

RESEARCH ARTICLE

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Impact of Double Expression of C-MYC/BCL2 Protein and Cell of Origin Subtypes on the Outcome among Patients with Diffuse Large B-Cell Lymphoma: a Single Asian Center Experience

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Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) with double expression of c-MYC and BCL2 protein is associated with dismal outcome after treatment with R-CHOP. Local data on disease burden and survival outcome in DLBCL is limited. We investigated the prognostic values of c-MYC/BCL2 protein co-expression and cell of origin subtypes using immunohistochemistry (IHC) and to determine their associations with multiethnic groups under resource limited setting. **Methods:** This was a retrospective study which recruited 104 patients in between June 2012 and December 2015 for IHC review and analysis. **Result:** We demonstrated that patients with high International Prognostic Index (IPI) (score 3-5) and co-expression of c-MYC/BCL2 protein had significant inferior overall survival (OS) and event free survival (EFS) respectively ($P < 0.05$). c-MYC/BCL2 protein co-expression was more common in non-germinal center B-cell (non-GCB) ($P = 0.048$) and contributed to adverse prognosis in this group of patients (OS, $P = 0.004$; EFS, $P = 0.005$). In multivariate analysis, double-protein co-expression was a significant independent predictor of inferior outcome after adjusted for IPI and cell of origin subtypes (OS hazard ratio [HR], 2.11; 95% CI, 1.01 to 4.04; $P = 0.048$; EFS HR, 2.31; 95% CI, 1.05 to 5.04; $P = 0.036$). In addition, non-GCB subtype was more common than GCB in Malays (60% vs 40%, $P = 0.106$) and Chinese (81.2% vs 18.8%, $P = 0.042$). Indians had more DLBCL without c-MYC/BCL2 protein co-expression compared to double-protein positive cases (66.7% vs 33.3%, $P = 0.414$). Otherwise, the prognostic impact of ethnicity on survival outcome was insignificant ($P = 0.961$). **Conclusion:** c-MYC/BCL2 protein co-expression in non-GCB subtype constituted a unique group with extremely inferior outcome regardless of ethnicity. Gene expression profile (GEP) may possibly provide insights into the cause of discrepancies in DLBCL subtypes and protein expression among the multiethnic groups.

Keywords: DLBCL- c-MYC/BCL2 protein co-expression- GCB- non-GCB

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the commonest type of non-Hodgkin lymphoma (NHL) worldwide, accounting for approximately 30-40% of all lymphoid malignancies (Fisher and Fisher, 2004). Rituximab in combination with cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP-21) has been the mainstay of treatment for DLBCL for the past 15 years with response rate range from 80-90% among patients with low risk disease (IPI score 0-2) (Feugier et al., 2005). Studies have shown that the addition of rituximab to CHOP resulted in both an improved event free survival (EFS) and overall survival (OS) (Coiffier et al., 2002; Pfreundschuh et al., 2006; Fu et al., 2008;

Sehn, 2010). Although DLBCL is potentially curable with conventional anthracycline-based chemotherapy, approximately 30-40% of patients relapse and 10% have refractory disease (Fisher et al., 1993; Perry and Goldstone, 1998). Variability in treatment response is often observed, further emphasizing heterogeneity of the disease.

The International Prognostic Index (IPI) remains one of the most important clinical prognostic tool to risk stratify the disease since 1993. Patients with extensive DLBCL often have high IPI score. As gene expression profile (GEP) techniques are costly, surrogate IHC-based algorithms have been proposed to sub-classify the disease base on the cell of origin (Hans et al., 2004; Choi et al., 2009). The Hans algorithm based on 3 IHC models

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(CD10, BCL6, MUM1) is the most widely used algorithm although its concordance rate with GEP does not exceed 80% (Hans et al., 2004). There are two distinct molecular subtypes of DLBCL, namely GCB and non-GCB. Higher proportion of non-GCB subtype was reported in patients over 60 years of age (Rosenwald et al., 2002). Many studies have shown that non-GCB is associated with more aggressive behaviour and worse outcome when treated with R-CHOP (Rosenwald et al., 2002; Lenz and Staudt, 2010; Alizadeh et al., 2011).

