Clinicopathological Study of 30 Cases of Peripheral T-cell Lymphoma with Hodgkin and Reed-Sternberg-like B-cells from Japan

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Abstract: The presence of Hodgkin and Reed-Sternberg (HRS)-like B-cells in peripheral T-cell lymphoma (PTCL) is rare and its clinicopathological features still remain unclear. Here, we describe 30 cases of PTCL with HRS-like B-cells from Japan. Twenty-three cases (77%) presented evidence of follicular T-helper phenotype (T_{FH}) derivation: 12 were angioimmunoblastic T-cell lymphoma and 11 PTCL with T_{FH} phenotype (PTCL-TFH). The remaining seven cases were diagnosed as PTCL, not otherwise specified (PTCL-NOS). Epstein-Barr virus (EBV) reactivation was detected in 25 cases (83%), but HRS-like B-cells were $EBER^+$ in only 20 cases (67%). The median age at diagnosis was 77 years (range, 39-91 y), including 24 patients (80%) were older than 60 years of age. Most of the patients presented at an advanced clinical stage and were associated with higher risk according to the International Prognostic Index. The 3-year overall and progression-free survival rates were 44% and 27%, respectively. No significant clinicopathological differences were detected between PTCL-TFH, PTCL-NOS and the angioimmunoblastic cases. Cases with $EBER^+$ HRS-like B-cells were associated with inferior overall and progression-free survival compared to those with $EBER^-$ HRS-like B-cells, but the difference was not significant. In conclusion, HRS-like B-cells were found in a subset of T-cell lymphomas, especially in association with the T_{FH} phenotype and EBV reactivation. These cells have a tendency to affect elderly patients and to be associated with advanced clinical stages and dismal prognosis. The EBV status of HRS-like B-cells does not seem to affect the clinicopathological features of this group of PTCLs.

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Peripheral T-cell lymphomas (PTCLs) encompass a heterogeneous group of lymphomas that vary in their clinical and pathological features.[1] Recently, a subset of PTCL, not otherwise specified (NOS), was reported to express the follicular helper T-cell (T_{FH}) phenotype, similar to angioimmunoblastic T-cell lymphoma (AITL),[2–4] and to share common genetic alterations with AITL.[5] Thus, the 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms included AITL and PTCL with T_{FH} phenotype (PTCL-TFH) under one heading, nodal PTCL with T_{FH} phenotype.[6]

The presence of a population of B-cells in the background of neoplastic T-cell proliferation was documented in AITL and PTCL-TFH and regarded as a diagnostic hallmark.[2,7,8] These B-cells are frequently Epstein-Barr virus (EBV)-positive and may express a monoclonal immunoglobulin gene rearrangement with subsequent progression to EBV^+ B-cell lymphoma in some cases.[7–10] This EBV^+ B-cell proliferation is secondary to the immune-deficient status, which commonly evolves during the course of these T-cell neoplasms.[8] In rare cases, these B-cells may closely resemble Hodgkin and Reed-Sternberg (HRS)-cells in morphology and immunohistochemistry, making the differential diagnosis of classical Hodgkin lymphoma (cHL) very challenging.[11–14] This issue has been addressed previously, but clinical findings and biological behaviors remain to be elucidated.

Here, we investigated the clinicopathological features of a series of 30 PTCL cases with scattered HRS-like cells of B-cell lineage from Japan.

MATERIALS AND METHODS

Patients and Samples

All cases of PTCL reported as Hodgkin-like or containing HRS-like cells between 2005 and 2015 were

selected from the consultation files of Nagoya University Hospital database. The Institutional Review Board of Nagoya University approved the study protocol. Diagnoses were established by histopathological and immunohistochemical criteria in accordance with the WHO classification. After the initial examination, two cases were excluded because the HRS-like cells were of T-cell origin: one case of PTCL-NOS with $CD3^+$/cytotoxic $molecule^+/CD20^-/PAX\text{-}5^-/EBV^-$ HRS-like cells and one case of PTCL-TFH with $CD3^+/PD\text{-}1^-/CD20^-/PAX\text{-}5^-/EBV^-$ HRS-like cells. Finally, 30 cases were identified: 12 AITL, 11 PTCL-TFH and 7 PTCL-NOS. In all PTCL-TFH cases, tumor cells expressed at least two of the T_{FH} cell-related antigens PD-1/CXCL-13/CD10 and BCL-6. PTCL-TFH cases were differentiated from AITL cases mainly by morphology due to an absence of AITL-characteristic features: a proliferation of high endothelial venules (HEVs) or follicular dendritic cells (FDCs), and an absence of reactive inflammatory background or monomorphic cytological features of tumor cells. In all cases, there were large atypical B-cells including scattered HRS-like cells without sheet formation, in a background of atypical T-cells. Any controversial cases were reassessed until consensus was reached on the diagnosis. Medical records with clinical and follow-up data were obtained from referring pathologists and hematologists.

Histological and Immunohistochemical Staining

Tissue samples were fixed in 10% formalin and embedded in paraffin. Sections (5-μm-thick) were stained with hematoxylin and eosin. Immunoperoxidase studies were performed on formalin-fixed paraffin-embedded sections. The monoclonal antibodies used were CD3, CD8, CD20, CD30, CD79a, FDC and latent membrane protein-1 (LMP-1) (DAKO, Santa Fe, CA); CD4, CD10, BCL-6, and perforin (Novocastra Laboratories, Newcastle, UK); PAX-5 (Biocare Medical, Walnut Creek, CA); programmed cell death 1 (PD-1) (Abcam, Cambridge, MA); CXCL-13 (R&D Systems, Minneapolis, MN); T-cell intracellular antigen (TIA-1) (Coulter Immunology, Hialeah, FL); granzyme B (Mososan, Uden, the Netherlands); CD15 and CCR4 (Becton Dickinson, Mountain View, CA); and βF1 [T-cell receptor (TCR)-β; T cell Science, Cambridge, MA]. All cases were stained for programmed cell death-ligand 1 (PD-L1) (Cell Signaling Technology, Danvers, MA) in order to examine its expression pattern in this subset of T-cell lymphomas.

