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B-Cell Lymphoma Unclassifiable with Features Intermediate between Diffuse Large B-Cell Lymphoma and Burkitt's Lymphoma: Comparison Study of Clinical Outcome and Treatment Response

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Abstract

The behavior of "B-Cell Lymphoma Unclassifiable (BCLU) with features intermediate between Diffuse Large B-cell Lymphoma (DLBL) and Burkitt Lymphoma (BL)" is just beginning to be examined. This is a ten-year retrospective examination of the clinical characteristics, survival, treatment response and presence of genetic alterations in MYC and BCL2 in BCLU (n=34) compared to conventional DLBL (n=97). Patients with BCLU had more frequent CNS involvement (p=0.01), and bulky disease (p=0.02) than those with DLBL. There was no significant difference in age, gender, International Prognostic Index, Ann Arbor stage, or bone marrow involvement. Median Overall Survival (OS) and progression-free survival (PFS) for BCLU was 330 and 221 days respectively, compared to 837 and 664 days for DLBL. The Hazard Ratio (HR) was 2.5 (95%CI 1.2-5.2, p=0.048) for OS and 2.0 (95%CI 1.0-3.9, p=0.048) for PFS. Four BCLU patients (12%) received BL chemotherapy regimes, while 24 (71%) received CHOP-based therapy. Disease progression while on treatment occurred in 9 (33%) of BCLU and 8 (10%) of DLBL (p=0.03). Nine of 24 (36%) BCLU tested had concurrent BCL2 and MYC genetic abnormalities, called Double Hits (DH). OS for DH was worse than non-DH, HR 13.8; (95%CI 2.3-83.6, p=0.004). Compared to DLBL patients, BCLU patients present with more advanced disease, progress while on treatment and have a poorer survival.

Keywords: Lymphoma; Neoplasia; Prognostication

Introduction

Diffuse Large B-cell Lymphoma (DLBL) and Burkitt Lymphoma (BL) are aggressive B-cell lymphomas that differ from each other with respect to histology, molecular alterations, prognosis and treatment. DLBL is composed of large lymphoid cells with abundant cytoplasm, large nuclei with open vesicular chromatin and has a moderate to high proliferation rate. In contrast, BL is composed of intermediate-sized cells with scant basophilic lipid containing cytoplasm, round nuclei with finely clumped chromatin, abundant mitosis and apoptosis with many tingible-body macrophages imparting a low-power "starry sky" appearance. The translocation t(14;18)(q32;q21), which juxtaposes the BCL2 gene to the Immunoglobulin Heavy Chain gene (IGH) enhancer is commonly found in DLBL but, by definition, is absent in BL, while translocations involving chromosome 8(q24) are the hallmark of BL, but are only found in a subset of DLBL [1-4]. Likewise, CHOP-based chemotherapy (cyclophospha-

mide, doxorubicin, vincristine, and prednisone) with rituximab is used to treat DLBL, resulting in a 45-60% 5-year overall survival, while BL responds best to intense chemotherapy regimens , resulting in 49% to >90% cure rates depending on risk category and the recent addition of rituximab [4-7].

It has long been recognized that certain lymphomas contain histologic and molecular features that overlap with both DLBL and BL [4, 8-10]. In the past, such lymphomas were assigned to the "best fitting" DLBL, BL or "Burkitt-Like" Lymphoma (BLL) category with no consensus as to the appropriate treatment. In 2008 the World Health Organization (WHO) dropped the term BLL and created a new category termed "B-cell lymphoma unclassifiable with features intermediate between BL and DLBL," hereafter referred to as BCLU [4]. These lymphomas are poorly characterized and not considered a specific diagnostic entity but instead encompass both BLL, as well as lymphomas with high grade morphologic features not fulfilling criteria for either BL or DLBL. They are a heterogeneous morphologically and immunohistochemically recognizable group with a spectrum of molecular abnormalities. From a molecular standpoint, these lymphomas may contain alterations in MYC, BCL2 and/or BCL6, and are more likely to harbor translocations involving two genes, so called "double-hit" lymphomas, as well as more complex karyotypes with multiple abnormalities [4, 11-13]. BCLU is believed to have a poor prognosis when treated with conventional therapies for DLBL [13-15]. Some early data is beginning to suggest that BCLU may respond better to intense chemotherapy, [16-18]. Overall, the clinical presentation, behavior and appropriate treatment for this new BCLU category are not well defined. In fact, the National Comprehensive Cancer Network Practice Guidelines for Non-Hodgkin Lymphoma recognizes the lack of evidence in this area, providing no specific suggestions as to the therapy of choice for patients with BCLU [7]. The aim of this study was to examine the clinical characteristics and survival outcome of patients with BCLU compared to those of DLBL. We also determined the frequency of MYC and BCL2 genetic alterations in BCLU and examined how these alterations related to patient survival. This study also examined protocols that have been used to treat BCLU patients and how the treatment response of these patients compared to that of DLBL patients.

