($P < 0.0001$), higher ratio of males ($P < 0.001$), less mediastinal involvement ($P < 0.001$), more involvement of paraaortic and retroperitoneal lymph nodes ($P = 0.038$ for both), and less occurrence of WBC count >15 000/mm^3 ($P = 0.009$).

Patients with EBV-positive CHL were significantly associated with aggressive clinical parameters, namely performance status (PS) >1 ($P = 0.026$), presence of B-symptoms ($P = 0.036$), and thrombocytopenia ($P = 0.025$). The international prognostic index (IPI) score was therefore significantly higher in the EBV-positive group ($P = 0.001$).

Histologically, the MC subtype was significantly more frequent in the EBV-positive group than in the EBV-negative group (80% vs. 24%, $P < 0.001$) while the NS subtype was significantly more frequent in the EBV-negative group (69% vs. 13%, $P < 0.001$).

Therapeutic response

A total of 265 patients received combination chemotherapy consisting of first-line treatment regimens as follows: doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD; 198 patients); cyclophosphamide, vincristine, procarbazine, and prednisone (C-MOPP; 18 patients); bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP; 6 patients); and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP; 19 patients). Twenty-four patients received other types of combination chemotherapy (Table 1).

One hundred and thirty-seven patients received radiation therapy, and 128 received both chemotherapy and radiation. In total, 80% patients (206 of 259) with CHL achieved a complete response with the initial therapy, 15% achieved partial response, 2% showed no response and 3% had progressive disease. There was no significant difference in response to therapy between the two groups.

Survival

There was no significant difference in OS between CD20-positive and -negative CHL cases ($P = 0.87$) and the 5-year OS rate was 75% and 79% respectively. Also, there was no significant difference in PFS between CD20-positive and -negative CHL cases ($P = 0.38$) and the 5-year PFS rate was 73% and 61% respectively. The rates of death within 24 months were 5% and 9%, respectively (Table 1). Also, when we analyzed the effect of CD20 status on survival within EBV-positive and -negative patient groups, there were no significant differences in OS between the CD20-positive and -negative cases (EBV-positive group, $P = 0.77$; EBV-negative group, $P = 0.75$).

Patients with EBV-positive CHL showed a tendency to poor prognosis in terms of OS compared with EBV-negative patients, but without statistical significance ($P = 0.09$). The PFS showed no significant difference between EBV-positive and -negative cases ($P = 0.3$) (Supplemental Table S2). IPI was successful in the stratification of the OS of CHL cases in this analysis ($P < 0.0001$).

Prognostic factors

Analyses of prognostic factors are shown in Tables 3 and 4. Univariate analysis identified 11 prognostic factors for OS in CHL patients: age ($P = 0.049$), involvement of more than one

Table 3 Risk factors for Overall Survival

	Univariate analysis			Multivariate analysis		
Variable	HR	95% CI	*P*	HR	95% CI	*P*
Age > 60 years	1.78	1.00–3.18	0.049*	0.66	0.15–2.93	0.583
Male sex	1.33	0.72–2.46	0.36			
Extranodal sites > 1	3	1.59–5.65	0.001*			
Stage III, IV	3.10	1.68–5.73	<0.001*	0.48	0.06–3.83	0.492
Presence of B symptoms	2.35	1.36–4.06	0.002*	21.2	1.32–340	0.031*
Performance status > 1	3.42	1.92–6.08	<0.001*	8.32	1.34–51.5	0.023*
WBC > 15000/mm^3	0.97	0.41–2.28	0.94			
Hemoglobin < 10.5 g/dL	2.41	1.35–4.28	0.003*	0.28	0.02–4.09	0.355
Platelet < 150 000/mm^3	2.32	1.02–5.26	0.044*	24.7	1.73–353	0.018*
Serum Albumin level < 3.5 g/dL	5.73	3.07–10.7	<0.001*	1.91	0.10–38.2	0.671
Elevated LDH	2.03	1.15–3.6	0.015*	13.8	1.37–138	0.026*
sIL-2R > 4000 U/mL	4.08	1.31–12.7	0.015*	1.22	0.28–5.35	0.788
IPI (HI/H)	3.35	1.87–6.02	<0.001*			
CD20	1.06	0.52–2.18	0.87	2.03	0.12–33.3	0.618
EBV	1.6	0.93–2.76	0.092	7.00	1.07–45.8	0.042*

*$P < 0.05$.

95% CI, 95% confidence interval; H, high risk; HI, high-intermediate risk; IPI, international prognostic index; LDH, lactate dehydrogenase; sIL-2R, soluble IL-2 receptor.

Table 4 Risk factors for Progression-Free Survival

	Univariate analysis			Multivariate analysis		
Variable	HR	95% CI	*P*	HR	95% CI	*P*
Age > 60 years	0.83	0.48–1.45	0.52			
Male sex	1.26	0.76–2.09	0.37			
Extranodal sites > 1	2.41	1.35–4.27	0.003*			
Stage III, IV	2.21	1.38–3.56	0.001*	2.07	1.14–3.73	0.016*
Presence of B symptoms	1.47	0.92–2.34	0.10			
Performance status > 1	1.78	1.01–3.16	0.047*	1.45	0.74–2.84	0.283
WBC > 15000/mm^3	0.95	0.43–2.09	0.90			
Hemoglobin < 10.5 g/dL	1.82	1.08–3.06	0.024*	1.15	0.58–2.32	0.686
Platelet < 150 000/mm^3	1.43	0.65–3.16	0.37			
Serum Albumin level < 3.5 g/dL	2.36	1.37–4.04	0.002*	1.47	0.74–2.91	0.274
Elevated LDH	1.15	0.71–1.87	0.58			
sIL-2R > 4000 U/mL	1.97	0.76–5.10	0.16			
IPI (HI/H)	1.58	0.92–2.73	0.099			
CD20	0.74	0.38–1.44	0.38	0.84	0.40–1.76	0.642
EBV	0.77	0.47–1.26	0.30	0.76	0.43–1.36	0.358

*$P < 0.05$.
95% CI, 95% confidence interval; H, high risk; HI, high-intermediate risk; IPI, international prognostic index; LDH, lactate dehydrogenase; sIL-2R, soluble IL-2 receptor.

extranodal site ($P = 0.001$), advanced clinical stage (III/IV; $P < 0.001$), presence of B symptoms ($P = 0.002$), performance status >1 ($P < 0.001$), hemoglobin level <10.5 g/dL ($P = 0.003$), thrombocytopenia ($P = 0.044$), serum albumin level <3.5 g/dL ($P < 0.001$), elevated serum lactate dehydrogenase (LDH) level ($P = 0.015$), elevated soluble IL-2 receptor >4000 U/mL ($P = 0.015$) and IPI score (high-intermediate and high risk; $P < 0.001$). Multivariate analysis found that the presence of B symptoms ($P = 0.031$), presence of performance status >1 ($P = 0.023$), thrombocytopenia ($P = 0.018$), elevated serum LDH level ($P = 0.026$), and EBV infection ($P = 0.042$) but not CD20-postivity were identified as significant prognostic factors for OS.

Univariate analysis identified five prognostic factors for PFS in CHL patients: involvement of more than one extranodal site ($P = 0.003$), advanced clinical stage (III/IV; $P = 0.001$), performance status >1 ($P = 0.047$), hemoglobin level <10.5 g/dL ($P = 0.024$), and serum albumin <3.5 g/dL ($P = 0.002$). On multivariate analysis, only advanced clinical stage (III/IV; $P = 0.016$) was proven to be of independent prognostic impact on PFS.

New prognostic model for CHL

The IPI could stratify the prognosis of CHL in this analysis; however, other factors, including EBV positivity (but not CD20 positivity), presence of B symptoms, and thrombocytopenia in addition to elevated serum LDH level and performance status >1 were identified as prognostic factors for OS on multivariate analysis. We thus attempted to construct a new prognostic model with these five prognostic factors. We classified patients into three risk groups with the use of the following terms: low risk, 0 or 1 adverse factor; intermediate risk, 2 or 3 factors; high risk, 4 or 5 factors. This novel prognostic model for CHL could stratify the prognosis of patients with CHL ($P < 0.0001$). For 144 patients (58%) classified into the low-risk group, 5-year OS was 91%. For 92 patients (37%) in the intermediate group, 5-year OS was 66%; for 11 patients (5%) in the high-risk group, 5-year OS was 36% ($P < 0.0001$; Fig. 2).

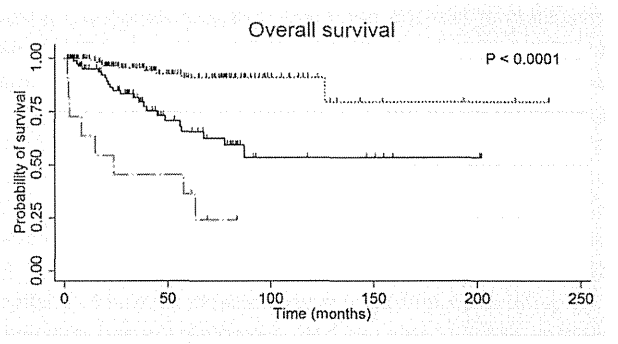

Figure 2 Overall survival of patients with CHL according to our new *prognostic model. This prognostic model could efficiently stratify the* outcomes into 3 groups: patients with 0 or 1 risk factor (low risk, $n = 144$), patients with 2 or 3 risk factors (intermediate risk, $n = 92$), and patients with 4 or 5 risk factors (high risk, $n = 11$). ·····, Low risk (0–1 adverse factor), $n = 144$; ———, Intermediate risk (2–3 adverse factors), $n = 92$; —·—·—, High risk (4–5 adverse factors), $n = 11$.

DISCUSSION

In this study, EBV positivity (but not CD20 positivity), the presence of B symptoms, presence of performance status >1,

Table 5 Summary of previous studies about the prognostic effect of CD20 expression in CHL

Reference	Country/region	Total number of cases	% of CD20$^+$ cases	Prognostic outcome measures	Prognosis of CD20$^+$ cases	% of EBV$^+$ cases
Tzankov *et al.*[28]	Innsbruck, Austria	119	20%	FFS, OS	Favorable	26%, No correlation with CD20 expression
Canioni *et al.*[32]	Paris, France	59	32%	Association with refractory status	Favorable	No data
Rassidakis *et al.*[31]	Texas, USA; Milan and Verona, Italy; Athens, Greece	598	22%	FFS	No prognostic effect	No data
Molot *et al.*[30]	Florida, USA	46 (only stage I & II who were treated by radiotherapy alone) including 3 cases of NLPHL	20%	FFS, OS	No prognostic effect	No data
Portlock *et al.*[29]	New York, USA	248	11%	TTF, OS	Unfavorable	No data
Aldred *et al.*[26]	Sao Paulo, Brazil	238	14%	FFS, OS	Unfavorable	41%, Significant positive correlation with CD20 expression

FFS, failure-free survival; OS, overall survival; TTF, time to treatment failure.

thrombocytopenia, and elevated serum LDH level were independent poor prognostic factors for OS in CHL patients. Our new prognostic model including these five adverse factors could stratify the prognosis of CHL patients with very high statistical significance ($P < 0.0001$). B symptoms (unexplained fever, night sweats, or weight loss) are associated with increased plasma levels of cytokines such as IL-6 which is reported to be associated with inferior survival in Hodgkin lymphoma patients.[23] Possible etiologies of thrombocytopenia during the course of the disease include the toxic effects of chemotherapy regimens, therapy-related myelodysplastic syndrome, heavy bone marrow infiltration and/or hypersplenism in patients with relapsed/refractory disease.[24] Elevated serum LDH reflects tumor burden and cellular turnover in several malignancies, including germ cell tumors, sarcomas and non-Hodgkin lymphoma. Therefore, it has been recognized as a poor prognostic factor and as a tumor marker.[25] EBV is an important factor involved in the pathogenesis of Hodgkin lymphoma and therefore worthy of its inclusion in a prognostic model for CHL. Our prognostic model was established in a single center cohort, therefore future investigations are thus warranted to determine whether it can be applied to other clinical cohorts.

