

# The Pattern of CD15, CD30 and Bcl-2 Expression in Diffuse Large B-Cell Lymphoma

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## Summary

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous entity. The pattern of CD15, CD30 and Bcl-2 expression is not well documented, especially in local population. We investigated 67 consecutive cases of DLBCL by immunohistochemistry on paraffin-embedded tissue. The male to female ratio was 1.2:1 with median age of 55 years, and more common nodal than extranodal in presentation. Only 3 of 67 cases expressed CD15. In addition, three cases showed weak membrane staining for CD30. Only one of these three cases was noted to have co-expression of CD15 and with occasional tumour cells showing weak CD30 expression. Bcl-2 protein was expressed in 43 of 67 (64%), more frequently in nodal than in extranodal tumours. In conclusion, CD15 and CD30 expressions are infrequent in DLBCL, and co-expression is rare. Bcl-2 protein expression is common in DLBCL.

**Key Words:** Diffuse large B-cell lymphoma, CD15, CD30, Bcl-2

## Introduction

Diffuse Large B-cell Lymphoma (DLBCL) is the most common type of adult non-Hodgkin's lymphoma (NHL). It accounts for 30-40% in the Western series<sup>1</sup>. In Malaysia, they were reported to constitute an even higher proportion, of approximately 60%<sup>2,3</sup>. This group of tumours is characterized by marked biological heterogeneity and a highly variable clinical course. It encompasses heterogeneous morphological, immunophenotypic, cytogenetic and molecular genetic features. The morphological variants of DLBCL include centroblastic, immunoblastic, T cell/histiocyte rich and anaplastic subtypes. However, there is no consensus on the usefulness of morphological subtyping of DLBCL<sup>4,5</sup>. These B cell malignancies can arise de novo, from normal lymphocytes at different stages of B cell differentiation, the germinal centre or post germinal center B cells, or they can occur following transformation from the less aggressive lymphomas such as follicular lymphoma or small B cell lymphocytic lymphoma.

Immunophenotypic characterizations of DLBCL has been attempted by many authors but have not helped to delineate distinctive morphologic subtypes. CD30 (Ki-1), an antigen that is expressed in lymphatic cells after activation, is expressed in a small proportion of large activated cells in normal lymphoid tissue, preferentially localized around B cell follicles and germinal center. However, it is also strongly expressed in the Hodgkin/Reed-Sternberg (H/R-S) cell, anaplastic large cell lymphoma and in some cases of DLBCL<sup>6</sup>. CD15, a differentiation antigen on human myelomonocytic cells, is present in more than 95% of mature peripheral blood eosinophils and neutrophils and low density on circulating monocytes. In normal lymphoid tissues, CD15 is expressed in granulocytes and macrophages. Both CD15 & CD30 were reported to be highly expressed in H/R-S cell. The latter is now known to be of B-cell origin, and therefore it can also express B-cell associated antigens, mimicking DLBCL. Hence, positive expressions of CD15 and/or CD30 in DLBCL may make definitive diagnosis difficult in some

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biopsy material. The frequency and expression pattern of CD15 and CD30 in B-NHL is not yet documented in local cases. Thus, it warrants studies to assess the probable extent of difficulties that may be encountered in differentiating DLBCL from Hodgkin's lymphomas, since incorporation of a panel of immunohistochemical stains has become a routine practice in reporting cases of malignant lymphomas.

Bcl-2 protein functions as an anti-apoptotic protein protecting cells from program cell death. It is widely expressed in normal lymphoid tissue, but is absent in germinal centre B cells. Bcl-2 protein expression is found in follicular lymphoma and some cases of DLCL. There had been reports implicating Bcl-2 protein expression in DLBCL as a marker of poor prognosis<sup>7</sup>.

This study aims to review consecutive cases of DLBCL, the most common subtype of NHL in Malaysia, to evaluate the usefulness of CD15 and CD30 expressions in differentiation of NHL and Hodgkin's Lymphoma, and to determine the frequency of Bcl-2 protein expression in local cases of DLBCL.

## Materials and Methods

### Case Selection

A total of 67 consecutive DLBCL cases and five reactive lymphoid tissues were obtained from the archives of the Department of Pathology, University Malaya Medical Centre for this study. The tissues were formalin fixed and embedded with paraffin wax. Hematoxylin-eosin stained sections were used for histomorphological study. Reconfirmation of DLBCL was based on the criteria recommended in the WHO classification of the lymphoid malignancies<sup>1</sup>. The patients' demographic data, site of presentation and biopsy was retrieved from the information volunteered by the referring clinician.