In situ Hybridization

The presence of EBV small ribonucleic acids was determined by in situ hybridization using EBV-encoded small nuclear early region (EBER) oligonucleotides on formalin fixed, paraffin-embedded sections.[15]

Polymerase Chain Reaction (PCR)

DNA was extracted from formalin-fixed tissues, and PCR of the T-cell receptor γ chain (TCR-γ) gene and immunoglobulin heavy chain (IgH) gene rearrangement performed using the BIOMED2 protocol as described elsewhere.[16]

Statistical Analysis

The differences in characteristics between the different groups were examined using Fisher's exact test and the Mann–Whitney U test. Patient survival data were analyzed using the Kaplan–Meier method. Differences in survival were tested by the log-rank test. Overall survival (OS) was calculated from the date of diagnosis to the date of death or the last date of follow-up. Progression-free survival (PFS) was calculated from the date of diagnosis to the first date of disease progression, relapse, or death as a result of any cause or the last date of follow-up. The results were considered statistically significant if $P < 0.05$.

RESULTS

Clinical Characteristics of the Patients

Table 1 summarizes the clinical characteristics of the 30 PTCL patients with HRS-like cells of B-cell lineage. They were 18 male and 12 female (male:female ratio was 1.5:1), ranging from 39 to 91 years with a median age of 77 years. 80% of patients were older than 60 years. 90% of patients were diagnosed with advanced clinical Ann-Arbor staging and 40% had B-symptoms. Laboratory data at presentation revealed increased white blood cells count, elevated lactate dehydrogenase level (LDH) and elevated level of soluble interleukin-2 receptor in 30%, 37% and 39% of patients, respectively. Anemia (hemoglobin level < 10.5 g/dL) was found in 9 (30%) and decreased level of serum albumin (< 3.5 g/dL) in 11 (37%). 53% of patients were classified as High-Intermediate and High risk groups according to the International Prognostic Index (IPI) and 50% of them as groups 3 and 4 according to the Prognostic Index of T-cell lymphoma (PIT). A history of autoimmune disease and administration of immunosuppressive therapy was present in three AITL and two PTCL-TFH patients, respectively. All cases were negative for Human T-cell leukemia virus type-1 antibody in sera. Human immunodeficiency virus antibody was not detected in the sera of all 19 examined patients.

AITL Cases

The 12 AITL cases presented with the proliferation of medium-sized tumor cells with pale cytoplasm and round to angulated nuclei surrounding hyperplastic HEVs. The background contained mixed reactive inflammatory cells. Large HRS-like cells were scattered in the background, with a variable number area by area. Tumor cells were $CD3^+$ (12/12, 100%), $CD10^+$ (6/10, 60%), $PD\text{-}1^+$ (10/12, 83%) and $CXCL\text{-}13^+$ (9/9, 100%). TCR-β chain was detected in five of the 12 cases (42%). The B-cell lineage of the HRS-like cells was maintained in all cases; nine of the 12 were $CD20^+$ and the $CD20^-$ cases were $PAX\text{-}5^+$. Nine of the 12 cases were $CD30^+$ while CD15 was positive in 3/12 cases. EBER was detected in HRS-like cells in 9/12 cases, with LMP-1 positivity in 7 of 8 examined

TABLE 1. Patients' Clinical Characteristics (N = 30)

	No. (n/N) (%)
Age	
Median, (range)	77, (39-91)
Age > 60 y	24/30 (80)
Sex, male	18/30 (60)
Bulky disease	2/30 (7)
Extranodal involvement > 1 site	9/30 (30)
Bone marrow involvement	7/30 (23)
Skin involvement	3/30 (10)
Stage III/IV	27/30 (90)
B symptoms	12/30 (40)
Performance status > 1	7/30 (23)
International Prognostic Index	
Low risk	6/30 (20)
Low-Intermediate	8/30 (27)
High-Intermediate	9/30 (30)
High	7/30 (23)
Prognostic Index of T-cell lymphoma	
Groups 3 and 4	15/30 (50)
WBC > 10,000/mm^3	9/30 (30)
Hemoglobin < 10.5 g/dL	9/30 (30)
Platelets < 150,000/mm^3	8/30 (27)
Serum Albumin level < 3.5 g/dL	11/30 (37)
Elevated serum LDH level	11/30 (37)
sIL-2R > 4000 U/mL	11/28 (39)
CRP > 2.00 mg/dL	10/29 (35)
EBV positivity	25/30 (83)
EBV-positive HRS-like cells	20/30 (67)
TCR-β phenotype	14/30 (47)
Past history of autoimmune disease or immunosuppressive therapy	5/30 (17)
Diagnosis	
AITL	12/30 (40)
PTCL-TFH	11/30 (37)
PTCL-NOS	7/30 (23)
Treatment	28/30 (93)
CHOP/THP-COP	25/28 (89)
ABVD	2/28 (7)
Steroids	1/28 (4)
Adjuvant Radiotherapy	1/28 (4)
BM stem cell transplantation	3/28 (11)
Response	
CR	14/28 (50)
PR	10/28 (36)
NC	1/28 (4)
PD	3/28 (11)
Relapse	11/28 (39)
Death	18/30 (60)

ABVD indicates doxorubicin, bleomycin, vinblastine, and dacarbazine; AITL, angioimmunoblastic T-cell lymphoma; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete remission; CRP, C-reactive protein; EBV, Epstein-Barr virus; HRS, Hodgkin and Reed-Sternberg; LDH, lactate dehydrogenase; NC, no change; NOS, not otherwise specified; PD, progressive disease; PR, partial remission; PTCL-TFH peripheral T-cell lymphoma with follicular helper phenotype; sIL-2R, soluble IL-2 receptor; TCR-β, T-cell receptor-β; THP-COP, cyclophosphamide, pirarubicin, vincristine, and prednisone; WBC, white blood cell.

cases (Fig. 1). One of the three negative cases had a few EBER$^+$ bystander B-cells in the background. Both CD30 and EBER were co-expressed in the HRS-like cells in 7 cases. However, they were inferiorly expressed by the scattered large B-cells in the background compared with the HRS-like cells.

