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Original article

Germinal Center B-Cell-Like versus Non-Germinal Center B-Cell-Like as Important Prognostic Factor for Localized Nodal DLBCL

Toshiyuki Habara,[1,4] Yasuharu Sato,[1] Katsuyoshi Takata,[1] Noriko Iwaki,[2] Hirokazu Okumura,[3] Hiroshi Sonobe,[4] Takehiro Tanaka,[5] Yorihisa Orita,[6] Lamia Abd Al-Kader,[1] Daisuke Ennishi,[7] Naoko Asano,[8] and Tadashi Yoshino[1]

Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin's lymphoma. Although many investigations have been performed on the prognostic factors of DLBCL, no reports have focused on localized nodal DLBCL. We examined the prognostic significance of 39 Japanese patients with localized nodal DLBCL with special reference to the germinal center B-cell-like (GCB) versus non-germinal center B-cell-like (NGCB) types. The median age was 65 years with 23 males and 16 females. Using Hans algorithm of immunohistochemistry, 18 patients (46%) exhibited GCB type and 21 (54%) exhibited NGCB type. Twenty-nine patients (74%) presented with disease in the neck (neck group) and 10 (26%) had disease in non-neck regions (non-neck group). Comparing Hans, Choi, and Muris algorithms, patients with GCB type showed statistically significant progression-free survival (PFS) only with Hans algorithm ($P = 0.022$, $P = 0.100$, and $P = 0.130$, respectively). Patient survival analyses revealed that GCB-type patients by Hans algorithm had a better PFS ($P = 0.012$), and neck-group patients had better PFS and overall survival (OS) ($P = 0.018$ and $P = 0.012$, respectively). Univariate analysis revealed that only neck vs. non-neck exhibited a significant difference in terms of OS ($P = 0.026$). Multivariate analysis revealed that GCB type by Hans algorithm and neck vs. non-neck were significantly different in terms of PFS ($P = 0.025$ and $P = 0.033$, respectively). Therefore, the subclassifications of GCB type vs. NGCB type and neck vs. non-neck are important predictive prognostic factors in localized nodal DLBCL. 〔*J Clin Exp Hematopathol 52(2) : 91-99, 2012*〕

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[1] Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan
[2] Department of Hematology and Oncology, Kanazawa University Hospital, 13-1 Takaramachi, Kanazawa 920-8641, Japan
[3] Department of Internal Medicine, Toyama Prefectural Central Hospital, 2-2-78 Nishinagae, Toyama 930-8550, Japan
[4] Department of Clinical Laboratory, Chugoku Central Hospital of the Mutual Aid Associations of Public School Teachers, 148-13 Miyuki-cho, Hiroshima 720-0001, Japan
[5] Department of Pathology, Okayama University Hospital, 2-5-1, Shikata-cho, Okayama 700-8558, Japan
[6] Department of Otolaryngology, Head and Neck Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan
[7] Centre for Lymphoid Cancer British Columbia Cancer Agency, 675 west 10 th Ave, Vancouver, BCV5Z1L3, Canada
[8] Department of Clinical Laboratory, Nagoya University Hospital, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8560, Japan
Address correspondence and reprint requests to : Dr. Yasuharu Sato, Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan
Email : satou-y@cc.okayama-u.ac.jp

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin's lymphoma (NHL).[1,2] Approximately 60% of all DLBCL cases primarily occur in the lymph node.[3,4] Previous studies have shown that patients with nodal DLBCL exhibited increased frequency of bulky growth, increased bone marrow metastasis, and high serum LDH titers compared with those with extranodal DLBCL.[4] Molecular studies, including those on Bcl-2, Bcl-6, and MYC, have also indicated significant phenotypic differences between nodal and extranodal DLBCL.[5-8] Studies have generally focused on both nodal and extranodal DLBCL ; however, there are no detailed studies characterizing nodal DLBCL, particularly localized I and II nodal DLBCL stages. DLBCL generally exhibits strong heterogeneity in morphology, immunophenotyping, genetics, and other clinical features. The International Prognostic Index (IPI) is one of the most important clinical indicators of prognosis in NHL cases.[9]

Recent analysis of the cDNA expression profile characterized two separate DLBCL types, germinal center B-cell-like (GCB) and activated B-cell-like (ABC). GCB type has significantly higher survival rate than ABC type.[10] Although several algorithms have divided DLBCLs into GCB, ABC, or non-germinal B-cell-like (NGCB) type using an immunohistochemical panel,[11–15] no study has investigated localized nodal DLBCL. Therefore, in this study, we clinicopathologically examined 39 Japanese patients with localized nodal DLBCL.

MATERIALS AND METHODS

Patients and materials

Thirty-nine Japanese patients with stage I and II nodal DLBCL were selected on the basis of the availability of clinical information and histological material. These were consecutive cases retrieved from the records of Chugoku Central Hospital and Toyama Prefectural Hospital, Japan, from 1997 to 2008. The histopathology of each DLBCL was reviewed by 3 pathologists (YS, TT, and TY). For accurate staging, the extent of the disease was determined by a standardized range of examinations, including neck, thoracic, abdominal, and pelvic computed tomography scans and/or positron emission tomography, as well as bone marrow biopsies. The study was conducted in accordance with the provisions of the Declaration of Helsinki and was approved by the ethics committee of Chugoku Central Hospital.

