**TITLE:** The role of MYC and BCL2 expression in a cohort of 43 patients with DLBCL: A retrospective study

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Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of high-grade non-Hodgkin lymphoma (NHL), representing a group of heterogeneous diseases with varied responses and prognosis. Although prognostication tools exist such as the International Prognostic Index (IPI), they do not account for underlying tumor biology and therefore marked differences exist in outcomes within each group. With the advent of genetic profiling, new subtypes have been recognised, however their application to the clinical setting has been limited due to cost of equipment and lack of expertise.

To improve prognostication and account for variable response in DLBCL, the role of *MYC* and *BCL2* oncogenes have been implicated in the pathogenesis of DLBCL1-5 using immunohistochemistry (IHC). Double-expresser lymphoma (DEL) indicates all patients in which upregulation of these proteins is evidenced using IHC, typically at ≥40% for *MYC* and >50-70% for *BCL2*. There remains controversy about firstly, whether co-expression of *MYC* and *BCL2* independent of their translocation status can predict prognosis1, 6-8 and secondly, what cut offs are clinically significant for *MYC* and *BCL2* expression.1, 6, 8 We have therefore investigated these in our cohort of 43 patients.

A comprehensive search was conducted on the local Merseyside Haemato-Oncology Diagnostic Service (HODS) database to identify new diagnosis of DLBCL between May 2013 and December 2015. Patients with a diagnosis of ‘diffuse large B-cell lymphoma’, ‘high grade B-cell non-Hodgkin lymphoma’ or ‘Burkitt’s lymphoma’ were included. Due to exposure of rituximab therapy influencing IHC, 18 patients with relapsed DLBCL were excluded and therefore only new cases were considered.

Data pertaining to patients’ age, gender and Ann Arbor staging were collected including clinical data relating to all components of the IPI score, performance status, therapy used and subsequent response achieved. Although majority of the patients were treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone), there were patients who had variation of this treatment in the form of attenuated rituximab (R), etoposide and omission of doxorubicin. Some patients were palliated either due to patient choice or after unsuccessful trial of steroids in the context of poor performance status. Patients were followed up for at least 2 years with a follow-up time of up to 4 years. The cell of origin (COO) subtype was defined using the Hans algorithm based on CD10, BCL6 and MUM1 expression into germinal center B-cell (GCB) or non-germinal center B-cell (non-GCB). In cases where the IHC markers were not available, this could not be defined fully.

*MYC* positivity was defined as >40% (Figure 1) and for *BCL2*, a cut-off of >70% was used for positivity. The IHC expressions were reviewed independently by two haemato-pathologists and any differences were resolved through discussion and achieving a consensus where required. Fluorescence in situ hybridization (FISH) analysis was performed using local protocol. At least 100 cells were examined for each probe used and images were captured using Applied Imaging Cytovision software.

From the cohort of 43 patients, 51% (22 of 43) were female with a median age of 70 (IQR 59-81) years. GCB subtype accounted for 56% (24 of 43) and non-GCB for 21% (9 of 43) of the cases with 23% (10 of 43) having unknown COO subtype due to incomplete documentation of expression profile. Most patients had advanced Ann Arbor staging of III (40%, 17 of 43) and IV (40%, 17 of 43). The involvement of extra nodal site, performance status, IPI score, therapy and response have been summarised in Table 1.

Median *MYC* expression was 40% (IQR 30-60%) for the 42 patients which had documented *MYC* expression levels with 62% (26 of 42) showing >40% *MYC* positivity. Cytogenetic data was available in 20 of 43 patients due to sample unavailability or insufficient sample. Of these, *MYC* translocation was seen in 20% (4 of 20). Using the >40% cut-off for protein expression, 75% (3 of 4) cases were *MYC* protein expression positive whereas out of the patients who did not have *MYC* translocation, 75% (12 of 16) were positive for *MYC* protein expression. Of the patients with known *BCL2* expression data, majority [78% (32 of 41)] expressed a high level (>70%). *BCL2* translocation was identified in 25% (5 of 20) cases. Ten percent (2 of 20) of patients had confirmed ‘double hits’ signified by concurrent *MYC* and *BCL2* translocations. Of the patients, with expression data for both *MYC* and *BCL2*, co-expression accounted for 46% (19 of 41) of cases using expression thresholds of >40% and >70% respectively (Table 1).

