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Case report

Combined use of pathological and genomic alteration analyses for the diagnosis of gray zone lymphoma

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Histiocytic sarcoma (HS) is a rare malignancy showing morphologic and immunophenotypic features of histiocytes. HS has morphologic overlap with many other diseases, including various kinds of lymphomas. Gray zone lymphoma (GZL) is a rare B-cell lymphoma subtype characterized by overlapping features between diffuse large B-cell lymphoma and classic Hodgkin lymphoma. The histologic overlap with other diverse diseases of HS and the pathological diversity of GZL make it difficult to render a diagnosis. A 44-year-old woman who was initially diagnosed with HS was diagnosed with GZL after reexamination, including a genetic alteration test. After 6 cycles of brentuximab vedotin, doxorubicin, vinblastine, and dacarbazine, she achieved a complete response. Genomic alteration assessment may be useful for the accurate diagnosis of malignant lymphomas, which are difficult to diagnose, such as GZL.

Keywords: histiocytic sarcoma, gray zone lymphoma, genomic alteration test

INTRODUCTION

Histiocytic sarcoma (HS) is a malignant proliferation of cells showing morphological and immunophenotypic features of mature tissue histiocytes.[1] As the histologic appearance of HS overlaps with other diverse diseases, a broad differential diagnosis is needed for HS, including malignant lymphomas, poorly differentiated or undifferentiated carcinomas, melanomas, and dendritic cell tumors.[2]

B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and classic Hodgkin lymphoma (cHL), is often referred to as gray zone lymphoma (GZL) and is a B-cell lymphoma subtype with overlapping clinical, morphological, and/or immunophenotypic features between cHL and DLBCL, especially primary mediastinal large B-cell lymphoma (PMBCL).[1] A large-scale DNA methylation analysis demonstrated a close relationship between GZL, cHL, and PMBCL, which are clearly different from DLBCL.[3] Patients with GZL present with primary mediastinal localization or systemic disease without mediastinal involvement. Patients with mediastinal disease are typically young adults in the third and fourth decades, whereas those with nonmediastinal disease are older and more often present with advanced-stage disease. The tumor cells exhibit an aberrant immunophenotype, and the diagnosis of GZL should be made by expert pathological evaluation. There are no standard management strategies for GZL patients, and they generally have higher relapse rates and poorer prognoses than cHL and PMBCL patients.[4] Here, we report a case of a patient with GZL who was diagnosed using a combination of pathological and genomic alteration analyses and was successfully treated with A+AVD (brentuximab vedotin, doxorubicin, vinblastine, and dacarbazine).

CASE REPORT

A 44-year-old asymptomatic Japanese woman received a regular medical checkup and mediastinal abnormalities were detected on her chest X-ray. A computed tomography (CT) scan showed multiple enlarged mediastinal and left supraclavicular lymph nodes. She received a diagnosis of histiocytic sarcoma (HS) after biopsy of the left supraclavicular lymph

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nodes in a community hospital. She was initially referred to the Department of Oncology in our hospital. As HS is a rare cancer, the oncologist submitted comprehensive cancer genomic profiling (FoundationOne CDx). Moreover, because HS is associated with hematological disorders, such as malignant lymphoma, she was admitted to the Department of Hematology in our hospital two weeks later. She had no medical history. On examination, her temperature was 36.4°C, and no hepatomegaly, splenomegaly, or rash were observed. She had multiple enlarged left neck and supraclavicular lymph nodes, which were nontender on palpation; the largest was 3 cm in diameter. Her Eastern Clinical Oncology Group performance status score was 0. She had no B symptoms. Laboratory tests showed no cytopenia. The serum lactate dehydrogenase level was not elevated (200 U/L; normal range 124-222 U/L). The serum C-reactive protein level was elevated to 2.93 mg/dL, and the erythrocyte sedimentation rate was increased to 54 mm/hour. Liver and renal functions were normal. Serological tests for Epstein-Barr virus (EBV) antibodies showed a past EBV infection. The serum soluble interleukin-2 receptor level was in the normal range (Table 1). A contrast-enhanced CT scan showed multiple enlarged left neck and supraclavicular lymph nodes, a solid mass extending from the mediastinum to the hilar region, and a mass above the left diaphragm. Fluorodeoxyglucose (FDG) positron emission tomography (PET)-CT showed abnormal accumulation with an SUV max of 14.24 in the mediastinal mass, which ran from the left supraclavicular fossa to the superior mediastinum and left pericardium (Figure 1). Upper gastrointestinal endoscopy showed no tumor, and a bone marrow examination did not show involvement of tumor cells. The histological findings of the left supraclavicular lymph node biopsy revealed prominent infiltration of histiocytic cells positive for CD4, CD68, CD163, and S100 with a background of fibrosis and infiltration of various kinds of inflammatory cells. *In situ* hybridization for EBV-encoded small RNA (EBER) was negative. Based on these findings, she was initially diagnosed with stage IVA HS.