Antibodies

The primary antibodies used were as follows : Bcl-2 (3.1, 1:400 ; Novocastra, Newcastle upon Tyne, UK), CD3 (LN10, 1:800 ; Novocastra), CD5 (4C7, 1:400 ; Novocastra), CD10 (56C6, 1:100 ; Novocastra), CD20 (L26, 1:200 ; Novocastra), MUM1 (MUM1p, 1:200 ; Dako, Glostrup, Denmark), Bcl-6 (polyclonal, 1:300 ; Dako), p53 (Pab1801, 1:2,000 ; Santa Cruz Biotechnology, Delaware Avenue, CA, USA), GCET1 (RAM341, 1:100 Abcam, Cambridge, UK), FOXP1 (JC12, 1:500 LifeSpan Biosciences, Seattle, WA, USA), and Ki-67 (MIB-1, 1:1,500 ; Novocastra).

Histological examination, immunohistochemistry, and in situ *hybridization (ISH)*

Surgically resected or biopsied specimens were fixed in 10% formaldehyde and routinely embedded in paraffin. Three-micrometer-thick serial sections were stained with hematoxylin-eosin. Immunohistochemistry was performed on paraffin sections using the Bond automated immunohistochemistry system (Leica Biosystems, Melbourne, Australia). For each section, 10 high-power fields were recorded, quantitated, and averaged for the estimated percentage of positively immunostained cells. DLBCL of at least 30% tumor cells was the cut-off for tumor positivity, except where noted in the Choi algorithm. Ki-67 immunoreactivity was quantified by determining the number of positive cells among the tumor cells.[16] ISH with Epstein-Barr virus-encoded small RNA (EBER) oligonucleotides was performed to test for the presence of EBER in formalin-fixed paraffin-embedded sections using the Bond automated immunohistochemistry system.

Statistical analysis

Differences in characteristics between the two groups were examined by the Chi-square test, Fisher's exact test, Student's t-test, and the Mann-Whitney U test as appropriate. Patient survival data were analyzed by the Kaplan-Meier method. Differences in survival rates were tested by the log-rank test.[17] Progression-free survival (PFS) rates were measured from the time of initial diagnosis to that of disease relapse. Overall survival (OS) rates were measured from the time of initial diagnosis to that of death or last follow-up. Univariate and multivariate analyses were performed with Cox proportional hazards regression models.[18] Results are expressed as hazard ratio and 95% confidence interval. All data were analyzed using STATA software (version 10.0, Stata Corp., Texas, USA).

RESULTS

Characterization of patients with localized stage I and II nodal DLBCL

The clinical findings are summarized in Table 1. The median age of the 39 patients (23 men and 16 women) with localized nodal DLBCL was 65 years (range 33-79 years). Twenty-nine patients (74%) presented with disease in the neck, four (10%) in the inguinal area, four (10%) in the axilla, one (3%) in the hilar, and one (3%) in the abdomen. Thirty-three patients (85%) were at low risk, five (12%) were at low-intermediate risk, and one (2%) was at high-intermediate risk according to IPI.[9] Seven patients (18%) exhibited elevated serum lactate dehydrogenase (LDH) titer levels, while 32 (82%) exhibited normal serum LDH titer levels. According to the Ann Arbor classification, 19 patients (49%) were at clinical stage I and 20 (51%) were at clinical stage II. Histopathologically, all 39 cases were classified as DLBCL. No patient had a history of prior therapy. They were initially and primarily treated with standard anthracycline combination chemotherapy with a predominant cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) regimen. Sixteen patients (41%) who received their first round of chemotherapy after September 2003 were treated with CHOP and rituximab.

Table 1. Summary of the clinical data of 39 localized stage I and II diffuse large B-cell lymphomas