### Immunohistochemistry

Lineage was determined in each case by review of immunohistochemically stained slides with antibodies to CD20 (L26, DakoCytomation, Denmark), CD79a (JCB 117, DakoCytomation) and CD3 (polyclonal, DakoCytomation). Three Tissue sections of three micrometer thickness were cut and mounted on poly-L-lysine coated slides. Tissue sections were pre-treated with microwave heat-induced antigen retrieval in 0.01M citrate buffer, pH6.0 at 99°C for 20 min. Following

which, the buffer was flushed off under running water. Monoclonal antibodies against CD15 (Leu-M1, Becton Dickinson, USA), CD30 (BerH2, DakoCytomation) and Bcl-2(124, DakoCytomation), known to be reactive in paraffin embedded tissue were applied. An Avidin-Biotin Complex (ABC) labeled with horseradish peroxidase detection system was used. Diaminobenzidine (DAB) as chromogenic substrate was used to form a brown reaction product. The sections were then counterstained with Harris haematoxylin.

A semi-quantitative evaluation of expression was performed. Tumour was considered to be Bcl-2 positive if more than 75% of the tumour cells stained positively for Bcl-2 protein. The cut-off point was established from background staining of hyperplastic lymphoid tissues to determine over-expression due to the tumour.

### EBER in situ hybridization

Three CD30-positive cases were further tested for the presence of Epstein-Barr virus (EBV) by in situ hybridization for EBV early RNAs (EBER) as described previously<sup>8</sup>. Briefly, EBV oligo probe labeled with fluoroisothiocyanate, FITC (NCL-EBV, Novocastra, United Kingdom) was employed and detected by alkaline phosphatase-conjugated rabbit anti-FITC antibodies (DakoCytomation). Color development was achieved by using the substrate, 4-nitro-blue-tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP, Boehringer Mannheim, Germany). A case of EBV-infected nasopharyngeal carcinoma was used as external positive control for the staining technique.

## Results

### Clinical characteristics

The gender ratio was close to unity, and the patients' age ranged from 12 to 86 years, with a median of 55 years. The disease presented more commonly in the nodal tissue (43, 64.2%), with nodal-extranodal ratio of 1: 0.6 (Table I).

### Immunohistomorphological pattern of DLBCL

All the cases fulfilled the morphological criteria for DLBCL, with diffuse infiltrate and collections of large tumour cells replacing normal tissue. These tumour cells strongly expressed CD20 and CD79a, confirming their B-cell lineage. There were varying numbers of small reactive T-cells (CD3+) in the tumour tissue.

**CD15 and CD30 expression**

The results of CD15 and CD30 expression in DLBCL are summarized in Table II. CD15 expression in the granulocytic cells was observed in all cases (Figure 1a). One of the 67 cases showed positive expression of CD15 in almost all the tumour cells (Figure 1b), and in addition, two cases showed heterogeneous staining of some scattered tumour cells (Figure 1c). Co-expression of weak CD30 in an occasional tumour cell was observed in one of these two cases, in which these cells were larger than the surrounding tumour cells, with Reed-Sternberg like morphology. In addition, two other cases showed weak CD30+ membrane staining (Figure 1d). In the remaining 64 cases evaluated for CD30 expression, all tumour cells were completely devoid of CD30 expression.

In the reactive lymphoid tissues, very strong CD15 membrane immunoreactivity was observed in granulocytic cells, and CD30 was expressed by scattered activated large lymphoid cells.

**Bcl-2 expression**

Bcl-2 protein expression was present in 43 of 67 (64%) DLBCL. There was a difference in the frequency of expression between nodal cases (31 of 43, 72%) and extranodal cases (12 of 24, 50%). Cytoplasmic staining pattern was observed in the positively stained tumour cells (Figure 1e). In addition, three cases showed only some scattered Bcl-2 positive tumour cells (Figure 1f). The results of Bcl-2 expression in DLBCL are summarized in Table III.

In the reactive lymphoid tissues, Bcl-2 was strongly expressed by most small cells, especially in the mantle zone but not germinal centre B cells.

**EBER in situ hybridisation**

All the three cases with CD30 expression showed negative result by EBER ISH, suggesting the absence of EBV infection. Presence of EBV was detected by EBER ISH with strong nuclear staining in the positive control case.

**Table I: Clinical presentation of Diffuse Large B-cell Lymphoma in 67 patients**

		No. (%)
Gender	Male	36 (53.7)
	Female	31 (46.3)
Race	Malay	26 (38.8)
	Chinese	33 (49.2)
	Indian	7 (10.5)
	Others	1 (1.5)
Site of presentation	Nodal	43 (64.2)
	Extranodal	24 (35.8)

**Table II: Immunoreactivity for CD15 and CD30 in DLBCL**

Antibody	Number of cases	Tumour cells (No. of cases)		
		+	+/-	-
CD15	67	1	2	64
CD30	67	2	1*	64

\* Co-expression of CD15 and CD 30 is observed in this case

**Table III: Bcl-2 protein expression in DLBCL**

Site	Bcl-2 expression No. (%)		
	+	+/-	-
Nodal	31 (72.1)	2 (4.6)	10 (23.3)
Extranodal	12 (50.0)	1 (4.2)	11 (45.8)
<b>Total</b>	<b>43 (64.2)</b>	<b>3 (4.5)</b>	<b>21 (31.3)</b>

## Discussion

The heterogeneity of DLBCL has aroused a great deal of interest among the researchers in the past decades, partly due to differences in treatment outcome of patients. Its higher prevalence has facilitated the ease of case collection for the study of this group of disease. In our series, DLBCL appears to have a preponderance of male than female (male to female ratio, 1.2:1) with median age of 55 years. This is similar to the findings reported in the Malaysia National Cancer Registry for NHL<sup>9</sup>, reflecting the sample population is probably representative of Malaysia population. Male preponderance for NHL has been documented as well, in Asia and Western country such as in Thailand, USA and Germany<sup>10, 11, 12</sup>.

