Symposium article

Burkitt lymphoma is immunophenotypically different from Burkitt-like lymphoma in young persons

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Summary

Introduction: Burkitt-like lymphoma (BLL) is a provisional category of B-cell lymphoma which is morphologically intermediate between Burkitt lymphoma (BL) and large B-cell lymphoma (LBCL). The clinical significance of this morphology is controversial.

Patients and methods: We examined 41 cases of pediatric B-cell lymphoma by immunohistochemistry for proteins associated with proto-oncogenes c-myc, BCL-2 and BCL-6 and a subset of cases (with adequate slides) for a proliferation-associated marker (Ki-67) and for apoptosis (Apop-Tag). Sixteen cases of BLL, thirteen cases of BL and twelve cases of LBCL were examined.

Results: Our results showed BCL-6 expression in 16 of 16 BLL, 4 of 13 BL, and 9 of 12 LBCL; c-myc expression in 14 of 15 BLL, 9 of 13 BL, and 12 of 12 LBCL; and BCL-2 expression in 2 of 16 BLL, 0 of 13 BL, and 6 of 12 LBCL. Mean apoptotic index for BLL was 10.3% (n = 6); for BL was 17.1% (n = 5); and for LBCL was 10.9% (n = 6). Ki-67 was diffusely reactive in all cases tested. There was a significantly higher proportion of BLL than BL which expressed BCL-6 (P = 0.0001).

Conclusions: Labeling for BCL-6 distinguishes BLL from BL. It is likely that in children in North America, BLL is biologically distinct from BL and more closely resembles a subset of LBCL.

Key words: BCL-2, BCL-6, Burkitt lymphoma, Burkitt-like lymphoma, large B-cell lymphoma, non-Hodgkin’s lymphoma

Background

Burkitt-like lymphoma (BLL) is a category of B-cell lymphoma morphologically intermediate between Burkitt lymphoma (BL) and large B-cell lymphoma (LBCL) (Figure 1) [1]. The clinical significance of this type has been previously addressed but is not resolved [2, 3]. Because of its intermediate histology, BLL may be classified as either BL or LBCL and diagnostic concordance has been previously demonstrated to be low [3]. It is difficult to compare therapeutic outcomes of various treatments for this disease because of diagnostic variation, although outcome for all pediatric B-cell lymphomas is generally good with modern therapies.

In a study of primarily adult patients, it was shown that BLL (or ‘non-Burkitt’s’ type) is more heterogeneous than sporadic BL, without rearrangement of the c-myc gene, but occasionally with BCL-2 rearrangement [4]. This has not been tested in children. Cytogenetic and molecular studies have been difficult to perform in large series of pediatric lymphomas because biopsy specimens, often obtained in primary care institutions prior to the patient’s referral to a pediatric oncology center, are frequently limited or not handled properly. In order to investigate potential markers for BLL as well as BL and LBCL, we tested routinely processed biopsies for immunohistochemical evidence of expression of several proto-oncogenes which have been associated with BL or LBCL (BCL-2, BCL-6 and c-myc) and also markers for proliferation and apoptosis.

Patients and methods

Paraffin sections of diagnostic biopsies from 41 patients with B-cell lymphomas enrolled on POG protocols (#9219-localized lymphoma, n = 16; #9317-advanced stage small non-cleaved cell lymphoma, n = 16; and #9315-advanced stage large-cell lymphoma, n = 9) were analyzed. Each case had been diagnosed at the treating institution and submitted for histologic review at the time of enrollment. Specimens consisted of paraffin blocks or previously cut slides.

Hematoxylin-eosin stained slides from each case were re-reviewed by two pathologists (REH and CF) and classified using the Revised European–American Lymphoma (R.E.A.L.) Classification [1] and the Working Formulation for Clinical Usage (WF) [5]. BL was identified by diffuse infiltrates of neoplastic lymphoid cells with relatively uniform medium size oval nuclei (similar to histiocyte nuclei) with moderately clumped chromatin in relatively clear parachromatin, high mitotic rate, moderate amphophilic cytoplasm, and admixed starry sky macrophages. LBCL was primarily identified by nuclei of which more
that 25% were larger than average histiocyte nuclei (or plump endothelial cells), and with nuclei which were round to sometimes clefted or elongate, sometimes with prominent single nucleoli, and sometimes diffuse fine fibrosis. BLL lymphoma cells were intermediate between BL and LBCL, with more size and shape variation than BL but less than 20% large cells. Starry sky macrophages were variably present.

Each case was labelled with antibodies to CD20 (L26), CD3, and BCL-2 (clone 124)(Dako, Carpentry CA), BCL-6 (clone P1F6) and c-myc (clone 9E11)(Novo Castra, Vector, Burlingame, CA). Seventeen cases (6 BL, 5 BLL and 6 LBCL) had adequate material to also assay for proliferation using antibody Ki-67 (Dako). Formalin or B5-fixed slides labelled using a Nexus automated immunostainer (Ventana, Tucson, AZ) with streptavidin-biotin-peroxidase technique following citrate buffer antigen retrieval. Scoring was by light microscopy with those showing > 20% positive cells considered positive.

