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Original Article

Prognostic significance of immunohistochemical subtypes based on the stage of B-cell differentiation in primary CNS lymphoma

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Abstract: Primary central nervous system lymphoma (PCNSL) has been immunohistochemically classified into two subtypes, germinal center (GC) B-cell and non-GC B-cell, but the prognostic impact of these subtypes remains debated. We investigated clinical features and prognostic significance of immunohistochemical subtypes that were identified by expression patterns of three B-cell differentiation markers in PCNSL. We also analyzed a factor related to responsiveness to high-dose methotrexate (HD-MTX) chemotherapy. Tumors from 32 PCNSL patients were immunohistochemically evaluated for expression of cluster of differentiation (CD) 10, B-cell lymphoma-6 (BCL-6), and multiple myeloma oncogene-1 (MUM-1) and classified into subtypes according to the expression patterns of these markers. Clinical features and prognostic outcome of these subtypes were investigated. Twenty-three patients were treated with HD-MTX-based chemotherapy followed by whole-brain radiation therapy (WBRT), and nine were treated with WBRT alone. Three immunohistochemical subtypes were identified, including A-type expressing CD10, BCL-6, and MUM-1 (12 patients), B-type expressing BCL-6 and MUM-1 (12 patients) and C-type expressing MUM-1 only (8 patients). Response rate in the HD-MTX therapy group was 57.1% (4/7) in A-type, 87.5% (7/8) in B-type, and 75% (6/8) in C-type. C-type with the lowest metabolic activity showed significantly longer overall survival than A-type with the higher uptake of methionine (71.6 versus 39.6 months) ($P<0.05$). Immunohistochemical identification of PCNSL based on the B-cell differentiation stage revealed three types of tumors, showing different metabolic activity and survival time. Refined immunohistochemical classification of PCNSL subtypes may become a useful tool for predicting more accurate prognosis and accessing sensitivity to HD-MTX therapy.

Keywords: Primary central nervous system lymphoma, CD10, BCL-6, MUM-1, high-dose methotrexate, PET study

Introduction

Primary central nervous system lymphoma (PCNSL) is a rare aggressive tumor confined to the brain, spinal cord, leptomeninges and eyes [1-3]. Most PCNSLs are diffuse large B-cell lymphoma (DLBCL), which are histologically indistinguishable from systemic DLBCLs. However, the biologic and prognostic features of PCNSLs are distinctly different from those of systemic DLBCLs [4, 5]. By gene expression profiling using a cDNA microarray, systemic DLBCLs can be divided into two major subgroups, germinal center (GC) B-cell-like (GCB) subgroup and activated B-cell-like (ABC) subgroup, the former of which has been shown to have a significantly better outcome than the latter [6, 7]. As it takes labor and cost to perform gene expression profiling for such a classification, various immunohistochemical (IHC) algorithms using B-cell differentiation markers have been developed to classify DLBCLs into subgroups [8, 9]. Among them, the Hans algorithm separated DLBCLs into germinal center B-cell (GCB) and non-ger-

minal center B-cell (non-GCB) subtypes by using antibodies against cluster of differentiation (CD) 10, B-cell lymphoma-6 (BCL-6), and multiple myeloma oncogene 1/interferon regulatory factor 4 (MUM-1/IRF4) [10]. Although many studies of the IHC subtyping of DLBCLs were performed, most studies in PCNSLs failed to demonstrate that the two IHC subtypes have as much of a significant difference in prognosis as that in systemic DLBCL.

On the other hand, previous IHC studies in PCNSL commonly presented a high percentage of MUM-1 expression in the tumor cells and co-expression of BCL-6 and MUM-1 in about half of the tumor cells [11, 12]. These expression patterns of B-cell differentiation markers indicate the late GC or early post-GC origin of PCNSL cells, suggesting that activated B cell-derived tumors may have critical influence on the prognosis of the patients with PCNSL. In the present study, immunohistochemical identification of the tumors by B-cell differentiation markers was divided into three types of the tumors based on the stage of B-cell differentiation in the GC and post-GC. We found that A-type, corresponding to the early time of the late GC stage, had a higher methionine uptake in Met-PET and shorter survival than C-type, corresponding to the post-GC stage. Refined analysis of IHC subtypes based on the histogenetic stage of PCNSL would clarify much more detailed biological features and enable to establish more accurate prognosis in subtypes of PCNSL.

Materials and methods

Patients and treatment protocol

The study was approved by the local ethics committee for clinical research, and all procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its later amendments.

Thirty-two patients whose histology was verified as DLBCL and who were treated in the Department of Neurosurgery of Ehime University Hospital (Matsuyama, Japan) between April 2002 and December 2015 were enrolled in this study. Consent was obtained from all participants after they were informed of the potential risks of the surgical procedures, radiation therapy, and chemotherapy. Tumor specimens were obtained from all 32 patients by biopsy. Before starting chemo-radiotherapy, board-certified pathologists confirmed the histologic diagnosis of the tumor as DLBCL using hematoxylin-eosin staining along with IHC with anti-CD20 and/or anti-CD79a antibodies. Immediately after the histologic diagnosis of the tumor was verified, systemic HD-MTX-based chemotherapy was performed in patients without severe renal dysfunction or a very low Karnofsky Performance Status (KPS) score (<20%). The chemo-radiotherapy consisted of three courses of rapid infusion of HD-MTX (3.0-3.5 g/m^2/3 hours) followed by whole-brain radiation at a dose of 30 Gy with a 10-Gy boost to the focal lesion. In patients at high risk for an adverse reaction to HD-MTX-based chemotherapy, only radiation therapy (RT) was given to the whole brain at a total dose of 40 Gy.

