ORIGINAL ARTICLE

Identification of MYC/BCL2 Double-expressers Lymphomas in Diffuse Large B Cell Lymphoma Patients and Association with their Prognostic Parameters and Survival Rates: A Single Centre Experience

Aliza Ahmad[1], Fauzah Abd Ghani[2], Junalina Jaafar[3], Maizaton Atmadini Abdullah[2]

[1] Faculty of Medicine and Health Science, Universiti Sains Islam Malaysia, Persiaran Ilmu, 71800 Nilai, Negeri Sembilan, Malaysia.
[2] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[3] Department of Pathology, Hospital Tuanku Ja'afar, 70300 Seremban, Negeri Sembilan, Malaysia.

ABSTRACT

Introduction: Diffuse large B cell lymphoma (DLBCL) is a heterogenous disease some of which show co-expression of c-MYC and BCL2 and they are known as double expressers (DE) lymphoma. DE lymphoma has been shown to be associated with aggressive clinical course and poor outcome in comparison to those without co-expression. The association of DE lymphomas with their prognostic parameters and the overall survival rate of DE compared to non-DE lymphoma patients were investigated. **Method:** 66 formalin-fixed paraffin-embedded DLBCL cases were subjected to c-MYC and BCL2 immunohistochemical staining. **Results:** Out of 66 cases, 25(37.9%) cases showed c-MYC and BCL2 co-expression. Majority of the patients were in age category of ≥ 60 years (68.0%). Male and female distribution were almost equal. DE lymphoma was significantly associated with ABC subtype in comparison to GCB subtype (p= 0.026). Median survival time for DE was 29 months and non-DE was 34 months. Log rank analysis showed there was no significant difference in the overall survival rate of DE and non-DE patients (p=0.401). **Conclusion:** This study showed significant association between DE lymphomas and ABC subtype. Identification of DE lymphoma with ABC subtype may guide the clinicians in identifying patients with poorer outcome hence a more aggressive treatment may be offered to this group.
Malaysian Journal of Medicine and Health Sciences (2022) 18(SUPP21): 86-91. doi:10.47836/mjmhs18.s21.14

Keywords: DLBCL, Double expressers, DE, Lymphoma, Prognostic

Corresponding Author:
Maizaton Atmadini Abdullah, PhD
Email: maizaton@upm.edu.my
Tel: +603-97692391

INTRODUCTION

Globally, recent attention has been given to an aggressive subset of lymphomas with rearrangements of MYC/BCL2 and/or BCL6 (i.e., double hit lymphoma (DHL) and triple hit lymphoma (THL). They form a separate entity called "High grade B-cell lymphoma (HGBL) with rearrangement of MYC and BCL2 and/ or BCL6" in the 2016 revised World Health Organization Classification of Lymphoid Neoplasm (1). WHO also recognises that approximately 20% of DLBCLs co-express both MYC and BCL2 proteins and these cases are designated as "double-expressers" (DE) (1).

Although a subset of these cases represent DHL, the majority actually do not carry the translocation. DLBCL cases which show MYC and BCL2 double expressions have been demonstrated to be an independent marker of poor prognosis (2). MYC expression is identified in a higher proportion of DLBCL (30%-50%) and co-expressed BCL2 in 20-35% of cases. Most studies used a cut off of more than 40% MYC-expressing cells (nuclear expression) to define these cases; while the cut off for BCL2 expression varied considerably in the literature, with a figure of 50% or more (membranous and cytoplasmic expression) is recommended. In few but not all studies, DE lymphomas have a poorer outcome than other DLBCL, NOS which do not co-express the protein but they are not as aggressive as the HGBL(1,3).

According to Green et al, DE lymphoma has a distinct clinical phenotype (4). They observed that patients with DE lymphoma had a median age of 71 years. Furthermore, DE patients were more likely to have poorer performance status, more advanced disease (stage III or IV), higher proliferative index (Ki67), intermediate to high-risk International Prognostic Index

(IPI) scores, widespread and multiple extra nodal sites disease, and more inferior response rate to R-CHOP. In comparison to DHL, DE is more commonly encountered in clinical practice as the method of detection is by immunohistochemical stains which are available in most laboratories. In a study by Johnson et al, 18% of the analysed patients showed co-expressions of BCL2 and MYC (5). Hu et al also demonstrated that 34% of the patients demonstrated these aberrations (6). In summary, it is estimated that 20% to 30% of DLBCL patients are DEs.

