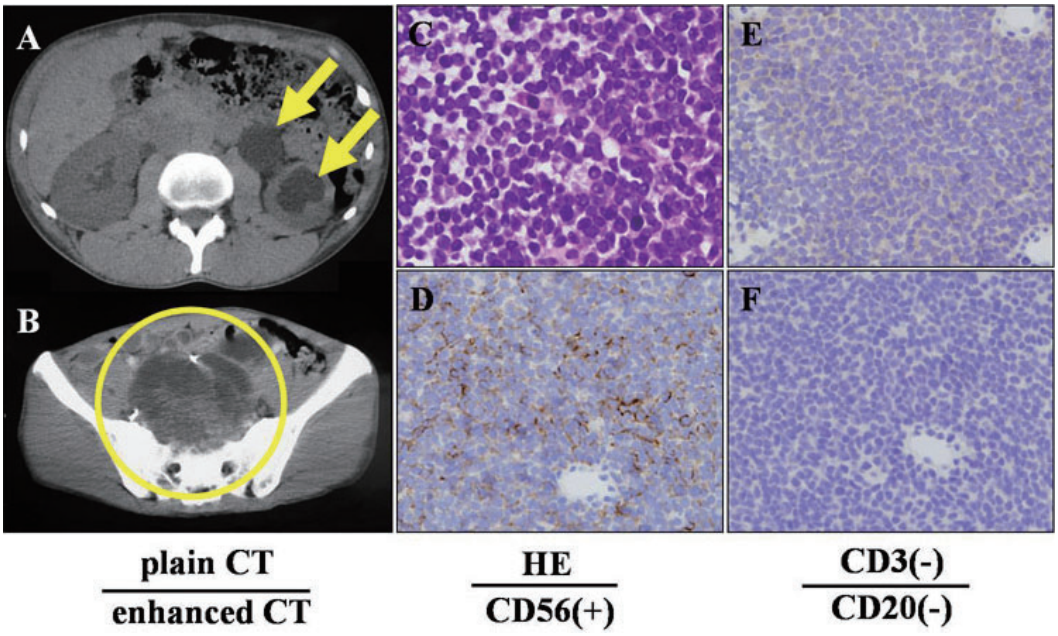

Figure 2. **Radiological and histological findings of the intrapelvic tumor. (A) A plain abdominal CT scan showed hydronephrosis (arrows). (B) A contrast-enhanced abdominal CT scan revealed a huge tumor (circle). The tumor was heterogeneously stained by contrast medium. (C-F) Histological findings of the tumor specimen obtained by needle biopsy. The tumor cells were round-shaped [C, Hematoxylin and Eosin staining] and positive for immunostaining against CD56 (D). However, CD3 (E) and CD20 (F) were both negative.**

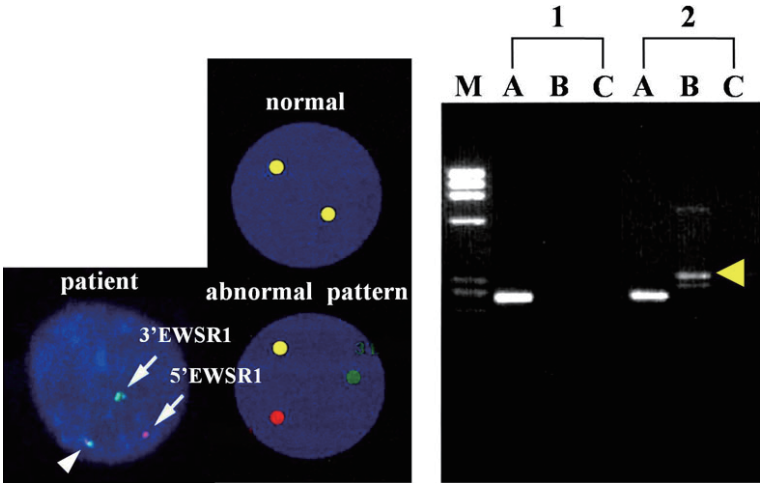

Figure 3. **Detection of a t(11;22) (q24;q12) translocation (EWS/FLI-1 fusion). Fluorescence *in situ* hybridization (FISH) (left panel) and reverse transcriptase-polymerase chain reaction (RT-PCR) (right panel) were performed as described in Methods (6). In the FISH assay, the 3′- and 5′ regions of the EWSR1 gene were labeled as a green and red signal, respectively, and normal cells showed a 1-yellow fusion signal (arrowhead). The tumor cells demonstrated one pair of split red and green signals caused by translocation of the EWSR1 gene. In the right panel, the PCR products of β-actin (A), EWS/FLI-1 (B), and EWS/ERG (C) genes were subjected to electrophoresis. The presence of aberrant EWS/FLI-1 gene fusion transcripts was confirmed in the tumor (arrowhead). 1: normal subject; 2: this patient; M: molecular weight marker.**

polymerase chain reaction (RT-PCR) (Fig. 3B) (6). Other histological markers suggestive of lymphoma or NK/T-cell lymphoma, such as CD45, CD34, terminal deoxynucleotidyl transferase (TdT), CD2, and EBER, were all negative. Based on these new findings, a revised diagnosis of intrapelvic ESFT was made. Since no distant metastasis was found, surgical removal of the tumor was attempted. Macroscopically, the tumor was dark red in color and smooth (Fig. 4). There