The patient was initially treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone). A CT scan performed after one cycle of CHOP showed stable disease. After 2 cycles of CHOP, we obtained the results of the cancer genome profiling test. The test revealed genetic mutations of *SOCS1* (c.477_497>G, p.Arg160fs*86) and *XPO1* (c.1711G>A, p.Glu571Lys), which are not seen in patients with HS, but are often seen in patients with malignant lymphoma, such as cHL, PMBCL, and GZL. These results led us to suspect that the patient had malignant lymphoma. We reexamined her lymph node biopsy with pathology and immunohistochemical tests. Additional immunostains highlighted atypical large cells positive for CD30 and PD-L1, weakly positive for PAX5, partially positive for CD20 and CD15, and negative for EBER (Figure 2). These large cells showed dense proliferation with a sheet-like pattern. Moreover, there were a lot of large cells morphologically similar to centroblastic/immunoblastic DLBCL with Hodgkin and Reed-Sternberg (HRS) cells. This morphological and immunohistochemical discordance suggested intermediate characteristics between cHL and large B-cell lymphoma. Considering all pathological and genomic findings, the numerous infiltrating histiocytes were interpreted to be reactive conditions. We corrected her diagnosis from HS to GZL and changed the therapeutic regimen to A+AVD. The interim PET-CT scan after 2 cycles of A+AVD showed no FDG uptake, and she achieved complete metabolic response (CMR). After a total of 6 cycles of A+AVD, she maintained CMR.

DISCUSSION

HS is rare and one of the most aggressive histiocytic neoplasms. A targeted next-generation sequencing approach

Table 1. Laboratory data on admission

Complete blood count		Serum biochemistry		Serological test	
WBC	7310/μL	TP	7.5 g/dL	CRP	2.98 mg/dL
Neutro	83.7%	Alb	3.4 g/dL	IgG	1637 mg/dL
Lym	6.3%	BUN	8.9 mg/dL	IgA	263 mg/dL
Mono	7.9%	Cr	0.48 mg/dL	IgM	235 mg/dL
Baso	1.0%	UA	4.2 mg/dL	ESR	54 mm/hr
Eosino	1.1%	AST	11 IU/L	EB/EA-IgG	<10
RBC	442×10^6/μL	ALT	7 IU/L	EB/EA-IgM	<10
Hb	11.3 g/dL	ALP	106 IU/L	EB/EBNA	160
Ht	35.8%	LDH	200 IU/L	EB.VCA-IgG	1280
MCV	81.0 fL	γ-GTP	15 IU/L	EB.VCA-IgM	<10
MCH	25.6 pg	T-Bil	0.5 mg/dL		
MCHC	31.6 g/dL	sIL-2R	481 U/mL		
Plt	53.0×10^4/μL			Blood coagulation	
				APTT	31.6 s
				PT-INR	1.01
				Fib	606 mg/dL
				FDP	5.4 μg/mL

