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Clinical outcome of Epstein–Barr virus-positive diffuse large B-cell lymphoma of the elderly in the rituximab era

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Key words

Diffuse large B-cell lymphoma, Epstein–Barr virus (EBV), prognosis

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Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of malignant lymphoma. The incidence of Epstein–Barr virus (EBV)-positive DLBCL in Asian and Latin American countries ranges from 8 to 10%. The prognosis of patients with EBV-positive DLBCL is controversial. To compare the clinical outcome of EBV-positive and EBV-negative patients with DLBCL in the rituximab era, we analyzed 239 patients with *de novo* DLBCL diagnosed between January 2007 and December 2011. The presence of EBV in lymphoma cells was detected using EBV-encoded RNA *in situ* hybridization, and it was found that 18 (6.9%) of 260 patients with diagnosed DLBCL tested positive. Among the 260 cases, 216 cases were treated with rituximab plus chemotherapy, as were 8 EBV-positive DLBCL patients. The median overall survival and progression-free survival times in patients with EBV-positive DLBCL were 8.7 months and 6.8 months, respectively. The median overall survival and progression-free survival could not be determined in EBV-negative DLBCL patients ($P = 0.0002$, $P < 0.0001$, respectively). The outcome of patients with EBV-positive DLBCL remains poor, even in the rituximab era.

Diffuse large B-cell lymphoma is the most common subtype of malignant lymphoma and accounts for 33% of all cases of malignant lymphoma in Japan.[1] Diffuse large B-cell lymphoma usually arises *de novo* in lymph nodes, but can also be derived from extranodal organs. The WHO classification describes various special types of DLBCL, and DLBCLs harboring EBV in patients older than 50 years are termed EBV-positive DLBCL of the elderly (EBV-DLBCL of the elderly) as a new category.[2,3] The EBV-DLBCL of the elderly category accounts for 8–10% of all DLBCL in Asian countries,[4] but <5% in Western countries.[5,6]

Epstein–Barr virus is the most common gamma herpes virus, and it has infected more than 90% of all adults. Most people are infected subclinically in childhood and maintain a latent infection throughout their life. During the process of infection, EBV attaches to B cells through the binding of viral gp350 protein to CD21 on the surface of B cells. Then, gp42 on EBV interacts with MHC class II molecules and triggers fusion with the host membrane.[7] The EBV is reactivated by various stimuli. Epstein–Barr virus-infected B cells are usually controlled by EBV-specific T cells, but they become uncontrolled when the host is immunodeficient. B cells infected with EBV sometimes become lymphoblastoid cell lines and obtain an unlimited ability to proliferate. Lymphoblastoid cell lines cause some lymphoid malignancies, including Burkitt lymphoma, extranodal natural killer/T-cell lymphoma, aggressive natural killer leukemia/lymphoma, angioimmunoblastic T-cell lymphoma, Hodgkin's lymphoma, immunodeficiency-associated lymphoproliferative disorders, and some DLBCLs.[8]

The standard treatment for DLBCL before the rituximab era was chemotherapy combined with CHOP. Since the introduction of rituximab into the clinic, R-CHOP has become the standard treatment for CD20-positive DLBCL.[9,10] The outcome of DLBCL patients is improved with R-CHOP, but the impact on the prognosis of EBV-positive DLBCL patients remains controversial.[11–15]

We investigated the clinical features of patients with EBV-positive DLBCL and showed that the outcome of elderly patients with EBV-positive DLBCL treated with R-CHOP was still worse than other groups in this study.

Materials and Methods

Patients. We reviewed the medical records of 289 patients who received a diagnosis of DLBCL at Tokai University Hospital (Isehara, Japan) and who were treated there and at affiliated hospitals between January 2007 and December 2011.

Table 1. Details of patients with diffuse large B-cell lymphoma (DLBCL) who were excluded from this analysis

	EBV-positive DLBCL	EBV-negative DLBCL
Total patients	18	242
Primary CNS DLBCL†	0	4
Immunodeficiency	3	2
Methotrexate	2	2
HIV infection	1	0
Unknown	1	11
No. of patients analyzed in this study	14	225

†Patients with primary central nervous system (CNS) DLBCL were excluded from analysis because rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone was not a treatment option. EBV, Epstein–Barr virus.

