Immunohistochemical profile and prognostic significance in primary central nervous system lymphoma: Analysis of 89 cases

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Abstract. The majority of primary central nervous system lymphomas (PCNSLs) are diffuse large B cell lymphoma, characterized by poor prognosis. In the present study, the expression of cluster of differentiation (CD)10, B cell lymphoma (BCL)-6, multiple myeloma-1 (MUM-1), BCL-2, CD138 and Ki-67 was analyzed by immunohistochemistry in 89 Chinese PCNSL cases, and the potential prognostic significance was evaluated. CD10, BCL-6, MUM-1, BCL-2 and CD138 were positive in 16.9 (15/89), 51.7 (46/89), 92.1 (82/89), 73.3 (63/86) and 0% (0/65) of all cases, respectively. According to the Hans algorithm, 71 patients (79.8%) were classified into the non-germinal center B cell-like (non-GCB) group, indicating a post-germinal center origin of PCNSL. The median follow-up time of 73 patients was 13 months [95% confidence interval (CI), 10.93-15.08]. The median overall survival (OS) time was 45.3 months (95% CI, 25.01-65.59) and the median progression-free survival (PFS) time was 30.0 months (95% CI, 13.43-46.57). Age (>60 years) was associated with a shorter OS time (P=0.009). Ki-67 (cutoff point 90%) was associated with shorter OS (P=0.037) and shorter PFS (P=0.039) times. No other immunohistochemical markers were associated with prognosis. On multivariate analysis, age (>60 years) was associated with shorter OS time (P=0.038), but immunophenotype and expression status of Ki-67, CD10, BCL-6 and BCL-2 did not predict prognosis. In conclusion, high Ki-67 expression may predict poor prognosis in PCNSL. The present study was limited by its sample size and short follow-up time. This requires more evidence to further clinical study.

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Introduction

Primary central nervous system lymphoma (PCNSL) is an aggressive neoplasm of the central nervous system with poor prognosis, accounting for 2-3% of all brain tumors worldwide (1,2). The incidence of PCNSL has increased markedly in immunocompetent patients for unknown reasons over the previous decades, whereas the incidence of human immunodeficiency virus (HIV)-associated PCNSLs has declined, possibly due to the development of highly active antiretroviral therapies (3,4). Morphologically, ~95% of these tumors are diffuse large B cell lymphoma (DLBCL), according to the new World Health Organization classification (5). Although the prognosis of PCNSL has been improved by optimal systemic treatment based on high-dose methotrexate (HD-MTX) (6-8), the overall survival (OS) of the majority of patients remains poor. This underlines the need to identify prognostic biomarkers for potential therapeutic targets and risk-stratified treatment.

In systemic DLBCL, based on cDNA microarray and immunohistochemical staining with various markers, including cluster of differentiation (CD)10, B cell lymphoma (BCL)-6 and multiple myeloma-1/interferon regulatory factor-4 (MUM-1), previous studies have identified two subtypes of DLBCL by Hans algorithm, namely, germinal center (GC) B cell-like (GCB) and non-GCB (9-13). Patients in the GCB subgroup showed an improved prognosis compared with ABC (activated B cell-like; including activated GCB and activated non-GCB, according to Chang's classification) (9,10,12,13). In PCNSL, numerous studies have been performed to observe the prognostic significance of the variable biological markers widely used in systemic DLBCL (14-24). The present study aimed to analyze the expression profile of immunohistochemical

markers and their potential prognostic significance in 89 Chinese PCNSL cases.

