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Prognostic significance of double expressor lymphoma subtype in patient with diffuse large B-cell lymphoma

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ABSTRACT

Background: DLBCL is the most common type of non-Hodgkin lymphoma in Asia and Indonesia. DLBCL, based on cell of origin is divided into germinal center B-cell-like (GCB) and non-GCB subtypes. 20% of patients have a molecular profile double expressor lymphoma (DEL), which has a worse prognosis. The study aims to determine the relationship between DEL subtypes and cell of origin subtypes with clinical stage and 3-year overall survival of double large B-cell lymphoma (DLBCL) patients in Kariadi General Hospital Semarang.

Methods: This study sample was 36 DLBCL patients in Kariadi General Hospital from January to September 2017. The data collection including age of diagnosis, location, stage, cell of origin subtype, DEL subtype and 3-year overall survival. Data analysis using chi-square test and Kaplan Meier curve.

Results: DLBCL DEL subtype patients were significantly associated with advanced-stage (p: 0.028). DLBCL non-GCB subtype and DEL subtype patients had a 3-year overall survival that was significantly worse than GCB subtype and non-DEL subtypes (p: 0.026 and p: 0.006, respectively), with a 3-year survival rate of non-GCB subtypes was 38.9% and DEL subtypes were 33.3%. DLBCL patients with advanced stages also have a 3-year overall survival significantly worse than the early stage (p: 0.000), with a 3-year survival rate of 14.3%.

Conclusion: DLBCL non-GCB subtype patients, DEL subtypes and advanced stages have a lower 3-year overall survival rate and thus have a worse prognosis.

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INTRODUCTION

Lymphoma is a malignant disease that arose from lymphocytes in lymphoid tissue, divided into two main groups: Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL). More than 85% of lymphoma in the world is NHL and more than 90% of NHL in the world arose from mature B cells. In Indonesia, NHL is the seventh rank most common cancers.[1,2] The most common type of mature B cell NHL types in Asia, including in Indonesia, is Diffuse Large B-Cell Lymphoma (DLBCL). Nearly 90% of all aggressive B cell type NHL in adults is DLBCL. DLBCL has the highest mortality rate among B cell NHL types and about 64% of DLBCL patients are mostly found in stage III and IV.[1,2,3]

Histologically, DLBCL has diffuse distribution of large malignant lymphoid cells expressing B cell markers (CD20, CD79a, CD19, CD22 and PAX5) and has high Ki-67 proliferation index.[2,3,4] DLBCL can be classified into 2 subtypes, based on the molecular profile of the cell of origin (Hans's criteria): Germinal Center B-cell-like (GCB) and Activated B-Cell-like (ABC) or non-Germinal Center B-cell-like (non-GCB), using immunohistochemistry (IHC) examination for CD10, BCL6 and MUM1.[2,5,6] Generally the GCB subtype DLBCL has better prognosis and overall survival than the non-GCB subtype.[2,6,7]

Some DLBCL patients also have genetic changes in the BCL2 and myelocytomatosis (MYC) genes. Translocation of the MYC gene provides a worse prognosis in DLBCL patients

treated with R-CHOP. Approximately 20% of DLBCL patients had a double expressor lymphoma (DEL) molecular profile, with high MYC and BCL2 protein expression. DEL type DLBCL is reported to have more aggressive clinical course and lower response to R-CHOP therapy.[8–12]

Data on lymphoid tissue malignancy cases in Indonesia have not been well published, and most DLBCL patients in Indonesia were not been diagnosed completely until its subtypes to support immunotherapy and predict prognosis. This study observed the prevalence of DLBCL and its subtypes, based on the cell origin and DEL subtype. This study's objective was to determine association between DEL subtypes and cell of origin subtypes with clinical profile and overall survival of DLBCL patients at Dr. Kariadi

Central General Hospital Indonesia.

METHOD

This is an observational analytic study with a cohort retrospective design. Samples were patients who had been diagnosed as DLBCL at anatomical pathology laboratory, Dr. Kariadi Central General Hospital, Semarang, Indonesia, based on histopathology and IHC examinations, which showed a high expression of diffuse CD20 and Ki-67 expression more than 30% of tumor cells, in the period of January - September 2017. A total of 36 patients were enrolled in this study and were followed for 3 year after diagnosis and treatment to see overall survival.

Clinical profile data were collected, including age at diagnosis which was then categorized into age> 50 years and age <50 years old, tumor location which was then categorized into nodal and extranodal, tumor stage using ann arbor staging which was then categorized into early-stage (stage I and II) and advanced stage (stage III and IV), cell of origin subtypes from IHC examination CD10, B-Cell lymphoma 6 (BCL6) and MUM1, DEL subtype based on IHC examination of B-cell lymphoma 2 (BCL2) and c-myelocytomatosis (c-MYC) which was then categorized into DEL if BCL2 expressed in ≥ 50% tumor cells and c-MYC expressed in ≥ 40 % tumor cells and non-DEL, 3-year overall survival rate of patient was determined by how many months patient can survive after diagnosis. Data analysis used chi-square test with a significance level of p<0.05, and kapplan meier curve analysis.

RESULT

This study shows that 36 DLBCL patients included were mostly diagnosed at over 50 years of age (52.8%) and more frequently had a primary extranodal location (75%). Extranodal locations of tumors in these patients included in the gastrointestinal tract, liver, spleen, kidneys, nasal cavity, mediastinum, tonsils, nasal cavity, palpebra, conjunctiva, central nervous system and femur, while nodal tumor sites were found in colli lymph. