Immunohistochemical and Genetic Evaluation of Double-Hit Lymphomas

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Abstract

Background
Diffuse large B-cell lymphoma and related entities are the most common type of non-Hodgkin lymphomas. According to the current WHO classification, double-hit lymphomas (DHL) comprise of a subset of aggressive B-cell lymphomas containing a *MYC* rearrangement in combination with either *BCL2* and/or *BCL6* (DHL-MYC) or a combination of *BCL2* and *BCL6* rearrangements (DHL-other). In this series, we attempt to further characterize DHL.

Design
760 cases were evaluated using an extensive panel of immunohistochemical stains (including CD20, CD3, CD5, CD10, Cyclin D1, BCL2, BCL6, EBER, Ki-67, and CD30) and a panel of FISH studies (including *MYC*, *IgH/BCL2*, and *BCL6*). Some cases were further evaluated with immunohistochemical stains MUM1, GCET1, LMO2, FOXP1, and CMYC. A total of 54 DHL [including 5 triple hit lymphomas (THL)] were identified, and their characteristics were compared to the larger diffuse B-cell lymphoma (DLBCL) group.

Results
Out of a total of 54 DHL cases, 30 were male and 24 were female and age ranged from 22 to 91 years old with an average age of 70 years. 35 cases were extranodal and 19 were nodal. Of all cases, 39/54 were DHL-MYC or THL, and 15/54 were DHL-other. Expression of CD10, BCL2, and MUM1 were comparable in DHL and DLBCL groups. All types of DHL had more frequent expression of GCET1, LMO2 and FOXP1 compared to DLBCL. DHL-other had a higher average age compared to DHL of all type (75.7 years compared to 69.5 years), and less frequent expression of CD10 (46% versus 73%). Nearly half of all DHL (47%) had >90% expression of CMYC. THL showed comparable findings to DHL-MYC and DHL of all types.

Conclusion
Our study demonstrates the immunophenotype heterogeneity in DHL cases and highlights similarities and differences from DLBCL cases. DHL associated with *MYC* gene abnormalities had similar immunophenotypic findings to those which had *IgH/BCL2* and *BCL6* translocations.

Background

Diffuse large B cell lymphoma (DLBCL) is the most commonly diagnosed subtype of lymphoma worldwide. The current World Health Organization (WHO) classification includes several subtypes which are based on a combination of clinical, immunohistochemical, and genetic differences. One aggressive variant of B-cell lymphomas include double hit lymphomas (DHL). The current WHO classification describes DHL as a subset of aggressive B-cell lymphomas containing a *MYC* rearrangement in combination with either *BCL2* and/or *BCL6* (DHL-MYC) or a combination of *BCL2* and *BCL6* rearrangements.

The presence of a variety of immunohistochemical, genetic and clinical features have an impact on prognosis of these patients. The goal of this study is to further characterize distinctive immunophenotypic and genetic findings of this subgroup.

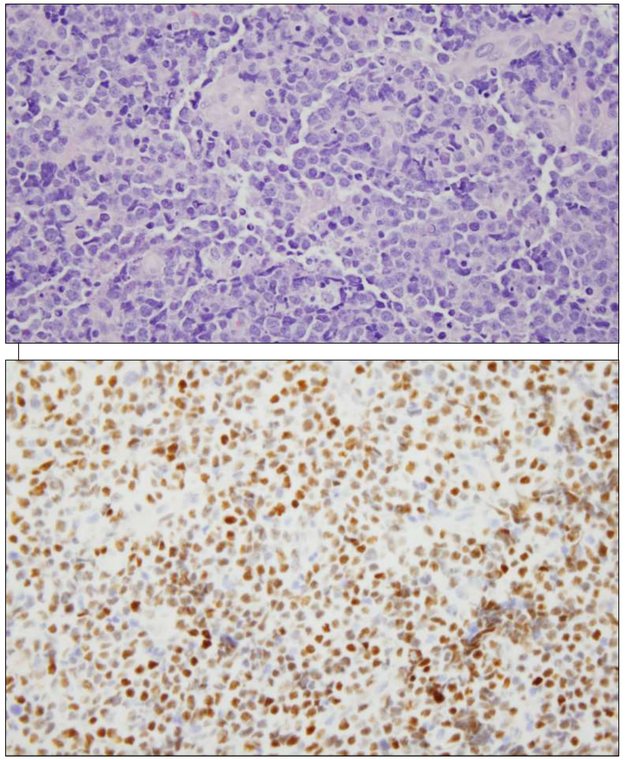
Materials and Methods

All cases were reviewed and diagnosed by DPO. The diagnoses were made in accordance with the 2008 WHO classification for hematopoietic and lymphoid tumors using a combination of immunohistochemical, genetic and other studies, as appropriate, to establish the diagnosis. The tissues were evaluated using both standard hematoxylin and eosin (H&E) staining and immunohistochemistry. Immunohistochemical stains were performed on a variety of platforms from Ventana (Tucson, AZ), Leica BioSystems (Buffalo Grove, IL), and Dako (Carpenteria, CA) using standard methodologies.

Most cases were evaluated using an extensive panel of immunohistochemical stains including CD20, CD3, CD5, CD10, cyclin D1, BCL2, BCL6, Ki67, and CD30. A subset of cases was further evaluated with immunohistochemical stains MUM1, GCET1, LMO2, FOXP1, and MYC. Fluorescence in situ hybridization (FISH) studies were performed in a subset of cases using standard methods (Abbott Molecular; Des Plaines, IL). This included lymphoma-associated translocations of MYC, IGH/MYC, IGH/BCL2 and BCL6 in most circumstances.

Indications for FISH testing were initially based on: aggressive appearing lymphomas including those with a Ki67 proliferation index of 90% or greater, the appearance of a Burkitt lymphoma-like morphology, or other clinical or histologic indicators of aggressive behavior. Approximately midway through the study, FISH testing was predicated on the presence of MYC immunohistochemical expression of >50%, except in cases with other indications of high-grade features.

TYPE	#	Mean age range	M:F	N:E	CD20	CD10	BCL6	MUM1	BCL2	Ki67 (avg)	GCET1	FOXP1	LMO2	CD30
DHL (+THL)	39	67 (44-91)	24:15 (1.6:1)	14:29 (1:1.8)	100% 39/39	83% 29/35	92% 36/39	42% 14/33	86% 31/36	82.6% 30-100	33% 6/18	100% 18/18	33% 6/18	5% 1/19
THL	5	64 (22-83)	4:1	1:4	100% 5/5	100% 4/4	100% 5/5	25% 1/4	100% 5/5	77.5% 70-100	67% 2/3	100% 3/3	67% 2/3	0% 0/3
BCL2/ BCL6	15	75 59-90	6:9 (1:1.5)	5:10 (1:2)	100% 15/15	46% 6/13	87% 13/15	57% 8/14	93% 14/15	66.7% 30-100	100% 3/3	100% 3/3	100% 3/3	0% 0/3



Upper: H&E double hit lymphoma
Figure 2: MYC IHC of double hit lymphoma

Results

We considered cases of DHL to have two or more identified driver mutations of lymphomas associated genes (IGH/BCL2, BCL6 or MYC). We subdivided these cases into DHL with MYC translocations (DHL-MYC), with a subset of these cases with three mutations, or THL (e.g. MYC, and IGH/BCL2 and BCL6). We identified 39 cases of DHL; 24 were male and 15 were female and ages ranged from 44 to 91 years old with an average age of 67 years. 25 cases were extranodal and 14 were nodal. Expression of CD10, BCL2, and MUM1 were comparable in DHL and DLBCL groups. Compared to DLBCL, DHL had more frequent expression of CD10 (83% vs. 40%), BCL6 (92% vs. 80%) and less frequent expression of MUM1 (42% vs. 59%) and LMO2 (33% vs. 62%). Half of all DHL (50%) had >90% expression of MYC. THL (5/54) showed comparable immunohistochemical findings to DHL. In DHL, 86% (31/36) were of GCB origin by Hans' method and 14% (5/36) were of NGC origin. Using the tally method, 38% (5/13) were of GCB origin and 62% (8/13) were of NGC center origin.

We identified 15 cases of BCL2/BCL6 lymphomas. These are included in the WHO 2008 BCLU category, but most authorities agree that these are not equivalent to DHL. Of these cases, there were 6 males and 9 females, with an average age of 75.7 years (range 59-90). There were 10 extranodal cases and 5 cases were nodal. In general, these cases did not differ significantly immunophenotypically from the larger group of DLBCL. In these lymphomas, 54% (7/13) were of GCB origin by Hans' method and 46% (6/13) were of NGC origin. Using the tally method, 67% (2/3) were of GCB origin and 33% (1/3) were of NGC center origin. In one discordant case, a Hans' method GCB case was switched to NGC by the tally method.

Discussion

- There is immunophenotypic heterogeneity in DHL cases
- DHL associated *MYC* gene abnormalities had similar immunophenotypic findings to those with *IgH/BCL2* and *BCL6* translocations
- Compared to DLBCL, DHL had more frequent expression of CD10, BCL6, and less frequent expression of MUM1 and LMO2