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Clinicopathological features of aggressive B-cell lymphomas including B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell and Burkitt lymphomas: a study of 44 patients from Argentina

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ABSTRACT

Aggressive B-cell lymphomas incorporate a wide spectrum of lymphomas that pose challenges in diagnosis as well as treatment. We evaluated the clinicopathological features of 44 patients with aggressive B-cell lymphomas which were classified into 3 groups based on the World Health Organization 2008 classification as follows: including 30 cases of diffuse large B-cell lymphoma (DLBCL), 8 cases of Burkitt lymphoma (BL) and 6 cases of B-cell lymphoma, unclassifiable, with features intermediate between Burkitt lymphoma and diffuse large B-cell lymphoma (BCLU). Male predominance was observed in BL and BCLU groups and the mean age varied from 29 years in BL, 61 years in DLBCL and 70 years in BCLU. Patients with BCLU presented at more advanced stages and had a higher international prognostic index. By immunohistochemistry, they shared characteristics of both BL (including more frequent expression of SOX11) and DLBCL. FISH analyses showed three cases with more than one rearrangement: one MYC/BCL2 and two BCL2/BCL6, in addition to which one case with BCL2/IGH translocation and another with MYC rearrangement were also detected. The mean follow-up survival time of BCLU was 6.6 months, which was significantly shorter in comparison to DLBCL (31 months) and BL (30 months), respectively. The importance of recognizing this BCLU group relies on its different clinical course, poor prognosis and shorter survival than DLBCL and BL. An accurate diagnosis is critical for risk stratification and to improve therapeutic approaches and outcomes.

1. Introduction

Aggressive B-cell lymphomas are a clinically and pathologically diverse group of hematopoietic neoplasm that derives from multiple different pathways of lymphoid transformation. The role of the pathologist in the diagnosis of this group is further complicated as the World Health Organization (WHO) 2008 classification incorporates more than 20 clinically and biologically recognized subgroups. The distinction of diffuse large B-cell lymphoma (DLBCL) from Burkitt lymphoma (BL) can be challenging for both pathologists as well as treating physicians. In the past, categories such as "atypical Burkitt" and "Burkitt-like lymphoma", among others, were used to describe lymphomas with characteristics resembling BL but with some unusual morphologic and/or immunohistologic features. In an attempt to refine diagnostic categories, the WHO 2008 classification proposed a new provisional entity, namely B-cell lymphoma, unclassifiable with features intermediate between DLBCL and BL (BCLU) [1]. As the designation implies, most of the cases of BCLU show morphological, immunohistochemical and molecular features that overlap between DLBCL and BL. BCLU is usually diagnosed at advanced stage with extranodal involvement and shows poor response to standard chemotherapy and a shorter survival time [2]. Diagnostic accuracy is therefore essential to risk stratify patients effectively.

To date, the majority of chromosomal abnormalities described in B-cell malignancies are reciprocal translocation involving the immunoglobulin genes (*IG*). *BCL2* gene at 18q21.3 and *MYC* gene at 8q24 are important examples of translocation partners of *IG* genes [3]. A specific translocation, however, does not necessarily correspond to a specific clinicopathological entity. In Burkitt lymphoma (BL) for instance, the typical but not exclusive abnormalities are those involving *MYC* and *IGH* or *IGk*/*IGλ*. There are rare B-cell lymphomas with concurrent *BCL2*/*IGH* and *MYC* rearrangements, also known as "double-hit" lymphomas [4], or preferentially named by the genetic alteration such as *MYC*/*BCL2 or*

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MYC/BCL6 lymphomas [5]. There are less than 300 cases of "double hit" lymphomas published, and many of these cases belong to a new provisional entity, the BCLU group.

In the current study, we evaluated 44 cases of aggressive mature B-cell lymphoma of large/intermediate-size cells from a single institution, including 30 cases of DLBCL, 8 cases of BL and 6 cases of BCLU, diagnosed by morphological, immunohistochemical and molecular/genetic according to the criteria proposed by the WHO (2008). Clinical, pathological and outcome data were compared among the three groups. Genetic abnormalities were studied by FISH in all cases, and include *MYC, BCL2* and *BCL6* gene rearrangements, to identify possible *MYC/BCL2* and/or *MYC/BCL6* lymphoma cases.

2. Materials and methods

2.1. Case selection, clinical data and demographic information

A total of 44 cases, including 30 cases of DLBCL, eight cases of BL and six cases of BCLU from children and adult patients, were obtained retrospectively from the files of the Pathology Department at Hospital Privado Centro Médico de Córdoba S. A., Córdoba, Argentina. The cases were received between January 2005 and June 2010. All cases had available hematoxylin-eosin sections for morphological review and included whole-tissue sections of the lymphomas. The WHO 2008 classification of hematopoietic neoplasms was applied in all cases for classification. Five of our BCLU cases were diagnosed previously as DLBCL, and one as BL. Medical records were reviewed to determine age at diagnosis, gender, anatomic location, international prognostic index (IPI) score, staging, treatment regimens, clinical outcome and overall survival (OS). Paraffin blocks were available from all 44 patients, and representative areas were selected for tissue microarray construction. Treatment regimens included chemotherapy with variable protocols including R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone), R-COP (rituximab, cyclophosmamide, vincristine and prednisone) and DA-EPOCH-R (dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab) for DLBCL. Patients with the diagnosis of BL were treated according to G.A.T.L.A group (Grupo Argentino de Tratamiento de la Leucemia Aguda). Clinical response information and complete follow-up were available for all patients.