Imaging analysis and measurement of tumor markers

Magnetic resonance imaging (1.5-tesla or 3-tesla MR scanner, Achieva, Philips, Best, The Netherlands) with and without gadolinium and computed tomography were performed in all patients at the time of admission. ^{11}C-methionine (MET)-positron emission tomography (PET) and ^{18}F-fluorodeoxyglucose (FDG)-PET were performed in patients with a good KPS score (≥60%). β2 microglobulin (MG) in cerebrospinal fluid (CSF), serum soluble interleukin 2 receptor (sIL2R), and lactate dehydrogenase (LDH) were measured at the time of admission. The clinical records and magnetic resonance imaging data of the patients were assessed at the time of admission, within 72 hours, and at 3 months after surgery by the same neurosurgeon and neuroradiologist.

Immunohistochemistry

Immunohistochemical expression of GCB-like markers (CD10 and BCL-6) and activated B-cell-like (ABC) markers (MUM-1, CD138) was investigated in all patients. Each tumor sample was fixed in formalin and embedded in paraffin. The blocks were sliced into 5 μm-thick sections, which were deparaffinized in Histo-Clear (Cosmo Bio), hydrated in a graded series of alcohols, and subjected to heat-activated antigen retrieval. After blocking endogenous peroxi-

Table 1. Clinical characteristics of patients with primary central nervous system lymphoma

Characteristics	Patients
Patients (n)	32
Age (years), median (range)	65.3 (44-87)
>60 years (n)	23
≤60 years (n)	9
Sex (Male/Female) (n/n)	23/9
KPS score (%), median (range)	50 (10-100)
≥60% (n (%))	14 (43.8%)
<60% (n (%))	18 (56.2%)
No. of lesions, single (n (%))	14 (43.8%)
multiple (≥2) (n (%))	18 (56.2%)
Serum sIL2R (n, mean ± SD)	30 (490.6 ± 287.0)
≥600 U/ml (n (%))	8 (26.7%)
<600 U/ml (n (%))	22 (73.3%)
Serum LDH (n, mean ± SD)	32 (205.3 ± 60.7)
≥240 U/L (n (%))	3 (9.4%)
<240 U/L (n (%))	29 (90.6%)
CSF β2-MG (n, mean ± SD)	25 (3847.2 ± 1650.6)
≥3000 μg/L (n (%))	16 (64%)
<3000 μg/L (n (%))	9 (36%)
Patients treated with HD-MTX (n (%))	23 (71.9%)
Patients treated with RT alone (n (%))	9 (28.1%)

n, number of patients; KPS, Karnofsky performance status; sIL2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; CSF, cerebrospinal fluid; β2-MG, beta2 microglobulin; SD, standard deviation; HD-MTX, high dose-methotrexate; RT, radiation therapy.

dase activity, the tissue was incubated with CD10 (mouse monoclonal antibody; clone 56C6; DAKO; ready to use), BCL-6 (mouse monoclonal antibody; clone PG-B6p; DAKO; ready to use), MUM-1 (mouse monoclonal antibody; clone MUM1p; DAKO; ready to use), and CD138 (mouse monoclonal antibody; clone MI15; DAKO; ready to use) antibodies for 4 hours at room temperature. Subsequently, the sections were washed and incubated with biotinylated secondary antibody for 30 minutes at room temperature. The reaction complexes were visualized with diaminobenzidine and counterstained with hematoxylin.

Statistical analysis

Parametric data were expressed as the mean ± standard deviation and compared using Student's t-test. Nonparametric data were expressed as the median value and compared using the Mann-Whitney U-test. The significance level was set at $P<0.05$. All analyses were performed using Office Excel 2016 software (Microsoft®, Redmond, WA, USA). PFS time was defined as the time from study entry to first progression or death from any cause. OS time was defined as the time from study entry to death or the last follow-up visit. PFS and OS times were estimated by the Kaplan-Meier method. For univariate analysis, survival distributions were compared by the log-rank test. Receiver operating characteristic analysis was used to evaluate the cut-off value for predicting the response to HD-MTX by analyzing the T/N ratio and maximum standardized uptake value (SUVmax) in MET-PET and FDG-PET.

Results

Clinical features

The median age of the 32 patients was 65.3 years (range 44-87 years), and they presented with a median KPS score of 50% (range 10-100%) (**Table 1**). All patients underwent open biopsy of tumors using an image-guided navigation system [13]. The tumors of all 32 patients were confirmed with histology as DLBCLs that showed positive immunostaining with both anti-CD20 and anti-CD79a antibodies. Among the 32 patients, 18 (56.3%) had multiple lesions. Sixteen of 25 patients (64%) examined presented with an abnormally high level (≥3,000 μg/L) of β2-MG in CSF. Eight of 30 patients examined (26.7%) showed a high value of serum sIL2R (≥600 U/ml). Three of 32 patients examined (9.38%) showed a high value of serum LDH (≥240 U/L).