This study was done on 389 consecutive cases of CHL. Seventy-four cases (19%) were CD20-positive which was within the reported range 5%–50% of CHL patients.[6,15–17] We found that the CD20-postive cases were different from CD20-negative cases regarding the age at onset and the frequency of EBV association with no significant difference in OS or PFS between the two groups. Aldred *et al.*[26] also found significant positive correlation between CD20 expression and the association with EBV while Bai *et al.*[27] suggested the possible down regulating effect of EBV infection on the expression of CD20 on HRS cells. One other report by Tzankov *et al.*[28] found no association between EBV infection and expression of CD20.

Aldred *et al.*[26] reported poor prognostic impact of CD20 expression in HRS cells on OS in patients aged 21–40 years. In our data, even after grouping the patients into different age groups, no prognostic value of CD20 could be detected regarding OS on univariate and multivariate analyses. We also analyzed the effect of CD20 status on OS according to the treatment regimens. For those patients who received ABVD combined chemotherapy and those who received other regimens, there was no significant difference in OS in CD20-positive and CD20-negative cases in both groups (ABVD group, $P = 0.98$; other chemotherapeutic regimens group, $P = 0.46$). Portlock *et al.*[29] reported poorer OS in CD20-positive CHL cases in ABVD chemotherapy receiving group ($P = 0.02$) but not in other chemotherapy receiving group ($P = 0.41$). The effect of CD20 status on survival was also investigated in EBV negative and EBV-positive CHL. Yet, there was no difference in survival between CD20-positive and -negative cases even after adjustment of age.

The effect of CD20 expression on the prognosis of patients with CHL is controversial, some reports found it related to worse prognosis,[26,29] others didn't detect a significant effect on prognosis,[30,31] while few other reports found it related to better prognosis.[28,32] Controversial data in the literature are possibly due to tissue sampling, different therapeutic approaches or may reflect geographic particularities (Table 5).

In our study, the frequency of CD20 expression in the MC subtype was higher than other subtypes; however, this did not reach statistical significance. This was in agreement with the study conducted by Tzankov *et al.*[6] and by Aldred *et al.*[26]

who in addition found significant positive association of CD20 expression with the MC subtype.

EBV infection is believed to play a crucial role in the pathogenesis of Hodgkin lymphoma being associated with activation of multiple signaling pathways, and the dysregulation of several transcription factors.[18] Despite East Asian countries have low incidence of Hodgkin lymphoma compared to USA and Europe, the association with EBV is higher in the East Asian patients than in the American and European patients.[33]

In this study, EBV-positive CHL constituted 44% of patients. The percent of EBV-positive CHL cases varies between developed and developing countries. In North America and European countries, the reported proportion of EBV-positive CHL cases varies from 26–50%,[19,34,35] while in developing countries the proportion of EBV-positive CHL is higher.[36,37]

EBV-positive patients were more likely to have MC subtype than EBV-negative patients who had more frequently NS subtype. This finding was in agreement with some other studies.[33,38–40] The clinical features of our EBV-positive CHL patients were in keeping with the results of previous reports.[34,41]

The effect of EBV association on the prognosis of CHL was previously investigated, but the results are controversial. Some reports found negative impact of EBV infection on the prognosis,[33,34,41–43] while others found no significant effect on prognosis.[44–47] Other reports found favorable prognostic impact of EBV association in CHL.[39,48–50] This might be due to the heterogeneous nature of the disease, the used therapeutic protocol and the age distribution of patients. In our series, we found a tendency to poor prognosis in term of OS in EBV-positive CHL patients compared with EBV-negative patients, but without significance by the log-rank test (P = 0.09). In multivariate analysis we identified EBV positivity (but not CD20 positivity), the presence of B symptoms, presence of performance status >1, thrombocytopenia, and elevated serum LDH level as independent poor prognostic factors for OS in CHL patients. The poor prognostic impact of EBV on OS in multivariate analysis was also reported in other studies.[41,42]

The IPI score was successful in stratification of the OS of CHL cases in this analysis. Only two of five prognostic factors in our analysis, namely elevated serum LDH level and the presence of performance status >1, were adopted in IPI; thus, the attempt to construct a new prognostic model with newly identified factors might be reasonable. Our novel prognostic model included EBV infection, which is an important factor involved in the pathogenesis of Hodgkin lymphoma and thus, it is quite reasonable to be included. EBV-positive CHL is significantly associated with older age at onset; thus, it may be more adequate to substitute the age >60 factor of IPI with the EBV status. Because EBV infection prevails in developing and East Asian countries, our new prognostic model may be more applicable for patients from these countries. We recommend examination of EBV association in CHL patients in routine pathologic practice especially in East Asian and developing countries.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Phenotypic characteristics of $CD20^+$ CHL and $CD20^-$ CHL.

Table S2 Clinical characteristics and histological subtypes of EBV^+ CHL and EBV^- CHL.

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Prognostic biomarkers in patients with localized natural killer/T-cell lymphoma treated with concurrent chemoradiotherapy

Motoko Yamaguchi,[1] Katsuyoshi Takata,[2] Tadashi Yoshino,[2] Naoki Ishizuka,[3] Masahiko Oguchi,[4] Yukio Kobayashi,[5] Yasushi Isobe,[6] Kenichi Ishizawa,[7] Nobuko Kubota,[8] Kuniaki Itoh,[9] Noriko Usui,[10] Kana Miyazaki,[1] Izumi Wasada,[11] Shigeo Nakamura,[12] Yoshihiro Matsuno,[13] Kazuo Oshimi,[14] Tomohiro Kinoshita,[15] Kunihiro Tsukasaki[9] and Kensei Tobinai[5]

[1]Department of Hematology and Oncology, Mie University Graduate School of Medicine, Tsu; [2]Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama; [3]Clinical Trial Department, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo; [4]Department of Radiation Oncology, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo; [5]Department of Hematology, National Cancer Center Hospital, Tokyo; [6]Department of Hematology, Juntendo University School of Medicine, Tokyo; [7]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai; [8]Department of Hematology, Saitama Cancer Center, Ina; [9]Division of Hematology and Oncology, National Cancer Center Hospital East, Kashiwa; [10]Division of Hematology and Oncology, Jikei University School of Medicine, Tokyo; [11]Department of Hematology, Tokai University, Isehara; [12]Department of Pathology and Laboratory Medicine, Nagoya University Hospital, Nagoya; [13]Department of Surgical Pathology, Hokkaido University Hospital, Sapporo; [14]Department of Internal Medicine, Kushiro Chuo Hospital, Kushiro; [15]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan

Key words

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Correspondence

Motoko Yamaguchi, Department of Hematology and Oncology, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan.

Tel: +81-59-231-5418; Fax: +81-59-231-5419;
E-mail: waniwani@clin.medic.mie-u.ac.jp

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Concurrent chemoradiotherapy has become one of the standard management approaches for newly diagnosed localized nasal natural killer (NK)/T-cell lymphoma (NKTCL). Few data are available on the prognostic biomarkers of NKTCL among patients treated with concurrent chemoradiotherapy. To evaluate the prognostic significance of immunophenotypic biomarkers for patients treated with concurrent chemoradiotherapy, latent membrane protein 1 (LMP1), cutaneous lymphocyte antigen (CLA) and cell origin were examined in samples from 32 patients who were enrolled in the Japan Clinical Oncology Group 0211 trial and treated with concurrent chemoradiotherapy. LMP1 and CLA were positive in 66% (19/29) and 29% (9/31) of the cases examined, respectively. The median follow-up duration was 68 months (range, 61–94). The patients with LMP1-positive tumors showed a better overall survival (OS) than the patients with LMP1-negative tumors (hazard ratio, 0.240; 95% confidence interval [CI], 0.057–1.013; 80% CI, 0.093–0.615; $P = 0.035$). All five patients with LMP1-negative tumors who experienced disease progression died of lymphoma, and both patients with local failure had LMP1-negative tumors. There was no significant difference in OS according to CLA expression. A total of 27 (84%) cases were of NK-cell origin, two were of αβ T-cell origin and three were of γδ T-cell origin. In contrast to those with tumors of NK-cell origin, all five patients with NKTCL of T-cell origin were alive without relapse at the last follow up. Our results indicate that LMP1 expression is a favorable prognostic marker and suggest that a T-cell origin of the tumor may be a favorable prognostic marker for patients with localized NKTCL treated with concurrent chemoradiotherapy.

Extranodal natural killer (NK)/T-cell lymphoma (NKTCL), nasal type, is a predominantly extranodal lymphoma associated with Epstein–Barr virus (EBV).[1,2] The tumor cells in most cases of NKTCL show an NK-cell phenotype,[1,2] while some cases show a T-cell phenotype, including γδ T-cell and αβ T-cell types.[3,4]

Tumor cells of NKTCL express P-glycoprotein, resulting in tumor multidrug resistance.[5–7] The outcomes after treatment with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) or with CHOP-like chemotherapy for localized nasal NKTCL are unsatisfactory.[8–10] Based on the results of clinical trials published in 2009,[11,12] concurrent chemoradiotherapy has been recognized as one of the standard management approaches for newly diagnosed localized NKTCL.[13–15] In Japan, the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG-LSG) conducted a phase I/II study (JCOG0211) of radiotherapy (RT) and dexamethasone, etoposide, ifosfamide and carboplatin (DeVIC) (RT-DeVIC) for newly diagnosed localized nasal NKTCL.[11,16] In patients who were treated with the recommended dose (RT-2/3DeVIC), the 5-year overall survival (OS) and the 5-year progression-free survival (PFS) were 70 and 63%, respectively.[16] Subgroup analysis further revealed that both the international prognostic index[17] and the NK/T-cell lymphoma prognostic

index[18] were not valid for the patient cohort of JCOG0211,[11] and similar results of a subgroup analysis were obtained in a phase II study of concurrent chemoradiotherapy in Korea.[12]

Latent membrane protein 1 (LMP1),[19–22] cutaneous lymphocyte antigen (CLA),[23,24] NK-cell origin[4,25–27] and EBV-encoded RNA (EBER) in the pretreatment bone marrow (BM), as detected by *in situ* hybridization,[28] have all been reported as prognostic biomarkers in patients with NKTCL who have been treated with conventional therapies. However, the prognostic significance of these biomarkers remains unclear, as most patients with NKTCL in previous studies were treated with heterogeneous treatment modalities. Because concurrent chemoradiotherapy is a new treatment modality for lymphoma, few data are available on the prognostic biomarkers of NKTCL among patients treated with concurrent chemoradiotherapy.