Among 289 patients, 29 patients were excluded because no paraffin-embedded samples were available. Therefore, 260 cases were examined for the presence of EBV using formalin-fixed paraffin-embedded tissue sections.

A suitably constituted Ethics Committee of our institution approved the protocol for this research project, and the work was carried out according to this protocol. Our study conformed to the provisions of the Declaration of Helsinki in 1995.

Epstein–Barr virus-encoded RNA *in situ* hybridization and IHC. Epstein–Barr virus-encoded RNA *in situ* hybridization was carried out using a fluorescein-conjugated EBER oligonucleotide probe and the purified IgG fraction of a mouse monoclonal anti-fluorescein antibody (Leica, Newcastle, UK). For IHC, mouse mAbs against CD3, CD5, CD10, CD15, CD20, CD79a, BCL-2, BCL-6, and MUM-1 (Novocastra, Newcastle upon Tyne, UK), and CD30 (Clone CON6D; Spanish National Cancer Research Centre (CNIO), Madrid, Spain) were used as primary antibodies. Detection of signals for EBER-ISH and IHC was carried out using the Leica BOND-MAX fully automatic IHC system with the BOND Polymer Refine detection kit according to the manufacturer's instructions using BOND Epitope Retrieval Solution for 20 min for antigen retrieval (DS9800 and AR9640; Leica Microsystems, Tokyo, Japan). For EBER-ISH-positive cases, LMP-1 (Novocastra) and EBNA-2 antibody (Novocastra) were examined with IHC.

When more than 30% of large-sized cells were positive, the case was deemed "EBV-positive". The DLBCL subtypes of GCB or non-GCB were categorized using CD10, BCL-6, and MUM-1 according to Hans' algorithm.[16] Cases that were unavailable for BCL-6 were categorized using CD10 and MUM-1 according to Chang's algorithm.[17] Epstein–Barr virus latency was classified as: latency I, LMP-1(−) EBNA-2(−); latency II, LMP-1(+) EBNA-2(−); and latency III, LMP-1(+) EBNA-2(+).

Clinical characteristics and statistical methods. Comparisons of characteristics between EBV-positive and EBV-negative cases were examined with Fisher's exact test or the non-parametric Mann–Whitney *U*-test, as appropriate. Tumor responses were assessed with computed tomography and PET. Patients were classified by the best tumor response according to the response criteria for malignant lymphoma.[18] Overall survival was defined as the duration from the date of diagnosis of DLBCL to the date of death of any cause. Progression-free survival was defined as the duration from the date of diagnosis to the date of progressive or relapsed disease. The OS and PFS probabilities were estimated using the Kaplan–Meier method, and patients who were alive at the last follow-up were censored. The log–rank test was used to compare pairs of subgroups regarding survival. Multivariate analyses were carried out using Cox's proportional hazards regression analysis. Statistical analyses were carried out using the GraphPad Prism 6.0 (GraphPad Software, San Diego, CA) and EZR version 3.0.2.[19]

Results

Patient selection, EBER-ISH, and IHC. The EBER-ISH analysis showed 18 cases of DLBCL that harbored EBV among the 260 cases examined (6.9%). Among these 260 cases, 21 cases were

Table 2. Summary of clinical data of patients with Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) (*n* = 14) and EBV-negative DLBCL (*n* = 225)