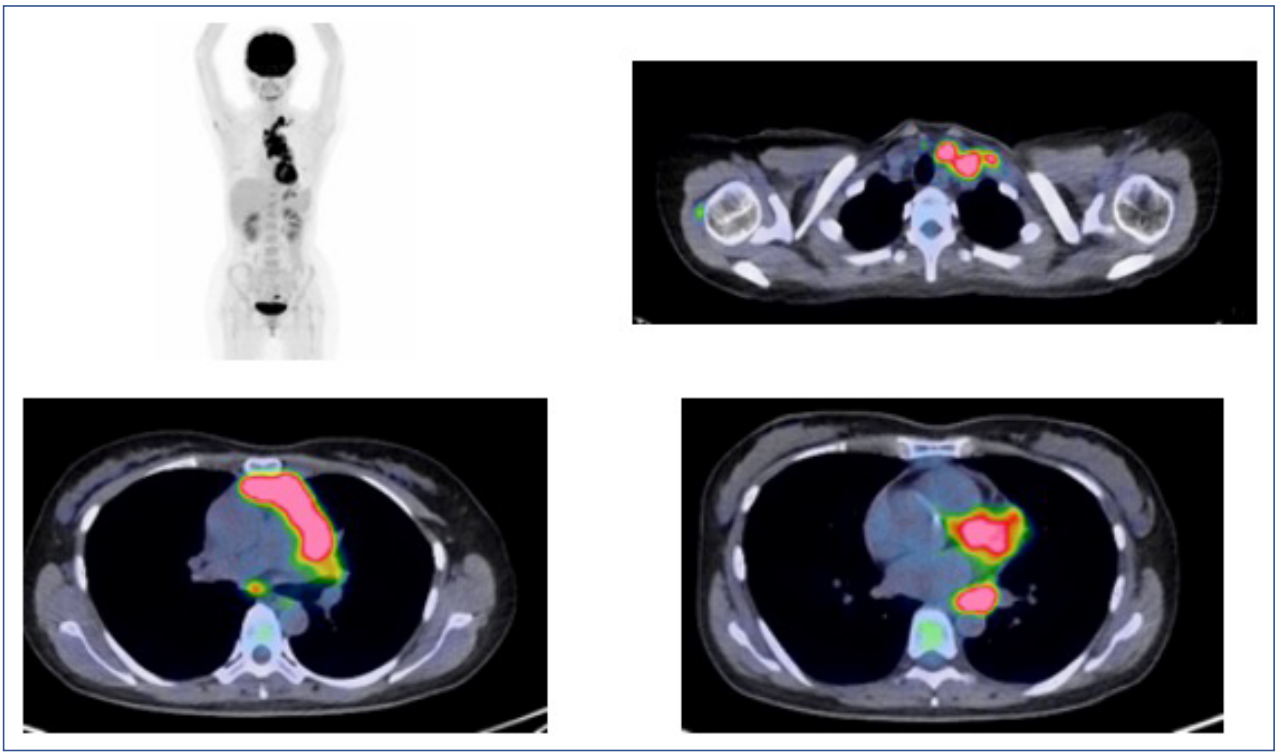

Fig. 1. PET-CT before treatment. The scan showed abnormal accumulation in the mediastinal mass.

identified that 57% of 28 patients with HS had mutations involving the RAS-MAPK signaling pathway (*MAP2K1*, *KRAS*, *NRAS*, *BRAF*, *PTPN11*, *NF1*, *CBL*) and 21% had mutations resulting in activation of the PI3K signaling pathway (*PTEN*, *MTOR*, *PIK3R1*, *PIK3CA*).[5] In our case, the patient was initially diagnosed with HS, but none of these mutations were detected. There is no standard therapy for HS. There are some reports that CHOP or CHOEP (CHOP plus etoposide) was effective for patients with HS.[6-8] We first treated the patient with CHOP, but it was not effective. After 2 cycles of CHOP, the mutations of *SOCS1* and *XPO1* were revealed.