Materials and methods

Patients and tumor specimens. The clinical data of 89 immunocompetent patients with PCNSL were retrospectively reviewed at the Department of Hematology, Beijing Tiantan Hospital, Capital Medical University (Beijing, China) and the Department of Neurosurgery, Navy General Hospital (Beijing, China), between July 2009 and April 2015. Of the total 89 patients, 53 were male and 36 were female (male-female sex ratio of 1.47:1). The median age was 56 years (range, 11-85 years; ≤60 years, 54 patients; >60 years, 35 patients). All specimens were obtained by stereotactic biopsy or surgery for pathological diagnosis prior to treatment. Diagnosis of DLBCL was made by histological review of all specimens by two pathologists using light microscopy. The pathologists assessed the immunohistochemical markers including CD20, CD10, BCL-6, BCL-2, MUM1, CD138 and Ki-67 independently and between 15 and 20 fields were analyzed/specimen (magnification, x400). A total of 16/89 patients were lost to follow-up. In the follow-up of the remaining 73 patients, 39 received HD-MTX plus cytarabine [3.5 g/m^2 intravenous (i.v.) in 3 h on day 1 + 0.5-1 g/m^2 i.v. on day 2 according to age and Karnofsky Performance Status] every 3 weeks, and the other patients received HD-MTX plus tomozolomide (3.5 g/m^2 i.v. in 3 h on day 1 + 100 mg/m^2 administered orally on days 1-5) every 3 weeks.

The present study protocol was approved by the Ethics Committees of Beijing Tiantan Hospital and Navy General Hospital. All patients gave written informed consent.

Immunohistochemical analysis. Tumor specimens were fixed with 10% formalin at room temperature for 24 h and paraffin-embedded. A series of 4-μm sections were obtained for conventional hematoxylin and eosin (H&E) and immunohistochemical staining. Sections were deparaffinized in xylene and dehydrated with ethanol. Endogenous peroxidase was blocked with 0.1% hydrogen peroxide-methanol for 30 min at room temperature. Sections were washed with PBS, and the specimens were then incubated for antigen retrieval in a microwave oven for 15 min, followed by washing with PBS. The sections were treated with 3% H_2O_2 for 5 min at room temperature to block endogenous peroxidase activity, and sections were then incubated with the working dilution of each monoclonal antibody in a moist box (100% humidity) at 4˚C overnight. Monoclonal antibodies against CD20 (UM800002, 1:100 dilution), CD10 (UM870127, 1:600 dilution), BCL-6 (TA804186, 1:150 dilution), MUM1 (TA327705; 1:100-1:500 dilution), BCL-2 (UM870117, 1:500 dilution), Ki-67 (TA352729, 1:100), CD138 (TA327619, 1:25-1:200) were used and all purchased from OriGene Technologies, Inc. (Rockville, MD, USA). Subsequent to washing the specimens with PBS, they were incubated with corresponding secondary antibodies at a dilution of 1:2,000 [horseradish peroxidase-conjugated monoclonal goat anti-mouse IgG (HS201-01) or horseradish peroxidase-conjugated monoclonal goat anti-rabbit IgG (HS101-01); TransGen Biotech Co., Ltd., Beijing, China] for 1 h at room temperature. The EnVision kit was purchased from OriGene Technologies, Inc., and immunohistochemistry (EnVision method) was performed according to the manufacturer's protocol. Systemic DLBCLs were used as positive controls.

The staining for each marker was scored by two pathologists independently. Images were captured using a LEITZ DMR microscope (between 15 and 20 fields; magnification, x400; Leica Microsystems GmbH, Wetzlar, Germany). Staining was considered positive for CD10, BCL-6, MUM-1 and CD138 when >30% of cells were positively stained (25). For BCL-2, staining was considered positive when >50% of cells were positively stained (26) (between 15 and 20 fields; magnification, x400). Ki-67 expression was evaluated by semi-quantitative method and on the basis of the proportion of positive tumor cells (between 0 and 100%). High expression was considered when >90% of cells were positively stained for Ki-67. Low expression was considered when ≤90% of cells were positively stained for Ki-67.

Immunophenotype classification

Hans' method. Using a decision tree, Hans *et al* (12) divided tumors into two main subgroups according to three markers: CD10, BCL-6 and MUM-1. All CD10$^+$ tumors and those with a CD10$^-$ BCL-6$^+$ MUM-1$^-$ phenotype were considered as the GCB subgroup. The non-GCB subgroup included CD10$^-$ BCL-6$^+$ MUM-1$^+$, CD10$^-$ BCL-6$^-$ MUM-1$^+$ and CD10$^-$ BCL-6$^-$ MUM-1$^-$ immunophenotypes.