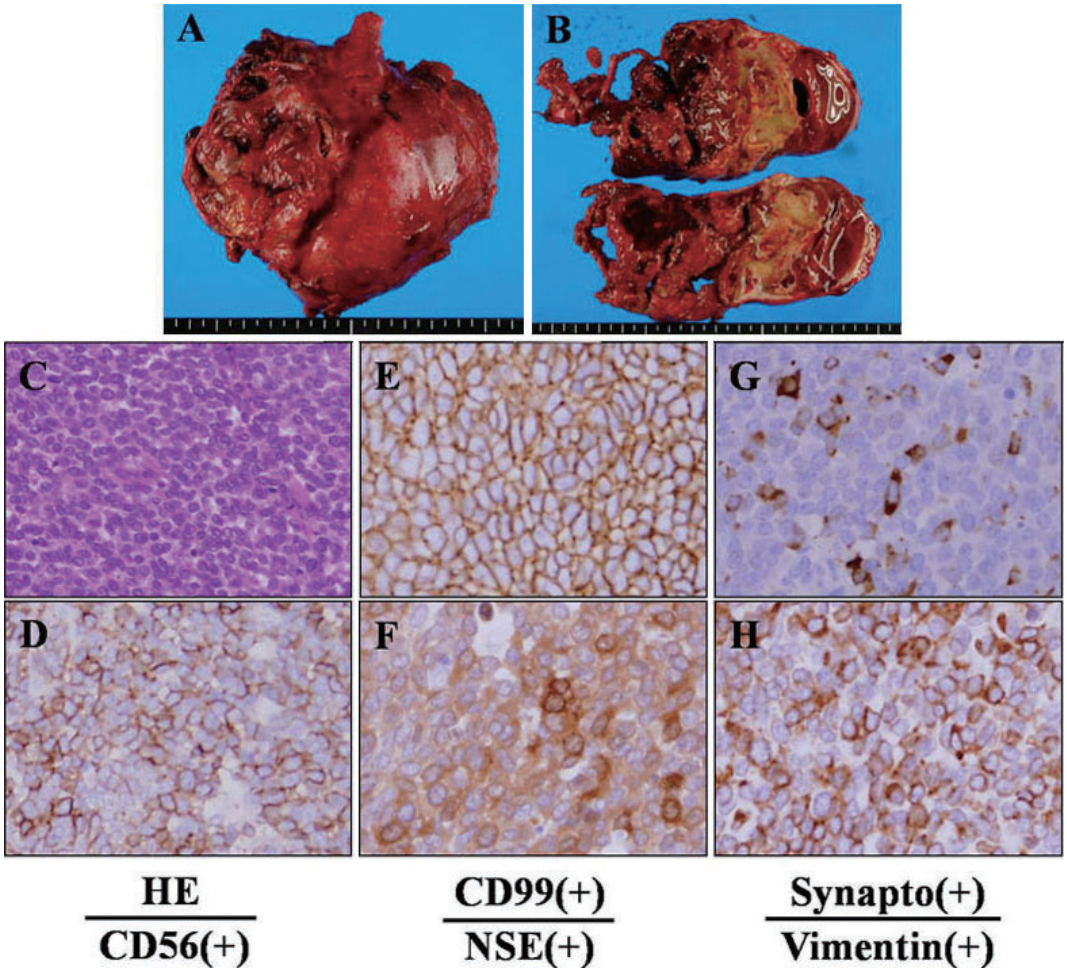

Figure 4. **Macroscopic and microscopic findings of the resected tumor. (A) Gross appearance of the tumor (10×10×6cm). (B) Hemorrhage and necrosis were found in the tumor. (C-H) Histological findings of the resected tumor. The tumor cells partially formed pseudorosettes [C, Hematoxylin and Eosin staining]. The cells were positive for immunostaining against CD56 (D), CD99 (E), NSE (F), synaptophysin (G), and vimentin (H).**

was no involvement of the iliac bones or lumbar vertebrae. The bilateral ureters were completely involved in the tumor. The tumor could be completely removed, but was seen to be partially adhered to the abdominal wall, suggesting the possibility of direct invasion. Histological sections stained with Hematoxylin and Eosin showed that the tumor cells were round-shaped and partially formed pseudorosettes. The cells were strongly positive for neuron-specific enolase (NSE), synaptophysin, and vimentin in addition to CD56 and CD99 (Fig. 4), which led to the final diagnosis of PNET. Considering the possibility of tumor residue, two types of systemic chemotherapies [ifosfamide, carboplatin, and etoposide (ICE) and cyclophosphamide, doxorubicine, and vincristine (CAV)] (Table 2) and regional radiation therapy were added after tumor resection. No serious adverse effects were observed during these treatments, and neither local recurrence nor distant metastasis has been found for over 3 years with careful monitoring.