Variable	EBV-positive DLBCL (*n* = 14)	EBV-negative DLBCL (*n* = 225)	*P*-value
Age, years, median (range)	71.5 (55–84)	68.0 (22–92)	0.3379‡
Gender (male/female)	8/6	122/103	1.0000†
	No. of cases (%)	No. of cases (%)	*P*-value
Over 60 years of age	11 (78.6)	170 (75.6)	1.0000†
ECOG PS 2–4	6 (43.9)	38/214 (17.8)	0.0223†
Ann Arbor stage III/IV	9 (64.3)	114/216 (52.8)	0.5819†
B symptoms, presence	6 (43.9)	57/208 (27.4)	0.1067†
Extranodal involvement (>1 site)	12 (85.7)	121/204 (59.3)	0.0856†
IPI, High intermediate/High	9 (64.3)	96/202 (47.5)	0.2749†
LDH, IU/L, median (range)	339.5 (154–1798)	262.0 (132–5310)	0.1803‡
LDH ≥ facility upper limit of normal	11 (78.6)	135 (60.0)	0.2580†
IL2R, U/mL, median (range)	2740 (374–6780)	1300 (164–68 800)	0.1146‡
IL2R ≥1000 U/mL	10 (71.4)	128/219 (58.4)	0.2501†
IgG, mg/dL, median (range)	1501 (561–2510)	1275 (300–3644)	0.3785‡
IgA, mg/dL, median (range)	226 (128–1473)	251 (33–952)	0.8541‡
IgM, mg/dL, median (range)	78 (20–176)	72 (8–1203)	0.9227‡
Pathological subtype			
GCB type	1 (8.3)	54 (25.0)	0.3021†
Activated B-cell (non-GCB) type	11 (91.7)	162 (75.0)	
NA†	2	9	

†Fisher's exact test. ‡Mann–Whitney *U*-test. ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B cell; IL2R, interleukin 2 receptor; IPI, international prognostic index; LDH, lactate dehydrogenase; NA, not available; PS, performance status.

excluded from analysis in this study for the following reasons: 1 case with HIV infection (EBV-positive); 4 cases with a history of methotrexate (EBV-positive, 2 cases; EBV-negative, 2 cases); 4 cases with primary large B-cell lymphoma of the central nervous system (EBV-negative); and 12 cases in which the clinical records were unavailable (EBV-positive, 1 case; EBV-negative, 11 cases). Finally, we analyzed 239 patients that included 14 cases of EBV-positive DLBCL and 225 cases of EBV-negative DLBCL, resulting in an EBV-positive rate of 6.0% (Table 1). Because all EBV-positive DLBCL patients were older than 50 years, they satisfied the criteria of EBV-DLBCL of the elderly.

Clinical data are summarized in Table 2. The median age was 71.5 years in EBV-positive patients and 68.0 years in EBV-negative patients ($P = 0.3379$). The percentages of patients aged over 60 years were 78.6% for EBV-positive and 75.6% for EBV-negative patients ($P = 1.0000$). The performance status was inferior in EBV-positive patients; the incidence of a performance status >2 in EBV-positive patients was higher than that in EBV-negative patients (43.9% *vs* 17.8%, respectively; $P = 0.0223$). Extranodal disease affecting more than two organs was found in 12/14 EBV-positive cases (85.7%) and 121/204 EBV-negative cases (59.3%) ($P = 0.0856$). Eleven out of 12 EBV-positive cases were non-GCB types (91.7%). In EBV-negative cases, GCB and non-GCB types were found in 54 patients (25.0%) and 162 patients (75.0%), respectively. In EBV-positive DLBCL, seven patients showed latency II and four showed latency III.

Table 3. Summary of therapy and treatment responses in patients with Epstein–Barr virus (EBV)-positive and EBV-negative diffuse large B-cell lymphoma (DLBCL)

	EBV-positive DLBCL	EBV-negative DLBCL	*P*-value
Immunocompetent	14	225	
No treatment	3	11	
Treatment	11	214	
Chemotherapy, no rituximab	3	8	
Radiation	0	3	
Rituximab only	0	5	
R plus chemotherapy	8	198	
R-CHOP	8	160	
R-CHOP-like	0	38	
R-COP	0	16	
R-THP-COP	0	14	
R-CHO	0	4	
R-CHP	0	2	
R-CO	0	2	
No. of chemotherapy cycles, median (range)	4.5 (1–8)	6 (1–8)	0.0201†
Response			
CR	2 (25.0%)	147 (74.2%)	0.0060‡
PR	2 (25.0%)	19 (9.6%)	
SD or PD	4 (50.0%)	29 (14.6%)	
NA	0	3	

†Mann–Whitney *U*-test. ‡χ2-test. C, cyclophospahmide; CR, complete remission; H, doxorubicin; NA, not available; O, vincristine; P, prednisolone; PD, progressive disease; PR, partial response; R, rituximab; SD, stable disease; THP, pirarubicin.