Many studies have observed that DE lymphoma is more frequently seen among the Activated B-cell (ABC) subtype in comparison to Germinal center B-cell (GCB) subtype (3,7). Gene expression profiling (GEP) study has stratified DLBCL into biologically meaningful and prognostically relevant subgroups which are GCB and ABC or non-GCB subtypes (4,5). GEP technology is expensive and not widely available, thus, simpler and widely available method to sub-classify DLBCL has been studied and has led to immunohistochemistry algorithm as a surrogate to GEP. A few algorithms were proposed namely Hans algorithm, Choi algorithm and new algorithm using 5 markers (8). The most commonly used algorithm is Hans algorithm as proposed by Hans et al. This algorithm described that GCB and ABC subtype of DLBCL can be accurately predicted by using three IHC panels, namely CD10, BCL6 and MUM1/IRF4 (9).

Single expresser DLBCL (either MYC or BCL2 overexpression) has been shown to have similar outcomes to those patients without such expression. Meanwhile, DE lymphoma patients demonstrated inferior 3-year progression-free survival (PFS)(39% vs 75%; p< 0.001) and 3-year overall survival (OS) (43% vs 86%; p< 0.001) in comparison to those without overexpression or single protein expression. These data demonstrate to us that double expressions is associated with adverse outcomes (5).

MATERIALS AND METHODS

Tissue samples

This study was approved by the Medical Research and Ethics Committee and Hospital Tuanku Jaafar (HTJS) Research Committee. A total of 100 formalin-fixed paraffin-embedded (FFPE) tissue specimens and slides of patients diagnosed as DLBCL from 2015 to 2018 were retrieved from the archive in the Department of Histopathology HTJS. These patients were followed up in the Medical Outpatient Department of HTJS. The samples collected were excluded if there was no H&E slide, missing formalin fixed paraffin embedded tissue, no histopathological report or patient data was not available in Lab information system (LIS). All patients were followed up until December 2018. After the application of the inclusion and exclusion criteria, a total of 66 cases were eligible for investigation. Cases were classified into MYC/BCL2 DE based on IHC panel done previously. Cases with incomplete IHC panel, were subjected for IHC staining for MYC and BCL2 accordingly. The approval and ethical clearance from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (MOH) was attained prior to commencement of the study [NMRR-19-3170-51172 (IIR)].

Immunohistochemical analysis

Commercially available, in vitro diagnostic (IVD) stain for anti-c-MYC (Y69) Rabbit Monoclonal Primary Antibody and anti-BCL-2 (SP66) rabbit monoclonal antibody were used to stain the FFPE using autostainer (BenchMark XT; Ventana Medical System). The immunohistochemistry was done according to the standard operating procedure. Cases which expressed > 40% MYC and >50% BCL2 were classified as MYC/BCL2 DE lymphoma (5,10). Using eye balling method, all intensity of protein expression (score 1+ to 3+) were considered positive if they were more than the cut off values. Cases that did not show co-expression of MYC/BCL2 were classified as others. Evaluation of IHC was done by two pathologists blinded of the patients' outcome.

Statistical analysis

All results were analysed by standard statistical software package, IBM SPSS statistics for windows, SPSS version 25.0. The distribution of demographic characteristics (age and ethnicity), and prevalence of DE lymphoma were determined using frequency distribution and percentage. To determine the association of DE lymphoma with its prognostic and clinicopathological parameters (stage of disease, lactate dehydrogenase (LDH) level, performance status (IPI), bone marrow involvement, and cell of origin), variables were compared using chi-square test. p value <0.05 was considered significant. Survival rate was calculated using Kaplan-Meier method. Survival rate between MYC/BCL2 DE and non-DE lymphoma were compared using log rank analysis.