Apoptotic cells were visualized using the ApopTag In Situ Apoptosis detection Kit (Oncor, Gaithersburg, MD) which utilizes the TUNEL method (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labeling) to identify DNA fragments of apoptotic cells. Seventeen cases (6 BL, 5 BLL and 6 LBCL) had adequate material for this assay.

The association between B-cell disease type (BL, BLL, or LBCL) and status (expressed or not expressed) for each of two proto-oncogenes BCL-6 or BCL-2 was predicted using the exact probability distribution of Fisher's exact test \([6]\) in StatXact. The utility of c-myc or Ki-67 was not investigated statistically due to high frequency in each group, nor was apoptosis, due to similarity of results across groups.

**Results**

Since January 1995 and through April 1998, there were 41 pediatric patients registered on POG protocols for small non-cleaved or large-cell lymphomas with adequate material for histology and immunohistochemistry. Thirteen cases were categorized as BL. Of these, 12 were males with a median age of 8.5 years (range 34 months to 17 years). Eleven had primary tumors in the abdomen and two in the head and neck. Median LDH was 1411 U/l. BCL-2 was positive in zero, BCL-6 in four and c-myc in nine. In five cases tested, the median apoptotic index was 17% (range 14%–21%) and each was diffusely positive for Ki-67.

Sixteen cases were categorized as BLL. Of these, 13 were males and 3 females with a median age of 8.5 years (range 35 months to 16 years). Eleven had abdominal primaries, three in the head and neck, and two in peripheral lymph nodes. Median LDH was 1024 U/l. BCL-2 was positive in 2, BCL-6 was positive in all 16, and c-myc in 13. In six cases tested, median apoptotic index was 13.5% (range 7%–19%) and each was diffusely positive for Ki-67.

Twelve cases were categorized as LBCL. Eight were males and five females, with a median age of 11.5 years (range 2–20 years). Six had abdominal primaries, three in the mediastinum, one in bone, one head and neck, and one listed as vascular primary. Median LDH was 680 U/l. Of these, 6 were positive for BCL-2, 9 for BCL-6 and 12 for c-myc. Of 7 tested, the median apoptotic index was 11% (range 6%–13%) and each was diffusely positive for Ki-67.

There was sufficient evidence to conclude that presence or absence of BCL-6 expression differs among the three types of lymphoma \((P = 0.0001)\) and that presence or absence of BCL-2 expression also differs among the three types \((P = 0.028)\). With no BCL-6 expression the probability that the patient had BLL is 0.0, that the patient had BL is 0.75 and that the patient had LBCL is 0.25. Given BCL-6 expression, the estimated probability that the patient had BLL is 0.552, that the patient had BL is 0.138 and that the patient had LBCL is 0.320. That is, based on our limited data, when BCL-6 = 1, the likelihood that the patient has BL is small and it is more likely that the patient has BLL. With positive BCL-6, the probability of a diagnosis of BLL is 0.5517, of BL is 0.1379, and that of LBCL is 0.3103. Thus, labeling for BCL-6 aids in the identification of BLL versus BL.

Similarly, estimates of the conditional probabilities of each of the B-cell lymphoma types given the degree of BCL-2 expression were computed. Given no BCL-2 expression \((BCL-2 = 0)\), the estimated probabilities are 0.394, 0.424, and 0.182 that the patient had BL, BLL or LBCL, respectively. Given BCL-2 = 1, the estimated probabilities are 0.0, 0.25 and 0.75 that the patient has BL, BLL, or LBCL, respectively. Estimates of the conditional probabilities of each of the disease types were computed for each of the four combinations of the presence or absence of each of BCL-2 and BCL-6.
Table 1. Conditional probabilities.

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<thead>
<tr>
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<th>BCL-6 = 1</th>
<th>BCL-6 = 0</th>
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<tr>
<td>BCL-2 = 1</td>
<td>P(BL) = 0.000</td>
<td>P(BL) = 0.000</td>
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<tr>
<td></td>
<td>P(BLL) = 0.286</td>
<td>P(BLL) = 0.000</td>
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<td></td>
<td>P(LBCL) = 0.714</td>
<td>P(LBCL) = 1.00</td>
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<tr>
<td>n = 7</td>
<td>n = 1</td>
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<tr>
<td>BCL-2 = 0</td>
<td>P(BL) = 0.182</td>
<td>P(BL) = 0.818</td>
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<tr>
<td></td>
<td>P(BLL) = 0.636</td>
<td>P(BLL) = 0.000</td>
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<tr>
<td></td>
<td>P(LBCL) = 0.182</td>
<td>P(LBCL) = 0.182</td>
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<td>n = 22</td>
<td>n = 11</td>
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Estimates of the conditional probabilities of each of the disease types were computed for each of the four combinations of BCL-2 and BCL-6. (In the cell of BCL-2 = 1/BCL-6 = 0, the number n = 1 is too small to be meaningful.)