Patient no.	Age	Sex	Primary site	IPI	LDH > normal values	Size (cm)	sIL-2R (IU/ml)	CS	Treatment	Relapse	Follow-up period (months)	Follow-up status
1	70	F	Neck	Low	No	4.8	1100	II	Epi-COP + RT	+	42	Dead
2	52	M	Neck	Low	No	3	921	II	CHOP + RT	+	64	Alive FOD
3	74	M	Inguinal	Low	No	2	491	I	Epi-COP + RT	+	35	Dead
4	72	M	Axilla	Low	No	3	954	I	Epi-COP + RT	+	17	Dead
5	72	F	Inguinal	LI	Yes	7	906	I	Epi-COP + RT	+	95	Alive FOD
6	65	M	Neck	LI	Yes	6	1080	II	CHOP + RT	+	81	Alive FOD
7	76	F	Neck	LI	Yes	2	3660	I	CHOP + RT	+	86	Alive FOD
8	78	M	Axilla	Low	No	5	502	I	R-THP-COP + RT	+	35	Dead
9	77	M	Neck	Low	No	6	1130	II	R-Epi-COP + RT	+	28	Dead
10	70	M	Axilla	Low	No	2.5	895	I	R-THP-COP + RT	+	28	Dead
11	75	M	Neck	Low	No	3	2550	II	R-THP-COP	+	49	Alive FOD
12	74	M	Neck	Low	No	2	406	I	R-Epi-COP + RT	+	49	Alive FOD
13	50	M	Inguinal	Low	No	8	2720	I	CHOP	+	13	LTF
14	61	F	Neck	Low	No	4	774	II	CHOP	+	20	Dead
15	79	F	Neck	Low	No	1.5	199	II	THP-COP	+	30	Dead
16	59	M	Hilar	Low	No	5	506	I	CHOP	+	37	Dead
17	55	M	Neck	Low	No	3	604	I	CHOP + RT	−	126	Alive FOD
18	56	M	Neck	Low	No	3	385	I	CHOP + RT	−	92	Alive FOD
19	79	M	Neck	Low	No	3	834	II	Epi-COP	−	71	LTF
20	72	M	Neck	Low	No	2	1740	II	THP-COP	−	103	Alive FOD
21	50	F	Neck	Low	No	3	491	I	CHOP + RT	−	73	LTF
22	79	M	Neck	Low	No	2	454	I	R-THP-COP + RT	−	59	Alive FOD
23	70	M	Neck	HI	Yes	5	10900	II	R-THP-COP + RT	−	33	Alive FOD
24	33	F	Axilla	Low	No	5	936	I	R-CHOP + RT	−	18	Alive FOD
25	79	M	Neck	Low	No	3.4	753	I	R-THP-COP + RT	−	17	Alive FOD
26	70	F	Neck	LI	Yes	1.8	956	II	Epi-COP + RT	−	128	Alive FOD
27	59	F	Inguinal	Low	Yes	2	319	I	RT	−	47	Alive FOD
28	68	F	Neck	Low	No	3	665	II	R-Epi-COP + RT	−	54	Alive FOD
29	66	F	Neck	Low	No	4	730	II	Epi-COP + RT	−	146	Alive FOD
30	53	F	Neck	Low	No	3	452	II	R-CHOP	−	25	Alive FOD
31	71	M	Neck	Low	No	3	507	I	CHOP	−	126	Alive FOD
32	60	F	Neck	Low	No	3	246	I	CHOP	−	103	LTF
33	52	F	Neck	Low	No	2	196	I	CHOP	−	99	LTF
34	77	M	Neck	Low	No	1.8	1423	II	THP-COP	−	4	LTF
35	57	F	Neck	Low	No	4	633	II	R-CHOP	−	58	Alive FOD
36	46	M	Abdomen	Low	No	5	740	II	R-CHOP	−	56	Alive FOD
37	52	M	Neck	Low	No	3.6	582	II	R-CHOP	−	10	LTF
38	65	M	Neck	Low	No	3	462	II	R-CHOP	−	29	Alive FOD
39	78	F	Neck	LI	Yes	1	553	II	R-THP-COP	−	15	Alive FOD

CHOP : cyclophosphamide, adriamycin, vincristine, prednisolone ; CS : clinical stage ; Epi-COP : epirubicin, cyclophospha-mide, vincristine, prednisolone ; F : female ; FOD : free of disease ; IPI : International Prognostic Index ; L : low ; LDH : lactat-e dehydrogenase ; LI : low-intermediate LTF : lost of follow up ; M : male ; R- : with rituximab ; RT : radiation therapy ; THP-COP : cyclophosphamide.

Immunohistochemical and ISH analyses of localized nodal DLBCL

Immunohistological positivity of tumor cells in > 30% of localized nodal DLBCL was observed for several antigens : CD5 in two cases (5%), CD10 in 16 cases (41%), CD20 in 39 cases (100%), Bcl-6 in 31 cases (79%), Bcl-2 in 25 cases (64%), p53 in 19 cases (49%), and MUM1 in 28 cases (72%). Immunohistological positivity of tumor cells in > 80% of localized nodal DLBCL was also observed for several antigens : including MUM1 in eight cases (21%), GCET1 in 17 cases (44%), FOXP1 in 32 cases (83%), and Ki-67 in 10 cases (26%) (Table 2).

Using Hans algorithm,[11] 18 cases (46%) were categorized as GCB type and 21 (54%) were categorized as NGCB type (Fig. 1). Using Choi algorithm,[12] 21 cases (54%) were categorized as GCB type and 18 (46%) were categorized as ABC type. Using Muris algorithm,[13] 25 cases (64%) were categorized as Group 1 (GCB) and 14 (36%) as Group 2 (ABC). No cases were positive for EBER by ISH.