RESULTS

Sociodemographic distribution of DLBCL

Out of 66 cases of DLBCL, 34(51.5%) of the cases were female and 32(48.5%) were male. 38(57.6%) of the cases were those more or equal to 60 years of age, and 28(42.4%) were less than 60 years of age. The disease was more commonly seen among Malay (50,75.8%) in comparison to Chinese (10,15.2%), Indian (5,7.6%) and others (1, 1.4%). Most of DLBCL patients presented with extranodal manifestations (56.1%) whereas the remaining (40.9%) showed nodal involvement.

Prevalence and sociodemographic distribution of DE lymphoma

In our study, we selected the cut off value of c-myc expression of >40% and BCL2 >50% for the cases to be considered double expressers. From the 66 cases

of DLBCL, 25 (37.9%) cases showed c-myc and BCL2 co-expressions. Other 41 (62.7%) cases either showed single expression of c-myc or BCL2, co-expressed c-myc and BCL6 or did not express both. 60.0% of patients presented with extranodal disease and the rest showed nodal involvement (40.0%) In our study, DE lymphomas were more commonly seen in patients age ≥60 years (68.0%). Male and female distribution was almost equal with 12 (48.0%) patients were male and 13 (52.0%) patients were female. Our study also demonstrated that DE lymphomas were more commonly seen in Malay (84.0%).

Association of DE lymphoma with their clinico-pathological parameters

In our analysis, DE lymphomas were significantly associated with ABC subtype in comparison to GCB subtype accounting for 72% and 28% respectively (p= 0.026, Table I). The classification was done according to Hans algorithm as stated earlier. Most of DE patients presented with high LDH level (88%), high to intermediate IPI score (88%) and advanced stage of disease (68.0%). However, we found that there was no significant association between stage of disease (p=0.653) and IPI index (p=0.086) with DE lymphoma. High LDH level and bone marrow involvement also showed no significant association. Figure 1 showed the morphology and immunohistochemistry of one of the DE lymphoma patients with ABC subtype.

Table I: Associations of DE lymphomas with their clinico-pathological parameters

MYC/BCL2 DE	Outcome n(%)		X^2 statistic	df	Fisher's exact test	p value
	Yes	**No**				
Cell of origin (COO):						
GCB	7(28.0)	23(56.1)	4.945	1		**0.026**
ABC	18(72.0)	18(43.9)				
LDH:						
≥UNL	22(88.0)	31(75.6)			0.340	
<UNL	3(12.0)	10(24.4)				
Bone marrow involvement:						
Involved	2(8.0)	5(12.2)			0.594	
Not involved	23(92.0)	36(87.8)				
IPI:						
High/ intermediate	17(68.0)	19(46.3)	2.938	1		0.086
Low	8(32.0)	22(53.7)				
Stage of disease:						
Stage III/IV	17(68.0)	30(73.2)	0.203	1		0.653
Stage I/II	8(32.0)	11(26.8)				

p <0.05 is considered statistically significant
GCB, Germinal center B-cell; LDH, Lactate dehydrogenase; UNL, Upper normal limit; IPI, International
Prognostic Index; ABC, Activated B-cell