A capture panel of 217 genes revealed that 40% and 16% of 50 patients with GZL had *SOCS1* mutation and *XPO1* mutation, respectively.[9] *SOCS1* is known to negatively regulate the signal transducer and activator of transcription-mediated expression of proliferation and survival-associated genes by interacting with Janus kinases.[10] *XPO1* mediates the translocation of several types of RNAs, ribonucleoprotein complexes, and cargoes, including tumor suppressors and regulatory proteins.[11] Mutations of *B2M* (32%), *GNA13* (24%), and *NFKBIA* (22%) were also identified.[9] Mutations of *SOCS1* and *XPO1* have also often been detected in patients with PMBCL[12] and cHL.[13] Gene expression profiling performed in a retrospective and multicenter GZL cohort, alongside EBV-positive DLBCL, cHL, and PMBCL samples, revealed that the gene expression profile of GZL was not significantly different from those of cHL and PMBCL.[14] *NBPF6* mutations were found in 13% of GZL patients, but were not found in PMBCL or cHL.[9] In our case, the patient had mutations in *SOCS1* and *XPO1,* but no mutations of *GNA13* or *NFKBIA*. *B2M* and *NBPF6* mutations were not assessed. Given the gene mutation findings, we re-examined the patient with pathological analysis and made an appropriate diagnosis. In the first immunohistochemical analysis, we detected atypical histiocytes that were positive for macrophage lineage markers, including CD163, CD68, and lysozyme, but it was possible that these histiocytes were in the microenvironment of the lymphoma, and this may lead us to misdiagnosis.

Sarkozy *et al.* proposed that GZL can be divided into 4 categories (groups 0 to 3) based on morphological and phenotypic charactoristics.[15] Our patient was categorized into group 0, which includes cases with morphological and cytologic characteristics suggestive of cHL, but with expression of CD20. GZL can also be divided into 2 subtypes based on whether the disease involves the anterior mediastinum (thymic) or not (nonthymic). Thymic GZL presents a gene expression profile similar to that of PMBCL. The tumor cells of thymic GZL also present a prominent immune escape phenotype with loss of MHC-I expression and expression of PD-L1 at the immunohistochemistry level.[14] On the other hand, the nonthymic type is similar to DLBCL and does not present with the immune escape phenotype and CD4 inhibitory markers.[14] Our patient was classified as having thymic-subtype GZL because anterior mediastinum involvement was detected upon CT scan and immunohistochemical analysis revealed the expression of PD-L1.

There is no standard therapy for patients with GZL. According to a retrospective study of 99 thymic GZL patients, 28% of patients presented with primary refractory disease [43% were treated with ABVD (doxorubicin, bleo-

