Age-Related EBV-Associated B-Cell Lymphoproliferative Disorders Constitute a Distinct Clinicopathologic Group: A Study of 96 Patients

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Abstract

Purpose: We have recently reported EBV+ B-cell lymphoproliferative disorders (LPD) occurring predominantly in elderly patients, which shared features of EBV+ B-cell neoplasms arising in the immunologically deteriorated patients despite no predisposing immunodeficiency and were named as senile or age-related EBV+ B-cell LPDs. To further characterize this disease, age-related EBV+ B-cell LPDs were compared with EBV-negative diffuse large B-cell lymphomas (DLBCL).

Experimental Design: Among 1,792 large B-cell LPD cases, 96 EBV+ cases with available clinical data set were enrolled for the present study. For the control group, 107 patients aged over 40 years with EBV-negative DLBCL were selected. We compared clinicopathologic data between two groups and determined prognostic factors by univariate and multivariate analysis.

Results: Patients with age-related EBV+ B-cell LPDs showed a higher age distribution and aggressive clinical features or parameters than EBV-negative DLBCLs: 44% with performance status >1, 58% with serum lactate dehydrogenase level higher than normal, 49% with B symptoms, and higher involvement of skin and lung. Overall survival was thus significantly inferior in age-related EBV+ group than in DLBCLs. Univariate and multivariate analyses further identified two factors, B symptoms and age older than 70 years, independently predictive for survival. A prognostic model using these two variables well defined three risk groups: low risk (no adverse factors), intermediate risk (one factor), and high risk (two factors).

Conclusions: These findings suggest that age-related EBV+ B-cell LPDs constitute a distinct group, and innovative therapeutic strategies such as EBV-targeted T-cell therapy should be developed for this uncommon disease.

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Diffuse large B-cell lymphoma (DLBCL) is the largest category of aggressive lymphomas and regarded as a heterogeneous group of lymphomas in terms of clinicopathologic profiles and biological properties (1). Recent advance in the lymphoma research shed the light on the distinct subgroups such as *de novo* CD5+ DLBCL (2), intravascular large B-cell lymphoma (Asian variant; ref. 3), primary effusion lymphoma (4), and pyothorax-associated lymphoma (5) under the nosologic term of DLBCL. In addition, we have recently identified a series of elderly patients of EBV+ B-cell lymphoproliferative disorders (LPD) and/or large-cell lymphomas without predisposing immunodeficiencies and named those senile or age-related EBV+ B-LPDs (6).

EBV is a ubiquitous γ-herpesvirus that infects more than 90% of worldwide adult population (7, 8). In contrast to its high prevalence, EBV is also well recognized as an apparent oncogenic agent (9). It transforms B cells into lymphoblastoid cell lines *in vitro*, and many human cancers, including Burkitt lymphoma (BL) (10), Hodgkin lymphoma (7), immunodeficiency-associated LPDs (11), and a part of diffuse large B-cell lymphoma (12), have close relation with EBV. Although the precise mechanism is not fully clarified, it is widely accepted that the T cell plays a crucial role for the suppression of EBV-associated oncogenesis (7). In fact, the use of strong

immunosuppressive agents in organ transplantation settings such as tacrolimus or cyclosporin A, or HIV infection, sometimes causes EBV-positive B-cell LPDs (13, 14). Four clinical settings of immunodeficiency associated with an increased incidence of lymphoma and other LPDs are recognized by the WHO classification: (*a*) primary immunodeficiency syndromes and other primary immune disorders; (*b*) infection by the HIV; (*c*) iatrogenic immunosuppression in patients who have received solid organ or bone marrow allografts; and (*d*) iatrogenic immunosuppression associated with methotrexate treatment, most commonly in patients with autoimmune disease (15).

We have highlighted the over-profile of senile EBV+ B-cell LPDs appearing analogous in many respects to that of immunodeficiency-associated B-cell LPDs, which were exemplified by a marked propensity to involve extranodal sites and a morphologic spectrum ranging from the precursor polymorphous proliferation of lymphoid cells to diverse types of lymphomas, although no evidence of underlying immunodeficiency was found (6). Therefore, it is speculated that this disease is related to an immunologic deterioration derived from the aging process, i.e., senescence in immunity. However, the detailed clinicopathlogic features and follow-up information of age-related EBV+ B-cell LPDs remain limited because of an inclusion of a small number of patients and the lack of the comparison with EBV-negative DLBCL. To address these issues further, we retrospectively assessed the clinicopathologic features of 96 cases with age-related EBV+ B-cell LPDs as a collaborative study.