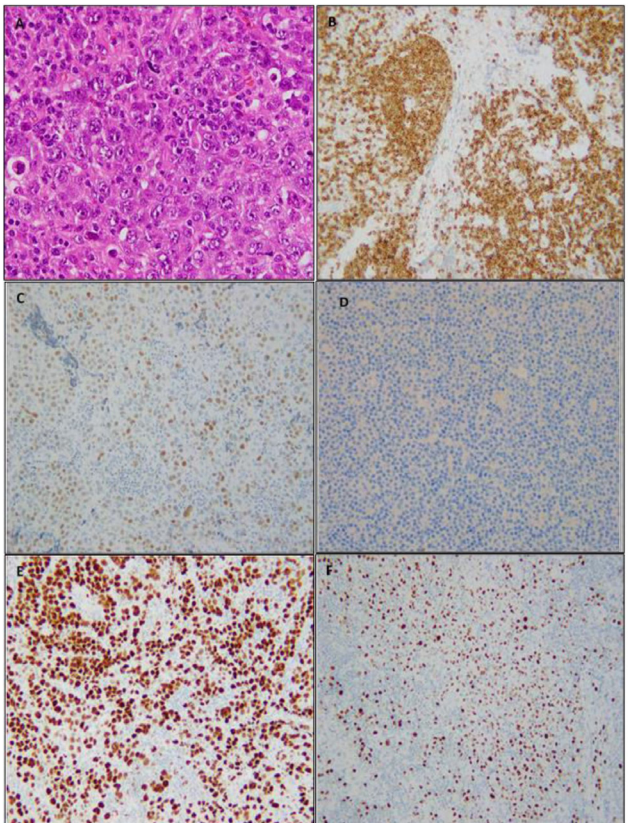

Figure 1: Picture on co-expressions of myc and BCL2 (A) H&E stain showing large neoplastic B cells in diffuse pattern (magnification x40) (B) strong membranous and cytoplasmic reactivity of neoplastic B cell towards BCL2, >50% of cells (C) variable nuclear c-myc positivity of the B cell >40% of cells (D) negative CD10 immunostain (E) nuclear reactivity towards BCL6 >30% (F) variable nuclear reactivity towards MUM1 >30% of cells; indicating ABC subtype (magnification B,C,D,E X200).

Comparison between overall survival rates of DE patients and non-DE patients

Univariate survival analysis showed median survival time for DE was 29 months and non-DE was 34 months. Log rank analysis was used to compare the overall survival rate and there was no significant difference in the overall survival rate of DE and non-DE patients (p=0.401) (Figure 2).

We also compared overall survival rate between ABC and GCB subtype DE lymphomas and there were no statistically significant differences in their overall survival rate (p=0.066) (Figure 3).

DISCUSSION

DLBCL is a neoplasm of medium to large B cells that exhibit a diffuse growth pattern and their nuclear sizes are more than twice of normal resting mature lymphocytes. It is the commonest type of NHL, accounting for almost 30-40% of B cell NHL (1,10). DLBCL is a heterogeneous disease in clinical, pathological and molecular aspect. DLBCL is a disease of the elderly (median age 7th decade)

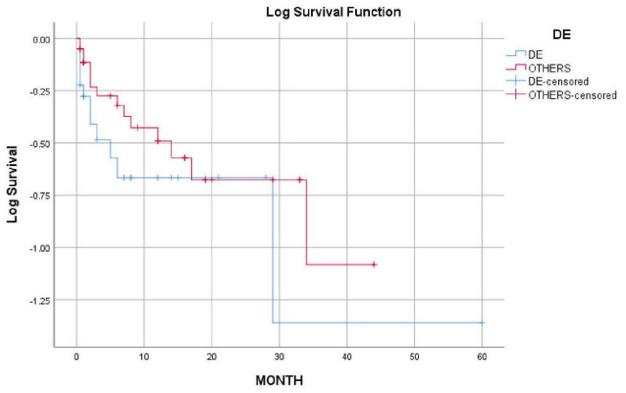

Figure 2: Overall survival of DE lymphoma patients and non-DE lymphoma patients (p=0.401)

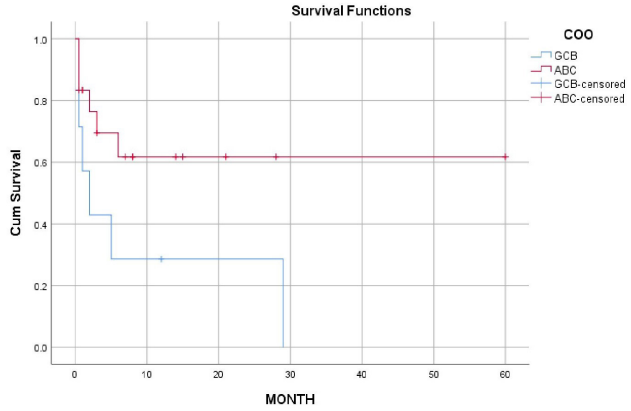

Figure 3: Overall survival of GCB and ABC subtype of DE lymphoma patients (p=0.066)

but can also be seen in children and young adults. It is more common in males than female. Our study also showed most of DBCL patients were ≥60 years of age (57.6%) and it was more commonly in Malay population accounting for 75.8%. Data published by National Cancer Registry also showed similar finding whereby lymphoma is more common in Malay (11). Our study showed, it is more commonly seen in female gender, in contrast to a study done by Clark et al, whereby 49% of DLBCL were detected in male patients (2). Data shown in National Cancer Registry also reported that lymphoma is more common in male (58.5%) in comparison to female (41.5%)(11). Few other studies including two local studies showed the disease was also slightly more common in males rather than females (2,3,12). The difference noted in our study could be attributed to our limited number of samples.