Chang's method. Using the immunohistochemical markers CD10 and BCL-6 for GCB markers, and MUM-1 and CD138 for ABC markers, Chang *et al* (13) classified the tumors as GCB and activated GBC subgroups (activated GCB and activated non-GCB subgroups). At least one positive GCB marker without the expression of activation markers was considered as the GCB subgroup. The activated GCB subgroup expressed CD10 and/or BCL-6 and one activation marker. The activated non-GCB subgroup expressed at least one activation marker without the expression of GCB markers.

Statistical analysis. OS time was counted from the start of treatment to the time of mortality due to any cause. Progression-free survival (PFS) time was counted from the start of treatment to the time of disease progression or mortality due to PCNSL. Kaplan-Meier survival curves were obtained, and differences in OS or PFS times were performed using the log-rank test. Multivariate analysis for OS and PFS times using the Cox proportional hazards regression models. Distribution of the characteristics of patients examined using the χ^2 test. All statistical analyses were performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics and clinical outcomes of patients. The characteristics of patients with PCNSL are described in Table I. All patients with PCNSL were immunocompetent patients who were HIV-negative. Of the total 89 patients, 53 were male and 36 were female (male-female sex ratio of 1.47:1). The median age was 56 years (range, 11-85 years; ≤60 years, 54 patients;

Table I. Clinical characteristics of patients with PCNSL.

Characteristics	Patients
Median age (range)	56 (11-85)
Age, n (%), years	
>60	35/89
≤60	54/89
Sex, n (%)	
Male	53/89 (59.6)
Female	36/89 (40.4)
ECOG, n (%)	
0-1	21/89 (23.6)
2-4	68/89 (76.4)
LDH, n (%)	
Elevated	30/76 (39.5)
Normal	46/76 (60.5)
No. of lesions, n (%)	
1	29/89 (32.6)
≥2	60/89 (67.4)
Deep brain lesions, n (%)	
Absent	28/89 (31.5)
Present	61/89 (68.5)
Chemotherapy, n (%)	
HD-MTX+Ara-C	39/73 (53.4)
HD-MTX+TMZ	34/73 (46.6)
Median OS (95% CI)	45.3 (25.01-65.59)
Median PFS (95% CI)	30.0 (13.43-46.57)

ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; HD-MTX + Ara-C, high-dose methotrexate + cytarabine; HD-MTX + TMZ, high-dose methotrexate + tomozolomide; OS, overall survival; PFS, progression-free survival; CI, confidence interval.

>60 years, 35 patients). In total, 21 patients (23.6%) had an Eastern Cooperative Oncology Group (ECOG) performance status (27) of 0 or 1 and 68 patients (76.4%) had an ECOG performance status of 2-4. Multiple brain lesions were observed in 67.4% (60/89) of patients and the presence of deep brain structures was observed in 68.5% (61/89) of patients. The concentration of serum lactate dehydrogenase (LDH) was elevated in 30 (39.5%) of 76 patients.

Follow-up was performed for 73 patients, as 16 were lost to follow-up. The median follow-up time was 13 months [95% confidence interval (CI), 10.93-15.08]. The median OS time was 45.3 months (95% CI, 25.01-65.59) and the median PFS time was 30.0 months (95% CI, 13.43-46.57).

Cytological and immunohistochemical analysis. In tumor cells with a diffuse distribution, the nuclei were 2 times greater than normal lymphocytes. CD20 staining showed diffuse patterns; CD20, CD10 and CD138 showed cell membrane staining; BCL-6, MUM-1 and Ki-67 showed nuclei staining; and BCL-2 showed cytoplasmic staining of cells (Fig. 1).

CD10, BCL-6, and MUM-1 were positive in 16.9 (15/89), 51.7 (46/89) and 92.1% (82/89) of patients. Among 86 tested samples, BCL-2 was positive in 73.3% (63/86). In total, 42 PCNSLs showed >90% Ki-67 expression. CD138 was negative in 100% (65/65).