Besides, there is also growing interest on double-hit lymphomas (DHL) and c-MYC/BCL2 protein co-expression lymphomas. c-MYC protein overexpression occurs in 29-64% of DLBCL while BCL2 protein overexpression has been reported in about 50% of the cases (Johnson et al., 2009; Green et al., 2012; Horn et al., 2013). Approximately 19-34% of DLBCL expressed both c-MYC and BCL2 protein (Johnson et al., 2009; Horn et al., 2013). Overexpression of both protein can occur in the absence of chromosomal rearrangements. Studies have demonstrated that DHL and lymphomas with double-protein co-expression have poorer prognosis than patients without these alterations. Importantly, the prognostic impact of c-MYC overexpression is found only in patients who co-expressed BCL2 protein (Bea et al., 2005; Johnson et al., 2009; Snuderl et al., 2010; Green et al., 2012; Li et al., 2012; Horn et al., 2013). Cytogenetic and fluorescence in situ hybridization (FISH) are used to detect MYC rearrangement (MYC-R) and BCL2 translocation in DHL while IHC is applied to demonstrate double expression of c-MYC and BCL2 protein in tumour cells. However, FISH is costly and not widely available in the public hospitals in Malaysia. Therefore, in resource limited countries, IHC is preferable in determining c-MYC/BCL2 protein co-expression compared to FISH for DHL to predict outcome and treatment options in the lymphoma patients.

Despite a large number of studies on DHL and double-protein co-expression lymphoma in the Western countries, unfortunately some of the lymphoma guidelines were not applicable to the local institutions in Asian countries due to huge disparities in the health care facilities, economy and accessibility of novel agents. To date, the data on DLBCL with c-MYC/BCL2 protein co-expression in Asian countries is limited. The objective of this study is to investigate the prognostic values of c-MYC/BCL2 protein co-expression by IHC in Asian population, identify cell of origin subtypes and their influence on the treatment response. We also assessed whether different ethnic backgrounds were associated with adverse outcome. This is important for prognostic purpose and risk stratified treatment in future.

Materials and Methods

Patient selection and protocol

This is a retrospective study that included patients aged ≥ 15 years diagnosed with DLBCL in Hospital Pulau Pinang between June 2012 and December 2015. Clinical and laboratory data were retrieved from medical records. Age at presentation, staging, lactate dehydrogenase (LDH)

level, performance status, presenting symptoms, nodal status, pathologic characteristics, response to treatment and complications were reviewed. LDH was considered high at ≥ 220 IU/L. All patients received R-CHOP chemotherapy as the initial treatment for DLBCL. End of treatment (EOT) evaluation was performed using computed tomography (CT) or positron emission tomography (PET). Patients positive for HIV were excluded from the study. All patients were follow-up until March 2017. The protocol was approved by the Medical Research and Ethics Committee (MREC) and the study was registered at National Medical Research Register (NMRR), Malaysia.

Immunohistochemistry

An immunohistochemical analysis of formalin fixed, paraffin-embedded tissues was performed using a panel of antibodies which include CD20, CD3, CD5, CD10, BCL6, MUM1, PAX5, c-MYC, BCL2 and ki67. c-MYC and BCL2 immunoreactivity were considered positive when they were expressed in at least 40% and 30% of tumour cells respectively (Green et al., 2012; Horn et al., 2013). The tumours were assigned into GCB or non-GCB phenotype using Hans algorithm. All the histological diagnosis was reviewed by experienced pathologists. Evaluation of IHC was performed independently without knowledge of patients' outcome.

Terminology

Double-protein co-expression negative DLBCL refers to either c-MYC positive BCL2 negative, c-MYC negative BCL2 positive or both c-MYC and BCL2 negative. Response to treatment was assessed according to the "International Workshop to Standardize Response Criteria for Non-Hodgkin Lymphoma" (Cheson et al., 1999). Complete response (CR) is defined as the disappearance of all lesions and of radiologic abnormalities observed at diagnosis. Partial response (PR) is defined as the regression of all measurable lesions by more than 50%, the disappearance of non-measurable lesion and the absence of new lesions. Progressive disease (PD) is defined as the appearance of a new lesion, any growth of the initial lesion by more than 25%, or growth of any measurable lesion that had regressed during treatment by more than 50% from its smallest dimensions. Stable disease (SD) is defined as a regression of any measurable lesion by 50% or less or no change for the non-measurable lesions but without growth of existing lesions. OS is defined as the interval between the date of diagnosis and the date of death as a result of any cause or date of last follow-up. EFS is defined as the interval between the date of diagnosis and the date of disease progression or death as a result of any cause.