Cox Proportional Hazard (Cox PH) models with a single explanatory variable were fitted and results are listed in Table 2. In total 44% (19 of 43) patients died (see Figure 2A and B for overall survival and progression-free survival for all patients). There was no statistically significant association seen in prognosis when *MYC* and/or *BCL2* translocation and protein expression data were correlated with OS and PFS. However, co-expression of *MYC* and *BCL2* using a combination of *MYC* >60% with *BCL2* >50% or >70% was associated with inferior PFS [HR 2.83 (1.12-7.20), p=0.035 and HR 2.84 (1.10-7.36), p=0.041, respectively] (Figure 2C and D). Other combination of cut-offs (data not shown) were not associated with inferior prognosis. When considering “event” (death and/or progression) as a binary outcome, *MYC* expressionof>60% predicted outcome (OR 5.18 (1.15-23.29), p=0.023).

The main limitation of this study was the small cohort size. This reduced the ability to analyse the data in different ways to understand the variables better. Furthermore, since the IHC and FISH analyses were not carried out specifically for this study and existing reports were extracted for data collection, this meant that there was missing data, leading to exclusion of some patients and limited interpretation of certain aspects of the data. This however on the other hand shows real world data outside of the context of a clinical trial.

In conclusion, our cohort showed evidence of *MYC* and *BCL2* predicting outcomes when considered as co-expressing using *MYC* >60% along with *BCL2* >50% or 70% cut-offs, which in context of other publications, supports their use for DLBCL prognostication tools.

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**Tables**

|  |  |
| --- | --- |
| **Characteristic** | **Number (%)** |
| Age, *years*  Median  Range | 70  59-81 |
| Sex  Female  Male | 22 (51%)  21 (49%) |
| COO subtypea  Non-GCB  GCB  NK | 9 (21%)  24 (56%)  10 (23%) |
| LDH (U/L)  Median  IQR  Range | 534  354-867  178-4855 |
| Ann Arbor Staging  I  II  III  IV | 4 (9%)  5 (12%)  17 (40%)  17 (40%) |
| No of Extranodal sites  ≤1  >1  NK | 32 (74%)  5 (14%)  6 (12%) |
| Performance Statusc  ≤2  >2  NK | 31 (72%)  11 (26%)  1 (2%) |
| IPIb  0 or 1  2  3  4  5  NK | 7 (16%)  11 (26%)  6 (14%)  12 (28%)  4 (9%)  3 (7%) |
| *MYC* Expression (%)  <40  ≥40  NK | 16 (37%)  26 (60%)  1 (2%) |
| *MYC* Translocation  Absent  Present | 16 (80%)\*  4 (20%)\* |
| *MYC* Translocation Present  *MYC* expression >40%  *MYC* expression <40%  *MYC* Translocation Absent  *MYC* expression >40%  *MYC* expression <40% | 3 (75%)  1 (25%)  12 (75%)  4 (25%) |
| *BCL2* expression (%)  <50  ≥50  <70  ≥70  NK | 6 (14%)  35 (82%)  9 (21%)  32 (74%)  2 (5%) |
| *BCL2* Translocation  Absent  Present | 15 (75%)  5 (25%) |
| *BCL2* Translocation Present  *BCL2* expression >70%  *BCL2* expression <70%  *BCL2* Translocation Absent  *BCL2* expression >70%  *BCL2* expression <70% | 5 (100%)  0 (0%)  12 (80%)  3 (20%) |
| *MYC* Expression>40 and *BCL2* >50 (%)  No  Yes  NK  *MYC* Expression>40 and *BCL2* >70 (%)  No  Yes  NK | 19 (44%)  22 (51%)  2 (5%)  22 (51%)  19 (44%)  2 (5%) |
| *MYC* Expression>60 and *BCL2* >50 (%)  No  Yes  NK  *MYC* Expression>60 and *BCL2* >70 (%)  No  Yes  NK | 22 (72%)  10 (23%)  2 (5%)  33 (77%)  8 (19%)  2 (5%) |
| Ki67 (%)  <90  >90 | 30 (70%)  13 (30%) |
| Double Hitd  No  Yes  NK | 19 (44%)  2 (5%)  22 (51%) |
| Therapy  No Rituximab  Rituximab containing | 7 (16%)  36 (84%) |
| Complete Responsee  No  Yes | 18 (42%)  25 (58%) |
| Relapsed-refractory after treatment  No  Yes  NA | 29 (67%)  12 (28%)  2 (5%) |
| Died  No  Yes | 24 (56%)  19 (44%) |