PTCL-TFH Cases

The tumor cells from the 11 PTCL-TFH patients ranged from mildly atypical small-to-medium-sized cells with hyperchromatic nuclei (n = 7) to more pleomorphic medium-to-large-sized cells (n = 4). Focal AITL-like features were detected in some cases, but the overall pattern was not that of AITL. Two cases presented small aggregates of histiocytes focally mimicking Lennert's lymphoma. One case had follicular proliferation of the small-to-medium-sized tumor T-cells, providing the diagnosis of PTCL-follicular variant. All cases had scattered large HRS-like cells among the tumor cells. Tumor cells were CD3$^+$ (11/11, 100%) and CD5$^+$ (5/5, 100%). A double PD-1$^+$ and CXCL-13$^+$ reaction was detected in 10 cases (91%). Tumor cells in the remaining case were CXCL-13$^+$ and BCL-6$^+$. TCR-β chain was detected in 6/11 cases (55%). HRS-like cells were of B-cell lineage in all cases, with nine CD20$^+$ cases and 11 CD30$^+$. The CD20$^-$ cases were CD79a$^+$ and PAX-5$^+$. Five of 10 cases examined were CD15$^+$. The HRS-like cells were EBER$^+$ in 8/11 cases, with LMP-1 positivity in 5 of 6 examined cases (Fig. 2). Two of the three negative cases had scattered EBER$^+$ bystander B-cells in the background.

PTCL-NOS Cases

Of the seven PTCL-NOS cases, four had a mixed cell pattern appearance containing medium-sized tumor cells, and the other three exhibited high grade morphology with pleomorphic medium-to-large-sized tumor cells. HRS-like cells were scattered throughout the lesions. Tumor cells were CD3$^+$ (7/7, 100%), CD5$^+$ (5/7, 71%) and cytotoxic molecule (CM)$^+$ (3/7, 43%). All cases were negative for both PD-1 and CXCL-13. TCR-β chain was detected in 3/7 cases (43%). HRS-like cells were of B-cell lineage with a CD20$^+$ and/or PAX-5$^+$ reaction in all seven cases and a CD30$^+$ reaction in 6/7 cases. CD15 was positive in 2/6 cases. The HRS-like cells were EBER$^+$ in 3/7 cases (Fig. 3). Two of the four negative cases had a few EBER$^+$ bystander B-cells in the background. Two cases only were examined for LMP-1 and both showed LMP-1$^+$ reaction in the HRS-like cells. Table 2 summarizes the clinicopathological features of PTCL-NOS patients.

Molecular Features

PCR studies for TCR-γ and IgH gene rearrangement were performed in 28 cases (11 AITL, 11 PTCL-TFH and 6 PTCL-NOS). Clonal TCR-γ gene rearrangement was detected in 16 cases and clonal IgH gene rearrangement in two cases. No significant difference was found regarding OS and PFS between cases with clonal TCR-γ gene rearrangement and other cases lacking clonal TCR-γ gene rearrangement ($P = 0.937$ and $P = 0.689$, respectively).

PD-L1 Expression

All 30 cases were negative for PD-L1 expression on both tumor cells and HRS-like cells. Only the scattered histiocytes in the background were positive for PD-L1.

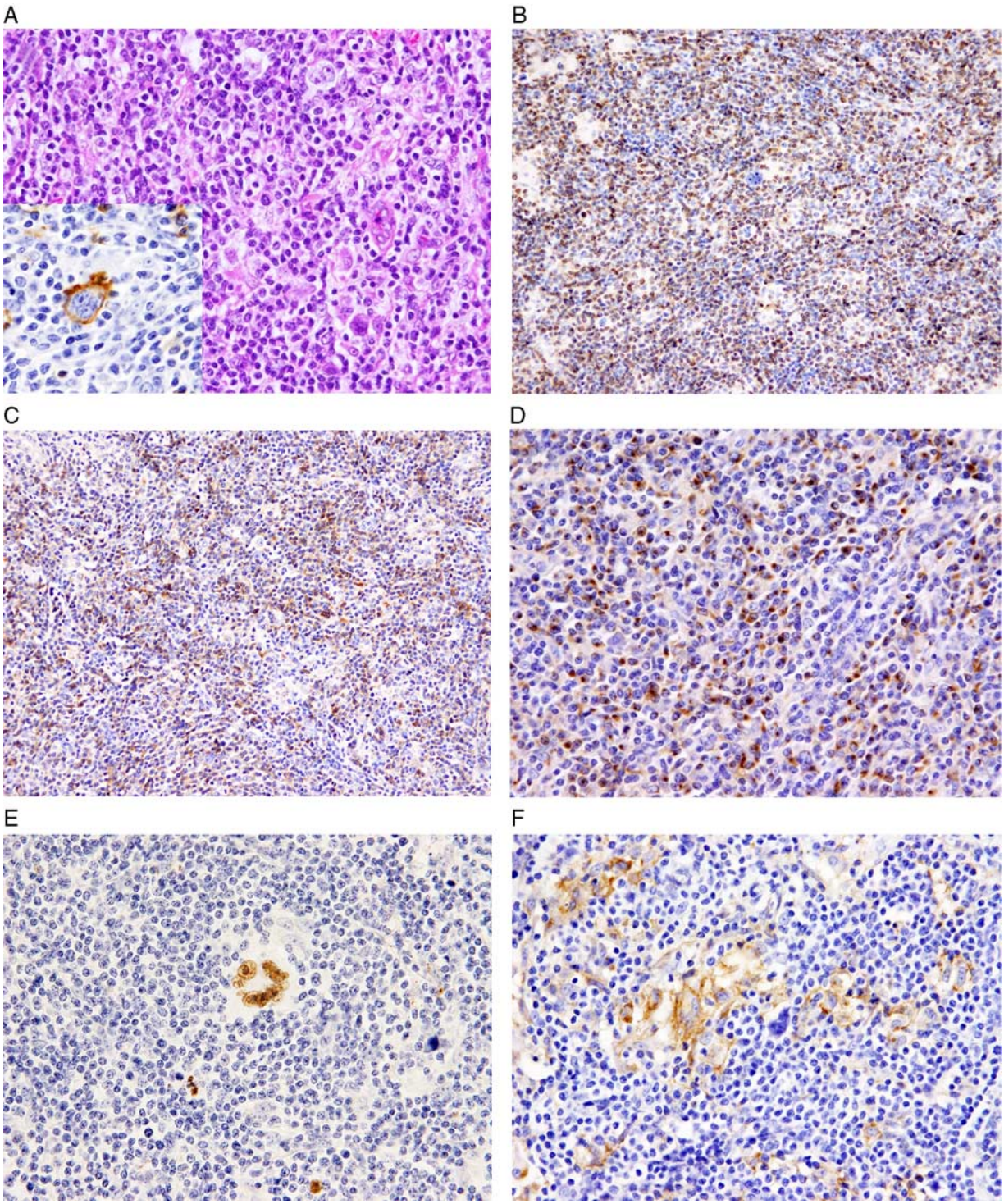

FIGURE 1. Angioimmunoblastic T-cell lymphoma. (A) Hematoxylin and eosin stain (400×) with CD20$^+$ Hodgkin and Reed-Sternberg (HRS)-like cells (inset). (B) Tumor cells stained for CD3 (40×), (C) PD-1 (200×), and (D) CXCL-13 (400×). (E) HRS-like cells stained for EBER (400×). (F) PD-L1-positive reaction in scattered histiocytes and a negative reaction in both tumor cells and HRS-like cells (400×).