Materials and Methods

Diagnosis. The diagnosis of age-related EBV-associated B-cell LPDs was made when more than 50% of the proliferating, often neoplastic-appearing cells showed both of the expression of one or more pan–B-cell antigens (CD20/CD79a) and/or light-chain restriction and positive signal for *in situ* hybridization using EBV-encoded small nuclear early region (EBER) oligonucleotides on paraffin section (Fig. 1) for patients more than 40 years of age without predisposing immunodeficiency such as HIV infection or past history of immunosuppressive agents (6) The cases <40 years old were excluded because we could not deny the possibility that they may be associated with any primary immune disorder or chronic active EBV infection (16, 17). In addition, pyothorax-associated lymphoma and EBV-associated lymphomas of T- or natural killer-cell phenotype were excluded from the present series because they were considered to constitute distinct clinicopathologic groups (5, 18). In particular, attention was given to the differential diagnoses of peripheral T-cell lymphoma with Reed-Sternberg–like cells of B-cell or angioimmunoblastic T-cell lymphoma with proliferation of large B cells (19). Only well-documented cases that had paraffin sections available for immunohistochemistry were included in this study. Each case was reviewed by five pathologists (authors K.T., K.O., N.N. T.Y., and S.N.) to confirm the diagnosis and immunophenotype. Among 149 cases fulfilling these criteria (Supplementary Table S1), 96 cases with available clinical data set were enrolled for the present study, including the 22 cases of senile EBV+ B-cell LPDs previously reported by us (6). For the control group, 107 patients aged over 40 years with EBV-negative DLBCL were selected from malignant lymphoma cases treated consecutively at Aichi Cancer Center between 1993 and 2000. This study was done by following the Ethical Guidelines for Clinical Studies and the Ethical Guideline for Epidemiological Research in Japan. The Institutional Review Board of the Aichi Cancer Center and the other institutes involved approved this study.

Histopathology. Tissue samples were fixed in 10% formalin and embedded in paraffin. Sections (5 μm thick) were stained with H&E, Elastica-van Gieson, silver impregnation, periodic acid-Schiff, May-Gruenwald-Giemsa, and methyl green-pyronine staining.

Immunohistochemistry. Immunoperoxidase studies were done on formalin-fixed paraffin sections with the avidin-biotin peroxidase complex method. A panel of monoclonal antibodies against human immunoglobulin light and heavy chains, CD3, CD8, UCHL-1/CD45RO, L26/CD20, Ber-H2/CD30, CD79a, latent membrane protein-1 (LMP-1), EBV-encoded nuclear antigen-2 (EBNA2; DAKO); CD2, CD4, CD5, CD56 (Novocastra Laboratories); LeuM1/CD15, Leu7/CD57

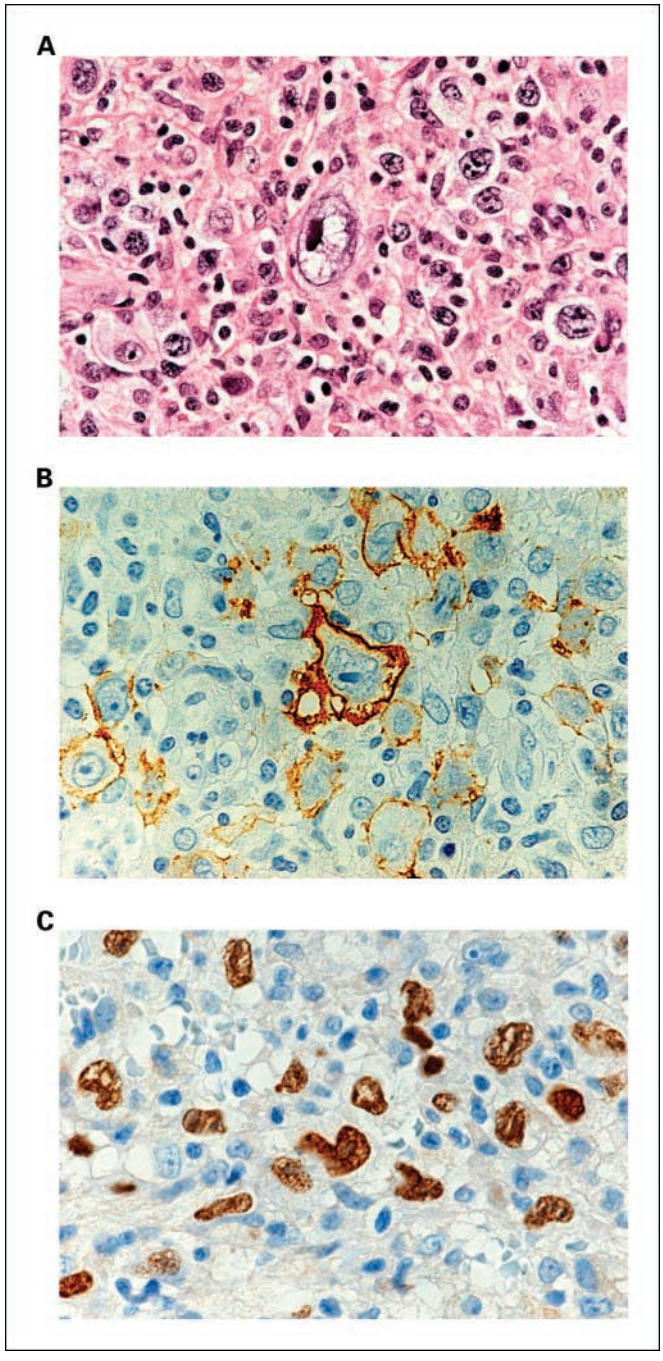

Fig. 1. Senile EBV-associated B-cell LPD, polymorphic subtype, arising in a 62-year-old male. The lesion reveals scattered distribution of Hodgkin and Reed-Sternberg–like giant cells (*A,* ×150), which are positive for CD20 (*B,* ×125). These large cells showed the expression of EBNA2 (*C,* ×125) in addition to the positive signals for EBV-encoded small RNAs (EBERs) *in situ* hybridization, indicating latency III status.