Materials and Methods

Patient selection

Search of the institutional computerized pathology database identified patients diagnosed with BL and DLBL from 1998 to 2008. Due to the variable terminology used to describe and diagnose lymphomas with BCLU characteristics prior to the 2008 WHO classification, the terms Burkitt, Burkitt-like, atypical BL, DLBL with Burkitt features, DLBL with high-grade features or high proliferation index were used to identify potential patients with BCLU and all patients with BL. The same number of DLBL cases, matched for year of diagnosis, was randomly selected for controls. No exclusion criteria were used at the time of initial case selection from the database. For diagnostic inclusion criteria for the classification of patients as having BL, DLBL or BCLU please see Pathology Review below.

Clinical information

Gender, age at diagnosis, International Prognostic Index (IPI), Eastern Cooperative Oncology Group (EGOC) performance status, Ann Arbor stage, presence of B-symptoms, Bone Marrow (BM) and Central Nervous System (CNS) involvement, presence of extranodal and bulky disease, were obtained through review of patient electronic records, paper charts and contact with the physicians most responsible for the patients' care. Overall Survival (OS) was calculated from the date of biopsy of diagnostic material to the date of last follow-up or death from any cause. Progression-free Survival (PFS) was calculated from the date of biopsy of diagnostic material to the date of last follow-up, disease progression or death from any cause. The response to induction chemotherapy was classified as Complete Response (CR), Partial Response (PR), Stable Disease (SD) or Progressive Disease (PD) based on the criteria outlined by Cheson, et al. [19]. Response duration was calculated from the time when criteria for CR or PR were met to the first documentation of relapse or disease progression.

Chemotherapy protocols were recorded and classified into one of three categories. DLBL-like regimens included CHOP with or without rituximab, as well as second-line therapy for patients proceeding to stem cell transplant e.g., gemcitabine, dexamethasone, cisplatin (GDP) [20] or dexamethasone, cisplatin, cytarabine (DHAP) with or without rituximab [21,22]. Burkitt-like regimes included the French LMB protocol (low dose cyclophosphamide, vincristine, and prednisone followed by two induction cycles with high doses of methotrexate, cyclophosphamide, vincristine, doxorubicin, and prednisone), [23] original or modified CODOX-M (cyclophosphamide, doxorubicin, vincristine with intrathecal methotrexate and cytarabine followed by high-dose systemic methotrexate alternating with IVAC, ifosfamide, cytarabine, etoposide and intrathecal methotrexate if indicated), [24,25] or HyperCVAD (cyclophosphamide, vincristine, doxorubicin and dexamethasone), both with or without rituximab [26,27]. Palliative regimens included radiation and non-anthracycline-based chemotherapy. Patients who died before any therapy was given were classified as receiving no treatment. Clinical reviewers were blinded to the results of the pathologic review (described below). This study was approved by the Hamilton Health Sciences Research Ethics Board, Hamilton, Ontario, Canada (REB no. 09-132-T).

Pathologic review

Only cases in which complete morphologic and immunohistochemical information could be obtained were included in the study. To reach a consensus agreement, three reviewers, blinded to all clinical information and previous pathologic diagnosis, together examined morphology and immunohistochemistry to classify each case as BL, DLBL or BCLU. Standard diagnostic criteria outlined by the WHO were used to classify cases as BL or DLBL [4].