Statistical analysis

Patient demographics, clinical characteristics and pathological evaluation were included for analysis using Statistical Package for the Social Sciences (SPSS) software, version 22.0. The Kaplan-Meier method was used to estimate OS and EFS. The log-rank test was used to compare survival distribution. Cox regression test was used in the multivariate analysis to evaluate if double

expression of c-MYC and BCL2 protein was predictive of OS and EFS after adjusting for IPI score and cell of origin. The statistical level of significance was defined as P value < 0.05.

Results

Clinical and immunohistochemical characteristics

A total of 104 patients were included for analysis. The median age at diagnosis was 58 years old (range 17-84 years). The majority of patients had at least stage III disease (60.6%), elevated LDH (71.2%) and B symptoms (66.3%) at presentation (Table 1). There were more Malay (53.8%) patients compared to Chinese (34.6%) and Indians (9.6%). The ethnic distribution in the cohort was consistent with the population distribution in Malaysia. Cell of origin subtype was assigned in 79 (75.9%) patients as some of the tissue specimens encountered incomplete fixation and staining. Non-GCB was more common than GCB (68.4% vs 31.6%) and the majority aged 60 years or more (76.3%). By using cut-off values of 40% for c-MYC and 30% for BCL2 positive tumor cells, we found that 45 (63.4%) cases were positive for c-MYC and 50 (70.4%) for BCL2 alone. 35 (49.3%) cases had c-MYC/BCL2 protein co-expression. Notably,

double-protein co-expression DLBCL was significantly more common in non-GCB group compared to GCB, accounting for 80% and 20% respectively (P = 0.048, Table 2).

Overall treatment response and outcome

The median cycles of chemotherapy was 6 (range 1-8) and the median follow-up time was 29 months (range 2-65). 97 (93.3%) patients received chemotherapy and 7 (6.7%) were excluded from survival analysis as some of them refused treatment or unfit for chemotherapy. 58 (59.8%) patients achieved CR, 11 (11.3%) had PR and 18 (18.6%) SD or PD. 10 (10.3%) patients were not evaluable as treatment was discontinued due to deterioration of general condition after chemotherapy. Figure 1 illustrates treatment response according to c-MYC/BCL2 protein co-expression. There was no significant difference in the response rate between ethnic groups (P = 0.678).

Among 97 patients who received chemotherapy, the estimated 3-year OS was 65.9% and EFS was 63.5% (figure 2A-B). The OS for Malays, Chinese and Indians at 3 years were 69.5%, 61.8% and 66.7% respectively (P = 0.961). At the time of analysis, 65 (67%) patients were still alive and 32 (33%) had died. As illustrated in Table 2, patients with extensive disease characterized by

Table 1. Clinicopathologic Characteristics among Different Ethnic Groups with DLBCL

Characteristics	Overall	Number of patients, n (%)				P value
	n (%)	Malays	Chinese	Indians	Other	
Age groups						
Age < 60	55 (52.9)	34 (60.7)	14 (38.9)	5 (50)	2 (100)	0.11
Age ≥ 60	49 (47.1)	22 (39.3)	22 (61.1)	5 (50)	0 (0)	
Gender						
Males	54 (51.9)	28 (50)	22 (61.1)	3 (30)	1 (50)	0.358
Females	50 (48.1)	28 (50)	14 (38.9)	7 (70)	1 (50)	
Staging						
I-II	41 (39.4)	21 (37.5)	17 (47.2)	2 (20)	1 (50)	0.444
III-IV	63 (60.6)	35 (62.5)	19 (52.8)	8 (80)	1 (50)	
B Symptoms						
No	35 (33.7)	20 (35.7)	11 (30.6)	3 (30)	1 (50)	0.905
Yes	69 (66.3)	36 (64.3)	25 (69.4)	7 (70)	1 (50)	
Serum LDH						
Normal	30 (28.8)	12 (21.4)	15 (41.7)	3 (30)	0 (0)	0.158
Elevated	74 (71.2)	44 (78.6)	21 (58.3)	7 (70)	2 (100)	
IPI Score						
Low (score 0-2)	59 (56.7)	31 (55.4)	20 (55.6)	6 (60)	2 (100)	0.652
High (score 3-5)	45 (43.3)	25 (44.6)	16 (44.4)	4 (40)	0 (0)	
Cell of origin						
GCB	25 (31.6)	16 (40)	6 (18.8)	3 (50)	0 (0)	0.161
Non-GCB	54 (68.4)	24 (60)	26 (81.2)	3 (50)	1 (100)	
c-MYC/BCL2 protein						
c-MYC+ / BCL2+	35 (49.3)	18 (50)	14 (50)	2 (33.3)	1 (100)	0.647
Other†	36 (50.7)	18 (50)	14 (50)	4 (66.7)	0 (0)	