Therapeutic and Survival Data

Follow-up data were available for all cases. The median follow-up period was 22 months (range, 1-65 mo). Twenty-eight patients received the following treatments: cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in 17 patients; cyclophosphamide, pirarubicin,

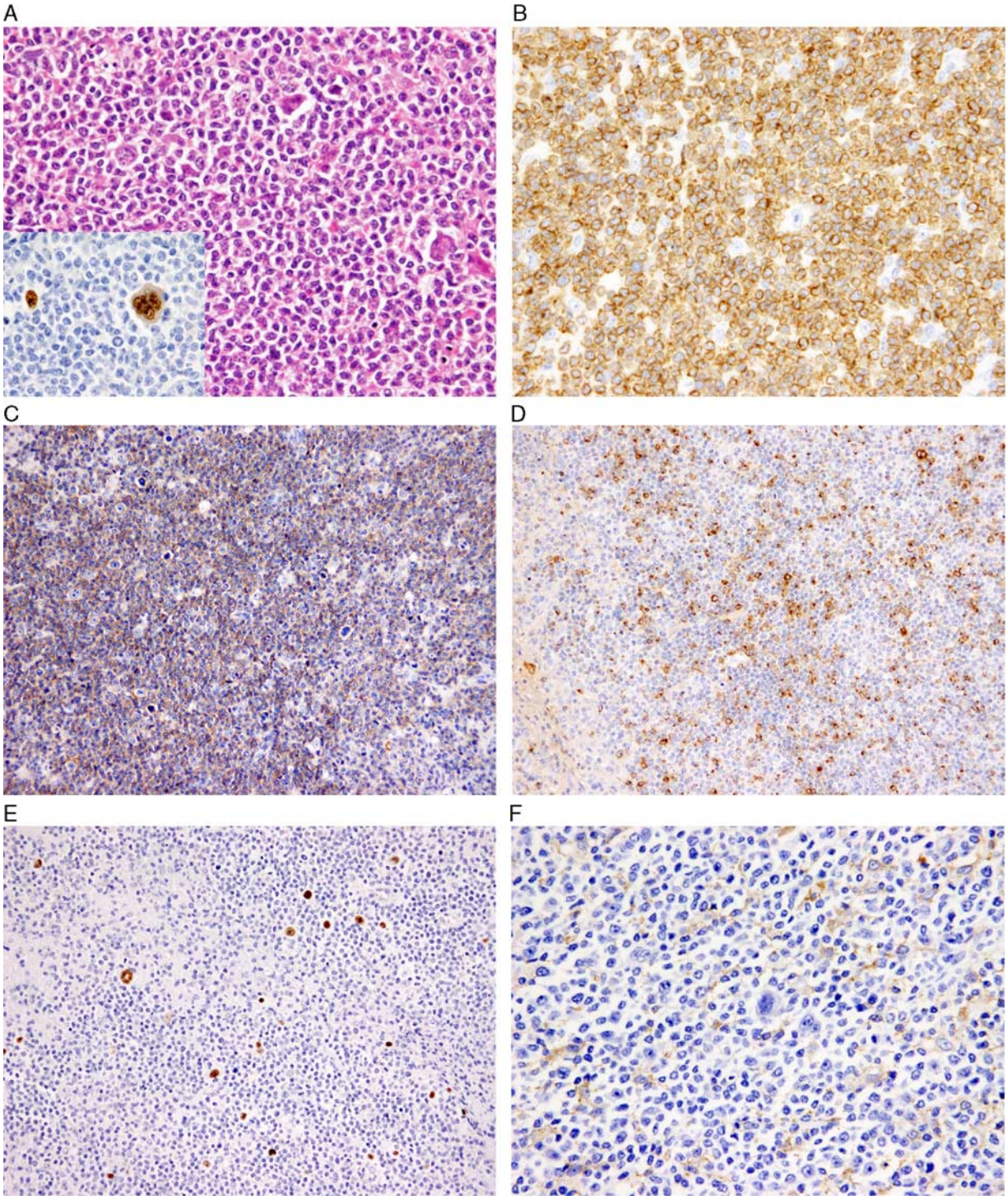

FIGURE 2. Peripheral T-cell lymphoma with T_{FH} phenotype. (A) Hematoxylin and eosin stain (400×) with PAX-5$^+$ Hodgkin and Reed-Sternberg (HRS)-like cells (inset). (B) Tumor cells stained for CD3 (40×), (C) PD-1 (200×), and (D) CXCL-13 (200×). (E) HRS-like cells stained for EBER (200×). (F) Faint PD-L1 reaction in the background and in occasional histiocytes and a negative reaction in both tumor cells and HRS-like cells (400×).

vincristine, and prednisone (THP-COP) in 5 patients; R (rituximab)-CHOP in 3 patients; doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) in 2 patients; and steroids in 1 patient. One patient received adjuvant radiotherapy and three patients underwent bone marrow stem cell transplantation. Complete remission (CR) was achieved