Immunohistochemical expression of markers for DLBCL

The tumors of all 32 patients were immunopositive for any markers of GCB and/or ABC. All tumors showed various degrees of positive expression for MUM-1 with immunostaining. Twelve tumors (37.5%) were immunopositive for CD10, and 24 tumors (75%) were immunopositive for BCL-6. Only two tumors showed immunopositive staining for CD138. The 32 patients were divided into one of the following three types according to expression patterns of markers CD10, BCL-6 and MUM-1. Twelve patients

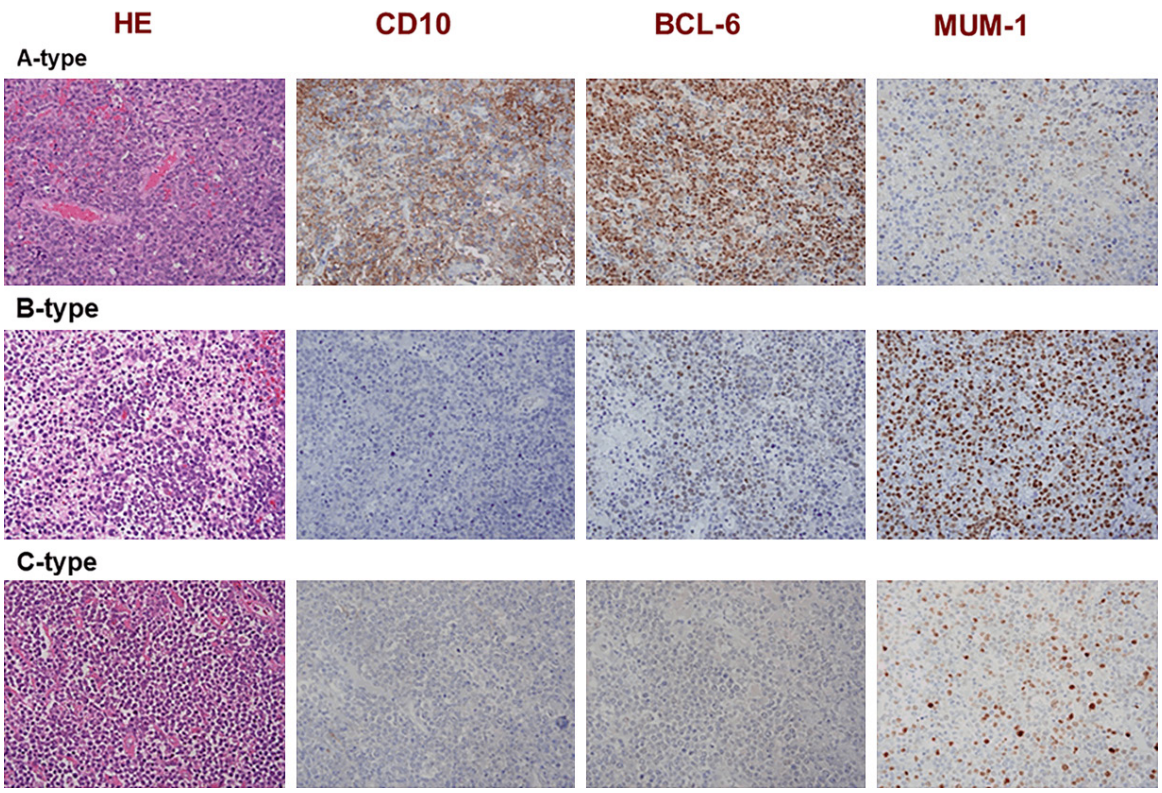

Figure 1. Histology (H&E: hematoxylin and eosin) and immunohistochemistry showing three different expression patterns of CD10, BCL-6, and MUM-1 in the representative case. We identified patients with primary central nervous system lymphoma (PCNSL) as one of three immunohistochemical subtypes. These three subtypes include A-type expressing CD10, BCL-6, and MUM-1, B-type expressing BCL-6 and MUM-1, and C-type expressing MUM-1 alone. Magnification ×200.

were CD10(+), BCL-6(+), MUM-1(+), A-type, 12 patients were CD10(-), BCL-6(+), MUM-1(+), B-type, and eight were CD10(-), BCL-6(-), MUM-1(+), C-type (**Figure 1**). Two patients with positive staining for CD138 showed positive staining for only MUM-1, so these were included in C-type. In the classification by the Hans algorithm [10], A-type corresponded to GCB subtype, while B- and C-types corresponded to non-GCB subtype.

Characteristic features of three immunohistochemical types

Clinical characteristics of 32 patients with tumors that were immunohistochemically classified into these three types are summarized in **Table 2**, including those classified by the Hans algorithm. We found no significant difference in age, sex, KPS score, CSF β2-MG, serum sIL2R, or LDH both among these three types and between the Hans subtypes. Although PET studies were not performed in all patients, both the T/N ratio and SUVmax in MET-PET showed much higher values in patients classified as A-type than in those classified as B-type or C-type. Similarly, both T/N ratio and SUVmax in MET-PET were much higher in patients of GCB subtype than those of non-GCB subtype. FDG-PET demonstrated no difference in activity of FDG uptake by tumors both among these three types and between the Hans subtypes (**Table 2**; **Figure 2**).