Cases were considered to be BCLU if their biopsy revealed lymphoma with a combination of morphologic and immunohistochemical features intermediate between DLBL and BL, according to the features outlined in the 2008 WHO clas-

Table 1: Immunohistochemical Panel

Antibody	Manufacturer	Dilution	Antigen Retrieval	Clone
CD20	Dakocytomaton	1/100	none	L26
CD10	Novacastra	1/25	Citrate, HIER	56C6
BCL2	Dakocytomaton	1/25	Citrate, HIER	124
BCL6	Biocare Medical	1/75	BORG, HIER	PG-B6p
Ki-67	Dakocytomaton	1/1000	Citrate, HIER	MIB-1
TdT	Novacastra	1/25	Dako lo, HIER	SEN28
CD34	Novacastra	1/50	none	QBend10
CD3	Cell Marque	1/500	Citrate, HIER	Polyclonal

HIER, Heat induced epitope retrieval; Citrate, 0.01M citrate buffer, pH 6.0; BORG and Dako lo, pre-treatment reagents supplied by the manufacturer

sification [4]. Some cases showed classic BL morphology with an immunophenotype inconsistent with BL, such as strong BCL2 immunostaining and/or Ki67 proliferation index of less than 90%. BCLU also included cases with cellular morphology intermediate between BL and DLBL with a monomorphic population of intermediate to large cells, many times with a component of "starry sky" appearance caused by tingible-body macrophages. Cells showed a more open chromatin pattern and more variation in nuclear size and shape than that seen in BL with more than occasional prominent single nucleoli. Mitoses were abundant and the proliferation index is characteristically very high (≥ 70%).

Immunohistochemistry

For cases in which the reviewed material showed that the immunohistochemical analysis was incomplete, immunohistochemistry was performed using the Labeled Streptavidin Biotin Technique (LSAB) on 3 μm thick deparaffinized sections of archived Formalin Fixed Paraffin Embedded Tissue (FFPET). Table 1 shows the list of antibodies and details of their protocols used in this study. For all cases the analysis included, but was not restricted to, the antibodies listed in Table 1 with standard positive and negative controls used for each case. Visualization of the antigen/antibody reaction was performed using either a polymer (Invitrogen, Carlsbad, CA) or labeled streptavidin biotin detection system (Invitrogen) with diaminobenzidine (Sigma) as the chromogen.

Molecular analysis

Cytogenetic abnormalities of MYC were detected using a Vysis LSI MYC dual color, break-apart rearrangement probe (Abbott Molecular, Abbott Park, Illinois, USA) by Fluorescence In Situ Hybridization (FISH) on FFPET, which identifies any MYC chromosomal rearrangement or polysomy. All BCL2-IGH t(14;18) testing done prior to this study as part of the original diagnostic workup was performed using multiplex polymerase chain reaction (PCR) with specific 5' primers for major breakpoint regions and minor cluster regions seen in t(14;18) [28]. For cases in which prior PCR testing was not performed, BCL2 t(14;18) break points were identified by FISH on FFPET using the LSI IGH/BCL2 dual color, dual fusion translocation probe (LSI IGH – SpG and LSI BCL2 – SpO; Abbott Molecular, Abbott Park, IL). Image capture for FISH was performed with a Zeiss microscope (Imager M1) equipped with the appropriate filters (DAPI, FITC and Spectrum Orange) and the Metasystem ISIS imaging software programs (MetaSystems, Altlussheim, Germany). A minimum of 200 interphase nuclei were examined and a cutoff of more than 5% positive cells for BCL2-IGH and 7% cells for MYC abnormalities was considered abnormal.

Statistical analysis

The sample size for this study was determined based on feasibility considerations since the study was primarily exploratory and the occurrence of BCLU is rare. The demographic and prognostic characteristics of the included patients were described by group using mean (standard deviation) for continuous variables and count (percent) for categorical variables. Chi-square and one-way analysis of variance were used respectively for categorical and continuous data to compare the baseline characteristics between groups. Univariate survival curves were plotted using the Kaplan-Meier estimates and compared using the log-rank test. The relationship between survival and group was estimated using multiple Cox regression analysis adjusting for IPI and treatment regimen. The parametric Weibull regression analysis was used to determine Hazard Ratios (HR) when the proportional hazards assumption for applying Cox regression was violated. The results are reported as estimate of HR, corresponding 95% Confidence Interval (CI) and associated p-value. All p-values are reported to three decimal places with those less than 0.001 reported as $p<0.001$. All tests were 2-sided with the criterion for statistical significance set at alpha = 0.05. We did not adjust the overall level of significance for multiple testing because the analyses were primarily exploratory. All statistical analyses were performed using either SAS 9.2 (Cary, NC).