CD15 was consistently absent in the tumour cells of DLBCL in this series except for three cases. This observation is in agreement with other reports, which also showed low frequency of CD15 expression in other NHL<sup>13</sup>. In the cases that expressed CD15, the positive stain was observed to be localised to cell membrane and it was granular. Only in one of three cases, co-expression of weak CD30 in an occasional tumour cells was observed. In this particular case, almost all the tumour cells expressed strong B cell markers (CD20 and CD79a) and the histomorphological pattern was consistent with DLBCL, although the CD15+CD30+ cells appear larger with H/R-S appearance. In the other two cases of CD15+, the tumour cells were CD20+CD3-CD30- and they do not appear like H/R-S cells. The reason for the expression of CD15 in the tumour cells of these three cases is not known. Beside H/R-S cell, the presence of Leu-M1 (CD15) antigen has been demonstrated in peripheral T-cell lymphomas and in a variety of nonhaemopoietic neoplasms such as adenocarcinomas<sup>14</sup>. CD15, a cell membrane carbohydrate antigen, is known to function as a ligand to selectins and to promote cell adhesion<sup>15</sup>. A small number of reports have shown a potential link between these molecules and intracellular signal transduction pathways, suggesting CD15 may mediate cell activation in neutrophils and macrophages<sup>16, 17</sup>. Hence, the expression of this cell membrane carbohydrate antigen may play a role in inflammatory response. There are some reports suggesting the expression of CD15 in the malignant HR-S cells is of favourable prognostic significance<sup>18, 19</sup>. In addition, an adverse outcome in treated HL patients has been shown to be associated with the expression of the sialyl-CD15 (sCD15) form<sup>18</sup>. Kadota A. et al has also

shown that the expression of sCD15 in non-small cell lung cancer is correlated with distant metastasis<sup>20</sup>. These findings suggest that CD15 moiety on the tumour cells may acquire a sialyl group, in the progression of malignant tumours towards a widely disseminated disease. However, the prognostic significance of CD15 in NHL has yet to be studied.

CD30 expression in subset of DLBCL is widely recognized, only 3 of 67 cases in our study expressed CD30. This observation also concurred with other reports, which showed low frequency of CD30 expression in NHL<sup>13</sup>. The observed CD30 expression in the large tumour cells might be indicative of the activation state of the tumour cells since CD30 is detected in activated T and B cells, and EBV infected immunoblasts<sup>18</sup>. In situ hybridisation for EBV was performed on these 3 CD30+ cases and there was no evidence of EBV infection in all cases.

In clinical diagnostic practice, distinction between classical Hodgkin's Lymphoma and NHL can be easily made often based on their characteristic morphologic findings on haematoxylin-eosin-stained section. Nevertheless, there can be some instances in which the morphologic distinction is difficult, such as T-cell/histiocyte rich large B-cell lymphomas from classical Hodgkin's Lymphoma. In our study, CD15 and CD30 were not frequently expressed in the tumour cells of DLBCL cases. Co-expression of both CD15 and CD30 is even more infrequent. Therefore, they remain as useful markers in distinguishing classical Hodgkin's Lymphoma and NHL.

Bcl-2 protein was expressed in 64% of DLBCL in our series, more frequently in nodal than in extranodal tumours. These findings appear to concur with many other reports<sup>19, 20</sup>. BCL-2 gene was originally discovered by virtue of its involvement in the t(14;18)(q32;q21) translocation. However, Bcl-2 protein over-expression in DLBCL can be either due to BCL-2 gene translocation or by BCL-2 gene amplification via other mechanism<sup>21</sup>. In some large scale studies, Bcl-2 protein expression was shown to be associated with decreased disease-free or overall survival in DLBCL<sup>7</sup>. However, the criteria used to classify Bcl-2-positive and Bcl-2-negative cases vary among investigators, as the pattern of staining and cut-off value used to classify positivity differed. Further study to assess the association of Bcl-2 protein expression pattern and clinical outcome for local population is required to determine its prognostic implications.

### Conclusion

CD15 and CD30 are not frequently expressed in the tumour cells of DLBCL cases, and hence these markers remain useful in distinguishing classical Hodgkin's Lymphoma and DLBCL in situations where morphological distinction is difficult. Bcl-2 protein expression is frequent in DLBCL, its prognostic implications for DLBCL in the local setting need to be further elucidated. However, the presence or absence

of Bcl-2 protein played no role in the diagnosis of DLBCL.

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