A variety of clinical, pathological and molecular markers have been developed aiming to help in characterisation of the disease. Currently, DLBCL are classified based on their morphological variant and cell of origin or molecular subtypes. DLBCL, NOS has a diverse morphology, and the disease can be divided into common and rare morphological variants. The affected lymph nodes may show partial or more commonly total architectural effacement. The neoplastic cells may exhibit enlarged nuclei with one central nucleoli resembling immunoblasts or multiple nucleoli resembling centroblasts. Morphologically, DLBCL cases in our study showed spectrum from immunoblastic, centroblastic as well as mixed morphology. A study by Horn et al showed that immunoblastic morphology had emerged as a negative prognostic value (13). The cell of origin also carries prognostic value and can be classified as GCB and ABC subtype. Most of our DLBCL cases were of GCB subtype (54.5%) which was actually known to carry a better prognosis in comparison to ABC subtype.

HGBL is gaining attention worldwide and the method of detection of MYC/BCL2 and or BCL6 translocation is by FISH. DE lymphomas are on the rise as the method of detection is by immunohistochemistry which is more readily available in many centres. Out of 66 cases of DLBCL in our study, 25 cases (37.9%) showed co-expressions of c-myc and BCL2. We applied the cut off value of >40% expression for c-myc and >50% expression for BCL2 in order to classify the cases as double expresser. Teoh et al in their study reported 35(49.3%) cases had c-MYC/BCL2 double expressions by using cut-off values of 40% for c-MYC and 30% for BCL2 (3).

WHO and many other studies also recommended similar value for classification of DE in their reports (6,13). The optimal value of classifying expression of c-myc and BCL2 is still not clearly defined. Few studies have used the value of >70% and >40% expression of BCL2 and c-myc respectively. Some other research used >50% and >40% or >30% and >50% (4,6,14). A study by Clark Schneider et al verified that the cut off value of >50% and >40% had successfully identified DE lymphoma with poor overall survival (OS) compared to those whom did not co-express both proteins at this level (2). Similar finding was also reported by Johnson et al (5).

Riedell et al emphasised on the necessity to screen all patients diagnosed with DLBCL for DHL at diagnosis in view of the known fact of DHL having poor OS and dismal outcome (15). They proposed a few strategies in choosing which patients' to be subjected for FISH study including limited FISH testing for MYC rearrangement only or those whom co-expressed MYC/BCL2 (15). In the Western countries, FISH was used to identify various molecular abnormalities in a variety of malignant neoplasms. In view of DE does not equate to DHL, immunohistochemistry alone will not be able to identify DHL. Thus, when resource appears to be an issue, strategy like subjecting only GCB subtype for ISH is a good step as >90% of DHL is associated with GCB subtype. Another strategy is by subjecting the tissue for MYC rearrangement testing first before

proceeding to BCL2 and/ BCL6. However, in countries with limited resources, identification of these patients by immunohistochemistry is more feasible.

In our study, most of the DE cases were seen in older population aged ≥60 years (68.0%) and more among the Malays. Another local study by Teoh et al also showed similar distribution (3). The male and female distribution was however almost equal. Approximately two third of DE cases were of ABC subtype (72.0%). Those DE cases were classified according to their cell of origin into GCB and ABC subtype based on the Hans algorithm using protein expression of CD10, BCL6 and MUM1. As suggested by Hans et al in their publication, we used the cut off value of >30% (9). Most laboratories applied the usage of immunohistochemistry for cell of origin (COO) classification. To date, RNA-based platforms using FFPE specimen are emerging as new gold standard and they offer a unique opportunity for assay standardisation for laboratories globally (16).