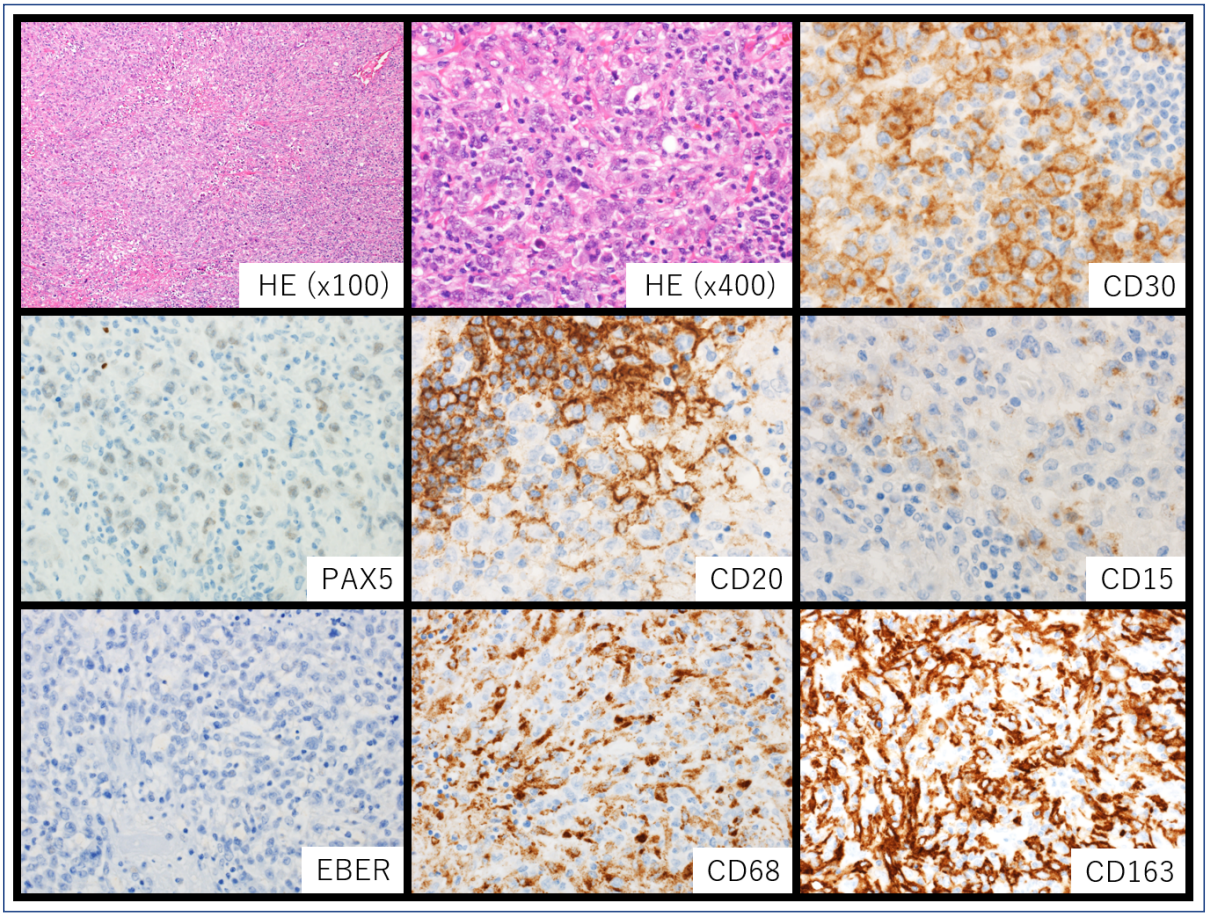

Fig. 2. Pathological findings of lymph node biopsy. Atypical large cells resembling centroblasts or immunoblasts showed dense proliferation with Hodgkin/Reed Sternberg cells. These atypical lymphoid cells were positive for CD30, weakly positive for PAX5, partially positive for CD20 and CD15, and negative for EBER. Numerous histiocytes were positive for CD68 and CD163.

mycin, vinblastine, and dacarbazine) or escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone), and 57% were treated with a CHOP-like regimen with or without rituximab]. The three-year event-free survival and overall survival (OS) rates were 63% and 80%, respectively.[16] In that report, there was no significant difference in the three-year OS rates between regimens. Some other reports suggested that the therapeutic regimens used for DLBCL [CHOP with or without rituximab and DA-EPOCH-R (dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin with rituximab)] were more effective for the treatment of GZL than the therapeutic regimen used for cHL (ABVD with or without rituximab).[4,17] The four pathological categories of GZL showed no significant differences in prognosis.[15] Patients with nonthymic GZL tended to have better outcomes than patients with thymic GZL.[15] Moderate to strong CD30 expression may be associated with inferior progression-free survival (PFS).[15] As our patient was categorized as having thymic GZL and the tumor cells showed strong expression of CD30, she was considered to have a high risk of poor prognosis.

In the results of a randomized phase 3 trial involving patients with previously untreated stage III or IV cHL, in which 664 received A+AVD and 670 received ABVD, the A+AVD group had a significantly better prognosis than the ABVD group (2-year modified PFS: 82.1% vs. 77.2%).[18] Based on this result, A+AVD became a treatment option for previously untreated advanced-stage cHL. Several retrospective studies had also revealed the efficacy of brentuximab vedotin for CD30-positive GZL.[19-21] Therefore, we changed the treatment regimen to A+AVD. Our patient was successfully treated with a total of 6 cycles of A+AVD. This case highlights that genomic alteration testing led us to reexamine the patient, who was initially diagnosed with HS, and helped us to make an accurate diagnosis.

CONCLUSION

The identification of specific genetic mutations contributed to the diagnosis of GZL. Further investigation of genetic abnormalities is warranted to diagnose lymphoma appropriately.

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