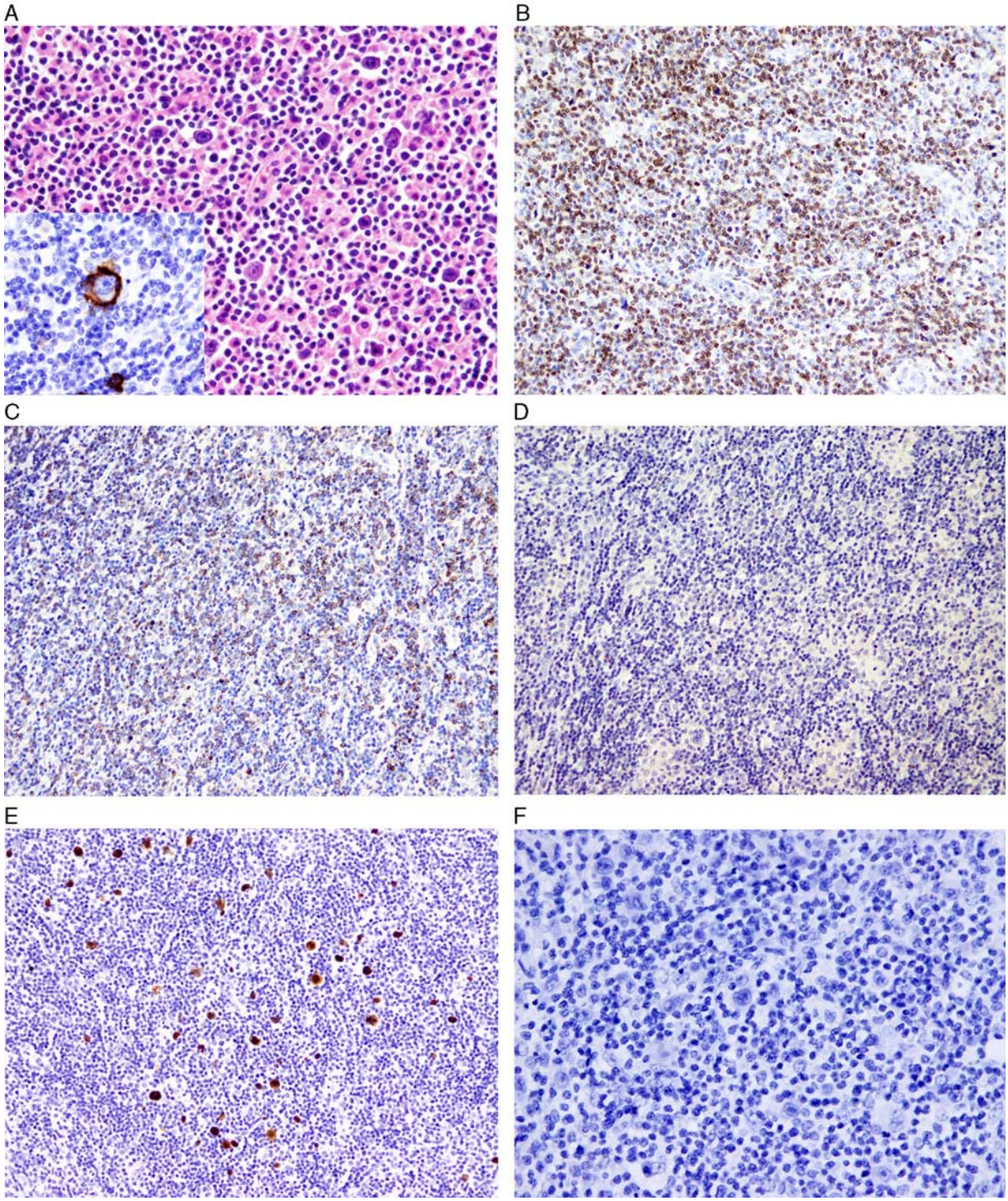

FIGURE 3. Peripheral T-cell lymphoma subset NOS. (A) Hematoxylin and eosin stain (400×) with $CD20^{+}$ Hodgkin and Reed-Sternberg (HRS)-like cells (inset). (B) Tumor cells stained for CD3 (200×) and (C) TIA-1 (200×), and (D) negative for PD-1 (200×). (E) HRS-like cells stained for EBER (200×). (F) EBER-negative HRS-like cells in another case with the same subset of disease (400×).

in 14 patients (50%), partial remission (PR) in 10 (35.7%), no response in 1 (3.6%) and progressive disease in 3 (10.7%). Eleven patients experienced relapse, and 18 died from the disease or its complications. One patient with PTCL-TFH relapsed as EBV^{-} diffuse large B-cell lymphoma (DLBCL) approximately 30 months after his first

TABLE 2. Clinical and Immunophenotypical Characteristics of PTCL-NOS with HRS-like Cells

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Sex/Age	M/71	M/68	M/55	F/77	M/72	M/82	F/61
Stage	IIIA	IVB	IA	IIIB	IVB	IIIA	IVA
IPI risk group	High-Intermediate	High-Intermediate	Low	High-Intermediate	High	Low-Intermediate	High-Intermediate
Appearance	Pleomorphic medium & large cells	Pleomorphic medium & large cells	Pleomorphic medium & large cells	Mixed cell pattern	Mixed cell pattern	Mixed cell pattern	Mixed cell pattern
Disease site	Cervical, supraclavicular, axillary, mesenteric, liver-hilar, para-aortic LN, tonsil	Cervical, supraclavicular, axillary, para-aortic, pelvic, inguinal LN, bone marrow	Pelvic LN	Cervical, supraclavicular, axillary, para-aortic, retroperitoneal, pelvic, inguinal LN	Cervical, supraclavicular, lung-hilar, liver-hilar, para-aortic LN, bone marrow, spleen	Cervical, liver-hilar, para-aortic LN	Para-aortic, pelvic, inguinal LN, skin
Tumor cells							
CD3	+	+	+	+	+	+	+
CD4	−	−	−	−	+	−	+
CD8	−	+	−	−	−	−	−
CD5	NA	−	+	−	NA	+	+
CD10	−	−	−	−	NA	−	NA
CD30	−	−	−	−	−	−	−
PD-1	−	−	−	−	−	−	−
CCXL-13	−	−	−	−	−	−	−
TIA-1	+	+	−	+	−	−	−
Granz. B	NA	+	−	−	−	−	−
Perforin	+	+	NA	+	NA	NA	NA
CCR4	NA	NA	+	NA	−	−	−
TCR-β	−	−	+	−	+	−	+
HRS-like cells							
CD20	−	+	+	+	+	+	−
PAX-5	+	NA	NA	NA	+	+	+
CD30	+	+	+	+	+	−	+
CD15	+	+	−	−	−	NA	−
EBER	−*	+	−	−*	+	+	−
Treatment	CHOP	CHOP	R-CHOP	THP-COP	CHOP + Radiotherapy	No treatment	CHOP
Clinical Course	Relapsed twice then DOD, 22 mo	Alive without disease, 65 mo	Relapsed once then DOD, 52 mo	Alive without disease, 62 mo	Relapsed once then DOD, 23 mo	Alive with disease, 12 mo	Allogenic BM stem cell transplantation, Alive without disease, 18 mo

*These two cases contain few scattered EBER+ bystander small B-cells in the background.