To evaluate the prognostic significance of immunophenotypic biomarkers among patients treated with concurrent chemoradiotherapy, we conducted an ancillary clinicopathologic study of the JCOG0211 trial.

Materials and Methods

Patients, treatment and tissue samples. The subjects in this study included 33 patients who were enrolled in the JCOG0211 trial. The design of the JCOG0211 trial has previously been described in detail.[11] Briefly, patients were eligible for the study if they were 20 to 69 years old and had previously untreated extranodal NKTCL, nasal type.[1] Patients were also required to have stage IE or contiguous stage IIE disease with cervical lymph node involvement and at least one measurable lymphomatous lesion in the nasal cavity and/or its adjacent sites. Patients received RT-DeVIC therapy consisting of RT of 50 Gy and three cycles of DeVIC chemotherapy. A two-thirds dose of DeVIC was selected for 27 patients who were evaluated in the phase II portion of JCOG0211. A full-dose of DeVIC was selected for six patients in the phase I portion. Among 33 cases, four cases had been included in a previous single-center study analyzing LMP1 expression in tumor cells of NKTCL.[21]

Sections of formalin-fixed, paraffin-embedded tissues of pretreatment lymphoma and BM samples were collected from the patients. The histological diagnoses of all patients were

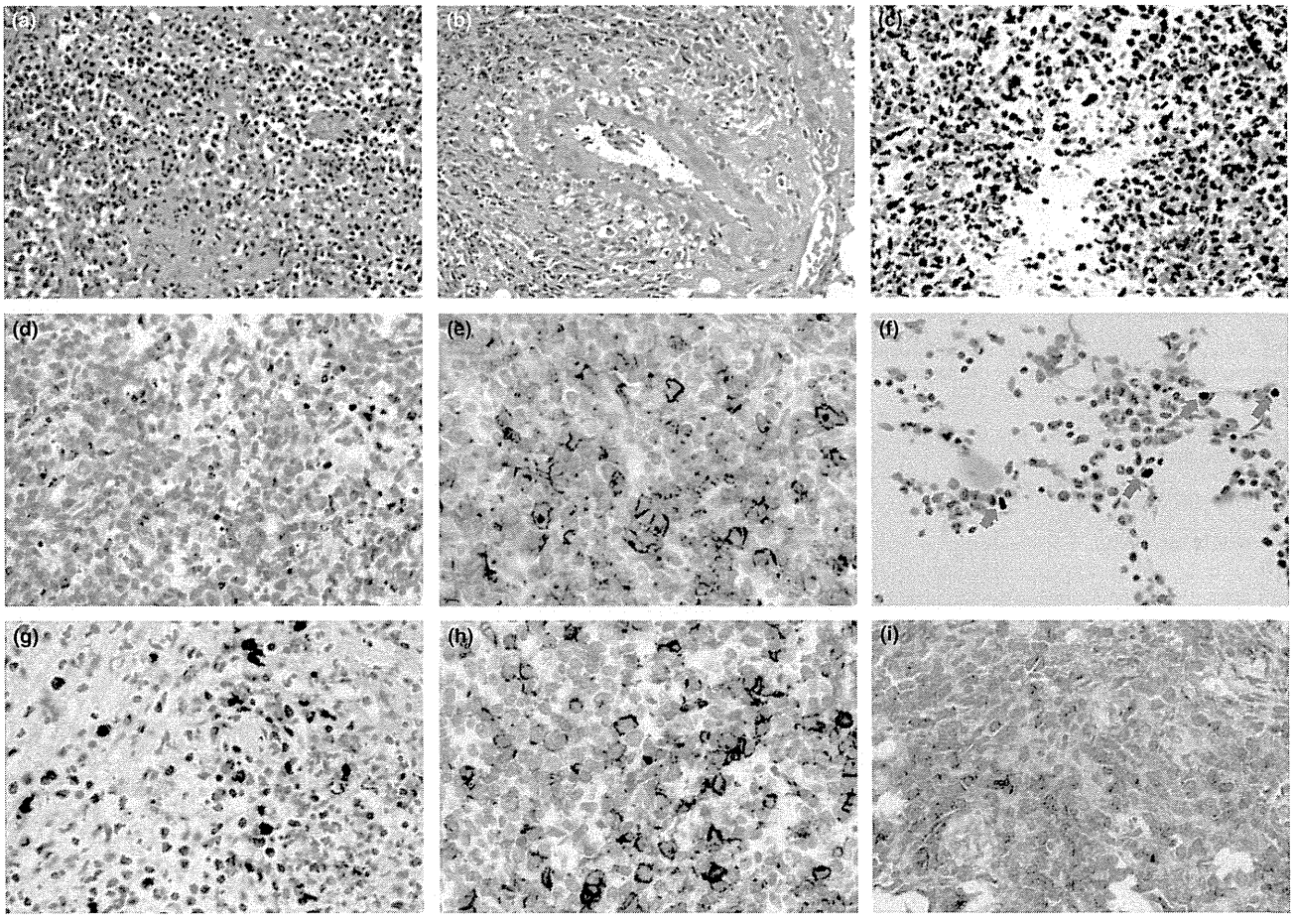

Fig. 1. (a) H&E staining in NK/T-cell lymphoma (NKTCL) of γδ T-cell origin (57-year-old male patient). Medium to large sized tumor cells with necrosis are seen. (b) H&E staining in NKTCL of γδ T-cell origin (34-year-old male patient). Tumor cells have an angiocentric angiodestructive quality. (c) *In situ* hybridization study for NKTCL of γδ T-cell origin (57-year-old male patient). Tumor cells are positive for Epstein–Barr virus encoded RNA (EBER). (d) Neoplastic tissue from the nasal cavity of a 39-year-old female patient with NKTCL of NK-cell origin. The neoplastic cells are positive for latent membrane protein 1 (LMP1). (e) Neoplastic tissue from the oral cavity of a 57-year-old male patient with NKTCL of NK-cell origin. The neoplastic cells are positive for cutaneous lymphocyte antigen (CLA). (f) Bone marrow tissue from a 34-year-old male patient with NKTCL of γδ T-cell origin. There are a small number of EBER-positive cells (red arrows). (g–i) Neoplastic tissue from the nasal cavity of a 55-year-old male patient with NKTCL of γδ T-cell origin. The neoplastic cells are positive for LMP1 (g), CLA (h) and T-cell receptor γ (i).

confirmed as extranodal NKTCL, nasal type by the Central Pathology Review Board.[11] All immunohistopathological examinations for the current ancillary study were performed at the Central Pathology Office of the ancillary study (Okayama University Hospital, Okayama, Japan).

The current study was approved by the JCOG Protocol Review Committee and the institutional review board at each study site. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki. All data on baseline features, treatment details, response and follow up were retrieved from the original JCOG0211 dataset.

Immunohistochemical analysis. Immunohistochemical staining was performed on sections of formalin-fixed, paraffin-embedded tissues of pretreatment lymphoma samples with heat-induced or trypsin-induced epitope retrieval using an avidin–biotin complex method and an automated immunostainer (Bond-max, Leica Biosystems, Melbourne, Vic., Australia), as previously described.[29] The following primary antibodies were used to assess these samples: LMP1 (CS1-4, 1:50, Novocastra, Newcastle-upon-Tyne, UK), T-cell receptor (TCR) β (βF1; TCR1151, 8A3, 1:50, Thermo Scientific, Waltham, MA, USA), TCRγ chain constant region (CγM1; TCR1153, γ3.20, 1:80, Thermo Scientific) and CLA (HECA452, 1:10) as previously described.[23,29]

For the LMP1 antigen, samples were determined to be positive when the lymphoma cells were positive according to the methods described by Kanemitsu *et al.* [21]. For βF1, CγM1 and CLA expression, samples in which 30% or more of the cells expressed the antigen were scored as positive, as previously described.[23,29] A preliminary evaluation for the study showed that staining for TCRγ and TCRδ was concordant in all cases.[29] Cases were considered to be of NK-cell origin if both TCRβ and TCRγ expression was not observed. Cases with positive staining for one or both of the antibodies (TCRβ and TCRγ) were determined to be of T-cell origin.[29]

***In situ* hybridization.** Pretreatment BM specimens from patients were examined for EBER. *In situ* hybridization with EBER-1 probes (INFORM EBER, Leica Biosystems, Melbourne, Victoria, Australia) was performed to detect EBV.[29]

Statistical analysis. Survival estimates were calculated using the Kaplan–Meier method. Hazard ratios (HR) and 80 and 95% confidence intervals (CI) were estimated using a Cox regression. All of the analyses were performed using IBM SPSS Statistics 20.0 (IBM Japan, Tokyo, Japan).

Results

Expression of biomarkers. Pathological samples from 32 out of 33 patients were available for this study. Tissue samples from the remaining patient were exhausted during the Central Pathology Review and, therefore, were no longer available for the current study. This patient was a 33-year-old woman with

Table 1. Clinical features according to each biomarker expression

	All Patients *n* = 32 (%)	LMP1		CLA		Cell Origin	
		Positive *n* = 19	Negative *n* = 10	Positive *n* = 9	Negative *n* = 22	NK-cell type *n* = 27	T-cell type *n* = 5
Age at Diagnosis, *n* (%)							
≤60	25 (78)	15 (79)	8 (80)	8 (89)	16 (73)	21 (78)	4 (80)
>60	7 (22)	4 (21)	2 (20)	1 (11)	6 (27)	6 (22)	1 (20)
Sex, *n* (%)							
Male	19 (59)	9 (47)	3 (30)	9 (100)	10 (45)	12 (44)	1 (20)
Female	13 (41)	10 (53)	7 (70)	0 (0)	12 (55)	15 (56)	4 (80)
PS, *n* (%)							
0 or 1	30 (94)	17 (89)	10 (100)	9 (100)	20 (91)	25 (93)	5 (100)
>1	2 (6)	2 (11)	0 (0)	0 (0)	2 (9)	2 (7)	0 (0)
Serum LDH Level, *n* (%)							
≤Normal	25 (78)	15 (79)	7 (70)	7 (78)	17 (77)	20 (74)	5 (100)
>Normal	7 (22)	4 (21)	3 (30)	2 (22)	5 (23)	7 (26)	0 (0)
B Symptoms, *n* (%)							
Absent	20 (63)	13 (68)	5 (50)	4 (44)	15 (68)	16 (59)	4 (80)
Present	12 (37)	6 (32)	5 (50)	5 (56)	7 (32)	11 (41)	1 (20)
Stage, *n* (%)							
IE	22 (69)	12 (63)	8 (80)	5 (56)	16 (73)	20 (74)	2 (40)
IIE	10 (31)	7 (37)	2 (20)	4 (44)	6 (27)	7 (26)	3 (60)
Skin Involvement, *n* (%)							
Absent	18 (56)	14 (74)	3 (30)	4 (44)	14 (64)	13 (48)	5 (100)
Present	14 (44)	5 (26)	7 (70)	5 (56)	8 (36)	14 (52)	0 (0)
IPI, *n* (%)							
0	19 (59)	11 (58)	6 (60)	6 (67)	12 (55)	15 (56)	4 (80)
1	10 (31)	6 (32)	3 (30)	3 (33)	7 (32)	9 (33)	1 (20)
2	3 (9)	2 (11)	1 (10)	0 (0)	3 (14)	3 (11)	0 (0)
NK-PI, *n* (%)							
0	11 (34)	7 (37)	3 (30)	1 (11)	9 (41)	9 (33)	2 (40)
1	9 (28)	4 (21)	4 (40)	5 (56)	4 (18)	8 (30)	1 (20)
2	9 (28)	7 (37)	1 (10)	2 (22)	7 (32)	7 (26)	2 (40)
3	3 (9)	1 (5)	2 (20)	1 (11)	2 (9)	3 (11)	0 (0)

CLA, cutaneous lymphocyte antigen; IPI, international prognostic index; LDH, lactate dehydrogenase; LMP1, latent membrane protein 1; NK-PI, NK/T-cell lymphoma prognostic index; PS, performance status.

stage IIE NKTCL who obtained a complete response (CR) by RT-(full-dose) DeVIC and was alive at the last follow-up examination (86 months). Pretreatment BM samples were obtained from 29 of the 32 patients.