2.2. Tissue microarray construction

Paraffin blocks of 44 cases were used for tissue microarray construction with the aid of a tissue arrayer (Beecher Instruments, Sun Prairie, WI). Three tumor cores of 0.6 mm taken from the original paraffin blocks represented each case. Serial sections of 3 μm were cut from the tissue array blocks and used for immunohistochemical analyses.

2.3. Immunohistochemistry

Immunohistochemical studies were performed on the tissue microarray using the Novolink polymer® (Novocastra, Newcastle Upon Tyne, UK) as the detection system, and an epitope-retrieval method was applied as needed for each specific antibody; diaminobenzidine was the chromogen. Primary antibodies directed against the following antigens were used: CD3, CD20, BCL2, BCL6, CD10, MUM1, Ki-67, SOX11, and FOXP1. Technical specifications of immunohistochemistry staining are shown in Table 1. Appropriate positive and negative controls were included for each antibody. MUM1, CD10, FOXP-1, BCL-6, and BCL-2 were considered positive above a 30% cut off. SOX11 was considered positive if more than 70% of neoplastic nuclei were stained. At least 10 high-power fields consisting of lymphoma were evaluated. The Ki-67 proliferative index was evaluated using the monoclonal MIB1 antibody and assigned a percentage value that was calculated by scoring 500 tumor cell nuclei.

Table 1
Primary antibodies and conditions used for immunohistologic studies

Marker	Clone	Dilution	Epitope retrieval	Source
CD20	L26	1:1,200	MWCB	Dako
CD3	SP7	1:200	SCB	Lab Vision
BCL-6	PG-B6P	1:100	T+ STRIS	Dako
Ki-67	MIB-1	1:4,800	PCCB	Dako
BCL-2	124	1:400	MWCB	Dako
MUM-1	MUM1P	1:1.200	SCB	Dako
CD10	270	1:200	SCB	Novocastra
FOXP1	EPR4113	1:400	SCB	Biogenex
SOX11	1159	1:400	SCB	Sigma

DAKO, Carpinteria, CA; Lab Vision Corporation, Fremont, CA; Novocastra; Biogenex, Fremont, CA; Sigma, St Louis, MI; MW: microwave oven; PC: pressure cooker; S: steamer; T: trypsin and CB: citrate buffer.

2.4. Fluorescence in situ hybridization (FISH)

FISH analysis was performed using a 3-μm-thick tissue section of the block and probes specific for *MYC, BCL2*, and *BCL6* rearrangements, as previously described [6,7] (Chuang, 2007 #82). For the detection of breakpoints (splits) in the *MYC* and *BCL6* loci, specific LSI Dual Color Break-apart Rearrangement probes (Vysis, Abbott, IL, USA) were applied. The t(14;18)(q32;q21) translocation was analyzed using a commercial Dual ISI *IGH/BCL2* probe (Vysis, Downers Grove, IL, USA). The slides were evaluated using spectrum orange and spectrum green filters (Chroma Technology GmbH, Fuerstenfeldbruck, Germany) on a Zeiss Axio Imager M1 fluorescence microscope (Carl Zeiss AG, Jena, Germany) using the assistance of Isis FISH Imaging Software (Metasystems, Altlussheim, Germany). A positive case was defined when the mean number of positive tiles detected was three standard deviations above the mean of a negative control (reactive lymphoid tissue). The threshold established was 2.19% for *MYC*, 15.9% for *BCL2* and 2.2% for *BCL6* (the mean of the negative control group was 0.73%, 7.8%, and 0.75, respectively.

2.5. Statistical analyses

All statistical analyses were performed using the software IBM SPSS Statistics version 19. Overall survival was calculated from date of diagnosis to date of death or last follow-up. Distribution of OS was

Table 2
Clinical characteristics of patients with DLBCL, BL, and BCLU

	DLBCL	BL	BCLU
Gender	14M/16F	7M/1F	5M/1F
Mean age (y)	59	29	70
Site at diagnosis	22 nodal – 8 extranodal*	3 nodal – 5 extranodal**	4 nodal – 2 extranodal***
Stage I	6	-	-
Stage II	10	3	-
Stage III	10	4	5
Stage IV	4	1	1
Low-IPI	9	4	-
Low-int-IPI	6	1	-
High-int-IPI	7	2	1
High-IPI	8	1	5

M, male; F, female. *Parotid gland (3), bone (2), uterine cervix (1), spleen (1) and stomach (1) **tonsil (2), gingiva (1), soft tissue (1) and thyroid (1) ***thyroid (1), small bowel (1).