Role of MYC and BCL2 expression in a cohort of 43 patients with DLBCL: a retrospective study

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of high-grade non-Hodgkin’s lymphoma, 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 tumour 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 has been implicated in the pathogenesis of DLBCL using immunohistochemistry (IHC). Double-expressor lymphoma 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 first, whether coexpression of MYC and BCL2 independent of their translocation status can predict prognosis and second, what cut-offs are clinically significant for MYC and BCL2 expression. We have therefore investigated these in our cohort of 43 patients.

A comprehensive search was conducted on the local Merseyside Haematology Oncology Diagnostic Service database to identify new diagnosis of DLBCL between May 2013 and December 2015. Cases with a diagnosis of ‘diffuse large B-cell lymphoma’, ‘high grade B-cell non-Hodgkin’s 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, hydroxydaunorubicin (doxorubicin), Oncovin (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 centre B-cell (GCB) or non-germinal centre 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 haematopathologists and any differences were resolved through discussion and achieving a consensus where required. Fluorescence in situ hybridisation (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 extranodal site, performance status, IPI score, therapy and response has been summarised in table 1.

Table 1  Summary statistics (categorical variables)

| Characteristic | Number (%) |
|---------------|------------|
| Age, years    |            |
| Median        | 70         |
| Range         | 59–81      |
| Sex           |            |
| Female        | 22 (51)    |
| Male          | 21 (49)    |
| COO subtype*  |            |
| Non-GCB       | 9 (21)     |
| GCB           | 24 (56)    |
| NK            | 10 (23)    |
| LDH (U/L)     |            |
| Median        | 534        |
| IQR           | 354–867    |
| Range         | 178–4855   |
| Ann Arbor Staging |        |
| I             | 4 (9)      |
| II            | 5 (12)     |
| III           | 17 (40)    |
| IV            | 17 (40)    |
| No of extranodal sites |        |
| ≤1            | 32 (74)    |
| >1            | 5 (14)     |
| NK            | 6 (12)     |
| Performance status† |      |
| ≤2            | 31 (72)    |
| >2            | 11 (26)    |
| NK            | 1 (2)      |
| IPI‡          |            |
| 0 or 1        | 7 (16)     |
| 2             | 11 (26)    |
| 3             | 6 (14)     |
| 4             | 12 (28)    |
| 5             | 4 (9)      |
| NK            | 3 (7)      |
| MYC expression (%) |        |
| <40           | 16 (37)    |
| ≥40           | 26 (60)    |
| NK            | 1 (2)      |
| MYC translocation |        |
| Absent        | 16 (80)$   |
| Present       | 4 (20)$    |
| MYC translocation present |    |
| MYC expression >40% | 3 (75)   |
| MYC expression <40% | 1 (25)    |
| MYC translocation absent | | |
| MYC expression >40% | 12 (25)   |
| MYC expression <40% | 4 (25)    |
| BCL2 expression (%) |        |
| <50           | 6 (14)     |
| ≥50           | 35 (82)    |
| <70           | 9 (21)     |
| ≥70           | 32 (74)    |
| NK            | 2 (5)      |
| BCL2 translocation |        |
| Absent        | 15 (75)    |
| Present       | 5 (25)     |
| BCL2 translocation present |   |
| BCL2 expression >70% | 5 (100) |
| BCL2 expression <70% | 0 (0)     |

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; 10×).
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 per cent (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, coexpression accounted for 46% (19 of 41) of cases using expression thresholds of ≥40% and >70%, respectively (table 1).

Cox proportional hazard 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, 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, coexpression of MYC and BCL2 using a combination of MYC ≥60% and BCL2 ≥50% was associated with a statistically significant increase in risk for death and/or progression event (HR 2.84, 95% CI 1.10 to 7.36, p = 0.041).

Table 1: Results of single variable Cox proportional hazard models with overall survival (OS) and progression-free survival (PFS) as outcome

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

The table reports the HR in terms of increased risk of death and/or progression event. P values highlighted in bold are statistically significant.

ECOG, Eastern Cooperative Oncology Group; GCB, germinal centre B-cell; IPI, International Prognostic Index; NA, not applicable.

Figure 2: Kaplan-Meier plot showing (A) overall survival (OS) and (B) progression-free survival (PFS) data of all patients. (C) and (D) The OS and PFS of patients who had coexpression of MYC ≥60% and BCL2 ≥50% compared with those who did not.
with BCL2 ≥50% or >70% was associated with inferior PFS (HR 2.83 (1.12 to 7.20), p=0.035 and HR 2.84 (1.10 to 7.36), p=0.041, respectively) (figure 2C,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 expression of ≥60% predicted outcome (OR 5.18 (1.15 to 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 were 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 coexpressing using MYC ≥60% along with BCL2 ≥50% or 70% cut-offs, which in context of other publications, supports their use for DLBCL prognostication tools.

**Contributors** UTK and MK were involved in acquisition of data, analysis, interpretation and writing of the manuscript. JD conducted detailed analyses on the data acquired. SF was involved in writing of the manuscript. BH, JS, AA, NK and AP were involved in data acquisition and coauthoring of the paper. MA conducted the FISH analysis on all the samples. AC was involved in writing of the paper. IR-A was involved in reviewing and reporting of the slides and provided figures for the paper. GM designed the overall study and coauthored the paper.

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