Immunophenotype classification. According to the Hans classification, 18 tumors (20.2%) were classified in the GCB subgroup: 8 (9.0%) were $CD10^+$ $BCL\text{-}6^+$ $MUM\text{-}1^+$; 2 (2.2%) were $CD10^+$ $BCL\text{-}6^+$ $MUM\text{-}1^-$; 5 (5.6%) were $CD10^+$ $BCL\text{-}6^-$ $MUM\text{-}1^+$; 3 (3.4%) were $CD10^-$ $BCL\text{-}6^+$ $MUM\text{-}1^-$; and none were $CD10^+$ $BCL\text{-}6^-$ $MUM\text{-}1^-$. A total of 71 tumors (79.8%) were considered as non-GCB: 33 (37.1%) were $CD10^-$ $BCL\text{-}6^+$ $MUM\text{-}1^+$; 36 (40.5%) were $MUM\text{-}1^+$ only; and 2 (2.2%) were negative for all markers tested.

According to the Chang classification, the 87 patients were classified into the three subgroups: 5 (5.7%) tumors were $CD10^+$ $BCL\text{-}6^{+/-}$ $MUM\text{-}1^-$ and $CD10^-$ $BCL\text{-}6^+$ $MUM\text{-}1^-$, and were classified as GCB; 46 (52.9%) tumors were $CD10^+$ $BCL\text{-}6^+$ $MUM\text{-}1^+$, $CD10^-$ $BCL\text{-}6^+$ $MUM\text{-}1^+$ or $CD10^+$ $BCL\text{-}6^-$ $MUM\text{-}1^+$ and were classified as activated GCB; and 36 (41.4%) tumors were $CD10^-$ $BCL\text{-}6^-$ $MUM\text{-}1^+$ and were classified as activated non-GCB.

Analysis of prognosis. Among the clinical characteristics of patients, an age >60 years was associated with a shorter OS time compared with an age of ≤60 years (univariate analysis, P=0.009; multivariate analysis, hazard ratio=0.229; 95% CI, 0.057-0.922; P=0.038; Fig. 2). Among the biological markers, based on univariate analysis, Ki-67 expression (>90%) was associated with a shorter OS (P=0.037) and shorter PFS (P=0.039) times, compared with ≤90% Ki-67 expression (Fig. 3). However, on multivariate analysis, no biological markers were associated with OS and PFS time. No significant prognostic effect on OS or PFS was observed for the other clinical or biological parameters (sex, ECOG, CD10, BCL-6, BCL-2, LDH concentration, number of lesions, chemotherapy regimens and GCB/non-GCB subgroups) (Table II).

Discussion

In the present study, it was revealed that, unlike systemic DLBCLs, the majority of PCNSLs originate from post-GC, with a low expression of the GC marker CD10, expression of the GC marker BCL-6 and high expression of the activated B cell-like marker MUM-1, which is consistent with the majority of previous studies (14-24,28,29) (Table III). In addition, the co-expression of BCL-6 and MUM-1 and the absence of late post-GC marker CD138 expression indicated the activated immunophenotype and the early post-GC origin of PCNSL, which was in accordance with Camilleri-Broët *et al* (24).

A number of previous studies (21,23,24,28-30) have utilized immunohistochemical markers to predict the prognosis of PCNSL. However, the significance of immunohistochemical markers on the prognosis of PCNSL remains questionable due to limitations, including a small sample size, heterogeneous treatment regimens or different methods and standards of immunohistochemistry. The present study analyzed the expression of biological markers and evaluated their prognostic significance in the largest retrospective studies