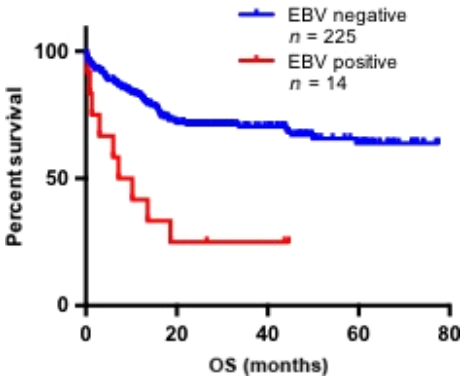

Fig. 1. Overall survival (OS) in immunocompetent Epstein–Barr virus (EBV)-positive versus EBV-negative patients with diffuse large B-cell lymphoma. The median OS in EBV-positive patients was 8.7 months; OS could not be determined in EBV-negative patients. Hazard ratio = 3.9; 95% confidence interval, 4.0–49.3; $P < 0.0001$.

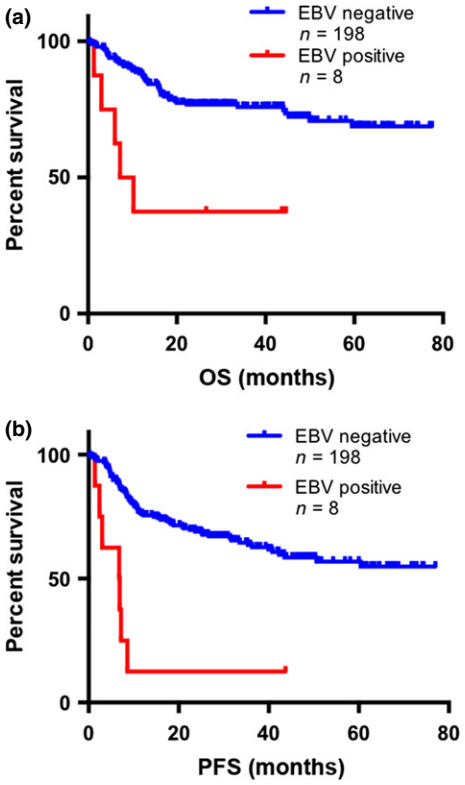

Fig. 2. Survival analysis in patients with diffuse large B-cell lymphoma (DLBCL) treated with chemotherapy regimens similar to rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone. (a) Overall survival (OS). The median OS in Epstein–Barr virus (EBV)-positive DLBCL patients was 8.7 months; OS could not be determined in EBV-negative patients. Hazard ratio = 4.3; 95% confidence interval, 3.6–121.6; $P = 0.0002$. (b) Progression-free survival (PFS). The median PFS in EBV-positive DLBCL patients was 6.8 months; median PFS could not be determined in EBV-negative patients. Hazard ratio = 5.6; 95% confidence interval, 13.0–384.6; $P < 0.0001$.

Treatment response. The various treatments are shown in Table 3. Both R-CHOP and R-CHOP-like regimens were used for chemotherapy.