Statistical analysis shown that there was a significant association between DE and ABC subtype (p=0.026). Study by Green et al, Johnson et al and Hu et al also showed that DE was associated with ABC subtype while 90 to 100% of DHL was associated with GCB subtype (4,6,17). This raised the question as to whether only GCB subtype need to be subjected for FISH testing as it is more common in DHL. The question is yet to be answered and needs further study. Currently no proper guidelines have been proposed regarding the criteria for FISH testing.

In our study, non-DE patients were shown to have a longer median survival (34 months) in comparison to DE patients (29 months). However, there was no significant difference in the overall survival rate of DE and non-DE patients (p=0.401) in our study. Teoh et al in their study showed that DE patients had statistically significant inferior OS(p=0.004)(3). This was also shown by Horn et al in their study (13). The possible reason for this maybe due to limited sample size in our study in comparison to the other studies. At the moment, no overall survival rate for DE lymphoma is available at national level. They also compared the OS of DE patients of GCB and ABC subtypes and concluded that there was a significant difference in the OS (estimated 3-year OS for GCB vs non-GCB, 74.5% vs 38.5%, p value=0.004). In our study, we did not observe significant difference in the survival rate of GCB and ABC subtypes of DE lymphoma (p=0.066). Rosenthal et al in their review stated that two studies had compared the biology of DE and DHL and they found that the characteristics did not differ with DE patients showing lower complete response (CR) with RCHOP (p=0.004) and shorter OS in comparison to non-DE patients. Thus, the author concluded that both DHL and DE do extremely poor and suggested that IHC being a sufficient method in identifying DLBCL cases with "double hit" biology (7). Even though the clinical relevance of DE is well understood, its application in our daily practice is still limited due to the uncertainty in IHC scoring thresholds and limited choices of treatment. To date, available data are still inadequate to evaluate the alternative treatment regimen for DE lymphoma even though some have observed that suboptimal outcome is seen with R-CHOP. In order to delineate an optimum treatment plan of DE lymphoma patients, a well-designed prospective clinical trial is necessary. In the current situation, either RCHOP or a more intensified regime like R-EPOCH seems to be a reasonable approach. A local study had mentioned that an intensified regimen of DA-EPOCH-R had been used in their center for young DE patients who have good performance status and high IPI score (3). However, more studies need to look into and evaluate the outcome of this group.

Our study was a cross sectional study, which was conducted in a single centre, therefore, the findings may not actually represent and reflect the actual picture of the disease burden in Malaysian population. Our findings may also be affected by our small sample size.

CONCLUSION

In our study, we have proven that DE lymphoma is associated with ABC subtype which carry a poorer prognosis. Detection of DE using IHC is a better option as it is widely available and more cost effective, however it is still debatable as to whether IHC can surrogate genetic testing and further research is warranted. Thus, referral of DE cases to other centres that provide ISH study is a good alternative. HGBCL are known to carry poor prognosis and dismal outcome. The method of MYC/BCL2 and BCL6 translocation detection using FISH which was not widely available in all laboratories limits their detection in our practice. To date, there is no proper criteria or guideline proposed on which patients should be tested for FISH. Theoretically all patients should undergo FISH testing for detection of translocation, but it is not possible especially in our setting. A cohort study will be useful to verify the IHC cut off value as well as to verify the association of DE with prognostic parameters. Future studies should also include association of DE lymphoma and prognostic parameters with various treatment modalities.

REFERENCES

1. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, Jaffe ES. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood, The Journal of the American Society of Hematology. 2016 May 19;127(20):2375-90. doi: 10.1182/blood-2016-01-643569
2. Clark Schneider KM, Banks PM, Collie AMB, Lanigan CP, Manilich E, Durkin LM, et al. Dual

expression of MYC and BCL2 proteins predicts worse outcomes in diffuse large B-cell lymphoma. Leuk Lymphoma. 2016;57(7):1640–8. doi: 10.3109/10428194.2015.1101099