Table 1 – Summary Statistics (categorical variables). COO, cell of origin; GCB, germinal centre B-like; NK, not known; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IPI, international prognostic index.

aCOO (cell of origin) based on Hans algorithm

bIPI is based on age, performance status, serum LDH, extent of extra-nodal involvement and Ann Arbor staging. Where the IPI score could not be calculated, the minimum IPI score was calculated and used

cPerformance status was calculated using ECOG (Eastern Cooperative Oncology Group) scoring and is based on the level of activity of the patient

dDouble hit denotes both translocation on *MYC* and *BCL2* gene

eIncludes CT-based and PET-CT-based assessment of response

\*Data is based on 20 patients with available cytogenetic data only

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Overall survival** | | **Progression free survival** | |
| **Explanatory Variable** | **HR (95% CI)** | **p-value** | **HR (95% CI)** | **p-value** |
| Sex (male) | 2.95 (1.12, 7.79) | **0.022** | 3.30 (1.27, 8.53) | **0.009** |
| Age | 1.04 (1.00,1.08) | **0.041** | 1.04 (1.00,1.08) | **0.018** |
| *MYC* translocation | - | 0.328 |  | 0.387 |
| *BCL2* translocation | - | 0.089 |  | 0.087 |
| Double Hit (Yes) | 3.46 (0.79, 15.13) | 0.157 | 3.79 (0.84, 17.16) | 0.139 |
| *MYC* expression (≥40%) | - | 0.708 | - | 0.577 |
| *BCL2* expression (≥70%) | - | 0.512 | - | 0.407 |
| *MYC* expression ≥60% *BCL2* ≥50% | - | 0.078 | 2.83 (1.12, 7.20) | **0.035** |
| *MYC* expression ≥60% *BCL2* ≥70% | - | 0.093 | 2.84 (1.10,7.36) | **0.041** |
| *Ki-67* expression (≥90%) | - | 0.797 | - | 0.868 |
| Relapsed-refractory | 3.34 (1.35,8.30) | **0.012** | NA | NA |
| R-containing therapy | 0.22 (0.08,0.57) | **0.006** | 0.27 (0.10,0.73) | **0.018** |
| IPI Score (≥3) | 8.82 (2.01, 38.78) | **<0.001** | 4.66 (1.53, 14.19) | **0.003** |
| Ann Arbor staging (≥3) | - | 0.584 | - | 0.406 |
| ECOG status (≥3) | 3.67 (1.48, 9.07) | **0.001** | 4.09 (1.63, 10.24) | **0.004** |
| GCB | - | 0.113 | 0.33 (0.12, 0.91) | **0.039** |

Table 2 – Results of single variable cox Proportional Hazard models with OS and PFS as outcome. The table reports the hazard ratios (HR) in terms of increased risk of death and/or progression event. Note: p-values highlighted in bold are statistically significant. NA, not applicable.

**Figure Legends**

Figure 1 – Immunohistochemistry staining of cases of diffuse large B-cell lymphoma with A) C-myc protein expression 0%, B) C-myc protein expression 40% and C) C-myc protein expression >60% (c-myc immunostains; 10x).

Figure 2 – Kaplan-Meier plot showing A) overall survival and B) progression-free survival data of all patients. C) and D) show the OS and PFS of patients who had co-expression of MYC >60% and BCL2 >50% compared with those who did not.