Among the 32 patients, 23 (71.9%) were treated with systemic HD-MTX-based chemotherapy followed by whole-brain RT. Nine patients (28.1%) were treated with whole-brain RT alone because five patients presented with very low values of creatinine clearance (lower than 50 ml/minute), three had a low KPS score (under 20%), and one was over 85 years old. In the HD-MTX therapy group, seven patients were classified as A-type, eight as B-type, and eight as C-type. In the RT alone group, five patients

Table 2. Clinical features of three immunohistochemical subtypes and Hans subtypes

Clinical Features	Classification based on the stage of B-cell differentiation			Hans classification	
	A-type	B-type	C-type	GCB subtype	non-GCB subtype
Number of patients (%)	12 (37.5%)	12 (37.5%)	8 (25%)	12 (37.5%)	20 (62.5%)
Age in years, median (range)	69.7 (54-87)	64.8 (41-77)	59.6 (44-73)	69.7 (54-87)	62.7 (41-77)
Sex (male/female)	10/2	9/3	4/4	10/2	13/7
KPS score (%), median (range)	80 (40-80)	55 (20-100)	55 (20-80)	80 (40-80)	55 (20-100)
Tumor markers					
β2-MG (μg/L) (mean ± SD) (n)	3200 ± 1400 (10)	4400 ± 2100 (8)	4200 ± 1300 (7)	3200 ± 1400 (10)	4299 ± 1702 (15)
sIL2R (U/ml) (mean ± SD) (n)	581.7 ± 299.9 (12)	478.1 ± 302.0 (10)	352.3 ± 208.9 (8)	581.7 ± 299.9 (12)	436.2 ± 263.2 (18)
LDH (U/L) (mean ± SD) (n)	217 ± 87.1 (12)	198 ± 39.0 (12)	199 ± 40.4 (8)	217 ± 87.1 (12)	198 ± 38.5 (20)
MET-PET, n	6	3	4	6	7
T/N ratio (mean ± SD)	5.39 ± 0.95	3.94 ± 0.97	3.62 ± 1.05	5.39 ± 0.95	3.75 ± 0.95
SUVmax (mean ± SD)	8.19 ± 0.93	5.58 ± 2.68	5.03 ± 2.36	8.19 ± 0.93	5.27 ± 2.30
FDG-PET, n	6	3	4	6	7
T/N ratio (mean ± SD)	3.28 ± 0.65	2.54 ± 0.92	2.78 ± 0.96	3.28 ± 0.65	2.68 ± 0.87
SUVmax (mean ± SD)	20.6 ± 1.41	16.8 ± 7.71	17.0 ± 6.32	20.6 ± 1.41	16.9 ± 6.30
HD-MTX therapy, n	7	8	8	7	16
Responsive to HD-MTX	4 (57.1%)	7 (87.5%)	6 (75%)	4 (57.1%)	13 (81.3%)
CR/PR, n (%)					
PFS (months), median	31.3	35.7	57.6	24	42
OS (months), median	39.6	46	71.6	24	59.5
RT alone, n	5	4	0	5	4
PFS (months), median	26.8	18.5	-	26.8	18.5
OS (months), median	35.2	24.25	-	35.2	24.25

KPS, Karnofsky performance status; β2-MG, beta2 microglobulin; sIL2R, soluble interleukin-2 receptor; MET-PET, methionine-positron emission tomography; FDG-PET, fluorodeoxyglucose-positron emission tomography; SD, standard deviation; SUV, standardized uptake value; HD-MTX, high-dose methotrexate; CR, complete response; PR, partial response; PFS, progression-free survival; OS, overall survival; RT, radiation therapy; n, number of patients.

were classified as A-type and four as B-type (**Table 2**).

Responsiveness to HD-MTX chemotherapy

In the group treated with HD-MTX chemotherapy, four (57.1%) of seven A-type patients, seven (87.5%) of eight B-type patients, and six (75.0%) of eight C-type patients responded to HD-MTX chemotherapy completely or partially. In the Hans subtype, the response rate was 57.1% in the patients with a GCB subtype and 81.3% in the patients with non-GCB subtype. Among the clinical variables, age, KPS score, levels of serum sIL2R and LDH, and values of β2-MG in CSF did not show a significant difference between responders and non-responders to HD-MTX (**Table 3**). On the other hand, responders to HD-MTX showed significantly lower T/N ratios and SUVmax with both MET-PET and FDG-PET than non-responders (**Table 3**). Receiver operating characteristic analysis showed that tumors with a T/N ratio ≤2.8 (cut-off value) on FDG-PET responded to HD-MTX chemotherapy with 100% sensitivity and 100% specificity.

Survival analysis

Progression-free survival

In the HD-MTX treatment group, median PFS times for A-type, B-type, and C-type patients were 31.3, 35.7, and 57.6 months, respectively. In the Hans' classification, median PFS times for GCB subtype and non-GCB subtype were 24.0 and 42 months, respectively. In the group treated with RT alone, PFS in A-type patients was 26.8 months, and for B-type patients, PFS was 18.5 months (**Table 2**). We found no significant difference in PFS both among the three subtypes and between GCB and non-GCB subtypes in the HD-MTX treatment group or between the two subtypes in the group that received RT alone.

