

Comparison of the Expression and Prognostic Significance of Differentiation Markers between Diffuse Large B-Cell Lymphoma of Central Nervous System Origin and Peripheral Nodal Origin

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Abstract Purpose: Whether diffuse large B-cell lymphoma (DLBCL) of primary central nervous system origin (PCNSL) is biologically different from DLBCL of peripheral nodal origin (NL) remains unclear. The purpose of this study was to compare the expression frequencies and prognostic significance of a panel of cell differentiation markers between these two disease entities.

Experimental Design: This study included HIV-unrelated patients with PCNSL ($n = 51$) and NL ($n = 72$) treated at four hospitals in Taiwan for whom archival tumor tissue was available. Immunohistochemistry for CD10, BCL-6, MUM-1, vs38c, CD138, and BCL-2 was done. CD10, BCL-6, and MUM-1 expression results were used to classify all cases into the germinal center B-cell (GCB) or the non-GCB subgroup. The prognostic significances of clinical and immunophenotypic markers were evaluated.

Results: Nuclear MUM-1 expression was significantly higher in PCNSL than in NL ($P < 0.001$; 84% versus 53%). PCNSL tumors were more frequently classified into the non-GCB subgroup than NL tumors ($P = 0.020$; 78% versus 62%). For patients with PCNSL, univariate analysis showed that patients with BCL-6 expression had a trend towards longer survival ($P = 0.073$; median survival, 25.3 versus 7.3 months), and multivariate analysis showed BCL-6 was an independent prognostic factor ($P = 0.026$). For patients with NL, both of univariate ($P = 0.003$) and multivariate analyses ($P = 0.002$) showed that GCB was significantly associated with favorable survival.

Conclusion: The higher frequency of non-GCB subclassification, which was mainly contributed by nuclear MUM-1 expression in PCNSL implies that it has a more differentiated cellular origin than NL. BCL-6 expression in patients with PCNSL and GCB subgroup in patients with NL were favorable prognostic factors.

Primary central nervous system lymphoma (PCNSL) is a rare disease, which has shown a dramatic increase in incidence in both immunocompetent and immunodeficient patient populations during the past two decades (1). The majority of PCNSLs in immunocompetent patients are diffuse large B-cell lymphomas (DLBCL), which are histologically indistinguish-

able from DLBCLs of peripheral nodal origin (NL). However, the clinical course and prognosis of PCNSLs are quite different from those of NLs, and the biological difference between these two entities has not been clearly defined (2).

The cellular origin of PCNSL is uncertain. Mutations in the 5' noncoding region of the *BCL-6* gene were identified in 13 of 22 (59.1%) tumors of immunocompetent patients with PCNSL (3). This result suggests a germinal center or post-germinal center origin for the majority of PCNSLs. Several antigens differentially expressed in germinal center and post-germinal center B cells (post-GCB) can now be identified by newly generated monoclonal antibodies suitable for immunohistochemical assay on paraffin sections. For example, CD10 and BCL-6 are markers for GCBs (4, 5); MUM-1 is a marker for late germinal center/early post-GCBs (6, 7); and CD138 and vs38c are markers for plasmacytic and/or post-germinal center differentiation (8, 9). However, previous studies attempting to clarify the cellular origin of PCNSL have made discrepant findings. For instance, the frequency of BCL-6 expression in PCNSLs ranged from 22% to 100% in different studies (3, 10, 11). Because it is difficult to compare the immunohistochemical results obtained by different methods and cutoff criteria for positive expression in these studies (7), a direct comparison of

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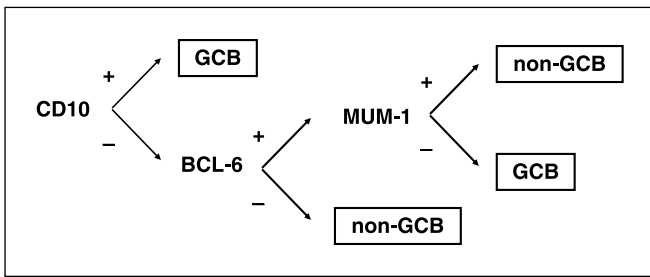


Fig. 1. Decision tree for immunohistochemical classification of DLBCL.

PCNSLs and NLs under the same immunohistochemical conditions is mandatory.

Furthermore, the identification of reliable and validated prognostic factors is an important issue in PCNSL. Traditional prognostic factors for NL, such as the Ann Arbor staging or International Prognostic Index scores, are not applicable to PCNSL because virtually all of these neoplasms represent stage I extranodal disease by definition. Therefore, evaluation of the prognostic significance of molecular markers is critically needed.

Our literature review found reports of two small studies in PCNSL and five large studies in NL, which had used immunohistochemistry to validate the prognostic significance of differentiation markers. For patients with PCNSL, BCL-6 expression was reported to be associated with favorable survival in one study of 33 patients (11) but was associated with unfavorable survival in another study of 14 patients (12). For patients with NL, CD10, BCL-6, and MUM-1 were generally used to classify cases into GCB and non-GCB groups. The resulting data are conflicting, with three studies showing a significantly better survival for the GCB group (13–15), whereas two others showing no difference in survival between the GCB and non-GCB groups (16, 17). In addition, BCL-2, an

Table 2. Histologic subclassification of PCNSLs and NLs according to GCB and non-GCB subgroups

Classification	PCNSL (n = 51)			NL (n = 72)		
	GCB (%)	Non-GCB (%)	P	GCB (%)	Non-GCB (%)	P
Centroblastic	7 (14)	24 (47)	1.000*	17 (24)	19 (26)	0.842
Polymorphic CB-1	4 (8)	16 (31)		2 (3)	5 (7)	
Polymorphic CB-2	0	0		9 (13)	13 (18)	
Immunoblastic	0	0		3 (4)	4 (6)	

*Computed only for a 2 × 2 table.

antiapoptotic protein, was reported to be a prognostic factor in NL in two of these studies (13, 16).

The primary objective of this study was to compare the expression of a panel of differentiation markers between PCNSL and NL. The second objective was to examine the prognostic significance of these differentiation markers and BCL-2 in patients with PCNSL or NL.

Patients and Methods

Patients. This study included HIV-unrelated patients with PCNSL (n = 51) or NL (n = 72) treated at four hospitals in Taiwan from 1991 to 2003 for whom archival tumor tissue was available. All tumors fulfilled the WHO criteria for the diagnosis of DLBCL (18). PCNSL was defined as lymphoma confined to the central nervous system with or without eye involvement at the time of diagnosis. All NL tumor specimens were obtained from peripheral lymph nodes, including the neck and inguinal areas. Information on patient demographics, Ann Arbor stage (for patients with NL), treatment, and survival was abstracted from medical charts.

Histologic features. Histologic slides were reviewed in all cases by two observers (K.T.K. and C.H.L.). Morphologic features were subclassified into the following categories based on the updated Kiel classification criteria (19): centroblastic, ≥90% of tumor cells were typical centroblasts; polymorphic centroblastic (polymorphic centroblastic), the proportion of immunoblasts ranged from 10% to 90% of tumor cells; and immunoblastic, ≥90% of tumor cells were immunoblasts. To allow more detailed comparisons among different groups in the statistical analysis, polymorphic centroblastic lymphomas were further subclassified into polymorphic CB-1 (≤50% immunoblasts) or polymorphic CB-2 (>50% immunoblasts) subtype as previously described (16).

Immunohistochemistry. Immunohistochemical studies were done on paraffin sections using an indirect biotin-avidin method. Sections were cut at 4-μm thickness, deparaffinized, and rehydrated. Endogenous peroxidase activity was blocked with hydrogen peroxide/methanol, and antigen retrieval was done in commercial buffer (Trilogy, Cell Marque, Hot Springs, AR) by autoclave for 10 minutes. All primary antibodies were incubated at a temperature of 4°C overnight. Antibody reactivity was detected using the Ventana iView DAB Detection System (Nexus IHC, Ventana Medical Systems, Tucson, AZ). Tissues known to express the determinants of interest were used as positive controls. The following mouse monoclonal antibodies were used: CD10 (clone 56C6; Novocastra, Newcastle upon Tyne, United Kingdom; dilution, 1:80), BCL-6 (clone PG-B6p; DAKO, Copenhagen, Denmark; dilution,

Table 1. Characteristics, treatments, and survival of patients with PCNSL and NL

Characteristics	PCNSL (n = 51)	NL (n = 72)	P
Age, y			0.758
Median	62	62	
Range	16-82	25-87	
Male/female ratio	29:22	41:31	0.993
Initial treatment			<0.001
High-dose methotrexate – based chemotherapy	29	0	
CHOP or CHOP-like	1	65	
Radiotherapy	15	0	
Supportive care	6	7	
Follow-up time, mo			<0.001
Median (95% CI)	22.0 (0-51.5)	62.9 (33.4-92.4)	
Overall survival, mo			0.011
Median (95% CI)	13.7 (9.8-17.6)	30.9 (0-66.8)	

Abbreviation: CI, confidence interval.