Results

A total of 140 cases were identified as having all the clinical and pathological material required for the study. The pathologic breakdown of these cases revealed 34 BCLU, 9 BL, and 97 DLBL. Representative examples of the morphologic criteria and immunohistochemical profile of each group are shown in Figure 1. While the initial objective of this study was to compare the characteristics and outcomes of patients with BCLU to both BL and DLBL, a meaningful comparison with the BL group was not possible due to the very small number of patients with BL, and so we confined this study to patients with BCLU and DLBL.

Patient characteristics

The clinical characteristics and treatment regimens of the patients in each group are presented in Table 2. Patients with BCLU presented at a mean age of 63.0 years (16.0 standard deviation), similar to DLBL patients who presented at a mean age of 64.5 years (14.5 standard deviation) (p=0.605). BCLU patients presented more frequently with CNS involvement

Table 2: Demographic and clinical characteristics at diagnosis and treatment regimens.

	DLBL (n=97)		BCLU (n=34)		
Variable	**n**	**%**	**n**	**(%)**	**P-value**
Male Gender	45	(46)	18	(53)	0.511
B symptoms	37	(41)	12	(40)	0.949
Positive BM	21	(26)	5	(19)	0.453
CNS involved	4	(5)	6	(20)	0.009
Bulky disease	18	(20)	12	(40)	0.031
Extranodal disease	35	(39)	14	(47)	0.427
IPI Score					0.219
0 1 2 3 4 5	6 16 19 18 11 14	(7) (19) (23) (21) (13) (17)	3 5 8 6 7 0	(10) (17) (28) (21) (24) -	
EGOC					0.434
0 1 2 3 4	31 27 12 15 6	(34) (30) (13) (17) (7)	11 10 6 1 2	(37) (33) (20) (3) (7)	
Ann Arbour stage					0.33
1 2 3 4	18 18 14 41	(20) (20) (15) (45)	8 5 8 9	(27) (17) (27) (30)	
Treatment regimen					
BL-like	4	(4)	4	(12)	
DLBL-like	74	(76)	24	(71)	
Palliative	13	(13)	1	(3)	
No treatment	6	(6)	5	(15)	

DLBL, Diffuse large B-cell lymphoma; BCLU, B-cell lymphoma unclassifiable with features intermediate between DLBL and BL; BL, Burkitt lymphoma; IPI, International Prognostic Index; ECOG, Eastern Cooperative Oncology Group Performance Status; BL-like refers to French LMB, CODOX-M or Hyper-CVAD protocols with or without rituximab; DLBL-like refers to CHOP, GDP or DHAP protocols with or without rituximab.

and bulky disease. There was no difference between the groups with respect to gender distribution, IPI, ECOG, Ann Arbor Stage, presence of B-symptoms, extranodal disease, or BM involvement.

The majority of the BCLU group (71%) received DL-BL-like regimens, which is comparable to 76% of the DLBL group. Four patients (12%) with BCLU and 4 patients (4%) with DLBL received BL-like chemotherapy. Only 3% of the BCLU group and 13% of the DLBL group received palliative therapy while 15% of BCLU and 6% of DLBL patients received no treatment.

Survival and treatment response

Figure. 2 shows the survival curves for OS and PFS comparing BCLU and DLBL. Patients with BCLU had a poorer OS (median 330 days) compared to DLBL patients (median 837 days) (HR=2.46, 95% CI 1.16-5.19; p=0.048). PFS was also significantly decreased in patients with BCLU (median 221 days)