The immunohistochemical features of these samples are shown in Figure 1. LMP1 and CLA were positive in 66% (19/29) and 29% (9/31) of the cases examined, respectively. Among the 32 cases that were examined to determine cell lineage, 27 (84%) cases were of NK-cell origin. Two (6%) cases were of αβ T-cell origin on the basis of TCRβ immunoreactivity but not TCRγ, and 3 (9%) cases were of γδ T-cell origin due to the presence of TCRγ immunoreactivity but not TCRβ immunoreactivity. Pretreatment BM samples were positive for EBER in 2 (7%) out of 29 cases examined. One of the two EBER-positive cases had two positive cells in a total field of view. Another case had three to five positive cells/high-power field. Positive cells were small to medium sized cells and were diffusely distributed in the latter case.

The Central Pathology Review of JCOG0211 confirmed that tumor cells in 29 out of the 32 cases were positive for EBER. Tissue samples of the remaining three cases were not evaluable for EBER. The current study revealed LMP1 expression in tumor cells of all the remaining three cases, indicating that all 32 cases were associated with EBV.

In the 29 patients whose samples were available for analysis of LMP1 and CLA expression, tumors from four patients were positive for both antigens, tumors from 15 patients were positive for LMP1 only, tumors from four patients were positive for CLA only, and tumors from six patients were negative for both antigens. In the three patients with NKTCL of γδ T-cell origin, two samples contained cells that were positive for both LMP1 and CLA, and one sample contained cells that were negative for both antigens. Of the two cases of αβ T-cell origin, one showed an LMP1-positive and CLA-negative immunophenotype, while another case was positive for both LMP1 and CLA. In two patients whose BM samples were positive for EBER, one had NKTCL of NK-cell origin that was negative for LMP1 and CLA, and the other had NKTCL of γδ T-cell origin that was positive for both LMP1 and CLA.

Clinical features according to the expression of each biomarker. The baseline clinical characteristics according to the expression of each biomarker are shown in Table 1. All CLA-positive tumors in the study were from male patients. Skin involvement prior to treatment was frequently observed in the patients with LMP1-negative tumors (odds ratio [OR], 6.533 [95% CI, 1.200–35.573]). All five patients with NKTCL of T-cell origin showed no skin involvement at baseline (OR, 0.722 [95% CI, 0.542–0.962]). No significant correlation was observed between the induction of CR and the expression of each biomarker (data not shown).

Survival analysis. The median follow-up duration was 68 months (range, 61–94). The OS was better in the LMP1-positive group than in the LMP1-negative group (HR, 0.240; 95% CI, 0.057–1.013; 80% CI, 0.093–0.615; $P = 0.035$, Fig. 2). The OS at 5 years was 84% in the LMP1-positive group and 50% in the LMP1-negative group. The PFS at 5 years was 74% in the LMP1-positive group and 50% in the LMP1-negative group. The HR of PFS among LMP1-positive tumors was 0.421 (95% CI, 0.121–1.463; 80% CI, 0.187–0.950). There were no significant differences in the OS and the PFS between the CLA-positive and CLA-negative groups (5-year OS, 56 *vs* 77%; 5-year PFS, 56 *vs* 68%, Fig. 2). All

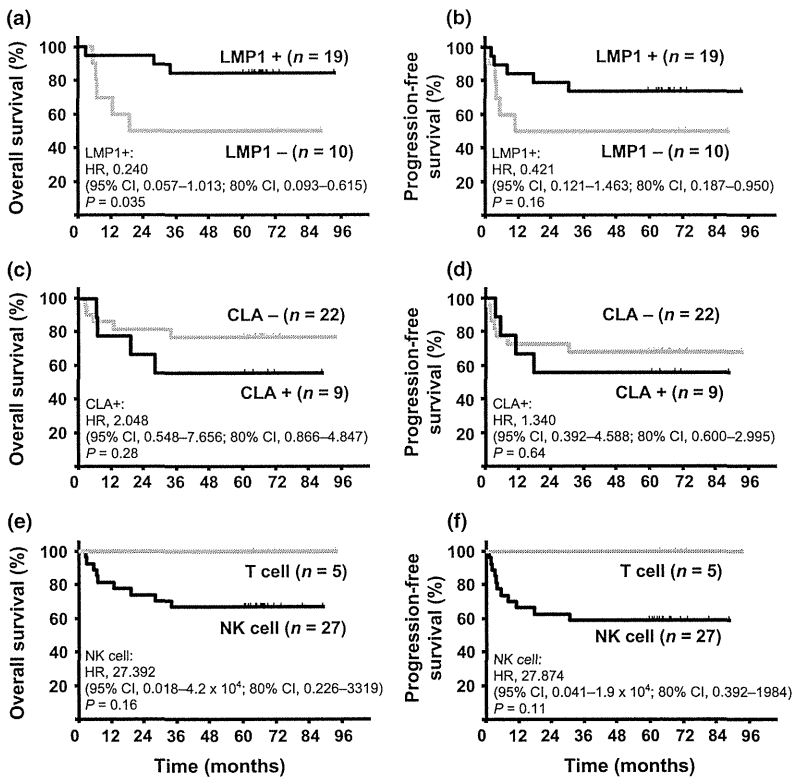

Fig. 2. (a,c,e) Overall survival curves of patients with NK/T-cell lymphoma (NKTCL) by latent membrane protein 1 (LMP1) expression (a), cutaneous lymphocyte antigen (CLA) expression (c) and cell-of-origin (e). (b,d,f) Progression-free survival curves of patients with NKTCL by LMP1 expression (b), CLA expression (d) and cell-of-origin (f).

Table 2. Characteristics and biomarker expression of the 11 patients who experienced disease progression or recurrence during follow-up

Age/Sex	Stage	LMP1	CLA	Overall response	Site(s) of recurrence	Salvage therapy	OS, mo	PFS, mo	Outcome
57/F	IIEA	+	–	PD	Liver, spleen, BM	None	3	2	DOD
21/M	IEA	+	–	PD	LN	Chemotherapy for ALL, CBT	69	3	AND
57/F	IIEA	+	–	CR	LN, stomach	L-asp/VCR/DMS, Allo PBSCT	68	8	AND
57/M	IIEB	+	–	CR	BM, PB	CMED, L-asp/VCR/DMS, EPOCH	34	31	DOD
38/M	IIEB	+	+	CR	LN, liver, spleen, ascites	mPSL, ETP, FCM	29	18	DOD
58/M	IEB	–	+	CR	Skin	ESHAP, RT, CHOP	19	11	DOD
63/F	IEA	–	–	PD	CNS, nasal cavity†, parotid gland, PB, subcutaneous tissue	MTX/AraC it, IVAC, HD-MTX, DeVIC, CHOP	13	1	DOD
57/M	IIEA	–	+	CR	LN, skin, BM	L-asp/VCR/DMS	7	4	DOD
58/F	IEA	–	–	PR	Skin, kidney, LN	ESHAP	6	4	DOD
60/M	IEB	–	+	SD	Eye, nasal cavity†, skin	None	7	6	DOD
45/M	IIEB	ND	–	PD	Gallbladder, BM	None	3	2	DOD

ALL, acute lymphoblastic leukemia; Allo PBSCT, allogeneic peripheral blood stem cell transplantation; AND, alive with no evidence of disease; AraC, cytarabine; BM, bone marrow; CBT, cord blood transplantation; CLA, cutaneous lymphocyte antigen; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CMED, cyclophosphamide, methotrexate, etoposide, and dexamethasone; CNS, central nervous system; CR, complete response; DeVIC, dexamethasone, etoposide, ifosfamide, and carboplatin; DOD, died of disease; EPOCH, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; ESHAP, etoposide, methylprednisolone, cytarabine, and cisplatin; ETP, etoposide; FCM, fludarabine, cyclophosphamide, and mitoxantrone; HD, high dose; it, intrathecal; IVAC, ifosfamide, etoposide, and cytarabine; L-asp/VCR/DMS, L-asparaginase, vincristine, and dexamethasone; LMP1, latent membrane protein 1; LN, lymph node, mPSL, methylprednisolone; MTX, methotrexate; ND, not done; OS, overall survival; PB, peripheral blood; PD, progressive disease; PFS, progression-free survival; PR, partial response; RT, radiotherapy; SD, stable disease. All 11 cases were of NK-cell origin. †Local failure.

five patients with NKTCL of T-cell origin achieved a CR by RT-DeVIC and were alive, without relapse, at the time of the last follow up (Fig. 2). The 5-year OS and PFS in the 27 patients with NKTCL of NK-cell origin were 67 and 59%, respectively. The two patients whose pretreatment BM samples were positive for EBER survived for more than 5 years without disease progression.

Characteristics and biomarker expression in patients who experienced disease progression or recurrence. During the follow-up period, 11 of the 33 patients who were enrolled in JCOG0211 experienced disease progression or recurrence. Detailed clinical information and biomarker expression for these cases are presented in Table 2. Among the 5 LMP1-positive patients, two patients achieved a second CR using L-asparaginase-containing chemotherapy followed by allogeneic hematopoietic stem cell transplantation and attained long-term survival. In contrast, all five patients whose tumors were LMP1-negative and who experienced disease progression died within 19 months of the initial registration. In JCOG0211, only two patients experienced local failure,[16] and the present study revealed that both of these cases were LMP1-negative (Table 2).

Discussion

The current study showed that LMP1 expression in tumor cells was associated with OS in patients with newly diagnosed localized NKTCL who were treated uniformly with concurrent chemoradiotherapy. Of note, all five patients with NKTCL of the T-cell type, including three patients with NKTCL of the γδ T-cell type, survived more than 5 years without recurrence.