The R-CHOP and R-CHOP-like regimens were given to 8/14 EBV-positive and 198/225 EBV-negative patients. The median number of R-CHOP cycles was 4 (range, 1–8) in

Table 4. Details of clinical data for patients with Epstein–Barr virus-positive diffuse large B-cell lymphoma (n = 14)

No.	Age, years/gender	PS	CS	IPI	Extranodal disease	CD20	CD15	CD30	EBER-ISH	LMP1	EBNA2	Latency	GCB/non-GCB	Morphological subtype	Others	LDH (IU/L)	IL2R (U/mL)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	Therapy	No. of cycles	Response	OS (months)	PFS (months)	Outcome
1	59/F	3	IVA	HI	Lung	++	−	+	++	++	−	II	Non-GCB	Poly	Necrosis	154	1250	1069	190	176	R-CHOP	5	CR	44.6	8.6	Relapsed, treated with Bendamustine, alive, 2nd CR
2	80/F	1	IIIB	HI	Skin, pleurae	+++	−	+	+++	+	−	II	Non-GCB	Large	Plasma differentiation	293	1990	1501	226	88	R-CHOP	4	PR	26.6	6.9	Progressive, treated with CEPP/VP16/rituximab, alive, 2nd CR
3	64/M	0	IIA	LI	Stomach	+++	−	−	++	++	−	II	Non-GCB	Poly	None	209	688	2510	396	78	R-CHO	6	CR	43.7	43.7	Alive, CR
4	78/F	0	IIA	LI	Intraoral ulcer	+++	−	−	+++	++	+	III	Non-GCB	Large	None	249	374	1279	238	119	R-CH (THP) OP	6	PD	10.2	6.7	Refractory to R-CPA-VP16/VP16/R-MIT-MCNU-VP16-Dex, died
5	77/F	1	IIIB	HI	Retroperitoneal tumor	+++	−	++	+++	+++	+	III	Non-GCB	Large	None	1677	3570	1421	149	52	R-CHOP	2	PD	6.0	2.4	Refractory to EPOCH/R-DeVIC, died
6	69/M	2	IIIB	LI	Liver	++	−	−	+	++	−	II	Non-GCB	Poly	HRS cell	219	6780	1573	1473	67	R-CHOP	3	PD	3.0	3.0	Sepsis, progressive disease, died
7	55/F	4	IIB	HI	Abdominal tumor (soft tissue)	+++	−	+++	+++	++	−	II	Non-GCB	Large	None	496	4520	634	174	71	R-CHOP	8	PR	7.2	7.2	Pneumonia, progressive disease, died
8	69/F	0	IA	LI	Intraoral ulcer	+++	−	−	+++	+++	+	III	Non-GCB	Large	None	363	1390	561	128	20	R-CHOP	1	PD	1.3	1.3	Pneumonia, alveolar hemorrhage, died
9	63/M	NA	IV*	H	None	++	−	++	+	++	−	II	Non-GCB	Poly	HRS cell	1798	9710	2001	189	113	No treatment	0	NA.	0.0	0.0	DIC, cerebral hemorrhage, died
10	76/M	4	IV*	H	Femur tumor	++	−	−	+++	++	+	III	Non-GCB	Poly	None	316	NA	NA	NA	NA	No treatment	0	NA.	0.4	0.4	Chose the best supportive care, died
11	79/M	0	IIA	LI	Left thoracic tumor, rib	−	NA	NA	+	NA	NA	NA	Non-GCB	Poly	None	370	952	2424	305	91	COP	8	PD	13.6	10.7	Relapse, chose the best supportive care after radiation, died
12	74/M	4	IVB	HI	Lung	−	+	+	+++	+	−	II	NA	Poly	Plasma differentiation	394	3530	1505	495	30	No treatment	0	NA.	0.0	0.0	Pneumonia by tumor invasion, died
13	59/M	4	IVA	H	Liver, small intestine	−	+	−	+++	NA	NA	NA	GCB	Large	None	768	12700	605	162	42	COP	1	PD	0.9	0.9	Progressive disease, died
14	84/M	1	IIIB	HI	None	++	−	+++	++	NA	NA	NA	NA	Poly	HRS cell	220	2740	2418	454	129	COP	6	CR	18.6	15.0	Relapse, chose the best supportive care, died