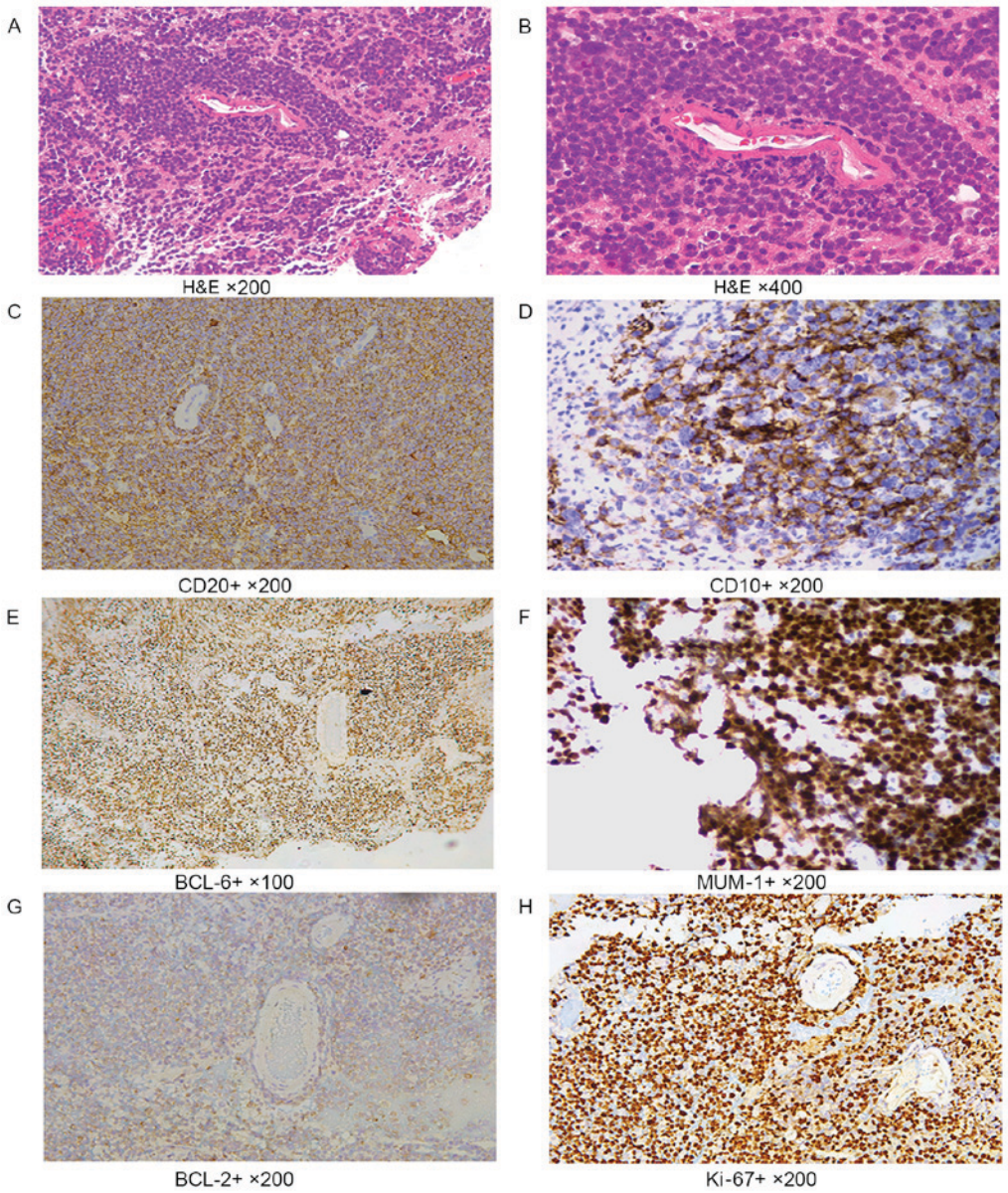

Figure 1. Immunohistochemical labeling. (A and B) H&E staining. In tumor cells with the diffuse distribution, the size of nuclei were 2 times greater than of normal lymphocytes. (A) Magnification, x200. (B) Magnification, x400. (C) CD20 cell membrane staining performed using the EnVision method. Magnification, x200. (D) CD10 cell membrane staining performed using the EnVision method. Magnification, x200. (E) BCL-6, nuclei staining, EnVision method, x100. (F) MUM-1 nuclei staining performed using the EnVision method. Magnification, x200. (G) BCL-2 cytoplasmic staining performed using the EnVision method. Magnification, x200. (H) Ki-67 nuclei staining performed using the EnVision method. Magnification, x200. H&E, hematoxylin and eosin; CD, cluster of differentiation; BCL, B cell lymphoma; MUM-1, multiple myeloma-1.