Overall survival

Univariate analysis of 32 patients showed that a KPS score ≥60%, serum level of sIL2-R antibody <600 U/ml, and negative immunostaining for BCL-6 were associated with a longer OS time (**Figures 3**, **4**). No prognostic impact on OS

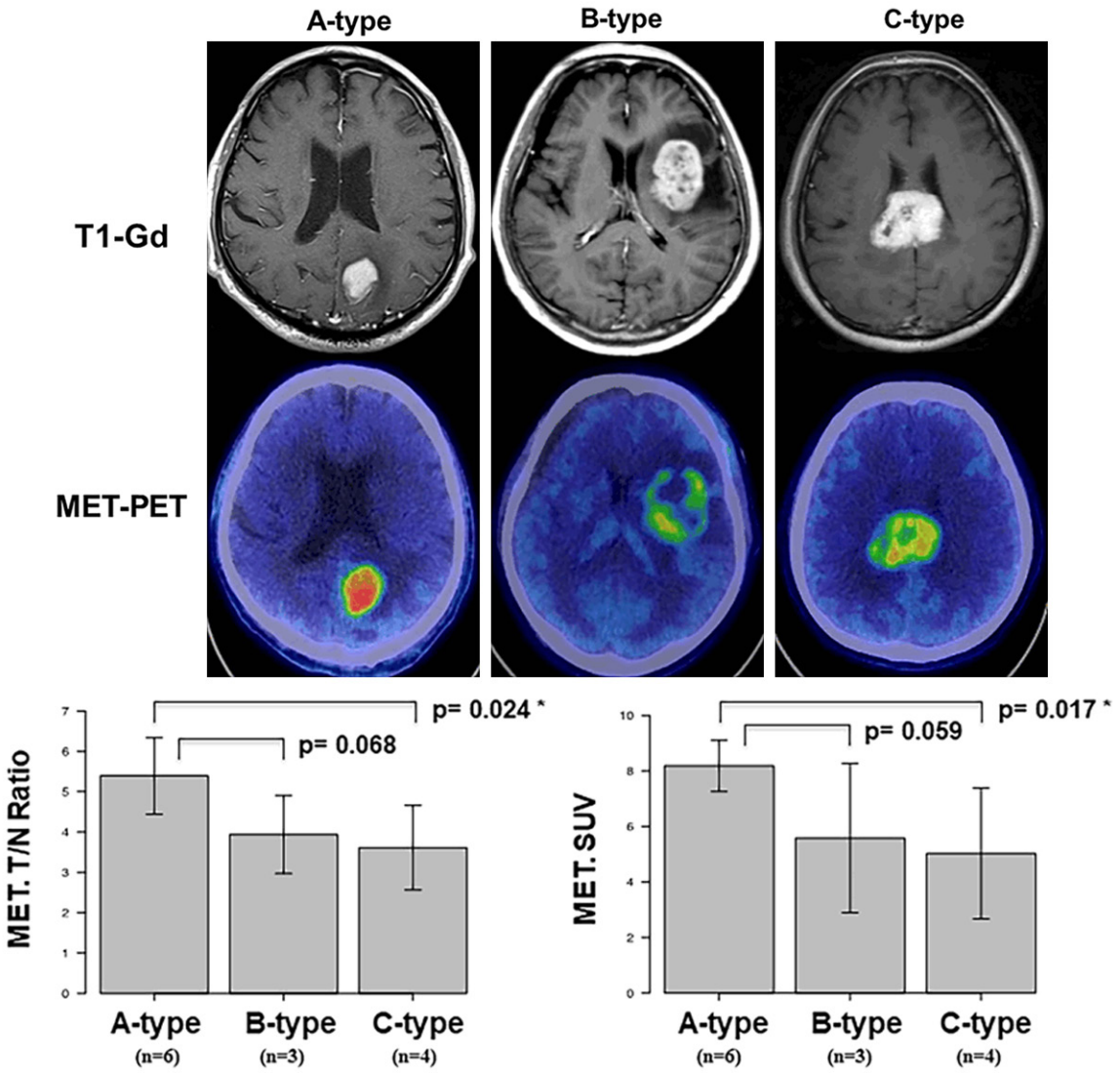

Figure 2. Magnetic resonance imaging and methionine (MET)-positron emission tomography (PET) study. Both the T/N ratio and maximum standardized uptake value (SUV) in MET-PET showed much higher values in patients of the A-type than the C-type.

time was found for other clinical-biologic variables tested (**Table 1**). As for the IHC subtype, in the HD-MTX treatment group, the median OS times of patients in the A-type, B-type, and C-type were 39.6, 46, and 71.6 months, respectively (**Table 2**). C-type patients showed a significantly longer median OS time than A-type patients (P=0.032), but we found no significant difference between C-type and B-type patients, or between A-type and B-type patients (**Figure 5**). On the other hand, in the Hans' classification, median OS times for GCB subtype and non-GCB subtype were 24.0 and 59.5 months, respectively, resulting in no significant difference between the two subtypes. In the RT alone group, median OS times of A-type and B-type patients were 35.2 and 24.3 months, respectively, demonstrating no difference between the two types in this treatment group, and no difference between the same IHC types who underwent HD-MTX chemotherapy or RT alone.