Table 2. Immunohistochemical findings of localized stage I and II diffuse large B-cell lymphomas

Patient no.	EBER	CD3	CD5	CD10	CD20	Bcl-6	Bcl-2	p53	MUM1 (> 30%)	MUM1 (> 80%)	GCET1	FOXP1	Ki-67 Labeling	Hans aigorithm	Choi algorithm	Muris algorithm
1	−	−	−	−	+	−	−	−	−	−	−	+	35	NGCB	ABC	group 1
2	−	−	−	−	+	−	−	−	−	−	−	+	52	NGCB	ABC	group 1
3	−	−	−	−	+	−	+	−	+	−	+	+	36	NGCB	GCB	group 2
4	−	−	−	−	+	+	+	+	−	−	−	+	86	GCB	ABC	group 1
5	−	−	−	−	+	+	+	+	+	−	−	−	68	NGCB	GCB	group 2
6	−	−	−	−	+	+	+	−	+	−	−	+	69	NGCB	ABC	group 2
7	−	−	+	−	+	+	+	+	+	−	−	+	67	NGCB	ABC	group 2
8	−	−	−	−	+	+	+	+	+	+	+	+	81	NGCB	ABC	group 2
9	−	−	−	−	+	+	+	+	+	−	+	+	80	NGCB	GCB	group 2
10	−	−	−	−	+	+	−	−	+	−	−	+	53	NGCB	ABC	group 1
11	−	−	−	−	+	+	−	−	+	−	−	+	86	NGCB	ABC	group 1
12	−	−	−	−	+	−	+	+	+	+	−	+	39	NGCB	ABC	group 2
13	−	−	−	+	+	+	+	−	+	−	+	+	48	GCB	GCB	group 1
14	−	−	−	+	+	+	+	−	−	−	+	+	60	GCB	GCB	group 1
15	−	−	−	−	+	+	+	+	+	+	+	+	80	NGCB	ABC	group 2
16	−	−	−	−	+	+	+	+	+	−	+	+	40	NGCB	GCB	group 2
17	−	−	−	+	+	+	−	−	+	−	+	+	76	GCB	GCB	group 1
18	−	−	−	+	+	+	+	−	+	−	−	+	71	GCB	GCB	group 1
19	−	−	−	−	+	+	+	−	+	+	−	+	48	NGCB	ABC	group 1
20	−	−	−	−	+	+	+	+	−	−	−	−	49	GCB	GCB	group 1
21	−	−	−	−	+	+	+	−	+	−	−	+	20	NGCB	ABC	group 2
22	−	−	−	+	+	+	−	−	+	+	+	+	80	GCB	ABC	group 1
23	−	−	−	−	+	−	+	+	+	+	−	+	89	NGCB	ABC	group 2
24	−	−	−	+	+	+	−	+	+	−	+	−	71	GCB	GCB	group 1
25	−	−	−	−	+	+	+	+	+	+	+	+	68	NGCB	ABC	group 2
26	−	−	−	+	+	+	−	+	−	−	−	−	60	GCB	GCB	group 1
27	−	−	−	+	+	+	−	−	+	−	−	−	80	GCB	GCB	group 1
28	−	−	−	−	+	−	+	+	−	−	−	+	77	NGCB	ABC	group 1
29	−	−	−	−	+	+	+	−	+	−	+	+	69	NGCB	GCB	group 2
30	−	−	−	+	+	+	+	−	+	−	+	+	84	GCB	GCB	group 1
31	−	−	+	−	+	−	+	−	+	−	−	+	86	NGCB	ABC	group 2
32	−	−	−	+	+	+	+	+	−	−	+	+	86	GCB	GCB	group 1
33	−	−	−	+	+	+	−	+	−	−	+	+	83	GCB	GCB	group 1
34	−	−	−	−	+	−	−	−	+	−	−	−	61	NGCB	ABC	group 1
35	−	−	−	+	+	+	−	+	−	−	+	+	90	GCB	GCB	group 1
36	−	−	−	+	+	+	+	−	+	+	−	+	84	GCB	GCB	group 1
37	−	−	−	+	+	+	−	−	−	−	+	−	37	GCB	GCB	group 1
38	−	−	−	+	+	+	+	+	+	−	−	+	61	GCB	GCB	group 1
39	−	−	−	+	+	+	−	+	+	−	−	+	59	GCB	GCB	group 1

Survival analysis of patients with localized stage I and II nodal DLBCL

The duration of the follow-up study ranged from 4 to 146 months (mean 56 months). Twenty-one patients were initially treated with chemotherapy plus irradiation, 17 with chemotherapy alone, and one with irradiation alone. All 39 patients had complete remission, but 16 patients relapsed thereafter (Table 1). Of the relapsed patients, 13 exhibited relapse at different primary nodes and/or organs (relapsed lesion of four patients also involved the primary site). The remaining three patients exhibited relapse at the primary site (Table 3).

Seven of the patients who exhibited relapse at the primary site underwent chemotherapy and/or radiation treatment. Three of 11 (27%) patients treated with chemotherapy plus irradiation exhibited relapse at the primary site, while four of five (80%) patients treated with only chemotherapy exhibited relapse.

According to the Kaplan-Meier method, the 5-year PFS rate was 56% and the 5-year OS rate was 71%. When the cases were categorized into GCB and NGCB types based on Hans algorithm, patients with GCB type had a better PFS rate than those with NGCB type ($P = 0.012$) (Fig. 2). When the cases were divided into neck and non-neck groups for the primary site, patients in the neck group had better 5-year PFS and OS rates than those in the non-neck group ($P = 0.018$ and $P = 0.012$, respectively) (Fig. 3a, 3b).