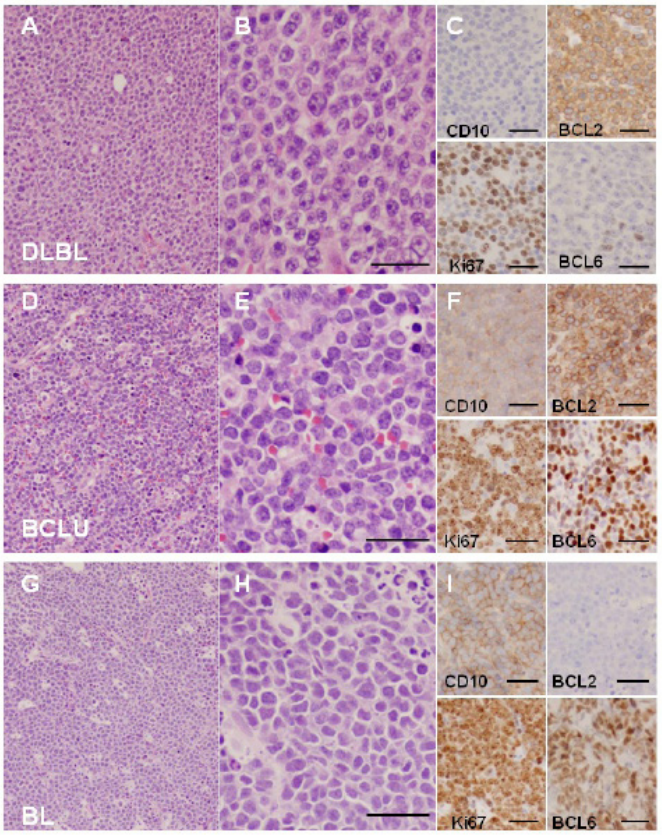

Figure 1: Morphologic and immunohistochemical features characteristic of DLBL, BCLU and BL. A-C: Low power (200x, A) and high power (400x, B) views of DLBL stained with H&E shows characteristic nuclear pleomorphism, prominent nucleoli and abundant cytoplasm. This example of DLBL shows strong immunohistochemical staining for BCL2 (Dakocytomaton) and weak staining for CD10 (Novacastra) and BCL6 (Biocare Medical) and has a Ki67 (Dakocytomaton) proliferation index of approximately 50-60% (C). D-F: Low (D) and high power (E) views of BCLU stained with H&E show intermediate sized cells with abundant tingible-body macrophages giving a low power "starry sky" appearance (D). The cells show some nuclear pleomorphism with only a portion of cells containing prominent nucleoli (E). This case of BCLU shows staining for BCL2, BCL6 and CD10, and a Ki67 proliferation index of approximately 80-90% (F). G-I: Low (G) and high power (H) view of BL stained with H&E shows the classic "starry sky" appearance of BL composed of intermediate sized cells with monotonous nuclei containing finely clumped chromatin and no prominent nucleoli. There is abundant apoptosis and necrosis (H). This case of BL shows strong staining for BCL6 and CD10, negative staining for BCL2 and has a Ki67 proliferation index approaching 100% (I). Low power views were taken at 200x magnification and high power views were taken at 400x magnification. Images were taken using an Olympus BX41 microscope with Olympus 20x/0.40 and 40x/0.65 lenses with an Olympus DP70 digital camera and recorded with scale bars embedded into representative images using Olympus DP Controller Version 3.1.1.267 imaging software. Images cropped from their original size have accompanying scale bars. All scale bars represent 50 μm. DLBL, Diffuse large B-cell lymphoma; BCLU, B-cell lymphoma unclassifiable with features intermediate between DLBL and BL; BL, Burkitt lymphoma; H&E, Hemetoxolin and Eosin.

compared to DLBL patients (median 664 days) (HR=1.96, 95% CI 1.01-3.93; p=0.048).

There was a significant difference in the proportion of patients classified as having CR, PR, SD and PD between BCLU and DLBL (p=0.026, Figure 3a). More DLBL patients obtained CR or PR while more BCLU patients showed SD or PD after induction chemotherapy. Of those BCLU and DLBL patients who obtained a CR or PR, there was no difference in response duration between the groups (Figure 3b) (HR=0.9, 95% CI 0.29-2.74; p=0.847).