Discussion

Most immunocompetent patients with PCNSL exhibit the histologic features of DLBCL characterized by poor prognosis compared with the systemic form, which can be classified into two IHC subtypes, the GCB type and the non-GCB

Table 3. Responsiveness to HD-MTX chemotherapy

Clinical Variables	Responder (n)	Non-responder (n)	*P* value	Cut-off value (sensitivity (%), specificity (%))
Age (years), mean ± SD	62.65 ± 9.6 (n = 17)	66.17 ± 7.3 (n = 6)	0.424	
KPS score (%) median (range)	60 (20-100) (n = 17)	60 (50-80) (n = 6)	0.589	
sIL2R (U/ml), mean ± SD	393.9 ± 176.0 (n = 15)	410.0 ± 188.2 (n = 6)	0.855	
LDH (U/L), mean ± SD	192.8 ± 28.6 (n = 17)	200.8 ± 41.1 (n = 6)	0.604	
β2-MG (μg/ml), mean ± SD	3,835 ± 1,367 (n = 14)	3,667 ± 1,776 (n = 6)	0.819	
MET-PET (T/N), mean ± SD	3.39 ± 0.83 (n = 5)	5.25 ± 0.84 (n = 5)	0.008	3.56 (100, 80)
MET-PET (SUV), mean ± SD	4.72 ± 2.67 (n = 5)	7.71 ± 1.08 (n = 5)	0.049	4.37 (100, 80)
FDG-PET (T/N), mean ± SD	2.20 ± 0.61 (n = 5)	3.46 ± 0.26 (n = 5)	0.003	2.8 (100, 100)
FDG-PET (SUV), mean ± SD	13.64 ± 4.50 (n = 4)	21.1 ± 1.14 (n = 5)	0.009	20.0 (80, 100)

KPS, Karnofsky performance status; β2-MG, beta2 microglobulin; sIL2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; MET-PET, methionine-positron emission tomography; FDG-PET, fluorodeoxyglucose-positron emission tomography; SD, standard deviation; SUV, standardized uptake value; HD-MTX, high-dose methotrexate; n, number of patients.

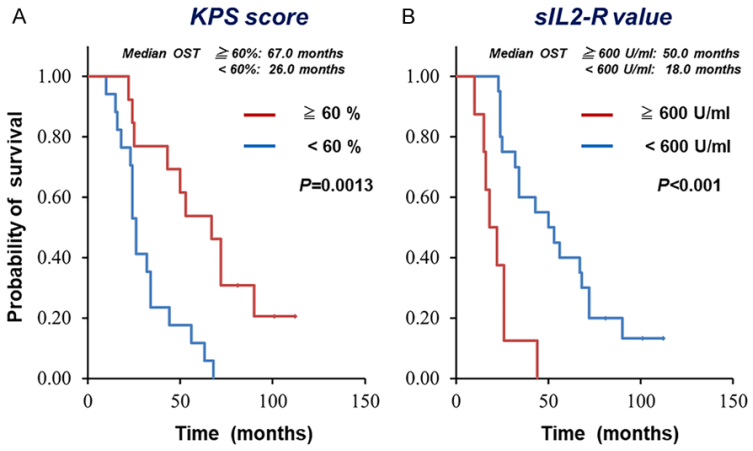

Figure 3. Kaplan-Meier survival analysis. A: Patients with high Karnofsky performance status (KPS) scores have a better OS time than those with a low KPS score (50% cumulative survival: 67.0 months for a high KPS score versus 26.0 months for a low KPS score, P=0.0013). B: Patients with low soluble interleukin 2 receptor (sIL2-R) antibody values have a better OS time than those with high sIL2-R antibody values (50% cumulative survival: 50.0 months for low sIL2-R antibody values and 18.0 months for high sIL2-R antibody values, P<0.001).

type [6, 7, 10, 14, 15]. In systemic DLBCL, the GCB subtype is associated with a better prognosis compared with the non-GCB subtype. However, the availability and the prognostic utility of this IHC subgrouping in PCNSL remains controversial. Thus, here we investigated the prognostic significance of factors related to B-cell differentiation in the histogenesis of PCNSL by analyzing the expression of markers corresponding to the period of B-cell differentiation and clarifying the clinical features of patients with subtypes of expression of particular markers.

We could divide the patients into three types A-type, B-type and C-type according to the difference in the patterns of IHC expression of three B-cell differentiation markers. In these three types, we investigated the clinical features and prognostic outcome along with those in the Hans subtypes. Survival analysis based on our present IHC typing showed that C-type patients had significantly longer OS time compared to A-type patients and showed a tendency to have much longer OS time than B-type. These results may indicate that each type has different biologic features, particularly between A-type and C-type. This may also be supported by the present PET studies demonstrating that responders to HD-MTX showed a lower T/N ratio and SUVmax in both MET-PET and FDG-PET and that the rate of non-responders to HD-MTX was higher in A-type patients with a high T/N ratio and SUVmax in both MET-PET and FDG-PET than in B- or C-type patients with a low T/N ratio and SUVmax in both PET studies. Consequently, the tumors of A-type patients may have much higher metabolic activity than tumors of B- or C-type patients. On the other hand, when survival analysis was performed based on Hans' classification, there was no significant difference in