Although NKTCL is associated with a type II EBV latency program,[30] the expression levels of LMP1 in NKTCL is variable at the single-cell level.[20,21,30] In addition, there have been conflicting results in previous studies analyzing the prognostic significance of LMP1 expression in NKTCL. A single-center study of 58 patients with NKTCL (advanced disease, $n = 12$) in China reported that patients with LMP1-positive tumors showed significantly worse survival.[19] Another study from China, which included 16 patients with NKTCL (advanced disease, $n = 2$), reported that patients whose tumors exhibited high LMP1 expression (81–100% of tumor cells) showed significantly shorter survival.[20] In contrast, one single-center study from Japan, which included 30 patients with NKTCL (advanced disease, $n = 7$), showed a favorable outcome for patients whose tumors were LMP1-positive compared to patients whose tumors were LMP1-negative.[21] Possible explanations for the incongruence in these reported results include the heterogeneous therapeutic approaches used, the differences in the incidence of advanced stage, and the use of different criteria for LMP1 positivity.

In the present study, the lack of LMP1 expression was associated with a short OS in patients with localized NKTCL who were treated with RT-DeVIC. Skin involvement was frequently observed in LMP1-negative cases. All five patients with LMP1-negative tumors who experienced disease progression died, and the two patients who experienced local failure in JCOG0211 had tumors that were LMP1-negative. Together, these results indicate that LMP1-negative NKTCL has an aggressive nature. The lack of detectable LMP1 in aggressive NK-cell leukemia[31] may also support this assertion. In LMP1-negative tumors, some researchers speculate that additional cellular genetic aberrations may be driving a more malignant tumor phenotype that no longer requires expression of the LMP1 oncogene.[32]

Differences in prognosis between NKTCL patients with the NK-cell type and the T-cell type have been the subject of previous research. Early studies revealed that patients with CD56-positive NKTCL show significantly worse survival than those with CD56-negative NKTCL.[25,26] A Chinese group reported that there was no difference in prognosis between these two groups.[27] However, patients in these studies were treated with various treatment modalities and different chemotherapeutic regimens. Recent immunohistochemical analyses using monoclonal antibodies against the TCRβ, γ and δ subunits have shown that some CD56-positive NKTCL cases are actually γδ

T-cell lymphoma and that NKTCL of T-cell origin exhibits a trend for better OS than NKTCL of NK-cell origin.[4] In the current study, patients were uniformly treated with RT-DeVIC. Moreover, the origins of the lymphoma cells were determined immunohistochemically with monoclonal antibodies against the TCRβ and γ subunits, which constitutes a more specific method of identifying cases of the T-cell type than the previous approaches. Our current study highlighted the favorable prognosis of patients with NKTCL of T-cell origin, which suggests that a more effective therapeutic strategy is needed for NKTCL of NK-cell origin.

CLA is a skin-homing receptor that functions in the adhesion of cells to the vascular endothelium.[33] CLA-positive lymphocytes that migrate to the skin consist mostly of T cells, but NK cells are also present in this population.[34] Patients with NKTCL often develop skin involvement during the clinical course of the disease.[1] In the present study, 5 (56%) out of nine patients with CLA-positive tumors also had skin lesions. Yoshino *et al.*[23] examined CLA expression in 52 patients with NKTCL, including 14 patients with advanced disease, and found that CLA expression in NKTCL was associated with skin involvement and a poor prognosis.[23] A retrospective analysis with a small number of patients showed similar results in terms of survival.[24] In the present study, CLA expression was not associated with a poor prognosis. One possible reason is that only patients with NKTCL localized within the nasal cavity or the nasopharyngeal region were included in our study. It is also possible that the prognosis of patients with CLA-positive tumors may have been improved by RT-DeVIC.

In the current study, we examined the prognostic significance of the presence of EBER in pretreatment BM samples from patients, partly because testing for BM EBER was not routinely performed in Japan during the study period (2003–2006) of the JCOG0211 trial. Two patients in our study with EBER-positive pretreatment BM survived more than 5 years without disease progression, which suggests that EBER-positive BM did not affect the prognosis of our JCOG0211 patient cohort.

The current study demonstrated, for the first time, the favorable impact of LMP1 expression on the OS of patients with newly diagnosed localized nasal NKTCL in a cohort of patients uniformly treated with concurrent chemoradiotherapy. The limitations of this study include the relatively small number of patients included, the lack of available data on the EBV DNA load in the peripheral blood, which has been reported as a useful biomarker of NKTCL,[35,36] and the possibility of intermingled TCR silent cases in our NKTCL cases of NK-cell origin. Although our evaluations were confined to a relatively small number of patients, these patients were derived from a uniform cohort and were prospectively treated with the same therapeutic strategy consisting of RT-DeVIC. The prognostic values of LMP1 expression and the T-cell origin warrant further evaluation in future studies with a larger number of patients with NKTCL who are treated with concurrent chemoradiotherapy.

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Disclosure Statement

K. Oshimi is an employee of Eisai. The remaining authors have no conflict of interest to declare.

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Prognostic significance of pleural or pericardial effusion and the implication of optimal treatment in primary mediastinal large B-cell lymphoma: a multicenter retrospective study in Japan

Tomohiro Aoki,[1,2] Koji Izutsu,[3] Ritsuro Suzuki,[4] Chiaki Nakaseko,[5] Hiroshi Arima,[6] Kazuyuki Shimada,[2] Akihiro Tomita,[2] Makoto Sasaki,[7] Jun Takizawa,[8] Kinuko Mitani,[9] Tadahiko Igarashi,[10] Yoshinobu Maeda,[11] Noriko Fukuhara,[12] Fumihiro Ishida,[13] Nozomi Niitsu,[14] Ken Ohmachi,[15] Hirotaka Takasaki,[16] Naoya Nakamura,[17] Tomohiro Kinoshita,[18] Shigeo Nakamura,[19] and Michinori Ogura[1,20]

[1]Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital; [2]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine; [3]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [4]Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine; [5]Department of Hematology, Chiba University Hospital; [6]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto; [7]Division of Hematology, Department of Internal Medicine, Juntendo University School of Medicine, Tokyo; [8]Department of Hematology, Endocrinology and Metabolism, Niigata University Faculty of Medicine; [9]Department of Hematology and Oncology, Dokkyo Medical University of Medicine, Tochigi; [10]Department of Hematology and Oncology, Gunma Cancer Center, Oota; [11]Department of Hematology and Oncology, Okayama University Hospital; [12]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai; [13]Department of Biomedical Laboratory Sciences, Department of Hematology, Shinshu University School of Medicine, Matsumoto; [14]Department of Hematology, International Medical Center, Saitama Medical University, Hidaka; [15]Department of Hematology, Tokai University, Isehara; [16]Department of Medical Oncology, Kanagawa Cancer Center, Yokohama; [17]Department of Pathology, Tokai University, Isehara; [18]Department of Hematology and Cell Therapy, Aichi Cancer Center, Nagoya; [19]Department of Pathology and Laboratory Medicine, Nagoya University Hospital; and [20]Department of Internal Medicine and Laboratory Medicine, National Hospital Organization Suzuka National Hospital, Japan

ABSTRACT

The prognosis of patients with primary mediastinal large B-cell lymphoma has improved over recent years. However, the optimal treatment strategy including the role of radiotherapy remains unknown. We retrospectively analyzed the clinical outcomes of 345 patients with newly diagnosed primary mediastinal large B-cell lymphoma in Japan. With a median follow up of 48 months, the overall survival at four years for patients treated with R-CHOP (n=187), CHOP (n=44), DA-EPOCH-R (n=9), 2nd- or 3rd-generation regimens, and chemotherapy followed by autologous stem cell transplantation were 90%, 67%, 100%, 91% and 92%, respectively. Focusing on patients treated with R-CHOP, a higher International Prognostic Index score and the presence of pleural or pericardial effusion were identified as adverse prognostic factors for overall survival in patients treated with R-CHOP without consolidative radiotherapy (IPI: hazard ratio 4.23, 95% confidence interval 1.48-12.13, P=0.007; effusion: hazard ratio 4.93, 95% confidence interval 1.37-17.69, P=0.015). Combined with the International Prognostic Index score and the presence of pleural or pericardial effusion for the stratification of patients treated with R-CHOP without radiotherapy, patients with lower International Prognostic Index score and the absence of effusion comprised approximately one-half of these patients and could be identified as curable patients (95% overall survival at 4 years). The DA-EPOCH-R regimen might overcome the effect of these adverse prognostic factors. Our simple indicators of International Prognostic Index score and the presence of pleural or pericardial effusion could stratify patients with primary mediastinal large B-cell lymphoma and help guide selection of treatment.

Introduction

Primary mediastinal large B-cell lymphoma (PMBL) is characterized by distinct clinical, pathological and genetic features and comprises a subtype of diffuse large B-cell lymphoma (DLBCL) according to the current World Health Organization (WHO) classification.[1] The disease is more common in younger females and often presents with bulky mediastinal mass without extrathoracic spread and pleural or pericardial effusion.[2-5]

Prior to the introduction of rituximab, the outcomes of patients treated with anthracycline-containing chemotherapies, including cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP), had a suboptimal progression-free survival (PFS) of 38%-52%.[5,6] Several retrospective analyses revealed that the outcomes of the 2nd- and 3rd-generation chemotherapeutic regimens, such as methotrexate, leucovorin, doxorubicin, cyclophosphamide, vincristine, bleomycin and prednisolone (MACOP-B), might be superior to those of CHOP regimens.[5,7-10] High-dose chemotherapy followed by autologous stem cell transplantation (HDT/ASCT) was also associated with encouraging results (PFS >75% for newly diagnosed PMBL patients).[3,11,12] These reports indicate that intensive regimens might be beneficial in a certain proportion of PMBL patients.

In the rituximab era, the combination of rituximab and

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Correspondence: iwbamasbytryf0305@yahoo.co.jp

chemotherapy has improved outcomes in various subtypes of B-cell lymphoma.[18-22] In the literature, more than 80% of patients with PMBL receiving immunochemotherapy with or without radiotherapy (RT) also achieved long-term overall survival (OS).[17-22] Despite the outstanding advances with R-CHOP, 20%-30% of patients still experience progression or relapse and have poor outcomes. Moreover, approximately 80% of long-term survivors treated with R-CHOP required consolidative RT for residual mediastinal disease.[20-23] Considering late adverse events induced by the mediastinal RT, namely the increased risk of secondary breast cancer and cardiac toxicity, the risk of RT should be minimized, especially for younger patients.[24-26]

Recently, Dunleavy *et al.* reported excellent outcomes for dose-adjusted etoposide, cyclophosphamide, doxorubicin, vincristine, prednisolone and rituximab (DA-EPOCH-R) when restricting candidates for RT according to the results of positron-emission tomography/computed tomography (PET/CT).[27] Although outcomes were reported from a phase II trial, the regimen might be a promising treatment strategy to reduce the risk of RT. Meanwhile, the DA-EPOCH-R regimen is somewhat complicated and expensive, requiring continuous infusion for 96 h in each cycle and frequent evaluation of complete blood counts. Considering R-CHOP-based regimens without RT could provide curative potential for a significant proportion of PMBL patients without hospitalization,[19,21] it would, therefore, be beneficial to identify the subset of patients that could be cured with this treatment strategy.

The goal of the present multicenter co-operative retrospective study in Japan was to investigate the optimal treatment strategy for PMBL patients by evaluating the clinical outcomes in response to various treatments and to assess a risk-stratified treatment strategy to minimize the risk of late adverse events in PMBL patients.