(Table 1). Because the sample sizes are small, it is recommended that caution be used in predicting disease type base on presence of absence of BCL-2 and BCL-6. The data are presented to create a basis for future study.

Discussion

Burkitt lymphoma (BL) was first noted in African Children by Dennis Burkitt in 1958 [7]. The endemic form is now known to be associated with the Epstein–Barr virus (EBV) [8]. Similar tumors occur sporadically worldwide without EBV-association while others occur in association with EBV commonly in human immunodeficiency virus (HIV) infection or other immunosuppression. Each group (endemic, sporadic and immunodeficiency-associated BL) shows differing epidemiology and molecular features.

When the histopathology of BL was defined by the World Health Organization (WHO) [9], morphologic overlap was noted with other hematopoietic tumors. Rapapport noted overlap of 'undifferentiated' lymphoma (including BL) with other types [10]. Lukes and Collins placed BL among the follicular center-cell (FCC) lymphomas with the term 'diffuse small noncleaved cell lymphoma' [11]. The Working Formulation subsequently utilized the term 'small non-cleaved (SNC), non-Burkitt' to describe cases which have some features of BL but more pleomorphism [5]. Carl Lennert's Kiel Classification clearly distinguished BL from the germinal center-cell lymphomas with the term 'lymphoblastic, Burkitt type' [12].

The R.E.A.L. Classification, an effort to standardize nomenclature, utilized the term 'Burkitt-like lymphoma' (BLL) as a provisional borderline category between BL and large-cell types [1].

A draft version of a WHO-sponsored revision of the R.E.A.L. Classification has subsequently recommended that all such tumors, in adults, be called large B-cell lymphoma [13]. This is based in part on studies which indicate a higher frequency of BCL-2 translocations in these tumors than in BL [4], but the data is derived primarily from tumors in adults.

We compared cases each of BL, BLL and large B-cell lymphoma treated on POG protocols using a panel of monoclonal antibodies directed against c-myc, BCL-2, BCL-6, and Ki-67 and an assay for apoptosis (Apop-Tag). C-myc is a proliferation-associated proto-oncogene product which is usually associated with BL [14], in which the coding gene is involved in translocations with an immunoglobulin gene. BCL-2 is a proto-oncogene product involved in apoptotic pathways and is upregulated in many lymphomas including follicular lymphomas and 30% of large B-cell types, often due to cytogenetic translocations involving the BCL-2 gene on chromosome 14 [15].

BCL-6 is a Kruppel type zinc finger protein involved in germinal center differentiation and is upregulated frequently by mutations or translocations involving the BCL-6 gene on chromosome band 3q27, constituting the most common genetic abnormalities in large B-cell lymphomas [16, 17]. BCL-6 mutations have also been found in BL [18]. Ki-67 is a proliferation-associated protein and Apop-Tag is a DNA end-labelling technique to identify apoptosis.

Our results showed that BCL-6 expression similar to that in normal germinal center cells was identified in most BLL but not in BL. A portion of large B-cell lymphomas also expressed BCL-6. BCL-2 was most often found in LBCL and in a portion of BLL, but not BL. BCL-6 and BCL-2 may, therefore, be helpful in distinguishing these entities. Proliferation and apoptosis were similar for each group, with slightly higher apoptosis in BL.

Our data for BCL-6 are in contrast to a report of Spina et al. [19], who found a higher frequency of BCL-6 in both endemic and sporadic BL than in BLL, which only rarely showed BCL-6 expression. Additionally, their study found a majority of BLL to express BCL-2. Lai et al. have also described BCL-2 positivity in BLL in four of six patients tested from a primarily adult cancer center [20]. We assume that the differences between those results and ours relates to the patient populations studied.

Spina studied 7 children and adolescents and 3 young adults with endemic EBV+ BL; 1 child, 2 adolescents and 7 young adults with sporadic BL (3 EBV+); and 12 patients with BLL, all but 1 (19 years old) of whom were adults. Comparing the data of these two studies, it appears that in terms of BCL-2 and BCL-6 expression, childhood BLL resembles both childhood endemic BL and adult sporadic BL more than it resembles adult BLL, which frequently (according to Spina) shows BCL-2 without BCL-6 expression.

Our results suggest that there are biologic differences between BL and BLL in children and these groups of disease may be separated by augmentation of morphology with one or more immunohistochemical assays. Larger numbers of cases are required for analysis in order to draw survival conclusions due to the generally favorable outcome of these disorders. In our group, four of five patients who failed therapy were BCL-6 positive. We plan additional studies to see if these markers may...
identify risk groups and possible targets for genetic therapy.

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References


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