3. Teoh CS, Lee SY, Chiang SK, Chew TK, Goh AS. 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. Asian Pacific journal of cancer prevention: APJCP. 2018;19(5):1229. DOI: 10.22034/APJCP.2018.19.5.1229
4. Green TM, Young KH, Visco C, Xu-Monette ZY, Orazi A, Go RS, Nielsen O, Gadeberg OV, Mourits-Andersen T, Frederiksen M, Pedersen LM. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2012;30(28):3460-7. doi: 10.1200/JCO.2011.41.4342
5. Sesques P, Johnson NA. Approach to the diagnosis and treatment of high-grade B-cell lymphomas with MYC and BCL2 and/or BCL6 rearrangements. Blood, The Journal of the American Society of Hematology. 2017 Jan 19;129(3):280-8. doi: 10.1182/blood-2016-02-636316
6. Hu, S., Xu-Monette, Z.Y., Tzankov, A., Green, T., Wu, L., Balasubramanyam, A., Liu, W.M., Visco, C., Li, Y., Miranda, R.N. and Montes-Moreno, S., 2013. MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. Blood, The Journal of the American Society of Hematology, 121(20), pp.4021-4031. doi: 10.1182/blood-2012-10-460063
7. Rosenthal A, Younes A. High grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6: Double hit and triple hit lymphomas and double expressing lymphoma. Blood reviews. 2017 Mar 1;31(2):37-42. DOI: 10.1016/j.blre.2016.09.004
8. Choi WW, Weisenburger DD, Greiner TC, Piris MA, Banham AH, Delabie J, Braziel RM, Geng H, Iqbal J, Lenz G, Vose JM. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clinical cancer research. 2009 Sep 1;15(17):5494-502. doi: 10.1158/1078-0432.CCR-09-0113
9. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Muller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, Pan Z. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004 Jan 1;103(1):275-82. doi: 10.1182/blood-2003-05-1545
10. Shankland KR, Armitage JO, Hancock BW. Non-hodgkin lymphoma. The Lancet. 2012 Sep 1;380(9844):848-57. doi: 10.1016/S0140-6736(12)60605-9
11. Azizah A, Hashimah B, Nirmal K, Siti Zubaidah A, Puteri N, Nabihah A, Sukumaran R, Balqis B, Nadia S, Sharifah S, Rahayu O. Malaysia National cancer registry report (MNCR). Available from: https://www.moh.gov.my/moh/resources/Penerbitan/Laporan/Umum/2012-2016%20(MNCRR)/MNCR_2012-2016_FINAL_(PUBLISHED_2019).pdf
12. Salam DS, Siew-Hoon T, Suat-Cheng P, Shiau-Chuen C. Retrospective Lymphoma Study from Malaysia Private Hospital. Int J Cancer Clin Res. 2020;7:140. Available from: https://clinmedjournals.org/articles/ijccr/international-journal-of-cancer-and-clinical-research-ijccr-7-140.php?jid=ijccr#:~:text=Of%20the%20210%20cases%20of,of%20Lymphocyte%20predominant%20nodular%20HL.
13. Horn H, Ziepert M, Becher C, Barth TF, Bernd HW, Feller AC, Klapper W, Hummel M, Stein H, Hansmann ML, Schmelter C. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood, The Journal of the American Society of Hematology. 2013 Mar 21;121(12):2253-63. doi: 10.1182/blood-2012-06-435842
14. Perry AM, Alvarado-Bernal Y, Laurini JA, et al. MYC and BCL2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with rituximab. Br J Haematol. 2014;165:382–91. doi: 10.1111/bjh.12763
15. Riedell PA, Smith SM. Double hit and double expressors in lymphoma: definition and treatment. Cancer. 2018 Dec 15;124(24):4622-32. doi: 10.1002/cncr.31646
16. Phang KC, Akhter A, Tizen NM, Abd Rahman F, Azma RZ, Elyamany G, Shabani-Rad MT, Masir N, Mansoor A. Comparison of protein-based cell-of-origin classification to the Lymph2Cx RNA assay in a cohort of diffuse large B-cell lymphomas in Malaysia. Journal of clinical pathology. 2018 Mar 1;71(3):215-20. doi: 10.1136/jclinpath-2017-204548
17. Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S, Scott DW, Tan KL, Steidl C, Sehn LH, Chan WC. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. Journal of clinical oncology. 2012 Oct 1;30(28):3452. doi: 10.1200/JCO.2011.41.0985