As shown in Tables 4 and 5, univariate Cox proportional hazards regression analysis revealed that GCB type, GCB or

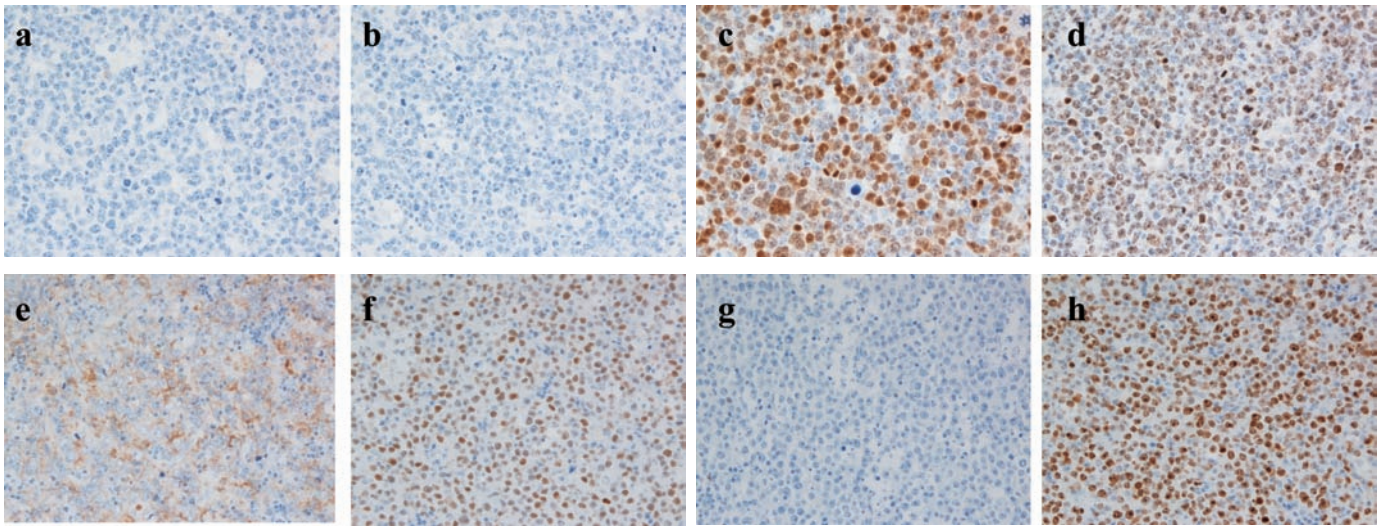

Fig. 1. Immunohistochemical findings of lymph nodes with CD 10, Bcl-6, MUM1, and Ki-67. Upper row case subclassified as non-germinal center B-cell type with negative CD10 expression (*1a*), Bcl-6 negative (*1b*), MUM1 positive (*1c*), and high proliferative activity as labeled by Ki-67 (*1d*). Lower row case subclassified as germinal center B-cell type with positive CD10 expression (*1e*), Bcl-6 positive (*1f*), MUM1 negative (*1g*), and high proliferative activity as labeled by Ki-67 (*1h*).

Table 3. Relapse sites and salvage treatment

Patient no.	Primary site	Treatment	Relapse site	Salvage treatment
1	Neck	Epi-COP + RT	LN (Mediastinium)	VP-16 + CPA
2	Neck	CHOP + RT	LN (Inguinal, Neck)	ESHAP
3	Inguinal	Epi-COP + RT	LN (Axilla, Mediastinium), Liver, Stomach	Epi-COP
4	Axilla	Epi-COP + RT	LN (Neck)	Devic
5	Inguinal	Epi-COP + RT	LN (Neck)	Epi-COP + RT
6	Neck	CHOP + RT	LN (Axilla)	R-THP-COP + RT
7	Neck	CHOP + RT	LN (Axilla)	R + Devic
8	Axilla	R-THP-COP + RT	LN (Paraaorta)	R-THP-COP + RT
9	Neck	R-Epi-COP + RT	LN (Neck, Axilla)	R-CHOP
10	Axilla	R-THP-COP + RT	LN (Neck)	R-THP-COP + RT
11	Neck	R-THP-COP	LN (Neck)	R-THP-COP + RT
12	Neck	R-Epi-COP + RT	LN (Neck, Axilla, Mediastinium, Paraaorta, Inguinal)	R-THP-COP + R-CHOP
13	Inguinal	CHOP	LN (Inguinal)	RT
14	Neck	CHOP	LN (Neck, Axilla)	TVBBM, MMMCCV
15	Neck	THP-COP	LN (Neck)	THP-COP
16	Hilar	CHOP	LN (Neck)	TVBBM

CPA : cyclophosphamide ; Devic : carboplatin, etoposide, ifosfamide, dexamethasone ; E-SHAP : etoposide, methylprednisolone, cisplatin, cytarabine ; MMMCCV : mitoxantrone, cyclophosphamide, vindesine, carboplatin, methotrexate, methylprednisolone ; TVBBM : pirarubicin, enocitabine, etoposide, bleomycin, methylprednisolone ; VP-16 : etoposide ; LN: lymph node.