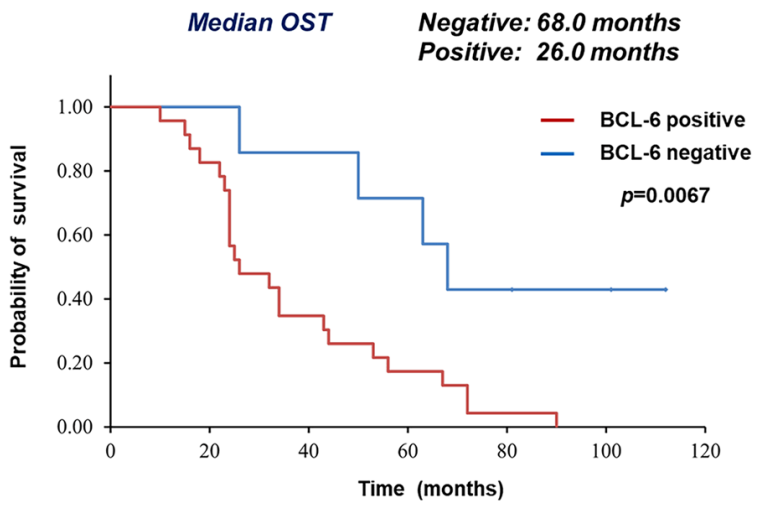

Figure 4. Kaplan-Meier survival analysis. Comparison of OS time according to BCL-6 staining between positive and negative patients. Patients with tumors with negative staining for BCL-6 have a better OS time than those with positive staining for BCL-6 (50% cumulative survival: 68.0 months for negative staining and 26.0 months for positive staining, P=0.0067).

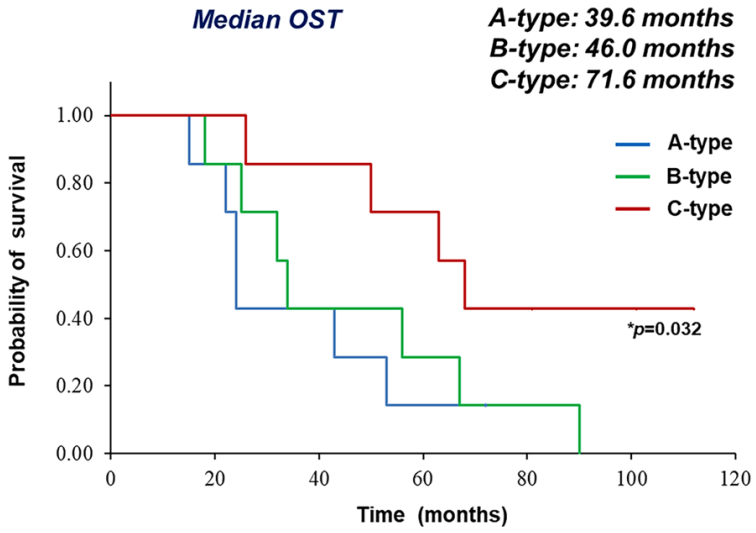

Figure 5. Kaplan-Meier survival analysis. Comparison of OS time according to A-type, B-type and C-type in patients treated with high-dose methotrexate (HD-MTX)-based chemo-radiation therapy. When comparing the median OS time between A-type and B-type and that between A-type and C-type, C-type showed significantly longer median OS time than A-type (50% cumulative survival: 71.6 months for C-type versus 39.6 months for A-type) (P=0.032), but B-type did not show significant difference in the OS time (46.0 months for B-type) compared to A-type.

either PFS or OS times between GCB and non-GCB subtypes.

From the expression patterns of B-cell differentiation markers, it is suggested that the tumors of A-type might derive from B-cells in the early time of the late GC stage when the B-cells had just begun to be activated. On the other hand, the tumors of B-type originated from the later period of the late GC stage, in which activated B-cells were on the way to just leave the GC (**Figure 6**). Tumors expressing CD10(+), BCL-6(-), and MUM-1(+) may be included in the same group as A-type. If there are tumors expressing the IHC patterns of CD10(+) and MUM-1(-), they would be classified to GCB-type in which B-cells are not activated and may have favorable prognosis. Among 393 PCNSL patients in the literature [5, 9, 14, 16-18] including our study, the numbers of patients who can be classified as GCB-type (non-activated), A-type (activated), B-type (activated), and C-type (activated) are 37 (9.4%), 44 (11.2%), 173 (44.0%), and 139 (35.4%), respectively. These frequency rates of immunosubtypes strongly support the previous hypothesis that most PCNSLs originate from activated B cells in the late GCB and early post-GC stages.