Table 1. Patient characteristics at diagnosis of age-related EBV-positive B-LPDs and EBV-negative DLBCL

Variable	Age-related EBV-positive LPD (n = 96)	EBV-negative DLBCL (n = 107)	P
Sex (male/female)	56/40 (1.4)	54/53 (1.02)	0.26
Age, median (range), y	71 (45-92)	62 (41-85)	<0.0001
	Number of cases (%)	**Number of cases (%)**	
Older than 60	79 (82%)	56 (52%)	<0.0001
ECOG PS 2-4	36 (44%)	18 (17%)	<0.0001
B-symptoms, presence	38 (49%)	18 (20%)	<0.0001
LDH level high	47 (58%)	46 (43%)	0.041
Ann Arbor stage III/IV	48 (58%)	49 (46%)	0.10
Extranodal involvement (>1 site)	28 (33%)	30 (28%)	0.43
Extranodal sites	n = 93	n = 107	
Skin	12 (13%)	5 (5%)	0.037
Lung	8 (9%)	3 (3%)	0.073
Pleural effusion	8 (9%)	5 (5%)	0.26
Stomach	8 (9%)	14 (13%)	0.31
Tonsil	7 (8%)	20 (19%)	0.021
Breast	0 (0%)	7 (7%)	0.012
IPI, High intermediate/high	43 (54%)	39 (37%)	0.017
Anti-EBV antibody titer category,*	18 (67%)	23 (24%)	<0.0001
Treatment			<0.0001
None or radiation only	9 (12%)	1 (1%)	
Ctx without anthracycline	7 (9%)	2 (2%)	
Ctx with anthracycline	62 (79%)	104 (97%)	
Response, in cases underwent Ctx with anthracycline			<0.0001
CR	37 (66%)	93 (91%)	
PR	8 (14%)	8 (8%)	
SD or PD	11 (20%)	1 (1%)	

Abbreviations: PS, performance status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; Ctx, chemotherapy; CR indicates complete response; PR, partial response; SD, stable disease; PD, progressive disease.
*Cases were determined as having abnormal serum anti-EBV antibody titer if anti-EBV viral capsid antigen antibody was 640-fold or higher, or anti-EBV nuclear antigen antibody was negative.

(Becton Dickinson); TIA-1 (Coulter Immunology); and granzyme B (Monosan) were used. All antibodies were applied after antigen retrieval following microwave oven heating treatment.

In situ *hybridization study.* The presence or absence of EBV small RNAs was assessed by means of *in situ* hybridization using EBER oligonucleotides and done on formalin-fixed paraffin embedded sections. Briefly, a DAKO hybridization kit was used with a cocktail of FITC-labeled EBER oligonucleotides (one oligonucleotide corresponding to EBER1 and one to EBER2, both 30 bases long; DAKO A/S code Y 017). Hybridization products were detected with mouse monoclonal anti-FITC (DAKO M878) and a Vectastain ABC Kit (Vector). RNase A or DNase I pretreatment was used for the negative controls and EBER-positive Hodgkin's disease specimens for positive controls.

Statistical analysis. Variables related to patients, treatment, and disease were compared among the two groups with the use of the χ^2 test or Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. The probability of survival was calculated with the use of the Kaplan-Meier estimator, and the log-rank test was used for comparisons. Univariate and multivariate analyses were done with the Cox proportional hazard regression model. All P values are two sided, with a type I error rate fixed at 0.05. Statistical analyses were done with the STATA version 9.

Results

Case selection. From the files of six collaborating institutions, during the period from January 1990 to December 2004, the positive signals for B-cell [pan–B-cell antigens (CD20/CD79a) and/or light-chain restriction] and EBV were detected on more than 50% of cells in 243 (14%) of 1,792 large B-cell LPD cases, mainly consisting of DLBCL, by EBERs *in situ* hybridization. They contained HIV-associated lymphomas (n = 17), autoimmune disease–associated LPDs (n = 10), secondary lymphoma with prior chemotherapy (n = 7), post-transplant LPDs (n = 10), pyothorax-associated lymphoma (n = 30), BL (n = 13), and cases without any documentation for predisposing immunodeficiency (n = 156; Supplementary Table S1). EBV was detected in 10% of HIV-negative patients with BL in this study, which was comparable to the reported frequency in nonendemic BL (20). A bimodal age distribution with an incidence peak in the 10- to 19-year range and a second peak in older adult aged 70 to 79 was evident for EBV-positive B-cell LPD patients without predisposing immunodeficiency (Supplementary Fig. S1*A*). The positive percentages of this group for all cases examined became higher in parallel with the elder patient populations (≥40 years), showing the highest peak at ages >90 years (Supplementary Fig. S1*B*).