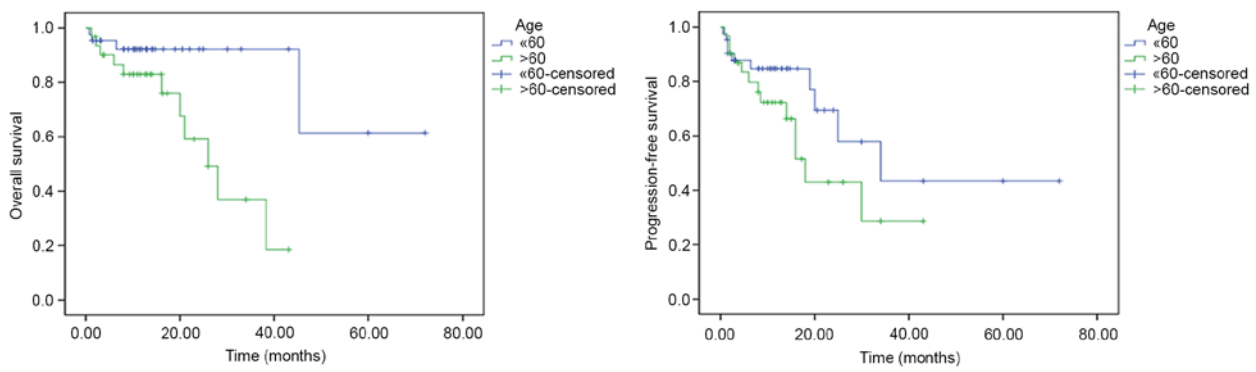

Figure 2. Comparison of OS and PFS time between age >60 and age ≤60 years by log-rank test. Univariate analysis revealed that younger age (≤60 years) was associated with a longer OS time (P=0.009) compared with older age (>60 years). No significant difference was observed for PFS time (P=0.141). OS, overall survival; PFS, progression-free survival.

Table II. Univariate and multivariate analyses for overall survival and progression-free survival.

A, Overall survival

Characteristic	Univariate analysis (log-rank test)	Multivariate analysis (Cox test)		
	P-value	HR	P-value	95% CI
Age (≤60 vs. >60 years)	0.009	0.229	0.038	0.057-0.922
Sex (male vs. female)	0.525	-	-	-
ECOG (0-1 vs. 2-4)	0.377	-	-	-
CD10 (positive vs. negative)	0.924	-	-	-
BCL-6 (positive vs. negative)	0.453	0.612	0.468	0.163-2.303
BCL-2 (positive vs. negative)	0.328	0.549	0.427	0.125-2.409
Ki-67 (>90 vs. ≤90%)	0.037	0.414	0.162	0.120-1.424
Immunophenotype (GCB vs. non-GCB)	0.410	0.506	0.365	0.116-2.209
Chemotherapy (HD-MTX+Ara-C vs. HD-MTX+TMZ)	0.671	0.993	0.990	0.309-3.191
LDH (elevated vs. normal)	0.442	-	-	-
No. of lesions (1 vs. ≥2)	0.592	0.880	0.835	0.262-2.954

B, Progression-free survival

Characteristic	Univariate analysis (log-rank test)	Multivariate analysis (Cox test)		
	P-value	HR	P-value	95% CI
Age (≤60 vs. >60 years)	0.141	0.566	0.237	0.220-1.456
Sex (male vs. female)	0.957	-	-	-
ECOG (0-1 vs. 2-4)	0.313	-	-	-
CD10 (positive vs. negative)	0.264	-	-	-
BCL-6 (positive vs. negative)	0.304	0.736	0.571	0.255-2.126
BCL-2 (positive vs. negative)	0.463	0.649	0.438	0.218-1.934
Ki-67 (>90 vs. ≤90%)	0.039	0.437	0.075	0.176-1.086
Immunophenotype (GCB vs. non-GCB)	0.131	0.398	0.109	0.129-1.228
Chemotherapy (HD-MTX+Ara-C vs. HD-MTX+TMZ)	0.459	1.063	0.898	0.422-2.675
LDH (elevated vs. normal)	0.779	-	-	-
No. of lesions (1 vs. ≥2)	0.740	1.021	0.967	0.387-2.696

ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; HD-MTX + Ara-C, high-dose methotrexate + cytarabine; HD-MTX + TMZ, high-dose methotrexate + tomozolomide; OS, overall survival; PFS, progression-free survival; CI, confidence interval; HR, hazard ratio; CD10, cluster of differentiation 10; BCL, B cell lymphoma.

