Primary Brain Lymphoma: A Report of Eight Cases from A Medical Center in Southern Taiwan

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Key Words
anaplastic large cell lymphoma; brain; lymphoma

Background. Primary brain lymphoma (PBL) in Taiwan has been reported only in three series with very limited immunophenotypic characterization.

Methods. We retrospectively studied PBL cases with history review, immunohistochemistry, and in situ hybridization (ISH) for Epstein-Barr virus-encoded mRNA (EBER) from a single institute in southern Taiwan during 1989-2000.

Results. We found eight cases of PBL including four males and four females with mean age of 64.1 years and median of 63.0. The major presenting symptoms were headache, poor memory, slurred speech, and hemiplegia in three patients each. All patients had stage I solitary tumor. Half of the patients received tumor excision, the other half, stereotactic biopsy. Seven cases were of diffuse large B-cell type (DLBL), with expression of bcl-2 in six cases. They were all negative for CD5, CD10, bcl-6, and EBER. The eighth patient had anaplastic large cell lymphoma (ALCL) of T-cell phenotype with expression of cytotoxic markers and was positive for EBER. Two were lost to follow up. The median follow-up time for the remaining six was 11.2 months (range, 5.5 - 25.0). They all received radiotherapy with initial complete remission. Two died of the disease, another of cardiopulmonary failure, and the other of stroke or recurrence. The remaining two were free of disease for 9.6 and 25.0 months after radiotherapy alone. The 1-year survival rate was 60%.

Conclusions. We have fully characterized eight cases of PBL, including seven DLBLs and one ALCL, in southern Taiwan that occurred in an older age group. Old age, immunophenotype (bcl-2-positivity and bcl-6-negativity), and lack of systemic chemotherapy were probably responsible for the shorter survival compared to other studies. Radiotherapy seems to be effective for inducing complete remission and even long-term survival in some patients, how ever, systemic chemotherapy should be administered to prevent recurrence and to achieve long-term survival. [Chin Med J (Taipei) 2002;65:172-179]
fuse poorly-differentiated lymphocytic lymphoma and T-cell lymphoblastic lymphoma. Limited immunophenotyping was described in only two cases. A recent report by Cheng et al stressed the efficacy of systemic chemotherapy with the BOMES protocol in treating 14 PBL. One was of T-cell lineage, the others, B-cell. The other recent report by Chen et al described 10 cerebrospinal NHL from southern Taiwan with focus on the efficacy of radiotherapy. The other recent report by Chen et al described 10 cerebral and three spinal NHL from southern Taiwan with focus on the efficacy of radiotherapy. They found that the initial response to radiation therapy was satisfactory but local recurrence frequently occurred. Diffuse large cell lymphoma was the most common histology type and accounted for 11 of 14 cases. However, immunophenotyping was not described in any of these cases. In a report of PBL in 18 Hong Kong Chinese patients, one case had post-transplant lymphoproliferative disorder, the remaining 17 immunocompetent patients had diffuse large B-cell lymphoma (DLBL) except one case of Burkitt’s lymphoma. For a better understanding of the clinicopathologic characterization of PBL in southern Taiwan, we retrospectively studied eight cases of PBL in our hospital with review of history and hematoxylin-eosin sections, immunohistochemical study, and in situ hybridization (ISH) for Epstein-Barr virus-encoded mRNA (EBER).

**Methods**

A total of eight cases of PBL during 1989 - 2000 were retrieved from the Pathology files of Chi-Mei Medical Center, located in Tainan, a city in southern Taiwan. The first five cases had been included in our previous study of the classification of malignant lymphoma in southern Taiwan. The eighth case was the subject of a case report. His story of all patients was reviewed. The Ann Arbor staging system for extranodal lymphoma was used for staging. The overall survival time was calculated from the date of diagnosis to the date of last follow-up or death.

The specimens from all the cases were fixed either in 10% unbuffered formalin or B-5 solution, processed by routine methods, and embedded in paraffin. All the original hematoxylin and eosin-stained sections were reviewed. Immunohistochemical stains were performed for each case based on the light microscopic and clinical findings. Sections of 4-μm thickness were used for hematoxylin-eosin, immunohistochemical stain, and in situ hybridization.