Methods

Patients

A total of 363 patients with PMBL newly diagnosed between May 1986 and September 2012 at one of any of the 65 participating hospitals in Japan were retrospectively analyzed. We registered consecutive patients who were diagnosed with PMBL at each institution in accordance with the WHO classification.[1] The time period during which we could collect the clinical data from each institution varied due to the differences in the length of time medical records are kept there. Medical record data since the 1980s were collected from three institutions, while data since the 1990s and 2000s were available from 10 and 65 institutions, respectively. In this study, PMBL was defined as patients with a dominant mass within the anterior mediastinum, irrespective of the tumor size. In addition, a central pathological review was performed by a hematopathologist (SN) for 196 patients for whom histological paraffin-embedded tissue materials could be provided. Eighteen of the 363 patients were excluded from analysis due to disease other than PMBL (n=10) by central pathological review or due to the absence of important clinical information (n=8). For the remaining patients who were not available for the central review, the histological diagnosis of PMBL was re-confirmed by a pathologist at each institution, according to the current WHO classification. Therefore, 345 patients were finally analyzed for the present study. Patients were treated according to each institution's treatment standards. The study protocol was approved by the institutional review boards of Nagoya Daini Red Cross Hospital where this study was organized and of each participating hospital. The study complied with all the provisions of the Declaration of Helsinki.

Immunohistochemistry

Immunohistochemistry was performed using formalin-fixed, paraffin-embedded tissue sections using the avidin-biotin peroxidase complex method. Monoclonal antibodies targeting the following proteins were used: CD20, CD30, CD3, CD10, BCL6, MUM1 and CD15 (Dako). In addition, programmed cell death ligand-1 (PDL1) was evaluated, as previously described.[28] To evaluate PDL1, we used a polyclonal rabbit antibody for CD274 (ab82059; Abcam) according to the manufacturer's instructions. The cut-off values for these markers were 20% for CD30, and 30% for Bcl-6, MUM1 and PDL1.[29-31]

Treatment

Initial treatments were performed based on the physicians' decisions at each institution, as there had been no uniform treatment guidelines for PMBL in Japan. Patients who received CHOP or a CHOP-like regimen, with or without rituximab, were categorized and analyzed as the R-CHOP or CHOP group, respectively. Patients who received 2nd-/3rd-generation treatments were categorized and analyzed as the 2nd-/3rd-generation regimen group, irrespective of the use of rituximab. Patients who received the DA-EPOCH-R regimen[27] were analyzed as the DA-EPOCH-R group. Patients who underwent consolidative HDT/ASCT after initial therapy were analyzed as the HDT/ASCT group, irrespective of the use of rituximab. CHOP- or R-CHOP-based regimens were mainly selected in 46 institutions. Physicians at six institutions selected 2nd-/3rd-generation chemotherapeutic regimens other than CHOP- or R-CHOP-based regimens as the first-line treatment. HDT/ASCT as the first-line treatment was performed at 13 institutions. Consolidative RT was performed according to the treatment strategy used at each institution.

Response assessment

Clinical data were collected from case report forms. In principle, an effusion was evaluated by CT and/or echocardiography, as per the usual pre-treatment evaluation. Responses were evaluated by each investigator in accordance with the 1999 International Workshop Criteria.[32]

Statistical analysis

Overall survival was defined as the period from diagnosis to death or last follow up. Progression-free survival (PFS) was defined as the period from diagnosis to disease progression, relapse, death from any cause, or last date of follow up. Patients who did not achieve a complete remission (CR) or partial response (PR) were considered to have primary refractory disease. Early relapse was defined as relapse occurring less than 12 months after diagnosis. PFS and OS were analyzed using Kaplan-Meier methods and results were compared using the log rank test. Univariate and multivariate Cox regression analyses were performed to assess the effects of prognostic factors. Multivariate analysis was built with a forward/backward, step-wise method using threshold values for removal from and addition to the model of P=0.20 and P=0.05, respectively. The individual factors of IPI were entered into the model in multivariate analysis. All probability values were two-sided and had an overall significance level of 0.05. Statistical analyses were performed with Stata SE 12 software (StataCorp LP, College Station, TX, USA).

Results

Patients' characteristics

Patients' characteristics are summarized in Table 1. Median age was 32 years (range 17-83 years) and females were predominant (58%). The median diameter of mediastinal mass was 10 cm (range 3-32 cm). Stage I/II disease, low-risk disease according to the International Prognostic Index (IPI), and performance status (PS) 0/1 were also predominant (67%, 52% and 75%, respectively). The presence of pleural or pericardial effusion, elevated lactate dehydrogenase (LDH) level and more than one extranodal lesion were observed in 46%, 80% and 9% of patients, respectively. For the patients who had extra-nodal involvement, major extra-nodal sites were lung (n=44), effusion (n=49) and cardiac (n=28). Pathological features are listed in Table 1. Lymphoma cells in all patients expressed CD20. Further, CD30, BCL6, and MUM1 expression was detected in 71%, 61%, and 96%, respectively. PDL1 was expressed in 62% of 110 evaluable patients.

Table 1. Patients' characteristics.

Characteristic	All		CHOP		R-CHOP		DA-EPOCH-R		2nd/3rd generation		HDT/ASCT	
	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%
Median follow up (months)	48		118		36		19		48		101	
Patient number	345		44		187		9		45		57	
Age at diagnosis (years)												
Median	32		31.5		33.5		30		31		27	
Range	17-83		17-77		17-83		24-64		18-76		17-63	
>60 years	47	14	10	23	30	16	1	11	3	7	3	5
Gender, male	146	42	18	41	85	45	4	44	12	27	27	47
PS, ≥2	84/338	25	12/42	29	40/182	22	3	33	8	18	20	3
Extranodal sites, >1	64/334	19	7/40	17	31/181	18	0	0	11	24	15/56	27
Stage, I/II	230/342	67	27	61	133/184	72	7	78	31	69	31	54
LDH at diagnosis, ≥ULN	270/337	80	35/41	85	134/183	73	8	89	37	82	54/56	96
B symptoms, present	90/337	27	15/42	36	40/183	22	2	22	11	24	22/55	40
IPI												
Low	175/334	52	19/40	48	103/181	57	5	56	26	58	21/56	38
Low-intermediate	84/334	25	11/40	28	44/181	24	3	33	9	20	16/56	29
High-intermediate	43/334	13	4/40	10	21/181	12	0	0	5	11	12/56	21
High	32/334	10	6/40	15	13/181	7	1	11	5	11	7/56	13
Bulky tumor size												
Median	10		10		9.2		12.6		10.5		10	
≥10 cm	166/324	51	20/36	56	84/180	47	6	67	26	59	30/56	58
s-IL2R after first-line therapy, ≥1000 U/mL	141/305	46	20/30	67	91/175	52	2/8	25	17/40	43	33/49	67
Presence of pleural or pericardial effusion	159/343	46	15/43	35	83/186	43	5	56	23	51	31	54
WBC, >10×10⁹/L	23/339	7	2/42	5	12/184	7	0	0	5	11	3/56	5
Hemoglobin, ≤12 g/dL	119/329	36	16/39	41	57/81	31	3	33	21	47	19/52	37
Platelet count, <150×10⁹/L	20/331	6	2/40	5	16/182	9	0	0	0	0	2/52	4
ALC at diagnosis, <0.5×10⁹/L	62/321	19	2/33	6	29/180	16	5	56	12	27	13/52	25
IHC staining, positive												
CD20	152/152	100	15/15	100	99/99	100	5/5	100	8/8	100	25/25	100
CD10	4/129	3	1/11	9	2/85	2	0/5	0	0/7	0	1/21	5
CD30	100/140	71	9/13	69	62/85	70	5/5	100	5/8	63	18/25	72
BCL6	72/116	61	8/11	73	46/75	61	2/5	40	4/6	67	12/19	63
MUM1	105/109	96	10/11	91	67/68	99	4/5	80	6/6	100	18/19	95
PDL-1	68/110	62	7/11	64	44/68	65	2/5	40	1/5	20	14/21	67
Treatment												
Administration of rituximab	267	77	0	0	187	100	9	100	28	62	43	75
Consolidation RT	145	42	21	48	64	34	4	44	30	67	24	42
Late adverse event												
Secondary cancer	7	2	1	2	4	2	0	0	0	0	2	4
Cardiac toxicity	10	3	0	0	9	5	0	0	0	0	1	2

CHOP: cyclophosphamide, adriamycin, vincristine and prednisone; R: rituximab; DA-EPOCH-R: dose-adjusted etoposide, cyclophosphosphamide, doxorubicin, vincristine, prednisolone and rituximab; HDT/ASCT: high-dose chemotherapy followed by autologous stem cell transplantation; PS: performance status; LDH: lactate dehydrogenase; ULN: upper limit of normal; IPI: international prognostic index; s-IL2R: soluble interleukin-2 receptor; WBC: white blood cell count; ALC: absolute lymphocyte count; IHC: immunohistochemical staining; RT: radiation therapy.

Treatment regimen

In all, 267 patients received rituximab-containing chemotherapy. CHOP and R-CHOP chemotherapy groups consisted of 44 and 187 patients, respectively. DA-EPOCH-R chemotherapy was administered to 9 patients. In the 2nd-/3rd-generation regimen group (n=45), 28 patients received MACOP-B with (n=18) or without (n=10) rituximab, 15 patients received cyclophosphamide, vincristine, bleomycin, etoposide, doxorubicin and prednisolone (CyclOBEAP)[33] with (n=12) or without (n=3) rituximab, and 2 patients received vincristine, cyclophosphamide, doxorubicin, ranimustine, vindesine, etoposide carboplatin and prednisone (JCOG-LSG15 study regimen).[34] In the HDT/ASCT group (n=57), 43 patients received rituximab-containing chemotherapy as the initial chemotherapy. Consolidative RT was given to 42% of all patients. After approval of the use of rituximab for DLBCL in Japan in 2003, the use of rituximab-containing regimens rapidly increased, as shown in *Online Supplementary Table S1*. There was a moderate decrease in the use of HDT/ASCT and radiation therapy after initial treatment. The DA-EPOCH-R regimen was selected in the latest period.

Clinical outcomes

With a median follow up of 48 months in surviving patients, the OS and PFS at four years were 87% and 70%, respectively (Figure 1A and B). The OS and PFS of patients treated with rituximab-containing chemotherapy were superior to those of patients receiving chemotherapy without rituximab (4-year OS: 91% *vs.* 77%, $P<0.001$; 4-year PFS: 75% *vs.* 54%, respectively, $P<0.001$). There was no difference in the risk of central nervous system (CNS) relapse between patients treated with and patients treated without rituximab as first-line treatment (3.8% *vs.* 1.3%; $P=0.251$). The OS at four years for patients treated with CHOP, R-CHOP, DA-EPOCH-R, the 2nd-/3rd-generation regimens, and HDT/ASCT was 67%, 90%, 100%, 91% and 92%, respectively, with median follow-up durations of 118 months, 36 months, 19 months, 48 months and 101 months, respectively ($P<0.001$) (Figure 1C); PFS at four years was 40%, 71%, 100%, 83% and 76%, respectively ($P<0.001$) (Figure 1D).