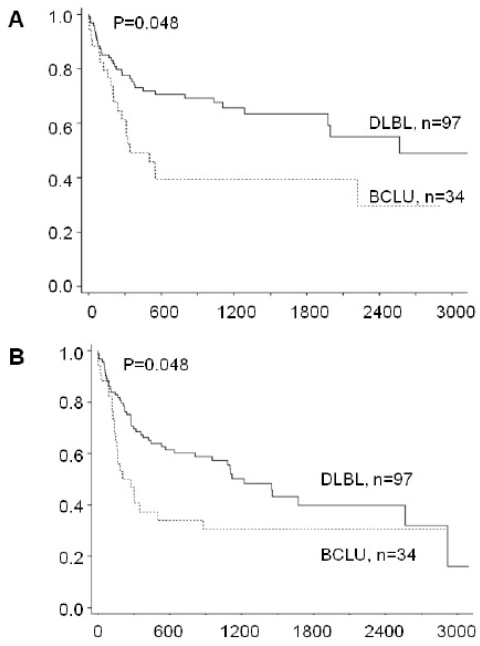

Figure 2

Figure 2: Overall and progression free survival curves of patents with BCLU and DLBL. (A) Overall survival of BCLU ( ) and DLBL ( ) patients. X-axis represents time (days); y-axis represents the cumulative survival. (B) Progression free survival of BCLU and DLBL patients. X-axis represents time (days); y-axis represents the cumulative survival. Analysis was performed using multiple Cox regression analysis adjusting for IPI and treatment regimen. Hazard Raito was 2.46 (95% CI 1.16-5.19) for overall survival (A) and 1.96 (95% CI 1.01-3.93) for progression free survival (B).

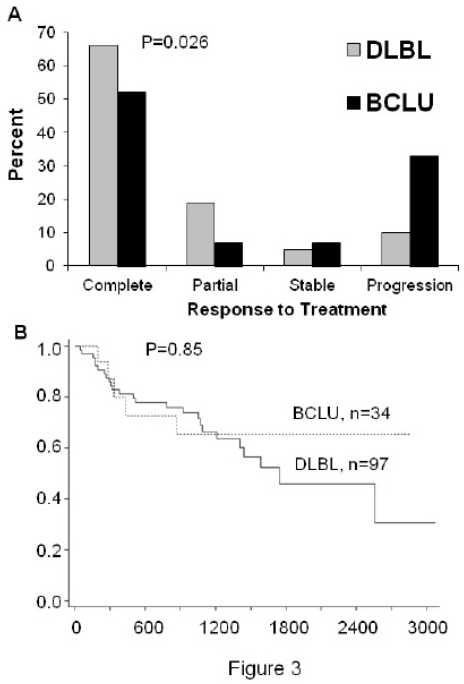

Figure 3

Figure 3: Response to treatment and response duration of treatment responders in BCLU and DLBL patients. (A) Histogram showing the percentage (y-axis) of BCLU and DLBL patients who were classified as showing a complete response (Complete), a partial response (Partial), no significant change in tumor burden (Stable) or progression of disease (Progression) after first-line chemotherapy. (B) Response duration, defined as the time when criteria for complete or partial response were met to the first documentation of relapse or disease progression, of BCLU ( ) and DLBL ( ) patients. X-axis represents time (days); y-axis represents the cumulative survival. Analysis was performed using multiple Cox regression analysis adjusting for IPI and treatment regimen. Hazard Raito was 0.9, 95% CI 0.29-2.74 (B).