LDH, lactate dehydrogenase; IPI, International Prognostic Index; GCB, germinal center B-cell; † refer to c-MYC positive, BCL2 negative or c-MYC negative, BCL2 positive or both negative.

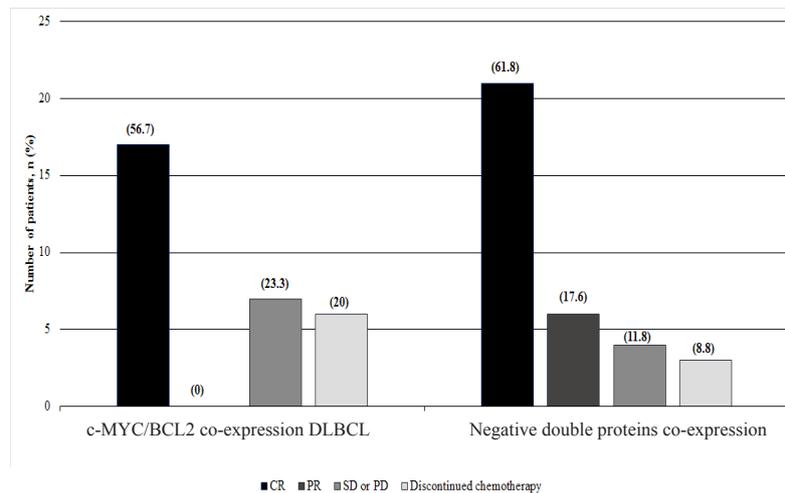


Figure 1. Treatment Response According to c-MYC and BCL2 Expression. DLBCL with c-MYC/BCL2 co-expression had lower CR, higher chemoresistant cases (SD or PD) and more complications that warrant discontinuation of chemotherapy compared to negative group (P = 0.046). DLBCL, diffuse large B-cell lymphoma; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

stage III-IV, presence of B symptoms and high LDH had significant inferior OS (P < 0.001) and EFS (P < 0.001). In addition, high IPI (score 3-5) was significantly associated with worse outcome compared to low IPI (score 0-2) (estimated 3-year OS, 44.9% vs 80.0%; P < 0.001; HR, 4.60; 95% CI, 2.03 to 10.4, P < 0.001; Figure 3A-B, Table 3). Likewise, patients with c-MYC/BCL2 protein co-expression had poor OS (estimated 3-year OS, 44.4% vs 69.3%; P = 0.038, Figure 3C-D) and EFS (P = 0.049) compared to double-protein negative group. The prognostic impact of GCB and non-GCB subtypes on survival was insignificant (estimated 3-year OS for non-GCB vs GCB: 56% vs 63.3%; P = 0.549; estimated 3-year EFS non-GCB vs GCB: 53.8% vs 57.5%; P = 0.705. Figure 3E-F).

Next, we investigated whether the prognostic value of c-MYC/BCL2 protein co-expression was dependent on cell of origin subtype and IPI. Survival analysis revealed that patients with double-protein co-expression had significant inferior OS (estimated 3-year OS for GCB vs non-GCB, 74.5% vs 38.5%; P = 0.004) and EFS (estimated 3-year EFS for GCB vs non-GCB, 73.2% vs 39.4%; P = 0.005) in non-GCB group (Figure 4C-D). However, no statistically significant difference was observed between IPI and c-MYC/BCL2 protein co-expression with respect to outcome. Multivariate analysis of OS and EFS that incorporated IPI, cell of origin subtypes and c-MYC/BCL2 protein co-expression showed that high IPI and double-protein co-expression were significant independent prognostic factors in DLBCL patients. c-MYC/BCL2 protein co-expression was a powerful predictor of inferior OS (P = 0.048) and EFS (P = 0.036, Table 3). The risk of death was two times greater for double-protein co-expression DLBCL compared to double-protein negative group.