Table 3. Frequencies of antigen expression and immunophenotypic subclassification in PCNSL and NL

Antigen	PCNSL (n = 51)	NL (n = 72)	P
CD10	9 (18%)	16 (22%)	0.535
BCL-6	30 (61%)	33 (46%)	0.156
MUM-1	43 (84%)	38 (53%)	<0.001
Vs38c	2 (4%)	5 (7%)	0.698
CD138	0 (0%)	0 (0%)	NE
BCL-2	25 (49%)	37 (51%)	0.796
GCB group	11 (22%)	31 (38%)	0.013

Abbreviation: NE, not evaluable.

1:20); MUM-1 (clone MUM-1p; DAKO; dilution, 1:50), vs38c (clone VS38c; DAKO; dilution, 1:50), CD138 (clone MI 15; DAKO; dilution, 1:50), BCL-2 (clone 124; DAKO; dilution, 1:80).

For all markers, positive expression was defined as positive staining in >20% of cells as previously described (15). For BCL-6 and MUM-1, only diffuse or granular nuclear staining was considered positive; for CD10 and CD138, only membrane staining was considered positive; for vs38c and BCL-2, only cytoplasmic staining was considered positive.

CD10, BCL-6, and MUM-1 expression results were used to classify all cases into GCB or non-GCB subgroup as previously described (Fig. 1; ref. 14).

Statistical analysis. Data on clinical characteristics were compared by Mann-Whitney *U* test for continuous variables and the χ^2 test for categorical variables (or two-tailed Fisher's exact test when expected

number of any cell smaller than five cases). To avoid spurious positive results in multiple comparisons of the expression frequencies of markers between PCNSL and NL, Bonferroni correction was used to preserve an overall type I error of 5% for the analysis of each marker.

The survival analysis included only PCNSL patients who received high-dose methotrexate-based polychemotherapy and NL patients who received cyclophosphamide, vincristine, doxorubicin, and prednisone (CHOP) or CHOP-like chemotherapy. Death from any cause was considered an event, and data on patients who were alive at the last follow-up contact were censored. The Kaplan-Meier method was used to estimate the probabilities of survival; the log-rank test was used for univariate comparisons; and the proportional hazards model was used to evaluate independent prognostic factors for survival. These analyses were done using SPSS for Windows version 11.0 software.

Results

Patient characteristics and treatment. The characteristics, treatment, and overall survival of 51 PCNSL patients and 72 NL patients are summarized in Table 1. The median age and male/female ratio were not significantly different between patients with PCNSL and NL. Among the patients with PCNSL, two had malignant lymphoid cells in cerebrospinal fluid at diagnosis, and none had ocular involvement. Among the patients with NL, 12 (16%), 22 (31%), 25 (35%), and 13 (18%) had Ann Arbor stage I, II, III, and IV disease, respectively. Upon diagnosis, 29 patients with PCNSL received high-dose methotrexate-based polychemotherapy, which included the BOMES regimen (20) for 21 patients and other

Table 4. Univariate and multivariate analyses of clinical and immunophenotypic variables for PCNSL patients who received high-dose methotrexate-based chemotherapy and for NL patients who received CHOP or CHOP-like chemotherapy as initial treatment

Variable	Category	PCNSL (n = 27)				NL (n = 65)			
		No. of patients	Median survival (95% confidence interval), mo	P	P*	No. of patients	5-y survival rate (%)	P	P*
Age (y)	<60	15	34.5 (0.6-68.4)	0.045	0.043	37	57	0.048	0.026
	≥60	14	8.9 (0.0-20.4)			28	32		
Sex	Male	18	25.3 (0.0-55.7)	0.338		38	42	0.619	
	Female	11	11.0 (2.8-19.2)			27	45		
Stage	I/II	29	19.8 (5.1-34.5)	NE		32	63	0.001	<0.001
	III/IV	0				33	25		
CD10	Positive	5	34.5 (0.0-82.7)	0.884	0.087	15	75	0.073	
	Negative	24	19.8 (7.8-31.7)			50	34		
BCL-6	Positive	19	25.3 (12.3-38.3)	0.073	0.026	30	56	0.156	
	Negative	10	7.5 (0.0-15.5)			35	36		
MUM-1	Positive	22	16.3 (7.7-24.9)	0.597		36	22	0.011	
	Negative	7	34.5 (4.3-64.7)			29	68		
Vs38C	Positive	0		NE		5	30	0.817	
	Negative	29	19.8 (5.1-34.5)			60	44		
BCL-2	Positive	13	43.4 (2.3-84.5)	0.835		34	29	0.063	
	Negative	16	16.3 (8.8-23.8)			31	58		
GCB	Yes	7	34.5 (4.3-64.7)	0.597		29	72	0.003	0.002
	No	22	11.0 (7.7-24.9)			36	31		

Abbreviation: NE, not evaluable.