Patient characteristics for age-related EBV-positive B-cell LPDs and EBV-negative DLBCL. In comparison with EBV-negative DLBCL, patients with age-related EBV-positive B-cell LPDs showed higher age distribution (median, 71 versus 62 years: $P < 0.0001$) and a closer association with aggressive clinical features or parameters: 79 patients older than 60 (82%,

$P < 0.0001$), 36 with performance status (PS) >1 (44%, $P < 0.0001$), 47 with serum lactate dehydrogenase (LDH) level higher than normal (58%, $P = 0.041$), 48 with stage III/IV disease at diagnosis (58%, $P = 0.10$), and 38 with B symptoms (49%, $P < 0.0001$; Table 1). As a result, the International Prognostic Index (IPI) score for patients with age-related EBV-positive B-cell LPDs was significantly higher than that for patients with EBV-negative DLBCL ($P = 0.0017$), with 43 (54%) of the EBV-positive group categorized in the IPI high or high intermediate-risk group. There was no statistical difference between two groups in the incidence of having more than one extranodal site.

At diagnosis, 67% of the cases with age-related EBV-positive B-cell LPDs showed abnormal anti-EBV antibody titer, which was defined if anti-EBV VCA immunoglobulin G (IgG) antibody was 640-fold or higher, or anti-EBNA antibody was negative, as compared with only 24% of cases with DLBCL that showed abnormality ($P < 0.0001$).

Sites of extranodal involvement. In 17 patients (20%) of the current EBV-positive series, the disease was limited to extranodal sites. Twenty-seven patients (31%) had only lymphadenopathies without extranodal involvement, and the remaining 43 (49%) had lymphadenopathies with extranodal involvement. The total incidence of extranodal involvement was similar between age-related EBV-positive B-cell LPDs and EBV-negative DLBCL (69% and 72%, respectively).

The main sites of extranodal involvement in age-related EBV-positive B-cell LPDs was skin ($n = 12$; 13%), lung ($n = 8$; 9%), pleural effusion ($n = 8$; 9%), stomach ($n = 8$; 9%), and tonsil ($n = 7$; 8%) in an order of the incidence (Table 1). A comparison with EBV-positive and EBV-negative groups showed that the incidence of cutaneous involvement was significantly higher in age-related EBV-positive B-cell LPDs than those of EBV-negative DLBCLs ($P = 0.027$, respectively). There is a tendency of difference in lung involvement, but no statistical significance (9% versus 3%, $P = 0.073$). Involvement of breast and tonsil occurred less frequently in age-related EBV-positive B-cell LPDs than in EBV-negative DLBCL ($P = 0.012$ and.021, respectively). There were no significant differences between these two groups in the incidence of involvement in the other extranodal sites (Supplementary Table S2).

Histologic features. Age-related EBV-positive LPDs generally showed a diffuse and polymorphic proliferation of large lymphoid cells with a varing degree of reactive components such as small lymphocytes, plasma cells, histocytes, and epithelioid cells and were sometimes accompanied by necrosis and an angiocentric pattern. These tumor cells were often featured by a broad range of B-cell maturation, containing morphologic centroblasts, immunoblasts, and Hodgkin and Reed-Sternberg (HRS)–like giant cells with distinct nucleoli (Fig. 1*A*). According to the previous report (6), the present series were morphologically divided into two subtypes: large-cell lymphoma (LCL) and polymorphic LPD subtypes. The former ($n = 34$) was characterized by having areas where large lymphoid cells with relatively monomorphic appearance were notably dominant. The remaining 62 cases were simply categorized as polymorphous subtype with the scattered distribution of large cells in the polymorphous composition. The histology was frequently varied from area to area, indicating a continuous spectrum between these two subgroups because several LCL cases had areas of polymorphic LPD in the same or other tissues. In contrast to morphologic divergence, there was no significant difference in any clinical characteristics and immunophenotype between these two groups (Supplementary Table S3).

We detected clonal B-cell population in 10 cases out of 12 cases tested: eight cases by PCR analysis, one case by Southern blot analysis, and one by lambda light-chain restriction. Polyclonal pattern was observed in one case, and no band was detected in the other. As to polymorphic LPDs, the presence of clonal B-cell population was identified in five cases out of seven samples.

Phenotypic features. According to the definition adopted for this study, all patients with age-related EBV-positive B-cell LPDs were positive for EBV and B-cell markers (CD20 and/or CD79a; Fig. 1*B*). Immunohistologic studies for the EBV-latent gene products on paraffin sections showed that LMP1 was positive on the large atypical cells in 67 (94%) out of 71 tested cases. EBNA2 was also detected in the nuclei of 16 (28%) of 57 tested cases (Fig. 1*C*, Supplementary Table S4), indicating latency type III. CD30 was stained more common in age-related EBV-positive B-cell LPDs than in EBV-negative DLBCL (75% versus 13%, $P < 0.0001$). In addition, a comparison of adjacent sections often disclosed an overlapping staining pattern of LMP1 and CD30. There was also a statistically significant difference in the incidence of CD10 expression (18% and 38%, respectively. $P = 0.015$), but not others (CD19, CD20, or CD79a) between age-related EBV-positive B-cell LPDs and DLBCLs (Supplementary Table S4).

Response to treatment and Kaplan-Meier survival estimates. Treatment of age-related EBV-positive B-cell LPDs consisted of chemotherapeutic regimens containing anthracycline for 62 patients (79%) and without anthracycline for 7 patients (9%; Table 1). A total of 40 (63%) of 64 evaluable patients with age-related EBV-positive B-cell LPDs achieved a complete remission (CR) with initial therapy, and the rest of the 24 cases (38%) failed to have a CR with initial chemotherapy. On the other hand, 95 (91%) of 104 evaluable cases with DLBCL achieved a CR, and only 9 cases (9%) were refractory (PR, SD, or PD) to initial chemotherapy ($P < 0.0001$). This difference, in response to treatment, was still in a significant level when compared in cases who received chemotherapy with anthracycline ($P < 0.0001$, Table 1).