Discussion

This is a retrospective study conducted in Malaysia under resource limited setting to evaluate the impact of c-MYC/BCL2 protein co-expression and cell of

origin subtypes on the outcome of DLBCL treated with R-CHOP. From the multivariate analysis, we found that double-protein co-expression was a significant predictor of inferior survival, independent of IPI score and cell of origin subtypes. This is consistent with the findings reported by Johnson et al., (2009) and Green et al., (2012). Double-protein co-expression was more common in non-GCB subtype and was associated with significant adverse outcome in our cohort. Hu et al., (2013)

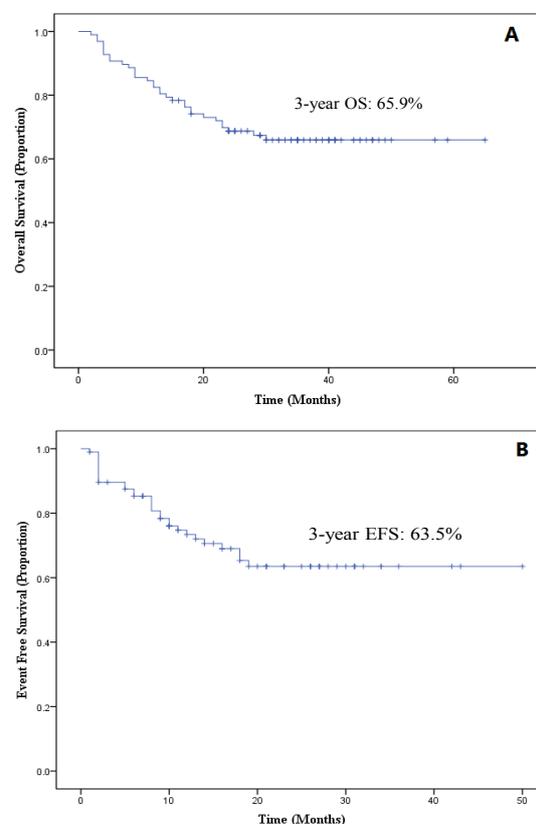


Figure 2. Overall Survival and Event Free Survival in DLBCL Treated with Chemotherapy. (A, B) 3-year OS (A) and EFS (B) in DLBCL patients treated with R-CHOP or CHOP-like chemotherapy was 65.9% and 63.5% respectively.