DOD indicates died of disease; F, female; HRS, Hodgkin and Reed-Sternberg; IPI, International Prognostic Index; LN, lymph node; M, male; NA, not available; PTCL-NOS, Peripheral T-cell lymphoma, not otherwise specified; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; THP-COP, cyclophosphamide, pirarubicin, vincristine, and prednisone.

diagnosis. He declined second-line medical treatment and died 1 year later. No clonal TCR-γ or IgH gene rearrangement was detected in this case. The 3-year OS and PFS rates were 44% and 27%, respectively.

Clinicopathological Features of the Three Groups

Expression of T_{FH}-related antigens was detected in 23 cases (12 AITL and 11 PTCL cases with HRS-like cells). Table 3 compares the AITL and PTCL-TFH cases. Both groups had overlapping clinicopathological features with no significant differences and tended to present in elderly patients, most with an advanced clinical stage. The incidence of high IPI and PIT scores was also comparable in both groups, as were their laboratory findings.

Furthermore, in a comparison of the 23 cases with T_{FH} phenotype and the 7 PTCL-NOS cases, no significant clinicopathological differences were found. Both groups tend to affect older patients and to present with an advanced clinical stage. However, patients with PTCL-NOS tended to have elevated serum LDH levels at the time of diagnosis compared to patients with lymphoma with T_{FH} phenotype, but the difference was not significant (Table 3). The OS and PFS curves demonstrated no significant differences among the three groups (Fig. 4A and B).

EBV Status and its Clinical Impact

HRS-like cells expressed EBER in most of the cases (67%) in this study. In order to investigate the clinical significance of HRS-like cells harboring EBV, we com-

TABLE 3. Clinical Characteristics of Different PTCL Groups

Variable	AITL with HRS Cells (n = 12)	PTCL-TFH with HRS-like Cells (n = 11)	*P* value	Both AITL and PTCL-TFH with HRS-like Cells (n = 23)	PTCL-NOS (n = 7)	*P* value
Age (y), median (range)	78 (39-87)	76 (49-91)	0.902*	78 (39-91)	71 (55-82)	0.302*
Age >60 y	8/12 (67%)	10/11 (91%)	0.317	18/23 (78%)	6/7 (86%)	1.000
Sex, Male	5/12 (42%)	8/11 (73%)	0.214	13/23 (57%)	5/7 (71%)	0.669
Bulky disease	0/12 (0%)	1/11 (9%)	0.478	1/23 (4.3%)	1/7 (14%)	0.418
Involvement of extranodal sites >1	3/12 (25%)	5/11 (46%)	0.400	8/23 (35%)	1/7 (14%)	0.393
Stage III/IV	11/12 (92%)	10/11 (91%)	1.000	21/23 (91%)	6/7 (86%)	1.000
B-symptoms	6/12 (50%)	3/11 (27%)	0.400	9/23 (39%)	3/7 (43%)	1.000
Performance status >1	5/12 (42%)	2/11 (18%)	0.371	7/23 (30%)	0/7 (0%)	0.154
IPI (HI/H)	5/12 (42%)	6/11 (55%)	0.684	11/23 (48%)	5/7 (71%)	0.399
PIT (groups 3 & 4)	5/12 (42%)	5/11 (46%)	1.000	10/23 (44%)	5/7 (71%)	0.390
WBC >10,000/mm^3	4/12 (33%)	4/11 (36%)	1.000	8/23 (35%)	1/7 (14%)	0.393
Hemoglobin <10.5 g/dL	4/12 (33%)	3/11 (27%)	1.000	7/23 (30%)	2/7 (29%)	1.000
Platelets <150,000/mm^3	1/12 (8%)	4/11 (36%)	0.155	5/23 (22%)	3/7 (43%)	0.345
Serum Albumin level <3.5 g/dL	4/12 (33%)	6/11 (55%)	0.414	10/23 (44%)	1/7 (14%)	0.215
Elevated serum LDH level	2/12 (17%)	4/11 (36%)	0.371	6/23 (26%)	5/7 (71%)	0.068
sIL-2R >4000 U/mL	5/11 (46%)	4/11 (36%)	1.000	9/22 (41%)	2/6 (33%)	1.000
CRP >2.00 mg/dL	3/12 (25%)	3/11 (27%)	1.000	6/23 (26%)	4/6 (67%)	0.143
EBV positivity	10/12 (83%)	10/11 (91%)	1.000	20/23 (87%)	5/7 (71%)	0.565
TCR-β phenotype	5/12 (42%)	6/11 (55%)	0.684	11/23 (48%)	3/7 (42.9%)	1.000

Fisher's exact test.
*Mann-Whitney test.
AITL indicates angioimmunoblastic T-cell lymphoma; CRP, C-reactive protein; EBV, Epstein-Barr virus; H, High; HI, High-Intermediate; HRS, Hodgkin and Reed-Sternberg; IPI, International Prognostic Index; LDH, lactate dehydrogenase; NOS, not otherwise specified; PIT, Prognostic Index of T-cell Lymphoma; PTCL-TFH peripheral T-cell lymphoma with follicular helper phenotype; sIL-2R, soluble IL-2 receptor; TCR-β, T-cell receptor-β; WBC, white blood cell.

pared EBER$^+$ and EBER$^-$ cases (Table 4). PTCL cases with EBER$^+$ HRS-like cells exhibited male predominance and tended to be associated with more than one extranodal site affected and B-symptoms, but without reaching significance. Other clinical parameters and laboratory data were comparable between the two groups. Regarding survival data, patients with EBER$^+$ HRS-like cells had inferior OS and PFS compared to those with EBER$^-$ HRS-like cells, but with no statistical significance ($P = 0.235$ and $P = 0.108$, respectively; Fig. 4C and D).