Immunohistochemical study was performed in the OptiMax Plus aut to matic cell stainer (BioGenex, San Ramon, CA, USA) using the labeled streptavidin-biotin peroxidase method (Horseradish peroxidase kit, BioGenex) with Super Sensitive Immunodetection System (BioGenex). An antigen retrieval technique was applied when needed for each individual antibody. The initial panel of antibodies for immunophenotyping included CD3, CD20, CD79a (DAKO, Carpinteria, CA, USA) and CD43 (BioGenex). Tumors were assigned to be B-cell lineage when the neoplastic cells were reactive for CD20 and/or CD79a but not CD3 and/or CD43. T-cell lineage was considered when the neoplastic cells expressed CD3 and/or CD43 but not CD20 and/or CD79a.

For a better understanding of the clinicopathologic characterization of PBL in southern Taiwan, we retrospectively studied eight cases of PBL in our hospital with review of history and hematoxylin-eosin sections, immunohistochemical study, and in situ hybridization (ISH) for Epstein-Barr virus-encoded mRNA (EBER).
Using the Kiel scheme into centroblastic lymphoma with four morphologic variants (monomorphic, polymorphic, multilobated, and centrocytoid) and immunoblastic lymphoma.10

Duration of follow-up was calculated from the time of diagnosis to either death or until March, 2001. The causes of death were recorded as either due to disease progression or other specified unrelated diseases. One-year survival rate was calculated by the number of patients surviving longer than one year divided by the number of deaths within this period plus the number of those surviving longer than one year.

Results

The clinical findings are summarized in Table 1. There were equal numbers of male and female patients. They were all immunocompetent without any sign of immunosuppression. The mean age was 64.1 with a median of 63.0. All patients had multiple neurological symptoms. The most common symptoms were headache, poor memory, slurred speech, and hemiplegia in three patients each. All patients had a single tumor and stage I disease at diagnosis of the disease.

Table 1. Clinical features of eight primary brain lymphomas

<table>
<thead>
<tr>
<th>Case</th>
<th>sex/age (y)</th>
<th>CC</th>
<th>Location</th>
<th>Size (cm)</th>
<th>Op</th>
<th>C/T</th>
<th>R/T</th>
<th>Outcome</th>
<th>Survival (mo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/76</td>
<td>Poor memory, slurred speech, R’t hemiplegia</td>
<td>L’t temporal</td>
<td>5</td>
<td>Ex twice</td>
<td>None</td>
<td>Yes, unknown dose</td>
<td>1st Recur 1 mo. after R/T, 2nd Recur 3 mo. after re-ex. DOD</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>2/F/53</td>
<td>Headache, vomiting</td>
<td>R’t temporal</td>
<td>4</td>
<td>Ex twice</td>
<td>MTX for Recur Unknown</td>
<td></td>
<td>Recur in left cerebellum in 0.7 mo.; LTF in 1 mo. after C/T</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3/M/72</td>
<td>R’t paralysys, stool and urine incontinence, aphasia</td>
<td>L’t caudate</td>
<td>4</td>
<td>Ex</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
<td>LTF</td>
<td>-</td>
</tr>
<tr>
<td>4/F/60</td>
<td>Conscious disturbance, urinary incontinence</td>
<td>L’t frontal</td>
<td>5</td>
<td>Ex</td>
<td>MTX for Recur</td>
<td>WB: 46; TBB: 8; WB: 18 for Recur</td>
<td>CR; Recur with ventricular seeding in 11 mo.; DOD</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>5/F/66</td>
<td>Poor memory, speech disturbance, general weakness</td>
<td>L’t frontal</td>
<td>4</td>
<td>Stereo bx</td>
<td>None</td>
<td>WB: 40; TBB: 20</td>
<td>CR</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>6/M/80</td>
<td>Conscious disturbance, poor memory, slurred speech</td>
<td>Bil basal ganglia</td>
<td>5</td>
<td>Stereo bx</td>
<td>none</td>
<td>WB: 45; TBB: 14</td>
<td>CR for 5 mo. then conscious change till death. DOD or DOUD (recurrence or stroke?)</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>7/M/60</td>
<td>Dizziness, headache</td>
<td>R’t occipital</td>
<td>3</td>
<td>Stereo bx</td>
<td>None</td>
<td>WB: 50.4; TBB: 14.4</td>
<td>CR</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>8/F/46</td>
<td>Headache, blurred vision, R’t hemiplegia</td>
<td>L’t occipitoparietal</td>
<td>5</td>
<td>Ex</td>
<td>None</td>
<td>WB: 50; TBB: 20</td>
<td>CR</td>
<td>25.0</td>
<td></td>
</tr>
</tbody>
</table>