CEPP, cyclophosphamide, etoposide, procarbazine, prednisolone; CHO, cyclophosphamide, doxorubicin, vincristine; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; CPA, cyclophosphamide; CR, complete remission; CS, clinical stage; DeVIC, carboplatin, etoposide, interferon, dexamethasone, granulocyte-colony stimulating factor; Dex, dexamethasone; EBER-ISH, Epstein–Barr virus-encoded RNA *in situ* hybridization; EBNA, Epstein–Barr virus nuclear antigen antibody; EPOCH, rituximab, etoposide, doxorubicin, vincristine, cyclophosphamide, prednisolone; F, female; GCB, germinal center B cell; H, high; HI, high intermediate; IL2R, interleukin 2 receptor; IPI, international prognostic index; Large, large-cell type; LDH, lactate dehydrogenase; LI, low intermediate; LMP, latent membrane protein; M, male; MCNU, ranimustine; MIT, mitoxantrone; NA, not available; OS, overall survival; PD, progressive disease; PFS, progression-free survival; Poly, polymorphous type; PR, partial response; PS, performance status; R, rituximab; THP, pirarubicin; VP16, etoposide.

EBV-positive patients and 6 (range, 1–8) in EBV-negative patients. Treatment was discontinued for various reasons in 5/8 EBV-positive patients and 30/198 EBV-negative patients (P = 0.0201). Among EBV-positive patients, two patients died of infection in the nadir phase during chemotherapy, one patient discontinued treatment due to PD, and one patient refused to continue chemotherapy due to an adverse drug reaction. Two EBV-positive patients showed CR (25%), two showed partial response (25%), and four showed stable disease/PD (50%). In contrast, 147 EBV-negative patients showed CR (74.2%), 19 patients showed partial response (9.6%), and 29 patients showed stable disease/PD (14.6%). The overall response rate was better in EBV-negative than EBV-positive patients (P = 0.0060).

Survival. The median follow-up time of surviving patients was 25.2 months (range, 0.8–71.3 months). Median OS was 8.7 months in EBV-positive patients and was not reached in EBV-negative patients (P < 0.0001; Fig. 1). Three EBV-positive patients could not receive chemotherapy because their general condition was poor and disease progression was rapid.

Median OS and PFS were 8.7 and 6.8 months, respectively, in EBV-positive patients treated with R-CHOP/R-CHOP-like regimens. Both OS and PFS were worse in EBV-positive patients than in EBV-negative patients (P = 0.0002, P < 0.0001, respectively; Fig. 2). Among eight patients who received R-CHOP/R-CHOP-like regimens, four died without achieving CR. All three patients with latency III died <1 year after diagnosis. Two of them were resistant to chemotherapy. No difference in OS or PFS was found between latency III and latency II (Table 4). We also did not find a difference in OS or PFS between the polymorphous type versus the large-cell type in EBV-positive DLBCL (Table 4). Among EBV-negative

Table 5. Summary of risk factors for prognosis in patients with diffuse large B-cell lymphoma, using multivariable analysis (n = 14)

	Univariate analysis†		Multivariate analysis‡	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age				
≥60 years	2.0 (1.1–3.8)	0.02039	1.6 (0.8–3.3)	0.1911
EBV				
Positive	4.2 (2.1–8.2)	<0.00010	4.6 (1.8–11.8)	0.0014
PS				
2–4	5.6 (3.4–9.1)	<0.00010	3.3 (1.7–6.7)	0.0007
Clinical stage				
III–IV	3.2 (1.9–5.5)	<0.00010	1.5 (0.7–3.1)	0.2616
B symptoms				
Present	2.5 (1.5–4.0)	0.00040	1.0 (0.5–2.0)	0.9379
Extranodal disease				
≥1 site	2.7 (1.6–4.7)	0.00030	1.0 (0.5–2.3)	0.9359
LDH levels				
≥Facility upper limit of normal	4.2 (2.6–6.7)	<0.00010	3.3 (1.4–7.7)	0.0070
IL2R levels				
≥1000 U/mL	4.2 (2.3–7.7)	<0.00010	1.2 (0.5–2.8)	0.6597
Subtype				
Non-GCB	2.2 (1.1–3.4)	0.02670	1.9 (0.8–4.2)	0.1324

†Log–rank test. ‡Cox's proportional hazards regression analysis. CI, confidence interval; GCB, germinal-center B cell; HR, hazard risk; IL2R, interleukin 2 receptor; LDH, lactate dehydrogenase; PS, performance status.

patients, 49 died and 149 were alive. Among the 149 alive patients, 115 maintained relapse-free survival after achieving the first CR.