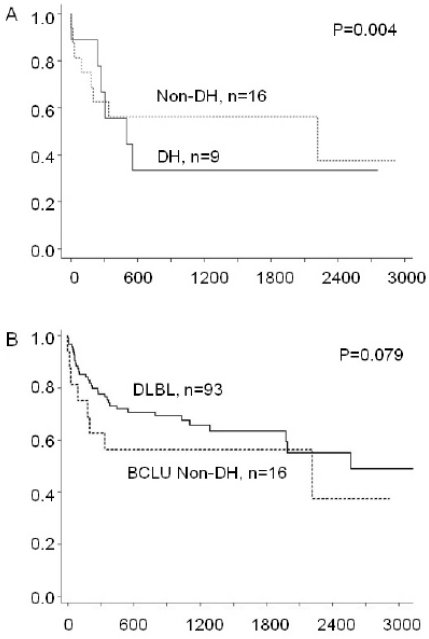

Figure 4

Figure 4: Overall survival curves of patents with BCLU according to MYC/BCL2 status and of BCLU patients without double-hit MYC/BCL2 compared to DLBL patients. (A) Overall survival of BCLU patients with double-hit MYC/BCL2 alterations (DH,) and without double-hit MYC/BCL2 (non-DH,). (B) Overall survival of BCLU without double-hit MYC/BCL2 alterations (BCLU non-DH,) compared to DLBL ( ) patients. X-axis represents time (days); y-axis represents the cumulative survival. Analysis was performed using multiple Cox regression analysis adjusting for IPI and treatment regimen. Hazard Raito was 13.8 (95% CI 2.3-83.6) for DH compared to non-DH (A) and 2.75 (95% CI 0.89-8.52) for BCLU non-DH compared to DLBL (B).

Molecular analysis

Molecular testing for rearrangements in both MYC and BCL2 genes was completed in 25 of the 34 BCLU cases. In the remaining 9 cases, molecular testing could not be performed either because there was insufficient material in the tissue block, or the block was not available. Nine cases (36%) contained genetic rearrangements involving MYC and BCL2-IGH, so called "Double-Hit" lymphomas (DH). Of the remaining 16 cases, 11 (44%) contained a MYC rearrangement only, 1 (4%) contained a BCL2-IGH rearrangement only and 4 (16%) showed no alterations in MYC and no BCL2-IGH rearrangement.

Figure 4a shows the survival curve for OS comparing those BCLU cases containing DH with those containing either a single or no rearrangements at these loci (non-DH). Patients with DH showed a significantly poorer OS compared to those with non-DH (HR=13.8, 95% CI 2.3-83.6; p=0.004). Due to the effect of DH on the survival of BCLU patients, a subset analysis was performed to compare OS of non-DH BCLU patients to DLBL patients (Figure 4b). The median OS for non-DH BCLU patients was 507 days compared to 836 days for DLBL patients (HR=2.75, 95% CI 0.89-8.52; p=0.079).

Discussion

The 2008 WHO category of BCLU contains a heterogeneous group of lymphomas that have morphologic, immunohistochemical and molecular features of both BL and DLBL. This is an expanded provisional category from the previous 2001

WHO category of BLL and few reports to date have specifically addressed the prognosis of BCLU. Earlier studies of BLL have shown it to have a poorer survival than DLBL and these patients may respond better to dose-intense chemotherapy than to CHOP-based therapy [8,16,19]. Studies examining the clinical characteristics and prognostic features of BLCU are just beginning to emerge. In this series, BCLU confers a worse OS and PFS than DLBL. BCLU patients presented at a similar age and with similar clinical characteristic to those of DLBL, except that they had more frequent CNS involvement and bulky disease. This is supported by Johnson, et al. [14] who found that within a population of lymphomas with concurrent MYC/BCL2 translocation, patients with BCLU phenotype had a worse OS compared to those with DLBL. Perry, et al. [13] described a case series of 39 BCLU patients which presented with advanced stage disease, high IPI scores and an OS and event free survival in the order of 4-9 months. This study is one of the first comparison studies looking at the clinical characteristics, survival and response to treatment of BCLU compared to DLBL. Our findings are in keeping with those of Burgesser, et al. [15] who compared 6 BCLU patients with 30 patients with DLBL and found BCLU patients present with more advanced stage disease, higher IPI and shorter OS compared to DLBL.

This is also one of the first comparison studies to examine the response to treatment of BCLU. In both BCLU and DLBL groups, greater than 70% of patients received DLBL-like therapy and approximately 18% received palliative therapy or no treatment. Overall, significantly fewer BCLU patients achieved a CR/PR after induction chemotherapy and more had SD/PD compared to DLBL patients. However, those BCLU patients who did show a response to treatment behaved similarly to DLBL patients with respect to response duration. The proportion of BLCU patients achieving CR/PR and SD/PD in this study is comparable to a similar sized retrospective case series of 39 BCLU patients reported by Perry, et al. [13]. In keeping with the results in this study, another comparison study showed BCLU had a higher proportion of incomplete response to treatment compared with DLBL [15].