Table 2. **Regimens of ICE-VAD Therapy in This Case**

ICE therapy		
Ifosfamide	1200 mg/m^2	days 1-5
Carboplatin	400 mg/m^2	day 1
Etoposide	100 mg/m^2	days 1-5
Mesna	1200 mg/m^2	days 1-5
CAV therapy		
Cyclophosphamaide	750 mg/m^2	days 1-5
Doxorubicin	75 mg/m^2/3days	days 1-3, continuous infusion
Vincristine	1.5 mg/m^2/3days	days 1-3, continuous infusion

Discussion

In Japan, ESFT, a generic disease term for Ewing sarcomas and PNET, is a relatively common sarcoma originating in the bones of patients younger than 20 years of age; approximately 80% of ESFT patients are children (4). As such, there are few reports regarding PNET in Japanese adults due to its rarity and difficulty in accurate pathological diagnosis.

Here, we report a rare case of adult PNET detected as a huge intrapelvic tumor.

In this case, the infrequency of PNET in adults and lack of careful histological examination delayed the correct diagnosis. The presence of a past history of EBV-related lymphoproliferative disorder and elevations in serum lactate dehydrogenase, β2-microglobulin, and soluble interleukin-2 receptor levels might have complicated the diagnosis as well. The patient's tumor cells were CD56-positive and CD3- and CD20-negative, so were initially diagnosed as NK/T-cell lymphoma. CD56, a neural cell adhesion molecule, is a hemophilic binding glycoprotein present on the surface of neurons, glia, skeletal muscle, and NK cells. Although CD56 has been established as a representative diagnostic marker of this type of lymphoma (7), it is also expressed in other non-hematological malignancies, such as olfactory neuroblastoma, neuroendocrine tumor, and alveolar rhabdomyosarcoma (8, 9). Thus, its positivity alone is insufficient to confirm the diagnosis of NK/T-cell lymphoma; if tumor cells are positive for CD56, additional immunostaining for other lymphocyte antigens, including CD45, CD34, and TdT, is required (10).

When NK/T-cell lymphoma can be ruled out in cases with a CD56-positive tumor, the possibility of ESFT should be taken into consideration. CD99 is often positive in ESFT (11), and other histological markers suggesting neuroectodermal differentiation, such as NSE, synaptophysin, and vimentin, may be positive as well, especially in PNET (12). Recently established genetic assays are also useful for clear differentiation between ESFT and other neurogenic tumors. For example, the t(11 ; 22) (q24 ; q12) translocation (EWS/FLI-1 fusion) is found in more than 85% of ESFT cases. Other translocations involving the EWS locus on chromosome 22, including t(21 ; 22) (q22 ; q12) and t(7 ; 22) (p22 ; p12) (EWS/ERG fusion), have also been identified (2, 3). Although EWS/FLI-1 and EWS/ERG fusions have been shown to promote the malignant transformation of cells (13), the precise function of fusion gene products remains to be elucidated.

The occurrence of a bulky mass or pelvic space, the presence of obvious metastasis, and advanced age are known as poor prognostic factors of adult ESFT (14-18). A retrospective study of 1,426 German ESFT patients demonstrated that an age of more than 15 years at the time of diagnosis and treatment elsewhere than in a pediatric oncology unit were associated with poor prognosis as well (19). The present patient had most of these unfavorable factors and the tumor was suspected to be directly invading the neighboring abdominal wall, which necessitated precautionary systemic chemotherapies and radiation therapy after surgical resection. Anti-cancer drugs, such as ifosfamide, etoposide, cyclophosphamide, vincristine, doxorubicin, carboplatin, and actinomycin D, have been proven to be effective for ESFT. Notably, the combination of ICE and CAV regimens has shown good results and is well-tolerated in adult patients with aggressive ESFT; the overall response rate to ICE-CAV therapy for advanced ESFT is reported to be 94%. Furthermore, it has also been shown that complete remission after ICE-CAV plus surgery is 95% and that the 3-year overall survival rate is estimated to be 67±12% (20).

The patient's negative results for CD56, CD99, and NSE in the abnormal cells of his EBV-related lymphoproliferative disorder, as evidenced by additional immunostaining, suggest a different strain from that of his PNET cells (Figs. 1, 4) and raises the possibility that the PNET in this case may have been a chemotherapy-associated secondary malignant neoplasm. In fact, a retrospective analysis of 11,183 patients who had undergone chemotherapies against primary malignant neoplasms during childhood or adolescence revealed a second malignancy in 479 patients. Of these, ESFT developed in 6 patients (21). Based on this report, the prevalence of ESFT after chemotherapy is estimated to be approximately 0.05%, which is considerably higher than that of idiopathic ESFT in the general population (0.0001-0.0002%). As far as we know, this is the first report indicating the possibility of ESFT as a second malignant neoplasm in the Japanese population. Accumulation of Japanese ESFT/PNET data will help clarify the novel aspect of ESFT/PNET as a second malignancy.

In conclusion, although ESFT/PNET is rare in adults, the possibility of this tumor should be kept in mind in patients with a CD56-positive tumor. The detection of CD99 expression and a t(11 ; 22) (q24 ; q12) translocation will yield the correct diagnosis of ESFT/PNET in such cases.

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