*P obtained by multivariate analysis.

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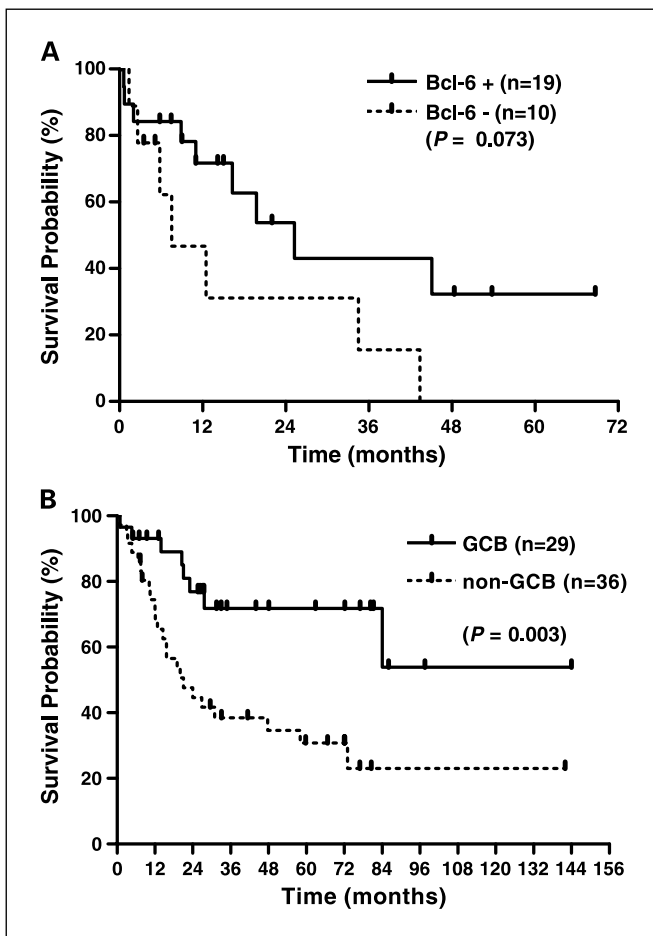


Fig. 2. Kaplan-Meier estimates of survival according to BCL-6 expression in PCNSL patients who received high-dose methotrexate-based chemotherapy as initial treatment (A) and GCB or non-GCB group in NL patients who received CHOP or CHOP-like chemotherapy as initial treatment (B).

modified regimens for eight patients. Upon diagnosis, 65 patients with NL received CHOP or CHOP-like chemotherapy. CHOP-like regimens included CEOP (doxorubicin replaced with epirubicin; four cases) and CNOP (doxorubicin replaced with mitoxantrone; two cases). Patients with advanced age or comorbidity were generally treated with radiotherapy, supportive care, or non-anthracycline-containing chemotherapy.

Morphologic study. The morphologic subclassification of PCNSLs and NLs according to GCB and non-GCB groups is listed in Table 2. None of PCNSLs fulfilled the criteria for immunoblastic or polymorphic CB-2. In contrast, 29 of 72 (40%) NLs were subclassified as immunoblastic or polymorphic CB-2 ($P < 0.0001$). No significant relationship was found between the morphologic subclassification and the GCB subclassification in PCNSL or NL.

Immunophenotypic profile. The frequencies of expression for CD10, BCL-6, MUM-1, vs38c, CD138, and BCL-2 are listed in Table 3. Nuclear MUM-1 expression was significantly higher in PCNSL than in NL ($P < 0.001$; 84% versus 53%). Although BCL-6 expression was also higher in PCNSL than in NL, this difference did not reach a significant level after Bonferroni correction. The frequency of non-GCB subgroup

was significantly higher in PCNSL than in NL ($P = 0.02$; 78% versus 62%).

Survival and prognostic analysis. The median survival of the 29 PCNSL patients who received high-dose methotrexate-based polychemotherapy was shorter than that of 65 NL patients who received CHOP or CHOP-like chemotherapy as first-line treatment ($P = 0.028$; median survival, 19.8 versus 58.0 months; 95% confidence interval, 5.1-34.5 versus 12.2-103.8 months).