NGCB type by Hans algorithm, and the primary node site (neck versus non-neck) exhibited significant differences in terms of 5-year PFS rate ($P = 0.022$ and $P = 0.027$, respectively). Using Choi and Muris algorithms, GCB type showed no significant difference in terms of PFS rate ($P = 0.100$ and $P = 0.130$, respectively). Only the primary node site (neck versus non-neck) showed significantly different 5-year OS rates ($P = 0.026$). Multivariate Cox proportional hazards regression analysis revealed that both GCB type and the primary node sites (neck versus non-neck) exhibited significant differences in terms of PFS rates ($P = 0.025$ and $P = 0.033$, respectively) (Table 6).

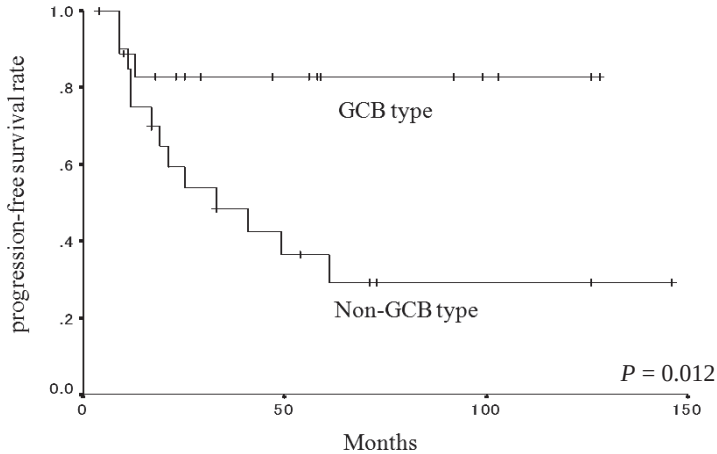

Fig. 2. The progression-free survival rate of localized stage I and II diffuse large B-cell lymphomas according to germinal center B-cell-like and non-germinal center B-cell-like phenotypes.

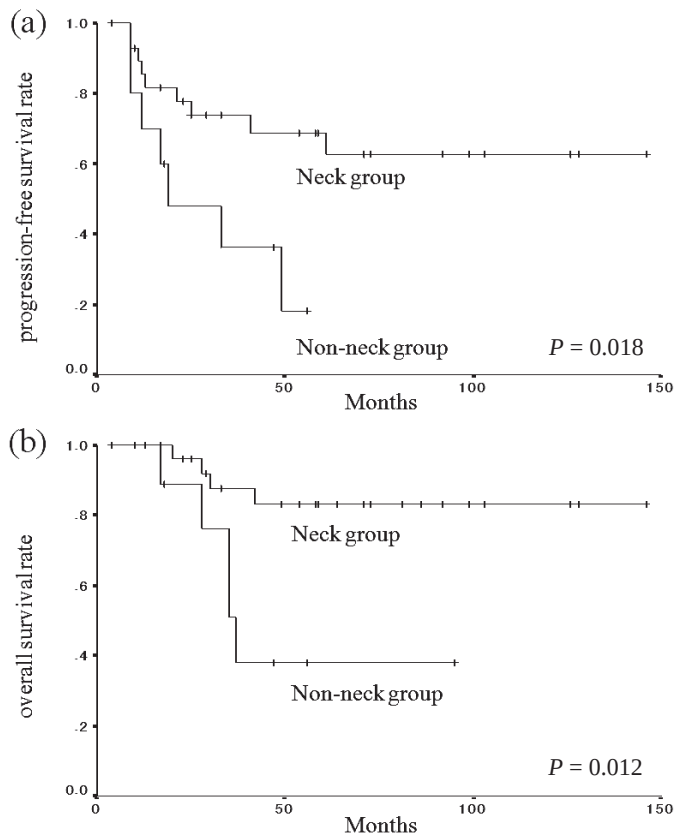

Fig. 3. (***3a***) The progression-free survival rate of localized stage I and II diffuse large B-cell lymphomas according to neck and non-neck groups. (***3b***) The overall survival rate of localized stage I and II diffuse large B-cell lymphomas according to the neck and non-neck groups.

Table 4. Univariate analysis of prognostic factors on progression free survival

Variable	No. of cases	p value	Exp (B)	95%CI	
				Lower Bound	Upper Bound
Male (vs female)	23/39	0.260	0.540	0.190	1.560
Stage I (vs stage II)	19/39	0.660	0.800	0.300	2.160
Rituximab yes (vs no)	16/39	0.960	1.030	0.260	4.180
Age < 60 (vs ≧ 60)	13/39	0.190	2.340	0.670	8.220
Primary site neck (vs non neck)	29/39	0.027	3.170	1.140	8.830
Bcl-2 negative (vs positive)	14/39	0.560	1.400	0.450	4.340
Hans algorithm GCB (vs NGCB)	18/39	0.022	0.150	0.070	0.820
Choi algorithm GCB (vs ABC)	21/39	0.100	0.430	0.160	1.180
Muris algorithm group 1 (vs group 2)	25/39	0.130	2.150	0.800	5.790