Multivariate analyses. We carried out a Cox's proportional hazard regression analysis that included the following variables: age; EBV present; PS; clinical stage; B symptoms present; extranodal disease; LDH levels; interleukin-2 receptor levels; and GCB or non-GCB subtypes. The EBV, PS, and LDH levels were independent risk factors ($P = 0.0014$, 0.0007, and 0.0070, respectively). Among them, EBV had the highest hazard ratio (HR: 4.6. 95% confidence interval: 1.8–11.8) (Table 5).

Discussion

We carried out a clinicopathological analysis of DLBCL with special interest in EBV. The percentage of patients with EBV infection (6.9%) among all cases of DLBCL in our data was equivalent to previously reported data from Asian countries.[8,11,12] All patients with EBV-positive DLBCL met the criteria of EBV-DLBCL of the elderly, proposed by WHO.

In our analysis, patient characteristics between those who were EBV-positive and those who were EBV-negative were almost equivalent except for the performance status ($P = 0.0223$) and a trend towards extranodal disease ($P = 0.0856$). Epstein–Barr virus-positive DLBCL tends to develop extranodal involvement in 70% of patients;[11,20,21] DLBCL generally involves extranodal organs such as the gastrointestinal tract, skin, lungs, and tonsils.[22] Differences in extranodal organ involvement between EBV-positive and EBV-negative cases are unknown.

Several studies regarding the prognosis of EBV-positive DLBCL have been reported. Morales *et al.*[12] reported that EBV is an independent prognostic factor associated with *de novo* nodal DLBCL, before the rituximab era. This study analyzed six patients with EBV-positive DLBCL and 51 patients with EBV-negative DLBCL. The median OS rates in EBV-positive DLBCL and EBV-negative DLBCL patients were 6.5 and 47 months, respectively, and patients with EBV-positive DLBCL showed an inferior prognosis compared to those with EBV-negative DLBCL ($P = 0.001$). Park *et al.*[8] reported that DLBCL patients who are EBER-ISH-positive show a more rapidly deteriorating clinical course with poorer treatment response, survival, and PFS. They analyzed 34 patients with EBV-positive DLBCL and 346 patients with EBV-negative DLBCL. Epstein–Barr virus-encoded RNA positivity was significantly associated with age older than 60 years ($P = 0.005$), more advanced stage ($P < 0.001$), involvement of more than one extranodal site ($P = 0.009$), higher international prognostic index (the international prognostic index includes age >60 years, PS >2, number of extranodal sites >2, stage >III, and LDH level >normal) ($P = 0.015$), presence of B symptoms ($P = 0.004$), and poorer outcome following initial treatment ($P = 0.006$). The EBER-positive patients with DLBCL showed significantly poorer OS (EBER-positive *vs* EBER-negative, $P = 0.026$) and PFS (EBER-positive *vs* EBER-negative, $P = 0.018$). Both reports arrived at the same conclusion: that the presence of EBV leads to a more rapidly deteriorating clinical course with poorer treatment response and survival.