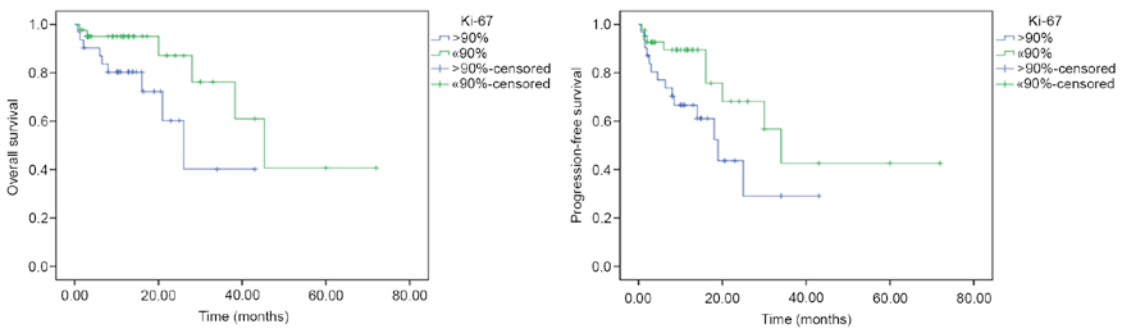

Figure 3. Comparison of OS and PFS time between Ki-67 expression >90 and ≤90% by log-rank test. Univariate analysis revealed that high expression of Ki-67 (>90%) was associated with a shorter OS (P=0.037) and PFS (P=0.039) times compared with low expression of Ki-67 (≤90%). OS, overall survival; PFS, progression-free survival.

Table III. Comparison of immunohistochemical marker expression in previous studies and the present study.

First author (year)	Country	Cases, no.	CD10, n (%)	BCL-6, n (%)	MUM-1, n (%)	BCL-2, n (%)	Algorithm	GCB, n (%)	Non-GCB, n (%)	(Refs.)
Mahadevan *et al* (2015)	India	24	0	12/24 (50.0)	22/24 (91.7)	-	Hans and Chang	-	22/24 (91.7)	(14)
Aki *et al* (2013)	Turkey	35	6/35 (17.1)	15/35 (42.8)	27/35 (77.1)	15/35 (42.8)	Hans	6/35 (17.1)	29/35 (82.9)	(15)
Hattab *et al* (2010)	USA	31	4/31 (12.9)	26/31 (83.9)	27/31 (87.1)	21/30 (70.0)	Hans	5/31 (16.1)	26/31 (83.9)	(16)
Raoux *et al* (2010)	France	39	8/39 (20.5)	14/39 (35.9)	23/39 (60.0)	33/39 (84.6)	Hans	13/39 (25.7)	26/39 (74.3)	(17)
Momota *et al* (2010)	Japan	27	6/27 (22.2)	13/27 (48.1)	22/27 (81.5)	-	Hans and Chang	1/23 (4.4)	22/23 (95.6)	(18)
Kinoshita *et al* (2010)	Japan	32	6/32 (18.8)	21/32 (65.6)	27/32 (84.4)	-	Hans and Chang	8/29 (27.6)	21/29 (72.4)	(19)
Bhagavathi *et al* (2008)	USA	21	1/21 (4.8)	19/21 (90.5)	19/21 (90.5)	17/21 (81.0)	Hans	2/21 (9.5)	19/21 (90.5)	(20)
Levy *et al* (2008)	USA	38	3/38 (7.9)	18/38 (47.4)	36/38 (94.7)	-	Hans	5/38 (13.0)	33/38 (87.0)	(21)
Cheng *et al* (2008)	China	47	3/47 (6.4)	25/47 (53.2)	43/47 (91.5)	-	Chang	4/47 (8.5)	43/47 (91.5)	(22)
Lin *et al* (2006)	Taiwan	51	9/51 (17.6)	30/51 (58.9)	43/51 (84.3)	25/51 (49.0)	Hans	11/51 (21.6)	40/51 (78.4)	(23)
Camilleri-Broët *et al* (2006)	France	82	2/82 (2.4)	45/81 (55.5)	75/81 (92.6)	45/81 (55.5)	Hans and Chang	3/82 (3.7)	79/82 (96.3)	(24)
Present study	China	89	15/89 (16.9)	46/89 (51.7)	82/89 (92.1)	63/86 (73.3)	Hans and Chang	18/89 (20.2)	71/89 (80.9)	