DISCUSSION

T/NK-cell neoplasms represent approximately 25% of all lymphoid neoplasms in Japan, with AITL and PTCL-NOS accounting for roughly 50% of these neoplasms.[17] The occasional presence of HRS-like cells of B-cell origin in PTCL was described previously, especially in AITL.[11–14] These cases should be distinguished from other subgroups of mature T-cell lymphoma that present HRS-like cells of T-cell origin.[18,19] The presence of B-cell proliferation in PTCL, which is mostly associated with EBV reactivation, is thought to be a secondary phenomenon due to a dysfunction in the patients' immune surveillance.[8] The derivation of tumor cells from T_{FH} cells in many cases could also explain their potential role in promoting the expansion of this secondary B-cell population.[20] The presence of the EBV oncoproteins was suggested to be a major contributor to the phenotypic alteration of these B-cells towards the HRS-like phenotype.[21]

In this study, we highlighted the presence of HRS-like cells of B-cell origin in a few PTCL subgroups among Japanese patients. Interestingly, the median ages of onset appeared to be much older than in those without HRS-like cells.[22–26] Many of the cases expressed T_{FH} phenotype on tumor cells and were associated with EBV reactivation as previously documented.[11–14] In our series, EBER$^+$ bystander B-cells were detected in 25 cases (83%), but EBV harboring was detected on HRS-like cells in 20 (67%) of these cases. Nicolae, et al[14] recently described the occasional occurrence of EBER$^-$ HRS-like cells in PTCL with T_{FH} phenotype. In their study, EBER$^+$ HRS-like cells were found in 52/57 (91%) of PTCL cases. The remaining cases (3 AITL and 2 PTCL, follicular variant) were accompanied with EBER$^-$ HRS-like cells, and with EBER$^+$ bystander B-cells in 2 of them. Previous Japanese and Western studies detected EBV in 66-76% and 40-57% of AITL and PTCL-NOS cases, respectively.[22–24,27,28] We considered that the higher incidence of EBV reactivation in our series (83% in AITL and 71% in PTCL-NOS) may be due to the exclusive inclusion of cases with HRS-like cells.

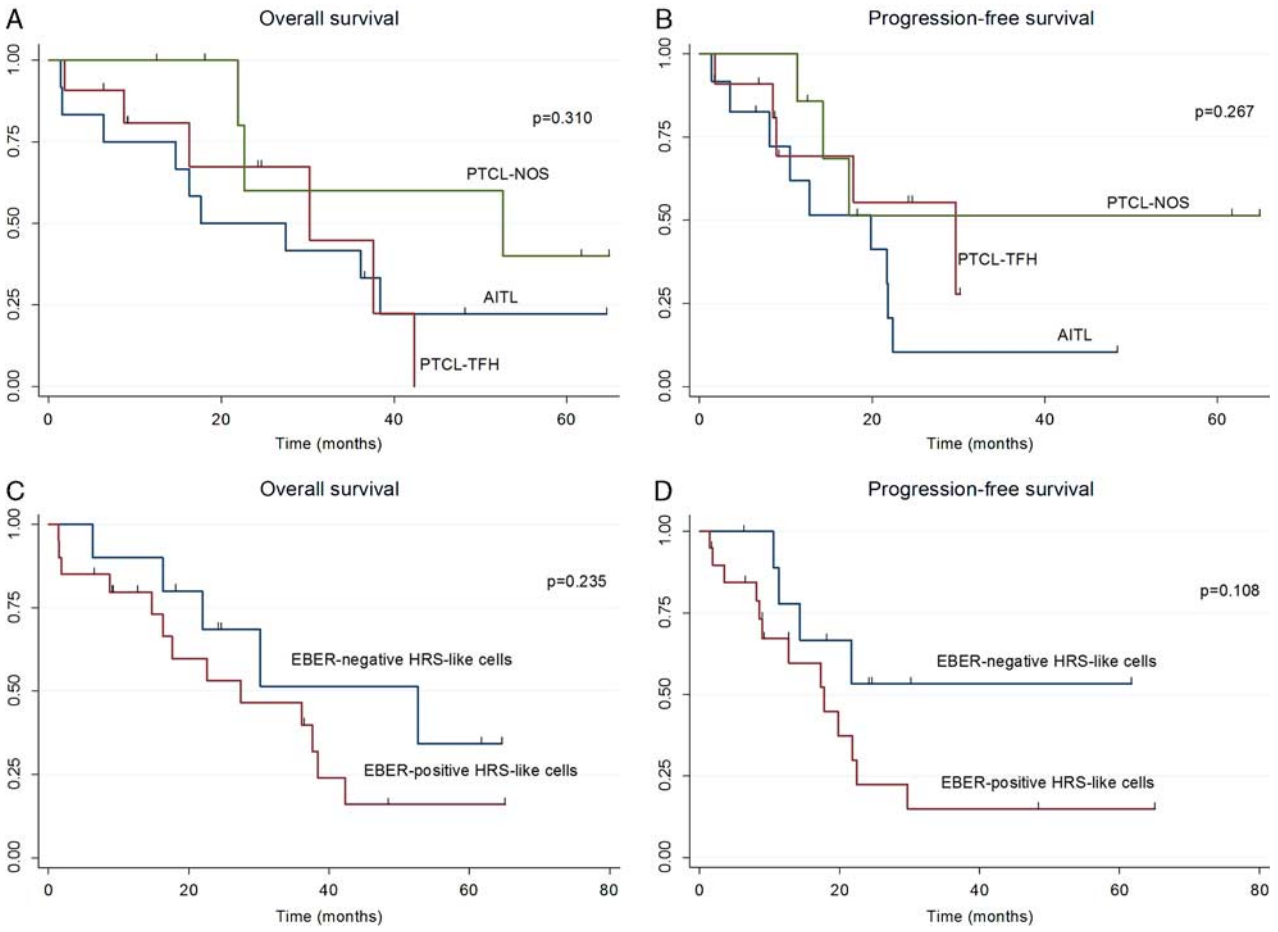

FIGURE 4. Survival curves for three subgroups of peripheral T-cell lymphoma (PTCL) with Hodgkin and Reed-Sternberg (HRS)-like cells. (A) Overall survival (OS). (B) Progression-free survival (PFS). (C and D) OS and PFS curves according to the EBER status of the HRS-like cells.