Table 5. Univariate analysis of prognostic factors on overall survival

Variable	No. of cases	p value	Exp (B)	95%CI	
				Lower Bound	Upper Bound
Male (vs female)	23/39	0.500	0.620	0.160	2.500
Stage I (vs stage II)	19/39	0.800	0.840	0.230	3.140
Rituximab yes (vs no)	16/39	0.960	1.030	0.260	4.180
Age < 60 (vs ≧ 60)	13/39	0.120	4.050	0.510	32.500
Primary site neck (vs non neck)	29/39	0.026	4.620	1.230	17.360
Bcl-2 negative (vs positive)	14/39	0.390	1.920	0.400	9.270
Hans algorithm GCB (vs NGCB)	18/39	0.240	0.420	0.090	2.000
Choi algorithm GCB (vs ABC)	21/39	0.680	0.760	0.200	2.830
Muris algorithm group 1 (vs group 2)	25/39	0.330	1.920	0.530	7.180

Table 6. Multivariate analysis of prognostic factors on progression free survival

Variable	No. of cases	p value	Exp (B)	95%CI	
				Lower Bound	Upper Bound
Hans algorithm GCB (vs non GCB)	18/39	0.025	0.240	0.067	0.830
Primary site neck (vs non neck)	29/39	0.033	3.060	1.100	8.530

DISCUSSION

Previous reports have indicated that not only IPI but also other serum markers, such as LDH, soluble interleukin-2 receptor, and albumin, have important roles.[19–21] In particular, a high LDH titer is detected in nodal lymphomas at a greater frequency than in extranodal lymphomas.[4] In the present study, LDH titer levels were within the normal range in 82% of the patients.

Many recent reports have mentioned that CHOP chemotherapy with rituximab had better outcomes than CHOP alone.[22–24] However, in the present study, we observed that patients treated with CHOP and rituximab were not significantly different from those given CHOP alone, demonstrating that rituximab did not improve prognosis. This finding suggests that the usefulness of rituximab should be reconsidered for localized nodal cases.

We also observed that, following chemotherapy, relapse in 13 of 16 patients occurred at sites other than the primary site. This result was similar to the results of previously published studies[25,26] and strongly indicates the importance of performing systemic medical examinations on patients after therapy, even during the early stages of nodal DLBCL.

It is well known that irradiation therapy reveals the effective local control of DLBCL.[27] In the present study, relapse at the primary site was more often seen in patients treated with chemotherapy alone than in those treated with chemotherapy plus irradiation.

Many investigators have also reported the use of various algorithms concerning GCB phenotypes. Meyer *et al*. stressed that the algorithms of Hans and Choi are useful in determining the cell origin and can separate patients into prognostic groups. In the present study, we compared the algorithms of Hans, Choi, and Muris. Hans algorithm utilizes

three antibodies, CD10, Bcl-6, and MUM1. Choi algorithm, along with the antibodies used in Hans algorithm, utilizes GCET1 and FOXP1 antibodies. Muris algorithm utilizes CD10, Bcl-2, and MUM1 antibodies. Univariate analysis of the three algorithms showed that only Hans algorithm retained its prognostic value in PFS. Multivariate analysis showed that Hans algorithm also remained an independent prognostic indicator. Therefore, this finding may be useful during diagnosis to identify patients who may need a more aggressive therapy.

In the present study, univariate analysis showed that the primary site of lymph nodes retained its prognostic value for both 5-year PFS and OS rates. Multivariate analysis also showed that the primary site of the lymph nodes can act as an independent prognostic indicator in the 5-year PFS rate. To the best of our knowledge, this is a novel finding on localized nodal DLBCL and was an unexpected discovery. There was no apparent difference between the neck and non-neck groups with regard to IPI, clinical stage, tumor size, proportion of patients treated with rituximab, or age. Because patients not treated with rituximab were included in the present study, further research should be conducted on a larger number of rituximab-treated patients in order to draw a clear conclusion.

We obtained clinical data of localized DLBCL from patients who underwent uniform treatment strategies at two separate institutes. It is well known that the prognosis of extranodal DLBCL is dependent on the primary site.[4] Similarly, in the nodal DLBCLs, the primary site may also be one of the more important prognostic factors. Therefore, we believe that classification of localized nodal DLBCL into the neck vs. non-neck subgroups is useful for predicting prognosis.

We also analyzed CD5 and EBER expression and detected two CD5-positive cases (5%), but EBER was not detected. CD5- and EBER-positive DLBCLs may be poor prognostic markers ;[28, 29] univariate analysis exhibited no significant difference in PFS and OS survival rates.

In conclusion, the subclassification of GCB type versus NGCB type and the primary node site is important in the prediction of prognosis in patients with localized nodal DLBCL.

DISCLOSURE STATEMENT

The authors declare no competing financial interest.