CC = chief complaint; Op = operative method; C/T = chemotherapy; R/T = radiotherapy; Ex = excision; DOD = died of disease; MTX = intrathecal methotrexate; LTF = loss to follow up; WB = whole brain; TBB = tumor bed boost; CR = complete remission; Stereo bx = stereotactic biopsy; DOUD = died of unrelated disease; Bil = bilateral; NED = no evidence of disease.
MRI showing ventricular cerebrospinal fluid (CSF) seeding with subependymal invasion at the trigone of the right frontal and occipital horns 11 months after operation. Numerous large lymphoma cells were identified in the CSF. Her disease progressed despite second course of radiotherapy to the whole brain and intrathecal methotrexate infusion. She died of lymphoma 13.7 months after diagnosis. Case 5 died of cardiopulmonary failure with complete remission of her lymphoma. Case 6 was 80 years-old and received radiotherapy for a total of 59 Gy. A CT scan taken 5 months later during his admission for transurethral resection of prostate revealed complete remission of the brain tumor. One month later, his conscious ness decreased progressively and he became bed-ridden with focal signs suggestive of stroke or tumor recurrence. He passed away 6.7 months after diagnosis with out autopsy. The remaining 2 patients (Case 7 and 8) were free of disease for 9.6 and 25.0 months after radiotherapy. The 1-year survival rate was 60%.

The neoplastic cells in Cases 1-7 were in a diffuse growth pattern and were composed mainly of large non-cleaved centroblasts with two to three distinct nuclei. Many neoplastic giant cells, with or without nuclear lobulation, were admixed with the centroblasts in Case 1 that was diagnosed accordingly as polymorphic variant of centroblastic lymphoma using the Kiel scheme (Fig. 1A). Cases 2-7 were classified as monomorphic variant of centroblastic lymphoma. The immunohistochemical findings of these cases are listed

<table>
<thead>
<tr>
<th>Case</th>
<th>Bcl-2</th>
<th>Bcl-6</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>43</th>
<th>79a</th>
<th>Cyc D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>P*</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
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<td>N</td>
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<td>P</td>
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<td>N</td>
<td>N</td>
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<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>N</td>
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<tr>
<td>6</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>P</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

CD = cluster designation; Cyc D = cyclin D1; P = positive; N = negative; P* = weak cytoplasmic positivity.

Table 2. Immunohistochemical results of the seven primary brain diffuse large B-cell lymphomas

![A](image1.png), ![B](image2.png), ![C](image3.png)

Fig. 1. Case 1. Diffuse large B-cell lymphoma of the polymorphic variant. (A) Multinucleated giant cells and large non-cleaved cells (Hematoxylin & Eosin stain, x132). (B) and (C) Positive immunoreactivity for CD79a, and bcl-2, respectively (Immunoperoxidase stain, x132).
Table 2. All seven cases were reactive for the B-cell markers CD20 and CD79a (Fig. 1B), confirming their B-cell phenotype and they were classified as DLBL according to the REAL scheme. All seven cases were reactive for bcl-2 (Fig. 1C) except Case 6. The immunostainings for bcl-6, CD3, CD5, CD10, CD30, CD43, and cyclin D1 were all negative except weak cytoplasmic positivity for CD30 in Case 1. All seven cases were negative for EBER by ISH.