Secondary malignancies and cardiac toxicity developed after treatment in 7 and 10 patients, respectively. The median age of these 17 patients was 62 years. Seven of 17

Table 2. **Risk factors for overall survival, progression-free survival and early relapse for patients treated with R-CHOP without consolidative radium therapy.**

	OS				PFS				Early relapse					
	univariate analysis			multivariate analysis			univariate analysis			multivariate analysis		
Variables	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Effusion present	4.93	1.37-17.69	0.015	4.67	2.28-9.57	<0.001	3.53	1.69-7.40	0.001	6.45	2.45-16.98	<0.001	6.11	2.30-16.24	<0.001
Age > 60 years	2.23	0.75-6.68	0.150	0.62	0.26-1.48	0.282	–	–	–	0.14	0.019-1.05	0.056	–	–	–
Sex Male	1.35	0.47-3.89	0.584	0.93	0.50-1.73	0.821	–	–	–	0.67	0.31-1.43	0.299	–	–	–
PS > 1	4.50	1.56-12.97	0.005	2.85	1.49-5.47	0.002	–	–	–	2.68	1.25-5.73	0.011	–	–	–
Extranodal sites > 1	2.47	0.83-7.37	0.106	2.28	1.16-4.51	0.017	–	–	–	2.38	1.08-5.27	0.032	1.75	0.79-3.91	0.169
Stage III/IV	1.75	0.61-5.06	0.300	2.76	1.47-5.18	0.002	2.16	1.14-4.11	0.018	2.89	1.37-6.09	0.005	–	–	–
LDH > ULN	1.80	0.50-6.46	0.369	3.72	1.45-9.53	0.006	2.28	0.86-6.00	0.096	3.02	1.05-8.71	0.041	–	–	–
B symptoms present	0.74	0.17-3.32	0.697	1.08	0.49-2.35	0.853	–	–	–	1.55	0.68-3.53	0.292	–	–	–
IPI ≥ 3	4.23	1.48-12.13	0.007	2.94	1.55-5.57	0.001	–	–	–	2.95	1.40-6.25	0.005	–	–	–
Tumor diameter ≥ 10 cm	1.31	0.44-3.90	0.150	2.40	1.26-4.60	0.088	–	–	–	3.69	1.61-8.43	0.002	–	–	–
s-IL2R > 1000 U/L	1.88	0.57-6.25	0.302	2.40	1.18-4.90	0.016	–	–	–	1.93	0.85-4.37	0.115	–	–	–
Serum albumin < 3.5 g/dL	1.82	0.56-5.89	0.322	1.46	0.69-3.10	0.321	–	–	–	1.80	0.79-4.10	0.159	–	–	–
ALC < 0.5×10⁹/L	1.15	0.26-5.15	0.855	1.17	0.49-2.79	0.728	–	–	–	1.33	0.50-3.50	0.566	–	–	–
Hemoglobin <12 g/dL	1.86	0.64-5.37	0.253	1.17	0.60-2.29	0.643	–	–	–	1.20	0.55-2.59	0.651	–	–	–
Platelet count <150×10⁹/L	2.15	0.48-9.65	0.316	1.82	0.71-4.67	0.316	–	–	–	0.93	0.22-3.91	0.919	–	–	–

OS: overall survival; PFS: progression-free survival; R-CHOP: rituximab, cyclophosphamide, adriamycin, vincristine and prednisone; RT: radiation therapy; HR: hazard ratio; CI: confidence interval; Effusion: pleural or pericardial effusion; PS: performance status; LDH: lactate dehydrogenase; ULN: upper limit of normal; IPI: international prognostic index; s-IL2R: soluble interleukin-2 receptor; ALC: absolute lymphocyte count.

patients received RT or ASCT as first-line treatment. In addition, 3 of 7 patients who developed secondary malignancies received RT during the initial series of treatment. Among the secondary malignancies, myelodysplastic syndrome (MDS) or acute myeloblastic leukemia (AML) was reported in 2 patients. The patient who developed MDS received HDT/ASCT as a first-line treatment. The patient who developed AML received CHOP as a first-line treatment and ICE as a salvage treatment. Among the 187 patients treated with R-CHOP, 9 experienced cardiac toxicity, and 4 developed a secondary cancer. The median time to development of a secondary malignancy was 40.5 months (range 9-200 months).

Patients' characteristics and clinical outcomes in the R-CHOP group

Detailed characteristics of patients in the R-CHOP group are shown in *Online Supplementary Table S2*. We divided this group into four subgroups according to the disease status after R-CHOP or R-CHOP-like regimen and the presence or absence of consolidative RT: namely, R-CHOP+RT with residual mass, R-CHOP+RT in CR, R-CHOP with residual mass and R-CHOP in CR. Among the 187 patients in the R-CHOP group, 64 patients received consolidative RT after R-CHOP (*Online Supplementary Table S3*). Elderly age and higher IPI score were less common in those who received consolidative RT. Thirty-three of 64 patients received consolidative RT with residual mass after R-CHOP, while 31 of 64 patients received RT in CR after R-CHOP. Among the remaining 123 patients without consolidative RT, 34 patients did not achieve CR after R-CHOP, and 89 patients were in CR after R-CHOP, respectively. Among 34 patients with residual mass who were treated with R-CHOP, 16 patients developed progressive disease (PD), and 4 patients received follow up without RT based on the negative findings on PET/CT after the initial series of treatment. Of 89 patients who achieved a CR after R-CHOP but did not receive RT, 14 patients experienced relapse. Among these 14 patients, 9 developed the relapsed disease in their mediastinum, while the remaining 5 relapsed in other sites. The OS and PFS at four years of patients receiving consolidative RT were 100% and 85%, respectively, in the group with residual mass, and 96% and 90%, respectively, in the

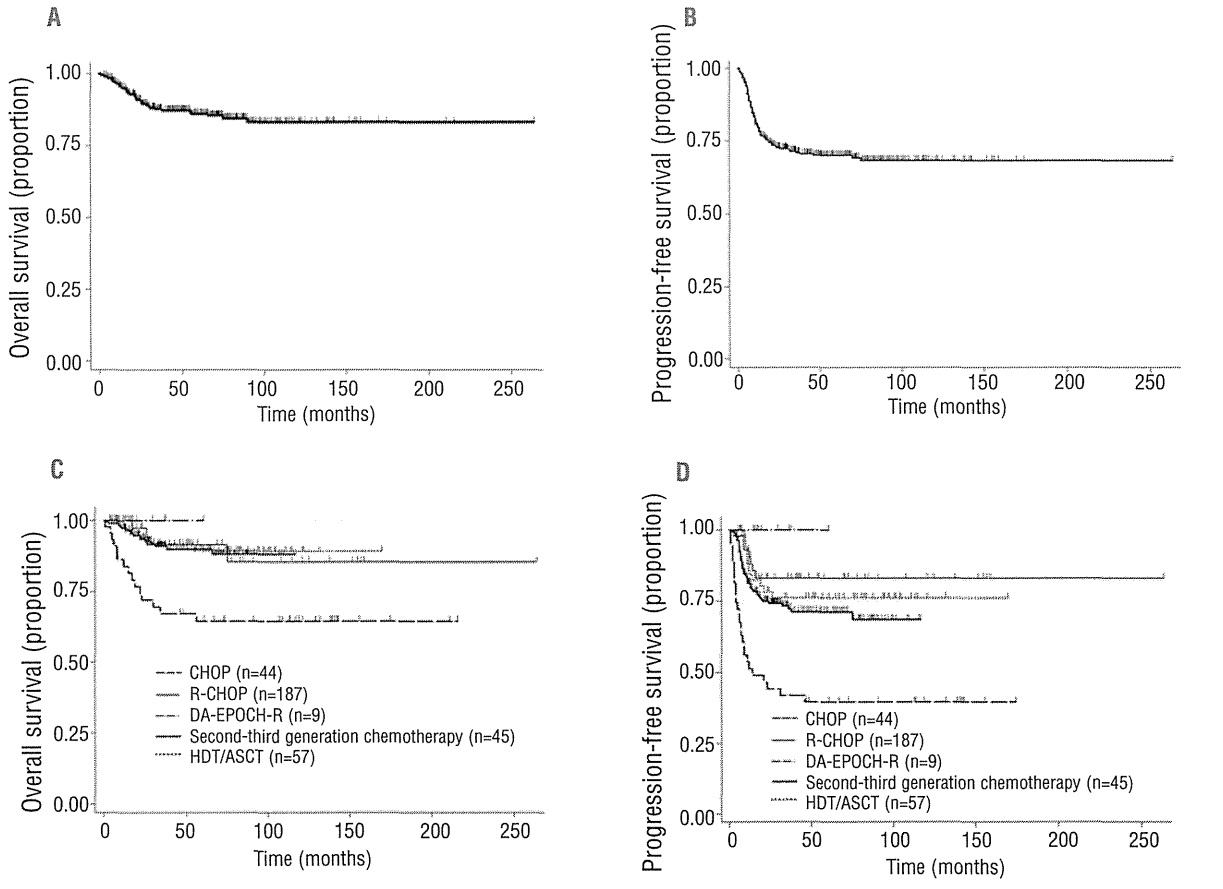

Figure 1. Survival of patients with primary mediastinal large B-cell lymphoma. (A) Overall survival (OS) of all patients with primary mediastinal large B-cell lymphoma (PMBL). (B) Progression-free survival (PFS) of all patients with PMBL. (C) OS of patients with PMBL treated with CHOP (n=44), R-CHOP (n=188), DA-EPOCH-R (n=9), 2nd- or 3rd-generation regimens (n=45), and HDT/ASCT (n=57). (D) PFS of patients with PMBL treated with CHOP (n=44), R-CHOP (n=188), DA-EPOCH-R (n=9), 2nd- or 3rd-generation regimens (n=45), and HDT/ASCT (n=57). CHOP: cyclophosphamide, adriamycin, vincristine and prednisone; R: rituximab; DA-EPOCH-R: dose-adjusted etoposide, cyclophosphosphamide, doxorubicin, vincristine, prednisolone and rituximab; HDT/ASCT: high-dose chemotherapy followed by autologous stem cell transplantation.

group in CR (OS: P=0.15; PFS: P=0.80) (*Online Supplementary Figures S1 and S2*). Meanwhile, the OS and PFS at four years of patients who did not receive consolidative RT were 64% and 35%, respectively, in the group with residual mass without disease progression, and 95% and 77%, respectively, in the group in CR (OS: P<0.001; PFS: P<0.001). Taken together, these data indicate that a significant proportion of patients achieving CR after R-CHOP can be cured without consolidative RT.