In this study, we observed 57 deaths in 96 cases of age-related EBV-positive B-cell LPDs and 34 deaths in 107 cases of DLBCL. The data on the causes of death were available for 47 cases for age-related EBV-positive B-cell LPDs and 29 for DLBCL. Deaths due to disease progression and complications such as infections were observed in 38 and 9 cases, respectively, in age-related EBV-positive B-cell LPDs, whereas 23 and 6 cases in EBV-DLBCL. The observed differences between two disease groups were not significant ($P = 0.870$). As to the cases of more than 70 years of age, 24 and 5 cases were dead due to disease progression, and seven and one were from complications in age-related EBV-positive B-cell LPDs and in DLBCL, respectively. Even in cases more than 70 years old, the observed differences were not significant ($P = 0.747$).

Unadjusted overall survival curves of both groups were shown in Fig. 2*A*. Age-related EBV-positive B-cell LPDs showed strikingly inferior survival to DLBCLs (median survival time, 24 months versus not reached, respectively;

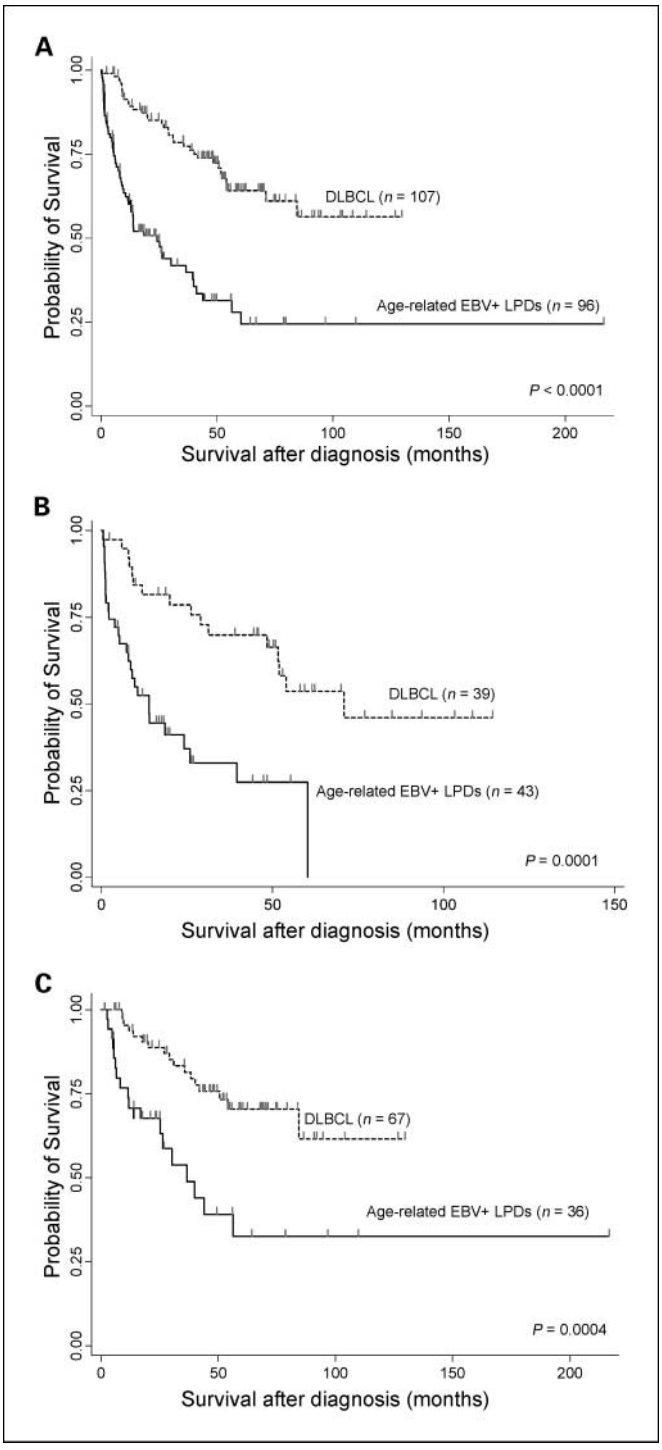

Fig. 2. Overall survival for patients with age-related EBV+ B-cell LPDs and EBV-negative DLBCLs. Age-related EBV+ B-cell LPDs ($n = 96$) show significantly worse survival than DLBCLs ($n = 107$) in all patients (*A*), patients with high-intermediate and high IPI risk ($n = 43$ and $n = 39$, respectively; *B*), and patients with low and low-intermediate IPI risk group ($n = 36$ and $n = 67$, respectively; *C*).

$P < 0.0001$). A significant difference was still found even when accounting for age (age ≤ 60, 60 < age ≤ 75, or age > 75) by performing the stratified log-rank test ($P < 0.0001$). Overall survival curves according to IPI are shown in Fig. 2*B* and *C*. Survival for age-related EBV-positive B-cell LPDs was significantly inferior to that for DLBCLs in both IPI subgroups. In this series, the IPI failed to separate age-related EBV+ B-cell LPD patients into groups with significantly different survivals ($P = 0.1$; Fig. 3*A*).