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REFERENCES

1 The Non-Hodgkin's Lymphoma Classification Project: A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. Blood 89:3909-3918, 1997

2 Aoki R, Karube K, Sugita Y, Nomura Y, Shimizu K, *et al*.: Distribution of malignant lymphoma in Japan : analysis of 2260 cases, 2001-2006. Pathol Int 58:174-182, 2008

3 Moller MB, Pedersen NT, Christensen BE: Diffuse large B-cell lymphoma : Clinical implications of extranodal versus nodal presentation -A population-based study of 1575 cases. Br J Haematol 124:151-159, 2004

4 López-Guillermo A, Colomo L, Jiménez M, Bosch F, Villamor N, *et al*.: Diffuse large B-cell lymphoma : Clinical and biological characterization and outcome according to the nodal or extranodal primary origin. J Clin Oncol 23:2797-2804, 2005

5 Kramer MH, Hermans J, Parker J, Krol AD, Kluin-Nelemans JC, *et al*.: Clinical significance of bcl2 and p53 protein expression in diffuse large B-cell lymphoma : a population based study. J Clin Oncol 14:2131-2138, 1996

6 Kramer MH, Hermans J, Wijburg E, Philippo K, Geelen E, *et al*.: Clinical relevance of BCL2, BCL6, and MYC rearrangements in diffuse large B-cell lymphoma. Blood 92:3152-3162, 1998

7 Raghoebier S, Kramer MHH, van Krieken JHJM, De Jong D, Limpens L, *et al*.: Essential differences in oncogene involvement between primary nodal and extranodal large cell lymphoma. Blood 78:2680-2685, 1991

8 Rao PH, Houldsworth J, Dyomina K, Parsa NZ, Cigudosa JC, *et al*.: Chromosomal and gene amplification in diffuse large B-cell lymphoma. Blood 92:234-240, 1998

9 The International Non-Hodgkin's Lymphoma Prognostic Factors Project: A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 329:987-994, 1993

10 Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, *et al*.: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403:503-511, 2000

11 Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, *et al*.: Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103:275-282, 2004

12 Choi WW, Weisenburger DD, Greiner TC, Piris MA, Banham AH, *et al*.: A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res 15:5494-5502, 2009

13 Muris JJ, Meijer CJ, Vos W, van Krieken JH, Jiwa NM, *et al*.: Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B cell lymphoma. J Pathol 208:714-723, 2006

14 Natkunam Y, Farinha P, Hsi ED, Hans CP, Tibshirani R, *et al*.: LMO2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with anthracycline-based chemotherapy with and without rituximab. J Clin Oncol 26:447-454, 2008

15 Nyman H, Jerkeman M, Karjalainen-Lindsberg ML, Banham AH, Leppä S, *et al*.: Prognostic impact of activated B-cell focused classification in diffuse large B-cell lymphoma patients treated with R-CHOP. Mod Pathol 22:1094-1101, 2009

16 Miller TP, Grogan TM, Dahlber S, Spier CM, Braziel RM, *et al*.: Prognostic significance of the Ki-67-associated proliferative antigen in aggressive non-Hodgkin's lymphomas : a prospective Southwest Oncology Group trial. Blood 83:1460-1466, 1994

17 Kaplan EL, Meier P: Non-parametric estimation from incomplete observation. Am J Stat Assoc 53:457-458, 1958

18 Cox DR: Regression models and life tables. J R Stat Soc (B) 34:187, 1972

19 Morito T, Fujiwara M, Asaoku A, Tari A, Sato Y, *et al*.: Serum soluble interleukin-2 receptor level and immunophenotype are prognostic factors for patients with diffuse large B-cell lymphoma. Cancer Sci 100:1255-1260, 2009

20 Ennishi D, Yokoyama M, Terui Y, Asai H, Sakajiri S, *et al*.: Soluble interleukin-2 receptor retains prognostic value in patients with diffuse large B-cell lymphoma receiving rituximab plus CHOP (RCHOP) therapy. Ann Oncol 20:526-533, 2009

21 Chihara D, Oki Y, Ine S, Kato H, Onoda H, *et al*.: Primary gastric diffuse large B-cell Lymphoma (DLBCL) : analyses of prognostic factors and value of pretreatment FDG-PET scan. Eur J Haematol 84:493-498, 2010

22 Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, *et al*.: CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 346:235-242, 2002

23 Sehn LH, Donaldson J, Chhanabhai M, Fitzgerald C, Gill K, *et al*.: Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. J Clin Oncol 23:5027-5033, 2005

24 Habermann TM, Weller EA, Morrison VA, Gascoyne RD, Cassileth PA, *et al*.: Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol 24:3121-3127, 2006

25 Velasquez WS, Fuller LM, Jagannath S, Tucker SL, North LB, *et al*.: Stages I and II diffuse large cell lymphomas : Prognostic factors and longterm results with CHOP-bleo and radiotherapy. Blood 77:942-947, 1991

26 Yu JI, Nam H, Ahn YC, Kim WS, Park K, *et al*.: Involved-lesion radiation therapy after chemotherapy in limited-stage head-and-neck diffuse large B cell lymphoma. Int J Radiat Oncol Biol Phys 78:507-512, 2010

27 Hoppe BS, Moskowitz CH, Zhang Z Maragulia JC, Rice RD, *et al*.: The role of FDG-PET imaging and involved field radiotherapy in relapsed or refractory diffuse large B-cell lymphoma. Bone Marrow Transplant 43:941-948, 2009

28 Yamaguchi M, Seto M, Okamoto M, Ichinohasama R, Nakamura N, *et al*.: *De novo* $CD5^+$ diffuse large B-cell lymphoma : a clinicopathologic study of 109 patients. Blood 99:815-821, 2002

29 Park S, Lee J, Ko YH, Han A, Jun HJ, *et al*.: The impact of Epstein-Barr virus status on clinical outcome in diffuse large B-cell lymphoma. Blood 110:972-978, 2007