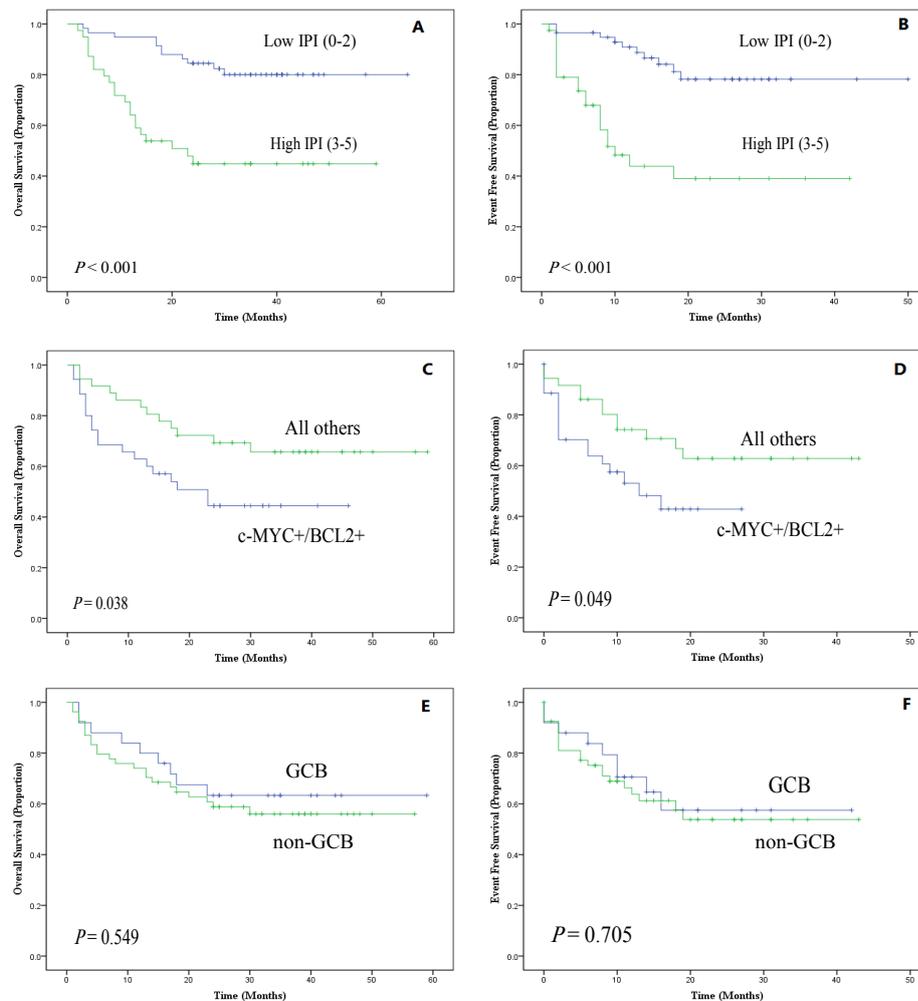


Figure 3. Prognostic Impact of IPI, c-MYC/BCL2 co-Expression and Cell of Origin in DLBCL. (A, B) OS (A) and EFS (B) according to IPI score. (C, D) OS (C) and EFS (D) according to c-MYC/BCL2 co-expression. (E, F) OS (E) and EFS (F) according to cell of origin subtypes: GCB versus non-GCB. The estimated 3-year OS for non-GCB vs GCB: 56% vs 63.3%; $P = 0.549$; the estimated 3-year EFS non-GCB vs GCB: 53.8% vs 57.5%; $P = 0.705$.

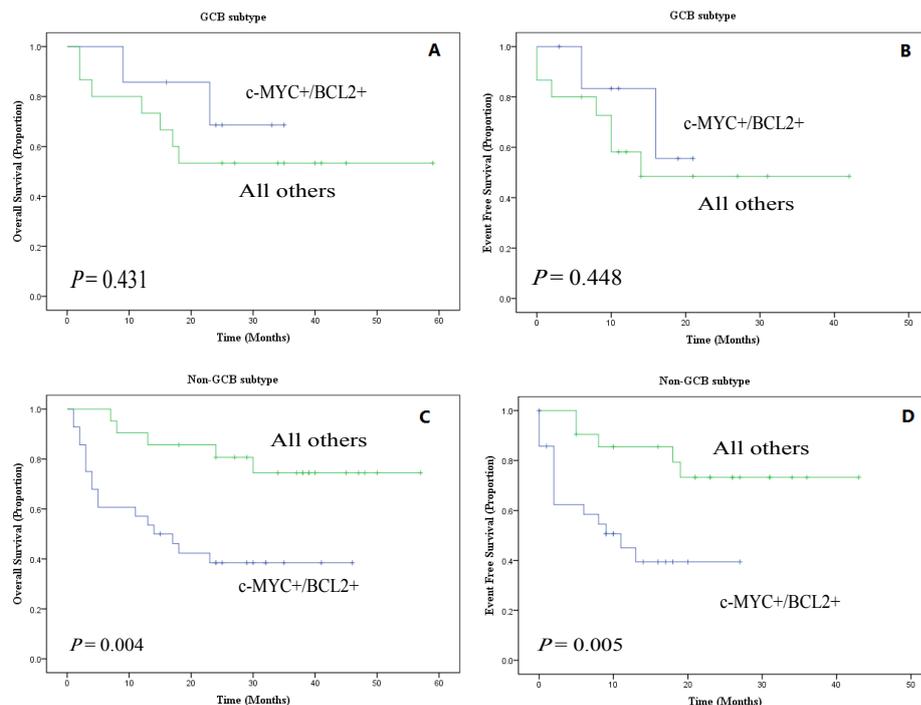


Figure 4. c-MYC/BCL2 co-expression contributes to inferior outcome in non-GCB DLBCL. (A, B) OS (A) and EFS (B) for c-MYC/BCL2 co-expression in GCB group. (C, D) OS (C) and EFS (D) for c-MYC/BCL2 co-expression in non-GCB group.