Survival of NL patients with centroblastic plus polymorphic CB-1 versus immunoblastic plus polymorphic CB-2 subclassification was not significantly different ($P = 0.386$; median survival, 73.0 versus 47.7 months; 95% confidence interval, 23.6-122.4 versus 0-99.6 months) and was not evaluable in PCNSL because none of PCNSLs fulfilled the criteria for subclassification as immunoblastic or polymorphic CB-2.

The results of univariate and multivariate analyses of prognostic factors and survival are shown in Table 4. In PCNSL, univariate analysis showed that age < 60 years was significantly associated with longer survival and that BCL-6⁺ patients had a trend towards longer survival (Fig. 2A). In the proportional hazards model, BCL-6⁺ and age < 60 years were independent prognostic factors for patients with PCNSL. In NL, univariate analysis showed that age < 60 years, stage I/II, MUM-1⁻, and GCB subgroup (Fig. 2B) were significantly associated with longer survival, and that CD10⁺ patients and BCL-2⁻ patients had a trend towards longer survival. In the proportional hazards model, age < 60 years, stage I/II, and GCB subgroup remained significant, but none of these immunophenotypic markers was an independent prognostic factor.

Discussion

The cellular origin of DLBCL arising in the CNS, a site devoid of resident lymphoid tissue, has not been directly compared with that arising in the peripheral lymph nodes. This study is the first direct comparison of these two entities with respect to cellular origin. We found significantly higher frequencies of centroblastic or polymorphic CB-1 subtype and nuclear MUM-1 expression in PCNSL than in NL. These morphologic findings are consistent with other studies of PCNSL (11) and NL (14). The frequencies of MUM-1 expression found in PCNSL (84%) and in NL (54%) are consistent with the rate of expression reported in previous studies of PCNSL (11) and NL (14, 21, 22). True plasmacytic differentiation as evidenced by membrane staining for CD138, however, was not found in either PCNSL or NL in this study.

In normal B cells, MUM-1 expression is thought to denote the final step of intra-GCB differentiation toward plasma cells. MUM-1 was strongly expressed in lymphoplasmacytoid lymphoma and multiple myeloma (6). Taken together, the immunophenotypic results of our study are suggestive of a late germinal center/post-germinal center stage of B-cell differentiation for most cases of PCNSL, which implies that PCNSL may have a more differentiated cellular origin than NL.

For patients with PCNSL, age and performance status are the only two universally accepted prognostic factors (23, 24). Although several other potential prognostic variables for PCNSL have been proposed, such as histologic type (25),

subtentorial localization (26, 27), and bilateral brain involvement (28), adequate confirmation of results was not obtained in subsequent studies (11, 12). Despite the lack of randomized trial, high-dose methotrexate-based chemotherapy at diagnosis has recently become the standard of care due to its association with increased survival (29). Age and performance status would considerably affect the choice of treatment. Because data on performance status obtained by chart review were incomplete, the effects of performance status could not be analyzed in this study. To minimize the bias induced by failure to consider performance status, prognostic analyses were done only for PCNSL patients who received high-dose methotrexate-based polychemotherapy and NL patients who received CHOP or CHOP-like chemotherapy. Because the resulting sample size was reduced, the prognostic value of BCL-6 in PCNSL was also reduced from being significant (data not shown) to exhibiting a trend ($P = 0.073$; Table 4) in the univariate analysis.

In PCNSL, multivariate analyses showed that age <60 years and BCL-6⁺ were independent favorable prognostic factors for survival. This result for BCL-6 strongly supports findings

reported by Braaten et al. (11). Because assay of BCL-6 is simple and widely available, this observation has potentially important clinical implications as it may be an important item for stratification of patients in randomized clinical trials comparing different treatments for this disease.

In NL, both univariate and multivariate analyses showed that age <60 years, Ann Arbor stage I/II and GCB group were significantly associated with longer survival. None of these immunophenotypic markers was an independent prognostic factor. These results are consistent with the finding of Barrans et al. (13), Hans et al. (14), and Chang et al. (15).

In summary, the higher frequency of non-GCB group in PCNSL than in NL was mainly contributed by nuclear MUM-1 expression. This result is suggestive of a more differentiated cellular origin of PCNSL than NL. Expression of BCL-6 represents a favorable prognostic marker for patients with PCNSL, whereas GCB subgroup does for patients with NL. Differences between PCNSL and NL in clinical presentation, morphology, immunophenotype, and prognosis might allow DLBCL of CNS origin to be distinguished from heterogeneous DLBCL group.

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