In contrast, Ahn *et al.* retrospectively analyzed 222 elderly patients (≥50 years) with DLBCL who received R-CHOP chemotherapy and evaluated the state of EBER. Eighteen cases (8.1%) were EBER-positive. At a median follow-up of 32.8 months, no significant difference was found in OS between the groups ($P = 0.627$). The EBV-positive DLBCL patients with early interruption of R-CHOP chemotherapy showed a trend toward a high EBV DNA titer (≥1000 copies/mL; $P = 0.091$). Thus, the EBV-positive tumoral status of elderly DLBCL patients who undergo R-CHOP chemotherapy may not predict their survival but their EBV status may contribute to the early interruption of R-CHOP chemotherapy.[15] In our study, we observed that OS and PFS of EBV-positive DLBCL patients were still lower than EBV-negative DLBCL, even after introduction of rituximab. Although the survival data are controversial between the two studies, they share some common features. For instance, both studies recognized that the overall response rate was worse in patients with EBV-positive DLBCL compared to those with EBV-negative DLBCL, and R-CHOP was interrupted early more frequently compared with EBV-negative DLBCL. Because the incidence of EBV-positive DLBCL of the elderly is low, a limitation is the small number of EBV-positive DLBCL patients in both studies. Based on these results, a multicenter study is needed to clarify the controversies regarding EBV-positive DLBCL.

Among 14 patients with EBV-positive DLBCL in our study, four patients showed latency III. Latency I is associated with EBV-related Burkitt lymphoma, latency II with classical Hodgkin's lymphoma and T-cell non-Hodgkin's lymphoma, and latency III occurs mainly in immunocompromised individuals suffering from post-transplant lymphoproliferative disorders and HIV-associated lymphoproliferative disorders and lymphoblastoid cell lines.[23] In general, a more intense immunosuppressive status is associated with a higher latency status. However, none of our patients was immunosuppressed. Patients with latency III died within 1 year of diagnosis, regardless of treatment with rituximab. Although no significant difference in survival was found, the outcome of latency III patients tended to be poor compared with that of latency II patients. Yoshino *et al.*[24] reported that EBV-positive DLBCL is resistant to standard chemotherapy. In this study, patients with latency III also showed resistance to chemotherapy.

In addition, we showed that the majority of patients classified as having EBV-positive DLBCL of the elderly were non-GCB types, which is a subtype with poor prognosis, and this may be another reason for the inferior prognosis. In EBV-positive DLBCL of the elderly, according to the WHO classification, age of more than 70 years and the presence of B symptoms are negative prognostic factors.[4,11,25] The proportion of the non-GCB type increases and reflects a change in the B-cell population during aging.[26] Thus, advanced age may lead to poor prognosis. Because age was not related to positive or negative EBV status in our study, we suggest that the latency status is a risk factor for developing the non-GCB type. Montes-Moreno *et al.*[27] reported that EBV infection may play a direct and additional role in activation of the nuclear factor-κB pathway. According to their report, EBV-positive DLBCL in the elderly is an aggressive and clonal B-cell neoplasm with prominent nuclear factor-κB pathway activation in the neoplastic cells. Epstein–Barr virus-encoded RNA positivity has an adverse impact on OS and PFS in patients with non-GCB DLBCL but not GCB DLBCL.[8] However, the detailed mechanism of conversion to more malignant clones in the presence of EBV is unknown. Infection with EBV in patients with non-GCB DLBCL may lead to further resistance to chemotherapy.

In summary, rituximab, which improved the outcome of DLBCL patients, did not show sufficient efficacy in EBV-

positive DLBCL patients. Patients with latency III were more resistant to chemotherapy. Further investigation of EBV-positive DLBCL patients based on their latency status and IHC phenotype is needed.

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Disclosure Statement

The authors have no conflict of interest.

Abbreviations

BCL	B-cell chronic lymphocytic leukemia/lymphoma
CHOP	cyclophosphamide, doxorubicin, vincristine, and prednisolone
CR	complete remission
DLBCL	diffuse large B-cell lymphoma
EBER	Epstein–Barr virus-encoded RNA
EBNA-2	Epstein–Barr virus nuclear antigen-2
EBV	Epstein–Barr virus
GCB	germinal center B cell
IHC	immunohistochemistry
ISH	*in situ* hybridization
LDH	lactate dehydrogenase
LMP-1	latent membrane protein-1
MUM-1	multiple myeloma oncogene-1
OS	overall survival
PD	progressive disease
PFS	progression-free survival
PS	performance status
R-CHOP	rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone
WHO	World Health Organization

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