Univariate and multivariate analysis for survival. Among a total of 203 patients with EBER-positive (age-related EBV-positive B-cell LPDs) and EBV-negative diseases (DLBCLs), univariate Cox analysis identified the following as prognostic factors: age > 60 years, clinical stage, PS, extranodal involvement of more than one site, LDH, IPI, B symptoms, and EBV association (Table 2). Multivariate analysis, including five IPI factors, B symptoms, and EBV association, showed high LDH level, the presence of B-symptoms, and EBV association to be significant factors (Table 2). When multivariate analysis was done for EBV association and IPI categories, both of them were recognized as independent significant prognostic factors (Table 2).

Among patients with age-related EBV+ B-cell LPDs, the clinical parameters associated with reduced survival in univariate analysis are listed in Table 3: age older than 70

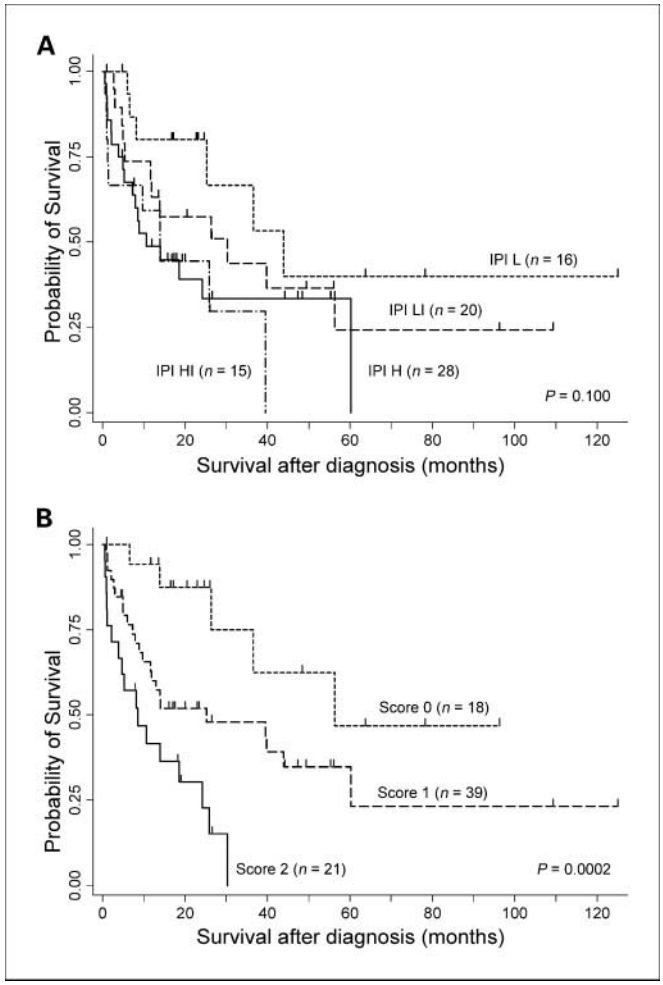

Fig. 3. Overall survival according to IPI (*A*) and prognostic model based on two simple clinical variables of age older than 70 y and the presence of B symptoms (*B*) in age-related EBV+ B-cell LPDs. This prognostic model is able to efficiently identify three groups of patients with different outcomes; patients with a score of 0 (*Score 0, n* = 18), no adverse factors; patients with a score of 1 (*Score 1, n* = 39), one factor; and patients with a score of 2 (*Score 2, n* = 21), two factors. Their median survival times were 56.3, 25.2, and 8.5 mo, respectively.

Table 2. Prognostic factors affecting overall survival of total entry series

Variables	Unfavorable factors	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Comparison with risk factors					
EBV	Positive	3.5 (2.3-5.5)	<0.0001	2.5 (1.5-4.1)	0.001
B symptom	Present	3.2 (2.0-5.1)	<0.0001	2.0 (1.2-3.5)	0.008
LDH	>normal	2.6 (1.6-4.1)	<0.0001	2.0 (1.2-3.4)	0.011
PS	2-4	2.4 (1.6-3.8)	<0.0001	—	—
Age	>60 y	2.0 (1.2-3.1)	0.006	—	—
Stage	III/IV	1.8 (1.1-2.8)	0.010	—	—
Extranodal disease	>1 site	1.5 (0.9-2.3)	0.083	—	—
Comparison with IPI category					
IPI	HI/H	2.1 (1.4-3.3)	0.001	2.0 (1.3-3.1)	0.003
EBV	Positive			3.3 (2.1-5.3)	<0.0001

Abbreviations: CI, confidential interval; LDH, lactate dehydrogenase; PS, performance status; IPI, International Prognostic Index.

years ($P = 0.0008$), the presence of B symptoms ($P = 0.0058$), and LDH level equal to or more than normal value ($P = 0.040$). Clinical stage, PS, and extranodal involvement of more than one site were nonsignificant factors. In multivariate analysis, the factors that turned out to correlate significantly with survival were B symptom ($P = 0.0026$) and age ($P = 0.0045$). Because the relative risk associated with each of the two factors was comparable, we constructed a prognostic model by combining these prognostic variables in the following way: patients with a score of 0 ($n = 18$), no adverse factors; patients with a score of 1 ($n = 39$), one factor; and patients with a score of 2, two factors ($n = 21$). This prognostic model for age-related EBV+ B-cell LPDs was able to efficiently identify three groups of patients with different outcomes (Fig. 3*B*; $P < 0.0001$). For the patients with scores of 0, 1, and 2, the median overall survival times were 56.3, 25.2, and 8.5 months, respectively.