In Case 8, the neoplastic cells were large, some with reniform or embryo-like nuclei, the so-called “hallmark cells” occurring in all morphologic variants of anaplastic large cell lymphoma (ALCL) as referred to by Benharroch et al. (Fig. 2A). Multinucleation of the neoplastic cells was focally noted. The immunohistochemical findings and T-cell receptor (TCR) gene rearrangement study are detailed elsewhere. In brief, the neoplastic cells expressed CD30, CD43, granzyme B, and T-cell intracellular antigen-1 (TIA-1). The immunostaining pattern for CD30 was membranous with accentuation in the Golgi area (Fig. 2B), while that for both TIA-1 and granzyme B was granular and cytoplasmic (Fig. 2C). The neoplastic cells were negative for CD3, CD15, CD20, CD45, CD45RO, CD79a, cytokeratin, and EMA. They were diffusely positive for EBER by ISH. Polymerase chain reaction study of formalin-fixed tissue showed clonal gene rearrangement of the TCR-γ chain (data not shown). The diagnosis was ALCL of T-cell lineage with cytotoxic phenotype.

**Discussion**

We have fully characterized the pathologic findings in eight cases of primary brain lymphoma in Southern Taiwan. Seven were DLBL. The major differentials for these B-cell lymphomas were follicular lymphoma (FL), mantle cell lymphoma (MCL) and plasmacytoma. FL was excluded by the diffuse growth pattern and negative immunostaining for CD10 in all cases. The possibility of MCL was excluded by morphology (large centroblasts) and negative immunostaining with cyclin D1. Plasmacytoma or multiple myeloma may invade the brain, especially in the set-
ting of immune deficiency state. Myeloma cells usually have abundant amphophilic cytoplasm and are non-reactive to CD20 and bcl-2.

The anaplastic and polymorphic morphology and weak cytoplasmic expression of CD30 of the neo plastic cells in Case 1 raised the possibility of a differential diagnosis of ALCL. This tumor was of B-cell lineage with expression of CD20 and CD79a. Based on extensive biologic and clinical studies, B-cell lymphoma expressing the CD30 antigen was not felt related to ALCL of T-cell or null cell phenotype as defined by the International Lymphoma Group in the REAL classification. Those B-cell lymphomas like our Case 1 with “anaplastic cytology” and CD30 expression were considered a morphologic variant of large B-cell lymphoma rather than ALCL.11

Bcl-2 protein is the gene product of bcl-2 gene on chromosome 18q21, with the major function of inhibiting apoptosis by suppressing cell death.12 The bcl-6 gene is located on chromosome 3q27. Both bcl-2 and bcl-6 expression are highly regulated during B-cell differentiation in the germinatal center (GC). The GC B-cells have a characteristic phenotype of bcl-2+/bcl-6-. Bcl-2 and bcl-6 protein expression are frequent in FL.13-15 It has been shown that the neoplastic cells in 30% of extranodal DLBLs expressed bcl-2 protein and 71% of DLBLs expressed bcl-6 protein.16 Low bcl-6 expression has been reported to be associated with a shorter disease-free survival time in DLBL, while high bcl-2 expression tended to be associated with worse survival, although the association was not statistically significant.17 Six of seven DLBLs in our study expressed bcl-2, but none of them expressed bcl-6. This bcl-2+/bcl-6- phenotype has been proved to be useful for differentiating primary lymphoid organs, formerly known as (bcl-2+/bcl-6-) in small lymphocytic lymphoma of chronic lymphocytic leukemia from the trapped normal GC in MCL that is bcl-2-/bcl-6+.15 The significance of bcl-2+/bcl-6- phenotype in DLBL is currently unknown. Further investigation based on large case numbers is needed to clarify this issue.

DLBL represents a heterogeneous group of lymphomas based on clinical, morphologic, immunophenotypic, and molecular genetic features. The frequency of CD5 expression (de novo CD5+ DLBL) has been reported to be between 5% and 10% among DLBL cases.18,19 De novo CD5+ DLBL has been reported to occur more frequently in elderly women and patients in whom there is extranodal involvement with poor treatment outcome.10 De novo CD5+ DLBL has been reported to be a heterogeneous group containing an unusual form of splenic lymphoma with a distinctive cordal infiltrating pattern in the red pulp.20 In our study of 20 cases of surgically resected primary lymphoma, neoplastic cells did not express CD5 (Chuang et al, manuscript submitted). Likewise, the neoplastic cells of these seven cases of PBL in this series did not express CD5. It seems that the relative frequency of CD5 expression in cases of extranodal DLBL in Taiwan is very low. Currently, we are conducting a large study to determine the relative frequency and possible clinical significance of CD5 expression in cases of nodal and extranodal DLBL in Taiwan.