Table 2. c-MYC/BCL2 Protein co-Expression and Outcome of DLBCL Treated with Chemotherapy

Clinical characteristics	c-MYC/BCL2 co-expression			n (%)	Overall	
	Yes	No	P value		OS	EFS
	n (%)	n (%)			P value	P value
Age groups						
Age < 60	19 (54.3)	18 (50)	0.718	52 (53.6)	0.125	0.122
Age ≥ 60	16 (45.7)	18 (50)		45 (47.4)		
Ethnicity						
Malay	18 (51.4)	18 (50)	0.647	53 (54.6)	0.961	0.758
Chinese	14 (40.0)	14 (38.9)		33 (34.0)		
Indian	2 (5.7)	4 (11.1)		9 (9.3)		
Other	1 (2.9)	0 (0)		2 (2.1)		
Gender						
Males	16 (45.7)	19 (52.8)	0.552	52 (53.6)	0.088	0.111
Females	19 (54.3)	17 (47.2)		45 (46.4)		
Staging						
I-II	12 (34.3)	16 (44.4)	0.381	40 (41.2)	0.007	0.008
III-IV	23 (65.7)	20 (55.6)		57 (58.8)		
B Symptoms						
No	9 (25.7)	11 (30.6)	0.65	34 (35.1)	<0.001	<0.001
Yes	26 (74.3)	25 (69.4)		63 (64.9)		
Serum LDH						
Normal	8 (22.9)	10 (27.8)	0.634	29 (29.9)	<0.001	<0.001
Elevated	27 (77.1)	26 (72.2)		68 (70.1)		
IPI category						
Low risk (score 0-2)	15 (42.9)	20 (55.6)	0.285	58 (59.8)	<0.001	<0.001
High risk (score 3-5)	20 (57.1)	16 (44.4)		39 (40.2)		
Cell of origin						
GCB	7 (20)	15 (41.7)	0.048	25 (31.6)	0.549	0.705
Non-GCB	28 (80)	21 (58.3)		54 (68.4)		
c-MYC/BCL2 co-expression						
No	-	-	-	36 (50.7)	0.038	0.049
Yes	-	-		35 (49.3)		

LDH, lactate dehydrogenase; IPI, International Prognostic Index; GCB, germinal center B-cell; OS, overall survival; EFS, event free survival.

Table 3. Multivariate Analysis of Different Variables in Patients with DLBCL

Variables	OS			EFS		
	HR	95% CI	P value	HR	95% CI	P value
IPI score						
Low (0-2)	1	-	-	1	-	-
High (3-5)	4.6	2.03-10.4	< 0.001	5.03	2.15-11.8	< 0.001
Cell of origin						
GCB	1	-	-	1	-	-
Non-GCB	1	0.46-2.20	0.996	0.82	0.37-1.83	0.629
c-MYC/BCL2 co-expression						
No	1	-	-	1	-	-
Yes	2.11	1.01-4.41	0.048	2.31	1.05-5.04	0.036

OS, overall survival; EFS, event free survival; HR, hazard ratio; CI, confidence interval; IPI, international prognostic index; GCB, germinal center B-cell.

reported similar findings and postulated that co-expression of c-MYC and BCL2 protein may contribute to poor prognosis in non-GCB group.

When we subtyped DLBCL according to cell of origin using Hans algorithm, approximately two-third of the cases were non-GCB (68.4%). Double-protein

co-expression was found in 49.3% of DLBCL while 80% of double-expressed lymphomas were of the non-GCB subtype. The frequency of double-protein co-expression DLBCL appears higher in our cohort compared to most studies which reported 19 to 34% (Johnson et al., 2009; Green et al., 2012; Hu et al., 2013). Most published data used a cut-off point of at least 40% for c-MYC and 50% (range from 30 to 70%) for BCL2 protein expression (Johnson et al., 2009; Green et al., 2012; Horn et al., 2013; Hu et al., 2013; Perry et al., 2014). However, the definition of c-MYC positivity by IHC is not universally standardized. In our cohort, the frequency of c-MYC protein overexpression alone was 63.4%, in line with the overall c-MYC positive rate of 64% observed by Hu et al., (2013). However, this result is different from Green et al., (2012) who reported 32% of c-MYC positive cells from 185 cases and 29% from 307 cases by Johnson et al., (2009). The cut-off value for BCL2 protein expression in the present study was 30%, similar to Perry et al. (2014) who concluded that the cut-off points of 30% for BCL2 was the best predictors of OS and EFS in their cohort of DLBCL treated with R-CHOP.