Discussion

We recently have documented 22 cases named as senile EBV-associated B-cell LPDs arising in elderly patients aged ≥60 years without predisposing immunodeficiencies, suggesting that this disease has a relationship with an immunologic deterioration derived from the aging process (6). Among 1,792 large B-cell LPD cases examined by EBERs *in situ* hybridization, 156 cases harbored EBV without underlying immunodeficiency-related diseases. This larger series revealed that 149 (96%) of these patients are more than 40 years of age, the increasing positive percentages of which were observed in parallel with the elder patient populations (≥40 years) for all cases examined and reached the highest peak at ages ≥90 years. These data provided additional evidence that EBV-positive B-cell LPDs without predisposing immunodeficiency mainly occur in elderly patients, although seven patients were found to be <40 years of age. Considering these rare cases, the term of "age related" may be more appropriate than that of senile for further understanding the overall age distribution of EBV-positive B-cell LPDs without predisposing immunodeficiency.

This study was predominantly a comparison of clinical features in age-related EBV+ B-cell LPDs and EBV-negative DLBCLs. An analysis of 96 patients with age-related EBV-positive B-cell LPDs, in which the clinical data were available, highlighted the clinical features of this disease—high age at onset, frequent association with poor prognostic components of IPI, and aggressive clinical course. These features were significantly different from those of EBV-negative DLBCL besides more frequent involvement of the skin, supporting the concept that age-related EBV-associated B-cell LPDs constitute a distinct disease with a broad spectrum. However, it could not be definitively concluded whether this disease

Table 3. Prognostic factors affecting overall survival of age-related EBV-positive B-cell LPDs

Variables	Unfavorable factors	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
B symptoms	Present	2.3 (1.3-4.3)	0.0058	2.6 (1.4-4.8)	0.0026
Age	>70 y	2.4 (1.4-4.3)	0.0008	2.5 (1.3-4.8)	0.0045
LDH	>normal	1.9 (1.0-3.4)	0.040	—	—
Stage	III/IV	1.8 (1.0-3.2)	0.062	—	—
PS	2-4	1.2 (0.7-2.1)	0.57	—	—
Extranodal disease	>1 site	1.3 (0.7-2.3)	0.38	—	—
IPI category	HI/H	1.8 (1.0-3.2)	0.064	—	—

Abbreviations: LPDs, lymphoproliferative disorders; CI, confidence interval; EBV, Epstein-Barr virus; LDH, lactate dehydrogenase; PS, performance status; IPI, International Prognostic Index.

represented a heterogeneous group of disorders including several lymphoma subtypes.

The morphologic spectrum of age-related EBV+ B-cell LPDs seems to be broader than has been previously realized (data not shown). This disease comprised a spectrum ranging from polymorphic proliferation, sometimes suggestive of a reactive process, to large-cell lymphomas mostly consisting of transformed cells and, therefore, was subdivided into two subtypes, i.e., polymorphic and large-cell lymphomas, based on morphology and conventional immunophenotyping in our previous report (6). However, in the present study, we failed to show any statistical difference in the clinical profiles between these two subgroups. Indeed, several cases had areas that seem more monomorphic in the same or other tissues, thus indicating a continuous spectrum between polymorphic and large-cell lymphoma subtypes. The results that we found in the histologic subgrouping of age-related EBV+ B-cell LPDs seemed to parallel those of the post-transplant LPDs, in which current classification schemes are not fully predictive of prognosis (15, 21). Further investigation should be done to refine the distinction of age-related EBV+ B-cell LPDs into more homogeneous categories with prognostic relevance.

The prognosis of age-related EBV+ B-cell LPDs was significantly poorer than that of EBV-negative tumors. One possible explanation is that the EBV association as a biological marker seemed to be closely associated with the higher IPI index because 35% of patients with this disease were categorized in the high-risk IPI group, which is higher than 15% of the present series of EBV-negative DLBCL or 19% of DLBCL reported by the Non-Hodgkin's Lymphoma Classification project (22, 23). The other is the age distribution and performance status of the patients (Table 1). Due to higher age or poorer PS, many patients with age-related EBV-positive B-cell LPDs might not maintain the intensity of chemotherapy. However, subgroup analyses by age or the IPI also showed that age-related EBV-positive B-LPDs had lower CR rate and inferior overall survival compared with EBV-negative DLBCLs. Multivariate analysis in all cases further identified EBV association and IPI category as an independent prognostic factor. These findings emphasized that age-related EBV-positive B-cell LPDs merits separate consideration because of the diagnostic and therapeutic problems it poses.