In this study, there were an insufficient number of BL patients and too few BCLU patients who underwent BL-like therapy for a meaningful comparison of response to intensified treatment protocols. Given the poor prognosis for BCLU patients, more study is needed to examine other treatment options that may improve survival. Studies are beginning to examine the potential benefit of more intense BL-like therapies for BCLU. Lin, et al. [18] examined 52 patients with BCLU and found those with MYC rearrangement who were treated with R-CHOP had a poorer OS than those treated with Rituximab with hyper CVAD or CODOX-M regimes. However, BCLU patients without MYC rearrangements did not show a survival benefit when treated with Rituximab with hyper CVAD or CODOX-M. Corazzelli, et al. [17] have shown that adult BL and BCLU patients treated with BL-like intense chemotherapy regimens had similar survival outcomes and the addition of Rituximab and intrathecal liposomal cytarabine to CODOX-M/IVAC (ifosfamide, etoposide and high-dose cytarabine) improved event free survival in adult patients with BL and BCLU compared to CODOX-M/IVAC alone.

Lymphomas with DH genetic alterations have been consistently associated with a poor prognosis [14, 29-34]. DH lymphomas present with high stage disease, higher IPI scores, more frequent extranodal disease, bone marrow and CNS involvement and have a median overall survival in the range of months rather than years [14,29,31,32,34]. Our study supports these findings as BCLU with DH had a worse overall survival compared to BCLU patients without DH.

DH molecular alterations occur in lymphomas of varying histologic categories including follicular lymphoma, lymphoblastic lymphoma, DLBL and BCLU [14, 29-35]. In our study the frequency of DH in BCLU is 36%, which is in keeping with the range of 10-50% reported in the literature [8, 9, 15, 18, 34]. The reported prevalence of DH in other lymphomas is usually much lower than that of BCLU and generally ranged from 2%-10% [4,33,35,36]. For laboratories under cost constraints, identification of the BCLU morphologic and immunohistochemical features provides pathologists a category for which ordering genetic testing would give high yield results. It is our suggestion, along with others, that any lymphomas with features of BCLU should undergo genetic testing for MCY and BCL2 alterations [8,32,33].

Just as not all DH lymphomas show BCLU morphology, not all BCLU are DH lymphomas. Due to the fact that BCLU has a high frequency of DH, the question still remains as to whether the poor prognosis of BCLU compared to DLBL is simply attributable to the high percentage of DH in this diagnostic category, or whether BCLU histology itself is an independent prognostic factor. Our study suggests that the OS of patients with BCLU without DH may be worse than that of DLBL patients. However, this comparison lacks a sufficient number of patients to make any definitive conclusions. Johnson, et al. [14] examined the prognostic significance of BCLU histology within a population of DH lymphomas of varying histological categories and also found that BCLU conferred a significantly poorer OS compared to DLBL. In contrast, Li, et al. [34] did not find a correlation between morphology and prognosis within a cohort of DH lymphomas, although this study compared DLBL to a collapsed group of non-DLBL lymphoma (87% of which were BCLU). Taken together, these studies are beginning to suggest that the BCLU category of lymphomas confers a poor prognosis, perhaps independently of the DH genetic profile. Further comparison studies with adequate sample sizes that address the effects of both histologic classification and DH genetic aberrations are necessary, as these two factors may be independent of one another. This information is of great importance to clinicians responsible for the care and treatment of these patients.

Although this study demonstrates potentially important prognostic findings to clinicians, it is limited in that it is a retrospective study with small sample sizes. A prospective cohort study with control of confounding variables such as genetic alterations, treatment, IPI or age would more reliably address the questions examined in this study.

In summary, this is one of the first comparison studies to examine the clinical characteristics and prognostic significance of the 2008 WHO category of BCLU. BCLU patients

present with similar clinical characteristics, but show a worse overall and progression free survival than DLBL patients. They also more frequently have stable or progressive disease after CHOP-type chemotherapy, but those who have a complete or partial response to therapy show a similar prognosis to DLBL patients. The DH genetic profile occurs in approximately one third of BCLU patients in this series and is a poor prognostic factor. This study and others suggest BCLU histology may be an independent poor prognostic factor [14]. However, more study is necessary to examine the prognostic significance of the BCLU category, factoring in the impact of DH genetic alterations, and whether or not more aggressive chemotherapy regimens may provide a superior response in these patients.

Disclosure and Conflicts-of-Interest Disclosure

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