The pathogenesis of HRS-like cells in PTCL is still unclear. Regarding with EBER$^+$ HRS-like cells, Vockerodt et al[21] reported that EBV oncoprotein, LMP-1, reprograms germinal center B-cells towards a HRS-like phenotype. Nicolae A, et al[14] suggested that HRS-like cells, especially when EBER$^-$, may contribute to an aberrant role of T_{FH} cells surpassing their normal function in supporting and promoting B-cell differentiation in germinal centers. For these EBER$^-$ HRS-like cases, another hypothesis was postulated based on the inconsistent presence of EBV infection through the disease course that the virus may persist or disappear during disease progression.[14]

EBV is a widespread lymphotropic virus infecting >90% of adults worldwide with life-long persistent infection.[29,30] Its association with AITL and a subset of PTCL-NOS has been discussed previously, but the prognostic significance of EBER$^+$ HRS-like cells in PTCL has not been addressed. According to our data, the EBER status of HRS-like cells did not significantly influence the clinical parameters of the PTCL cases, whereas patients with EBER$^+$ HRS-like cells tended to have more than one affected extranodal site and B-symptoms. Moreover, no significant difference in OS or PFS was found, though PTCL cases with EBER$^+$ HRS-like cells had inferior curves compared to cases with EBER$^-$ HRS-like cells. These findings appear to be in line with previous studies indicating that EBV status does not influence the survival of AITL patients.[31,32]

In contrast to the well-defined entity of AITL, which is known to be derived from T_{FH} cells,[33,34] PTCL-NOS represents a heterogeneous group of T-cell lymphomas with different morphological, clinical and molecular features.[1] Recently, another subgroup of PTCL-NOS with T_{FH} phenotype besides the follicular variant was recognized, which showed overlapping features with AITL and may represent a stage in the spectrum of AITL.[2–5] This overlap between both groups was also demonstrated in this study. According to our data, both AITL and PTCL-TFH with HRS-like cells have an elderly onset (78 and 76 y, respectively), and they have similar clinical parameters, including staging, IPI and PIT scores, and other laboratory data. Indeed, patients of both groups showed an overlapping OS and PFS curves. These findings provide

TABLE 4. Clinical Characteristics According to EBV-Status of HRS-like Cells

Variable	EBER-positive HRS-like Cells (n = 20)	EBER-negative HRS-like Cells (n = 10)	*P* value
Age (y), median (range)	77 (39-91)	76 (48-87)	0.659*
Sex, Male	15/20 (75%)	3/10 (30%)	0.045
Involvement of extranodal sites > 1	8/20 (40%)	1/10 (10%)	0.204
Stage III/IV	18/20 (90%)	9/10 (00%)	1.000
B-symptoms	10/20 (50%)	2/10 (20%)	0.235
Performance status > 1	5/20 (25%)	2/10 (20%)	1.000
IPI (HI/H)	11/20 (55%)	5/10 (50%)	1.000
PIT (groups 3 & 4)	10/20 (50%)	5/10 (50%)	1.000
WBC > 10,000/mm^3	6/20 (30%)	3/10 (30%)	1.000
Hemoglobin < 10.5 g/dL	8/20 (40%)	1/10 (10%)	0.204
Platelets < 150,000/mm^3	7/20 (35%)	1/10 (10%)	0.210
Serum Albumin level < 3.5 g/dL	8/20 (40%)	3/10 (30%)	0.702
Elevated serum LDH level	7/20 (35%)	4/10 (40%)	1.000
sIL-2R > 4000 U/mL	7/18 (39%)	4/10 (40%)	1.000
CRP > 2.00 mg/dL	6/19 (32%)	4/10 (40%)	0.698
CR response	8/18 (44%)	6/10 (60%)	0.695
OS			0.235†
PFS			0.108†

Fisher's exact test.
*Mann-Whitney test.
†Log-Rank test.
CR indicates complete remission; CRP, C-reactive protein; EBV, Epstein-Barr virus; H, High; HI, High-Intermediate; HRS, Hodgkin and Reed-Sternberg; IPI, International Prognostic Index; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival; PIT, Prognostic Index of T-cell Lymphoma; sIL-2R, soluble IL-2 receptor; WBC, white blood cell.

additional support for the assertion that AITL and PTCL-TFH constitute a continuous spectrum of disease, even among cases with HRS-like cells.

In this study, seven cases of PTCL-NOS with HRS-like cells were identified; three expressed CM and one expressed the T-cell regulatory marker CCR4. Our group recently documented a series of nodal high grade EBV$^+$ CM$^+$ PTCL with an aggressive clinical course[35] and another series of PTCL with T-cell regulatory phenotype which featured a frequent occurrence of EBER$^+$ bystander B-cells.[36] However, both of these series had no cases with HRS-like cells. In this study, an EBER$^+$ reaction was detected in 5/7 PTCL-NOS cases (71%), compared to 20/23 (87%) AITL and PTCL-TFH cases. Clinically, no significant differences were detected between PTCL-NOS patients and other patients who expressed the T_{FH} phenotype on their tumor cells, except for the tendency of the former group to present with more elevated serum LDH levels. In this study, PTCL-NOS cases had a similar prognosis as AITL and PTCL-TFH cases. Of course, it is too soon to draw any definite conclusion because of the limited number of enrolled cases. Further studies are needed to validate our findings.

The presence of secondary monoclonal B-cell proliferation, which is frequently EBV-related, has been reported in AITL and PTCL-NOS cases, with subsequent progression to DLBCL in a fraction of cases.[7–10] In this study, monoclonal rearrangement of the IgH gene was detected in two of 28 examined cases. The remaining cases showed polyclonal rearrangement including the one case with subsequent progression to EBV$^-$ DLBCL. In this study, the presence of HRS-like cells did not seem to reflect a higher incidence of progression to B-cell lymphoma, but the absence of follow-up biopsy in most relapsed cases prevents us from reaching a definite conclusion.

The interaction between the lymphoma cells and the surrounding microenvironment plays an important role in determining the biological and clinical behaviour of malignant lymphoma.[37,38] Recently, the interaction between PD-1 and its ligand PD-L1 attracted the attention for its role in suppressing T-cell immunity in cases of cHL and other virus-associated lymphomas, providing a potential tool for immunotherapy.[39,40] We investigated PD-L1 expression in all cases but none of the tumor cells and HRS-like cells were positive. The significance of this negative result is unclear.

In summary, we reported 30 cases of PTCL with HRS-like cells of B-cell lineage from Japan. Many of these cases expressed the T_{FH} phenotype and were associated with EBV reactivation. They had a tendency to affect elderly patients and to be associated with advanced clinical stages and dismal prognosis, but the presence of HRS-like cells was not necessarily an indication for a subsequent development of secondary B-cell lymphoma or adverse prognosis. Further studies should focus on this rare phenomenon in order to elucidate its pathogenesis and significance. Furthermore, in routine practice, careful attention should be paid to lymphoma cases with HRS-like cells in order to avoid misclassifications.

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