Indeed, in multivariate analysis, two host-related factors, i.e., age older than 70 years and the presence of B symptoms, were prognostically significant. In the present series of age-related EBV+ B-cell LPDs, the IPI scoring system did not seem to work with the same efficacy as in DLBCLs for identifying subsets of patients with different prognoses. However, the extension of the disease (clinical stage and extranodal involvement of more than one site) and the biology or cell turnover of the tumor (LDH level) were no longer significant. These findings further supported our assertion that this disease is distinct from DLBCLs and significantly influenced by the host immune status in outcome of patients. Our prognostic model based on the two simple clinical variables of age older than 70 years and the presence of B symptoms also seemed to better define the clinical outcome of age-related EBV+ B-cell LPDs categorized as a single group with an overall superior predictive capacity as compared with IPI (log-rank, 0.0002 versus 0.1). Of course, an external validation study should be done on the larger series of cases in the future.

It is presumed that the pathogenesis of age-related EBV-positive B-cell LPDs has a close relation with an immunologic deterioration or senescence in immunity derived from the aging process because this disease seemed analogous in many respects to that immunodeficiency-associated LPDs, such as EBV association, waxing and waning of disease, and polymorphic proliferation of large bizarre B cells (16). Aging in humans is known to be associated with impaired immune status such as increased infections, the more global phenomenon termed "immune senescence" (24). Indeed, in the present series, 28% of the age-related EBV+ B-cell LPD cases examined were immunohistochemically positive for EBNA2, indicating the reduced immunity to EBV, i.e., type III latency which is believed to occur only in the setting of profound immunodeficiency (25). EBV DNA in peripheral blood mononuclear cells was more frequently detected in healthy individuals older than 70 years of age (8 of 9, 89%) than in ones <70 years (1 of 11, 9%) using real-time PCR (26). Yanagi et al. also showed that EBNA-2 IgG antibodies evoked in young children by asymptomatic primary EBV infections remain elevated throughout life using sera, suggesting the intervention of reactivation of latent and/or exogenous EBV superinfection (27). These data provided additional support on the speculation that age-related decline in immunity may be contributing to the pathogenesis of age-related EBV+ B-cell LPDs.

Biological interfaces may be assumed between age-related EBV+ B-cell LPDs and other EBV-associated B-cell neoplasms such as lymphomatoid granulomatosis and plasmablastic lymphoma, the distinction of which is currently based on the constellation of clinical, morphologic, and immunophenotypic features (28, 29). In our series, nine cases showed pulmonary involvement and four ones had gingival lesions at presentation, posing the differential diagnostic problems from lymphomatoid granulomatosis and plasmablastic lymphoma, respectively, although they were not prototypic in morphology as the latter two. Classic Hodgkin lymphoma (CHL) is also well known to have EBV harboring in 30% to 50% of the cases with achieving a general consensus of the B-cell derivation of the H-RS cells in most (30, 31). Interestingly, three population-based studies of Clarke et al. (32), Stark et al. (33), and more recently, Jarrett et al. (34), without selection bias documented that a marked survival disadvantage in older EBV-positive CHL patients as compared with EBV-negative CHL cases, which was contrasted with no effect of EBV status on the clinical outcome of HL patients selectively enrolled in clinical trails, with a tendency of their relatively younger age distribution (35, 36). As the interpretation for this age-related influence of EBV on clinical outcome of CHL patients, Gandhi et al. (37) and Jarrett et al. (34) clearly indicated that a decline in cellular immunity to EBV with age may contribute to the pathogenesis of EBV+ CHL in older patients. This standpoint is tempting to speculate that EBV+ CHL and age-related EBV+ B-cell LPDs may constitute a continuous spectrum. Our study may also raise an even more fundamental question: whether biological properties, such as an interaction or balance between latent EBV infection and host immunity, precede the morphologic and immunophenotypic evaluation for further understanding the overall clinicopathologic profiles of EBV-associated B-cell LPDs and/or lymphomas. Much still needs to be learned about the detailed clinicopathologic

features, the immunology, and the molecular biology of these diseases in a further study.

Innovative therapeutic strategies such as immunotherapy against EBV should be explored for age-related EBV+ B-cell LPD patients (38, 39), because conventional combination chemotherapy had only a limited effect in an analysis of this larger series. For poor risk patients with aggressive lymphomas such as DLBCL, the superiority of high dose chemotherapy with stem cell support over conventional method is now under confirmation (40–42). This therapeutic approach may not, however, be suitable for age-related EBV+ B-cell LPDs because the older age distribution of the patients, many (70%) of which were more than 65 years old, made the application of high-dose chemotherapy difficult enough. Rituximab is a non-cytotoxic drug that showed efficacy when adding to cyclophosphamide-Adriamycin-vincristine-prednisone (CHOP) on elderly patients with DLBCL (43). In our present series, only one case was documented to have received chemotherapy combined with rituximab for an initial treatment, preliminarily providing a good efficacy of this agent on age-related EBV+ B-cell LPD. Now, we are conducting prospective clinical trials to test the efficacy of chemotherapy with rituximab as a multi-institutional study on age-related EBV+ B-cell LPD patients.

In conclusion, the current study elucidates that age-related EBV-associated B-cell LPDs constitute a distinct clinicopathologic group in contrast with EBV-negative DLBCLs, in which conventional chemotherapy has a limited efficacy for this disease. A study to test the efficacy of rituximab with chemotherapy for age-related EBV+ is now ongoing. In the future, less toxic treatment strategy such as a cell therapy for EBV-specific viral antigens will be needed and should be evaluated in clinical trials.

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