Prognostic factors and survival for patients treated with R-CHOP and without consolidative radiotherapy

One hundred and twenty-three patients receiving R-CHOP without consolidative RT were analyzed. The analysis of potential prognostic factors is shown in Table 2. On univariate analysis, the presence of pleural or pericardial effusion, performance status (PS) over 1 and higher IPI were adverse prognostic factors for OS, and the presence of pleural or pericardial effusion, advanced stage, extranodal involvement, PS, LDH, soluble interleukin-2 receptor (sIL-2R), and higher IPI were adverse prognostic factors for PFS. On multivariate analysis, we could not identify significant prognostic factors for OS. The presence of pleural or pericardial effusion [hazard ratio (HR), 3.53; 95% confidence interval (CI), 1.69-7.40; P=0.001] and advanced stage (stage III/IV; HR, 2.16; 95%CI: 1.14-4.11; P=0.018) were identified as adverse prognostic factors for PFS. As almost all the patients with progression after R-CHOP developed disease within 12 months after diagnosis, we performed Cox regression analyses to determine the predictive factors for primary refractory or early relapse within 12 months after diagnosis. On multivariate analysis, only the presence of pleural or pericardial effusion was predictive of primary refractory or early relapse within 12 months (HR, 6.11; 95%CI: 2.30-16.24; P<0.001). In this cohort, only 5 (8%) of 65 patients without pleural or pericardial effusion experienced primary refractory or early relapse within 12 months; meanwhile, 25 (43%) of 58 patients with pleural or pericardial effusion (P<0.001) had refractory or early relapsed disease.

As IPI and the presence of pleural or pericardial effusion were prognostic factors for OS on univariate analysis, and these were not related (correlation coefficient = 0.39), the OS and PFS in patients receiving R-CHOP without RT were analyzed according to these prognostic factors. The OS and PFS in patients receiving R-CHOP without RT were analyzed according to the presence of pleural or pericardial effusion and IPI. As expected (Figure 2A and B), the best OS and PFS were observed in patients with low IPI and without pleural or pericardial effusion. The OS and

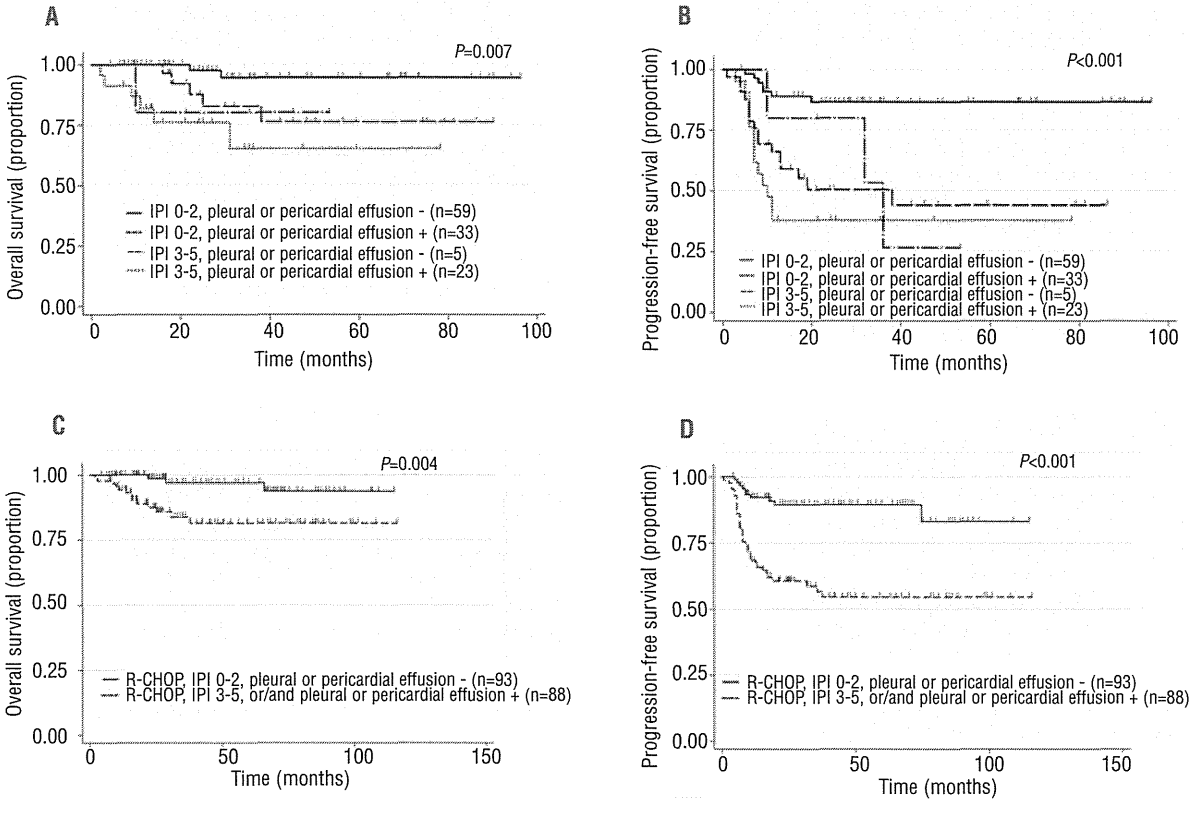

Figure 2. **Survival of patients with primary mediastinal large B-cell lymphoma according to the International Prognostic Index and the presence pleural or pericardial effusion.** (A) **Overall survival (OS) of patients with primary mediastinal large B-cell lymphoma (PMBL) treated with R-CHOP without radiation therapy (RT) according to the international prognostic index (IPI) and the presence pleural or pericardial effusion.** (B) **Progression-free survival (PFS) of patients with PMBL treated with R-CHOP without RT according to the IPI and the presence pleural or pericardial effusion.** (C) **OS of patients with PMBL treated with R-CHOP according to the IPI and the presence pleural or pericardial effusion.** (D) **PFS of patients with PMBL treated with R-CHOP according to the IPI and the presence pleural or pericardial effusion. R-CHOP: rituximab, cyclophosphamide, adriamycin, vincristine and prednisone; RT: radiation therapy.**

PFS at four years of these 58 patients were 95% and 87%, respectively. Meanwhile, based on individual factors of LDH, B symptom, and pleural or pericardial effusion identified on multivariate analysis for PFS, the OS and PFS were also analyzed (*Online Supplementary Figures S3 and S4*). Although the OS and PFS could be well stratified, the number of patients categorized into the well stratified low-risk category was lower than that of patients under the stratification using IPI and effusion. Taken together, these data indicate that a significant proportion of patients with low IPI and without pleural or pericardial effusion at the time of diagnosis can be potentially cured by the R-CHOP regimen without consolidative RT.

Meanwhile, the treatment should be considered for patients with higher IPI and the presence of pleural or pericardial effusion. As shown in Figure 2C and D, the outcomes of R-CHOP were not satisfactory in patients with higher IPI and/or the presence of pleural or pericardial effusion (4-year OS: 97% *vs.* 81%, P=0.004; 4-year PFS: 89% *vs.* 54%, P<0.001, respectively).

Discussion

It is important to establish a more effective and less toxic standard treatment for PMBL, as affected patients tended to be young and can be cured when properly treated. The present study investigated a larger cohort than other studies and indicated that almost all PMBL patients with lower IPI and the absence of the pleural or pericardial effusion could be cured by the R-CHOP regimen without consolidative RT. Considering the excellent outcomes of the recent promising regimen DA-EPOCH-R, reported by Dunleavy *et al.*,[27] the initial treatment regimen for PMBL could be stratified according to our simple indicators of IPI score and the presence of pleural or pericardial effusion; DA-EPOCH-R or R-CHOP could be selected for high- or low-risk PMBL patients, respectively.

Consistent with other studies, patients who received rituximab-containing chemotherapies showed better outcomes.[17-22,27,35] HDT/ASCT and 2nd-/3rd-generation regimens that were more intensive and that have been historically used as first-line treatment for PMBL resulted in better outcomes than those seen in response to CHOP chemotherapy.[11,17,18,36] In the present study, similar OS and PFS was observed among patients treated with a 2nd-/3rd-generation regimen, HDT/ASCT, and R-CHOP. This suggests that R-CHOP regimen might have curative potential in a significant proportion of PMBL patients without utilizing 2nd-/3rd-generation regimen or HDT/ASCT and thereby avoiding their associated toxicities.

Late toxicities are another important issue to consider when weighing the benefits of different curative regimens. In the current study, 17 patients had late adverse events (secondary cancer, n=7; cardiac toxicity, n=10). Previous reports indicated that RT to the mediastinum significantly increased the risk of breast cancer and cardiac toxicity.[24-26,37] Although longer follow up is required to evaluate for late toxicities, we investigated whether we could omit the consolidative RT from the current treatment strategies. We analyzed the outcomes of patients treated with R-CHOP without consolidative RT, and identified higher IPI and the presence of pleural or pericardial effusion as adverse risk factors for OS. Moreover, the presence of the effusion was identified as an adverse risk factor for early relapse. Considering that previous studies had reported that the presence of pleural effusion was associated with poor outcomes in patients with PMBL and Hodgkin lymphoma,[21,38] our results might be universal. Our simple indicators could identify patients who could be cured in response to R-CHOP without consolidative RT; however, patients with these factors comprised only approximately one-half of patients receiving R-CHOP. This means the remaining patients should be treated with an alternative regimen. The fact that excellent outcomes were seen in patients with higher IPI and the presence of the effusion receiving DA-EPOCH-R regimen in this study, as well as in another recent report,[27] suggests that it may be reasonable to use this approach in high-risk PMBL patients. A prospective trial of this strategy is warranted.

Another approach to stratify PMBL patients is currently being investigated in Europe. The prospective IELSG-37 trial is investigating whether consolidative RT could be omitted according to the presence or absence of FDG-PET or PET/CT findings after the initial series of treatments. In clinical practice, we frequently encounter patients in whom it is difficult to judge FDG-PET positivity.[39,40] Unfortunately, we could not evaluate the role of PET/CT in this study because of retrospective settings. Meanwhile, the very recent report from the IELSG-26 study clarified the role of PET/CT after treatment in PMBL patients.[41] Considering the difficulty of re-biopsy of the suspected mediastinal mass after treatment, using the optimal cut-off value on PET/CT after treatment reported by IELSG could be an important tool to assess the risk of treatment failure.

This study has several limitations. First, its retrospective nature might have unrecognized biases and the results should be interpreted with care. Regarding evaluation of response, evaluation of the residual mass might have been heterogeneous at each institution because of the retrospective setting. Therefore, the CR rate in our study could be over-estimated. Second, patients received various treatment regimens and consolidative RT according to each institution's preferred strategy; thus, treatment outcomes might have been over-estimated or under-estimated. In particular, patients who did not receive consolidative RT might have had clinical indicators that physicians considered favorable, resulting in an overestimation of the clinical outcomes in response to R-CHOP without consolidative RT. However, in the present analysis, the proportion of patients with higher IPI and with the presence of effusion was not low in patients who did not receive consolidative RT compared with that in patients who did receive RT. This suggests that the base-line characteristics and outcomes of patients without consolidative RT were not necessarily favorable and that they might not have been over-estimated. Finally, we carried out a central pathological review for only 196 patients. We tried to collect as much pathological histological paraffin-embedded tissue materials as possible. However, in some cases, sufficient materials were not available because they were too old. In addition, the period during which data could be submitted differed because clinical data were kept for different lengths of time at the different institutions. Therefore, the number of institutions who could submit clinical data in the 1980s and 1990s was smaller than in the 2000s: 10 and 65 institutions before and after the year 2000, respectively. Furthermore, although gene expression or methylation profiling can help to diagnose PMBL correctly, for the moment we cannot use these tools in routine clinical prac-