The base for the classification of malignant lymphomas has been evolving from morphology and immunology to the incorporation of clinical and genetic data. The Working Formulation (WF) was proposed in 1982, not as a new classification but as a means of translating among the 6 major classification systems and to facilitate clinical comparison of case reports and therapeutic trials.21 Morphologic criteria alone were used to separate the diseases into 10 categories and a group of miscellaneous entities. However, there are some problems with WF, in particular, the immunophenotyping of neoplastic cells was not incorporated, and different diseases are grouped together. Since the proposal of WF, much more new in formation has been come available, especially in the advancement of immunophenotyping, resulting in recognition of new entities and refinement of the previously recognized categories. The International Lymphoma Study Group (ILSG) proposed the REAL scheme in 1994 with the concept that the most practical approach to lymphoma categorization was to define the diseases with the currently available morphologic, immunologic, and genetic techniques.9 A large-series study using the REAL proposal for testing its clinical significance and practical utility was published in 1997.22
This study revealed high diagnostic accuracy and reproducibility of the REAL classification. The REAL scheme is also applicable to the community hospital, with high diagnostic concordance rate between pathologists in community hospitals and those in medical centers. It is now world-wide accepted and is the most commonly used classification scheme in recent research on malignant lymphomas. The upcoming WHO classification for lymphomas is similar to the REAL scheme, with minor modifications.

Primary cerebral ALCL is extremely rare and may pose a diagnostic challenge in brain tumors. Case 8 is the seventh case of primary cerebral ALCL in the literature. The large neoplastic cells with reniform or embryonic nuclei were admixed with abundant eosinophils and histiocytes and some small lymphocytes, similar to the rare variant of ALCL with extensive eosinophilic or neutrophilic infiltration described by McCullogh et al. Of the eight patients reported, four had a peripheral neutrophilia and one a peripheral eosinophilia. Case 8 in our study did not have either neutrophilia or eosinophilia at diagnosis and throughout the follow-up period. Her neoplastic cells expressed CD30 and cytotoxic markers, as do the tumor cells in usual ALCL. They were positive for EBER by ISH, which is a rare phenomenon in ALCL. The T-cell lineage was confirmed by poly merase chain reaction study of formalin-fixed tissue showing clonal gene rearrangement of the T-cell receptor-γ chain. The patient received cranial irradiation and is alive with out disease at 25 months of follow-up.

The prognosis of PBL is poor, with a mean survival around 11.6 - 20 months and 5-yr survival around 20%. Cranial irradiation is usually effective in achieving initial complete remission but with a high recurrence rate. Chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) has been re ported to have no role in the postradiotherapy treatment of PBL. Using univariate analysis based on 248 cases, Bataille et al identified age younger than 60 years, radiotherapy, and chemotherapy as favorable prognostic factors and partial excision an unfavorable prognostic factor. They recommend the treatment of PBL with the following sequence: stereotactic biopsy, chemotherapy with a methotrexate-and-anthracycline-based regimen, followed by cranial irradiation. In our current study, the median age (63.0 years) of the eight patients was old. All six patients with follow-up in formalin-fixed tissue achieved radiotherapy with initial complete remission and sub sequent recurrence in two or three patients. Although our case number is limited, it seems that radiotherapy is effective in achieving complete remission. The relatively short survival time of our series is probably because systemic chemotherapy was not administered. Whether the bcl-2-positive, bcl-6-negative immunophenotype played a role in survival needs to be evaluated by a large-scale, probably nation-wide study. Chemotherapy with a methotrexate-and-antracycline-based regimen as suggested by Bataille et al might be administered to prevent recurrence and for long-term remission.

Acknowledgements

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