CD10, cluster of differentiation 10; BCL, B cell lymphoma; MUM-1, multiple myeloma-1; GCN, germinal center B cell-like.

at present. All the patients uniformly received HD-MTX based chemotherapy as the first line of treatment. The Ki-67 proliferative index, a nuclear antigen present in all stages of the cell cycle, with the exception of G_0, represents the active growth fraction of the tumor (31-33). Ki-67 is a valuable immunohistochemical marker to distinguish indolent from aggressive lymphomas, particularly in small needle biopsies where exact typing may not be possible (31). Several studies have demonstrated that high expression of Ki-67 is an adverse prognostic marker in systemic DLBCL (34-36). In the present study, it was also observed that Ki-67 expression is a significant prognostic parameter of poor prognosis in patients with PCNSL. Unlike systemic DLBCLs, the mean Ki-67 index for PCNSL was high (mean, 88%). Patel *et al* (28) revealed that the proliferative index was high (60-98%) in their study of 73 PCNSL cases. Hashmi *et al* (31) showed that the mean Ki-67 index for indolent non-Hodgkin lymphoma (NHL) included 23% for small cell, 25% for mantle cell, 28.5% for marginal zone and 34.6% for follicular lymphoma. By contrast, the mean Ki-67 index for aggressive lymphomas was 66.4, 66.9, 80.3, 83.3 and 94.4% for DLBCL, T cell, anaplastic large cell, lymphoblastic and Burkitt's lymphoma, respectively (31). A uniform high expression of Ki-67 is a notable feature of PCNSL, which may explain the poor outcome of PCNSLs. Previous studies did not reveal the prognostic significance of Ki-67, which may be due to the small number of patients with a uniform high expression of Ki-67 or the different immunohistochemical methods used.

CD10 is expressed in pre-B cells and germinal center B cells (37,38). MUM-1 performs an important role in the terminal stages of B cell differentiation and can be used as a post-GC cell or activation marker (39,40). Due to the low expression of CD10 and the high expression of MUM-1, CD10 and MUM-1 where considered to be characteristics of PCNSL, but not prognostic indicators.

BCL-2, a proto-oncogene, localizes to mitochondria and enhances cell survival by blocking programmed cell death (41). BCL-2 protein expression is an important independent predictor of survival in patients with systemic DLBCL (42,43). However, in the present study, no association between BCL-2 and prognosis was observed, which is in accordance with the studies by Krogh-Jensen *et al* (44) and Preusser *et al* (45).

The BCL-6 gene encoding a nuclear-located Krüppel-type zinc finger protein is rearranged in ~30% of DLBCLs and is expressed predominantly in normal GCB cells and associated lymphomas (46,47). BCL-6 may have an important role in regulating the differentiation of normal GCB cells, and its deregulated expression may contribute to lymphomagenesis (30). Previous studies about the prognostic significance of BCL-6 expression remain controversial. Survival analyses revealed BCL-6 expression as an independent prognostic parameter of DLBCL associated with favorable outcomes, and its positivity indicates an improved disease course (21,29,45). The CALGB 50202 study of the prospective G-PCNSL-SG1 trial disclosed that BCL-6 may assume clinical relevance as an unfavorable prognostic biomarker in PCNSL (7,30). In the present study, BCL-6 expression was not associated with OS or PFS. The present study is a retrospective study with a short follow-up time, and these limitations may explain the discrepancy between the present study and previous studies.

In the present study, no significant difference was observed between GCB and non-GCB subgroups on OS or PFS time, which is in accordance with earlier studies (12,17,18,24,30). These results may indicate that PCNSL has a common immunophenotype classification, but this subtype classification may not have an effect on prognosis.

In conclusion, the present study confirmed the activated immunophenotype and the early post-GC origin of PCNSL, and determined that older age (>60 years) was associated with a shorter OS time. In addition, high Ki-67 expression was found to be a valuable biological marker for poor prognosis. Considering the short follow-up time of the present retrospective study and the controversial results of previous studies, additional prospective studies are required.

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