Correspondence

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[1–5] using immunohistochemistry (IHC). Double-expresser 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[1 6–8] and second, 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 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'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.

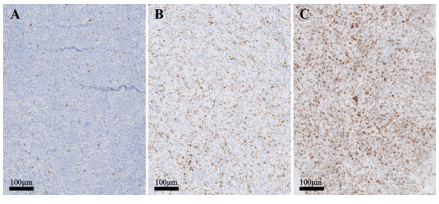

Figure 1 Immunohistochemistry staining of cases of diffuse large B-cell lymphoma with (A) C-myc protein expression 0%, (B) C-myc protein expression 40% and (C) C-myc protein expression >60% (c-myc immunostains; 10×).

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.

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 were available in 20 of 43 patients due to sample unavailability or insufficient sample. Of these,

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 (75)
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)

Continued

Table 1 Continued

Characteristic	Number (%)
BCL2 translocation absent	
BCL2 expression >70%	12 (80)
BCL2 expression <70%	3 (20)
MYC expression >40 and BCL2 >50 (%)	
No	19 (44)
Yes	22 (51)
NK	2 (5)
MYC expression >40 and BCL2 >70 (%)	
No	22 (51)
Yes	19 (44)
NK	2 (5)
MYC expression >60 and BCL2 >50 (%)	
No	22 (72)
Yes	10 (23)
NK	2 (5)
MYC expression >60 and BCL2 >70 (%)	
No	33 (77)
Yes	8 (19)
NK	2 (5)
Ki67 (%)	
<90	30 (70)
>90	13 (30)
Double hit¶	
No	19 (44)
Yes	2 (5)
NK	22 (51)
Therapy	
No rituximab	7 (16)
Rituximab containing	36 (84)
Complete response**	
No	18 (42)
Yes	25 (58)
Relapsed-refractory after treatment	
No	29 (67)
Yes	12 (28)
NA	2 (5)
Died	
No	24 (56)
Yes	19 (44)

*Cell of origin (COO) based on the Hans algorithm.
†Performance status was calculated using Eastern Cooperative Oncology Group (ECOG) scoring and is based on the level of activity of the patient.
‡International Prognostic Index (IPI) is based on age, performance status, serum lactate dehydrogenase (LDH), extent of extranodal involvement and Ann Arbor staging. Where the IPI score could not be calculated, the minimum IPI score was calculated and used.
§Data are based on 20 patients with available cytogenetic data only.
¶Double hit denotes both translocation on *MYC* and *BCL2* gene.
**Includes CT-based and Positron Emission Tomography - Computed Tomography (PET-CT)-based assessment of response.
GCB, germinal centre B-like; NA, not applicable; NK, not known.

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%

Table 2 Results of single variable Cox proportional hazard models with overall survival (OS) and progression-free survival (PFS) as outcome

	OS		PFS	
Explanatory variable	**HR (95% CI)**	**P value**	**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% *BCL2* ≥50%	–	0.078	2.83 (1.12 to 7.20)	**0.035**
MYC expression ≥60% *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.

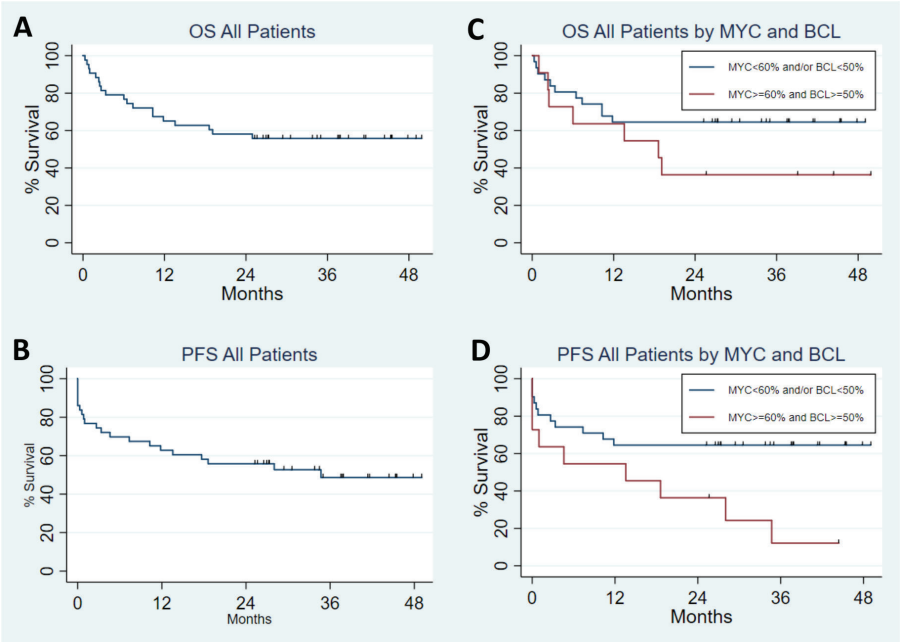

Figure 2 Kaplan-Meier plot showing (A) overall survival (OS) and (B) progression-free survival (PFS) data of all patients. (C) and (D) The OS and PFS of patients who had coexpression of MYC ≥60% and BCL2 ≥50% compared with those who did not.

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.

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