In the present study, all PCNSLs showed positive staining for MUM-1. Most previous studies reported a high percentage of MUM-1 expression in PCNSL (381/445 (85.6%), range: 60-94.7%). On the other hand, expression of CD10 is infrequent (60/445 (13.5%), range: 2.4-22.2%) [9, 14, 16-23]. These reports coincide with the previously described histogenetic stage of PCNSL, thus implying that most PCNSLs belong to the subgroup of the ABC phenotype and that very few patients have the GCB phenotype and the associated favorable prognosis. This may be the reason PCNSL is considered to have a much worse prognosis than systemic DLBCL. On the other hand, PCNSL

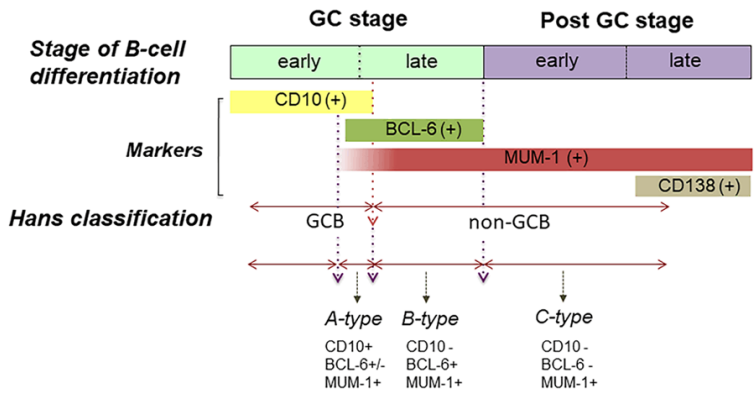

Figure 6. Hypothetical model for histogenesis of PCNSL based on B-cell differentiation stages. Immunohistochemical analysis demonstrated that tumors from A-type patients expressed CD10, BCL-6, and MUM-1, whereas tumors from B-type patients expressed BCL-6 and MUM-1, meaning that tumors from A-type patients were in the early period of the late germinal center (GC) transit stage, when activation of centrocytes has just begun. Patients with tumors expressing CD10(+), BCL-6(-), and MUM-1(+) may be included in the same group as A-type patients, if MUM-1 could be expressed in the more early period in the GC stage as shown in the illustration of marker expression of MUM-1 (faint red). On the other hand, tumors from B-type patients have activated centrocytes in the later period of the late GC stage as shown in the histogenetic model of PCNSL. IHC: immunohistochemistry.

patients with the post-GCB subtype tend to show a favorable prognosis compared to those with the activated GCB subtype [9, 17]. If the activated GCB subtype can be classified more precisely based on the histogenetic stage of the tumor, this may tell us the prognostic impact of the IHC subtypes of PCNSL.

Our study demonstrated that negative expression of BCL-6 was associated with a longer survival time. In systemic DLBCL, many studies have reported that BCL-6 expression is associated with favorable prognosis [7, 24, 25], but its prognostic value in PCNSL remains controversial. Braaten et al. showed that BCL-6 expression in PCNSL is associated with a favorable prognosis [24]. Other reports also described that BCL-6 expression in PCNSL is an independent prognostic factor for favorable outcomes [21, 26]. In contrast, the CALGB 50202 prospective study reported that high BCL-6 expression could become an unfavorable prognostic biomarker in PCNSL [5, 27]. Another prospective trial, G-PCNSL-SG1, the largest study including 119 patients with PCNSL who were homogeneously treated with HD-MTX-based chemotherapy, showed association of positive expression of BCL-6 with shorter PFS and OS [5]. These results suggest that positive expression of BCL-6 tends to be an unfavorable prognostic marker in PCNSL, but further investigation to clarify a significant role of BCL-6 in the tumorigenesis of PCNSL would be required.

In the current study, A-type patients whose tumors show positive expression of CD10 and BCL-6 have a worse OS than C-type patients whose tumors show negative expression of both markers. In addition, tumors from A-type patients showed a much higher T/N ratio and SUVmax in MET-PET and FDG-PET than tumors from C-type patients. Analysis of the metabolic activity among subtypes may enable a more accurate prognosis and clarify the cause of resistance to HD-MTX chemotherapy. Among various clinical factors, the T/N ratio and SUVmax in MET-PET in tumors from A-type patients were significantly higher than those from C-type patients. On the other hand, neither the T/N ratio nor the SUVmax in FDG-PET was different between A-type and C-type patients. These results suggest that differences in metabolic activity other than proliferative activity may exist between these subtypes.

The present retrospective study consisted of a relatively small number of patients. Further prospective and if possible, cohort studies with an increased number of patients are necessary to validate our results. In the future, MET-PET and FDG-PET studies in addition to precise IHC subtyping of PCNSL are expected to be useful tools not only to more accurately establish the prognosis of patients with PCNSL but also to clarify the biological features of subtypes of PCNSL.

In summary, in the present study, we immunohistochemically identified 32 patients with PCNSL as one of three types. These three types included A-type expressing CD10, BCL-6, and MUM-1 in 12 patients (37.5%), B-type expressing BCL-6 and MUM-1 in 12 patients (37.5%), and C-type expressing only MUM-1 in eight patients (25%). Among the 23 patients who were treated with HD-MTX-based chemoradiotherapy, C-type patients showed a much longer OS time than A-type patients. In addition, we found a significant difference in methionine

uptake in MET-PET between A- and C-types. These results indicate the significance of more refined classification of PCNSL based on the histogenetic stage that is assessed by expression patterns of B-cell differentiation markers for accurately establishing the prognosis of PCNSL patients and developing a novel treatment strategy for this type of tumor.

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Disclosure of conflict of interest

None.

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