It has been shown in many studies that patients with double-protein co-expression DLBCL treated with R-CHOP have dismal outcome with 3-year OS of 43% (Green et al., 2012). Another two studies Johnson et al., 2009 and Hu et al., (2013) reported 5-year OS of 30%. In the present study, the overall response (OR) to first line chemotherapy was 59.8%. Subgroup analysis showed that patients with double-protein co-expression had an OR of 56.7% and the estimated 3-year OS was 44.4%. When looking at the c-MYC/BCL2 protein co-expression in non-GCB group, the outcome is worse with estimated 3-year OS 38.5%. The majority of patients (66.6%) who discontinued treatment came from non-GCB and double-protein co-expression groups. We found that most of them were males, age of 60 years or more with B symptoms, elevated LDH and high IPI at presentation. Rosenwald et al., (2002) reported significant better outcomes for patients with GCB subtype after standard chemotherapy compared to non-GCB with 5-year OS 59% for the former and 30% for the later. However, this was not the case in our cohort. Although non-GCB group showed lower estimated 3-year OS (56% vs 63.3%) and EFS (53.8% vs 57.5%) compared to GCB, the difference was not significant (Figure 3E-F).

We observed that Malay and Chinese had more non-GCB subtype compared to GCB, accounting for 60% and 81.2% respectively. Both double-protein co-expression positive and negative cases were equally distributed in Malay (50%) and Chinese (50%) patients. Conversely, Indians had higher number of double-protein negative cases (66.7%) compared to double-protein positive DLBCL (33.3%). These interesting findings postulate a possible shared GEP for DLBCL among Malay and Chinese patients but not Indians. DLBCL signature gene set among ethnic groups may provide insights into the cause of these discrepancies. Nevertheless, the prognostic impact of ethnicity on survival outcome was insignificant.

There is currently no standard treatment for DLBCL

with c-MYC/BCL2 protein co-expression. Various alternative regimens have been suggested. The infusional regimen of dose adjusted etoposide, cyclophosphamide, doxorubicin, vincristine, prednisolone and rituximab (DA-EPOCH-R) has shown promising preliminary results in double-protein co-expression DLBCL (Wilson et al., 2008; 2012). Similarly, a retrospective review of DLBCL patients treated with DA-EPOCH-R suggested that this regimen may ameliorate the negative impact of c-MYC/BCL2 protein co-expression (Dunleavy et al., 2013). Wilson et al., (2008); (2012) postulated the use of two topoisomerase inhibitors (doxorubicin and etoposide) given as continuous infusion may potentially improve outcome in high risk DLBCL. There are limitations in this study. Firstly, this is a retrospective study conducted in a single institution. There is lack of full IHC data for all included patients. IHC studies can be hampered by technical difficulties that are related to fixation methods, quality of specimens and scoring of staining.

In conclusion, we have confirmed that high IPI and c-MYC/BCL2 protein co-expression are independent significant predictors of inferior outcome in DLBCL after treatment with R-CHOP. We demonstrated that patients with non-GCB subtype harboring double-protein co-expression had poor survival. The current findings suggest that the biology of disease is heterogeneous and although Malaysia is a country with different ethnicities, the adverse prognostic factors and outcome among Asian patients treated with chemotherapy do not differ much from those in the Western countries. It is feasible to apply IHC in resource limited institutions to identify double-protein co-expression DLBCL, which is generally more aggressive than those without double-protein co-expression. In addition, IHC is widely available, rapid and inexpensive. As a practical point, c-MYC/BCL2 protein co-expression could be used as a prognostic marker to identify high risk group particularly those with non-GCB subtype in which alternative strategies should be considered. Again, accessibility to novel agents is limited in most institutions in Asian countries. Currently, we incorporate the infusional regimen of DA-EPOCH-R in young DLBCL patients who have good performance status, high IPI score and double-protein co-expression at diagnosis. Further study is recommended to evaluate the outcome in this group of patients.

Statement conflict of interest

The authors declare no conflict of interest.

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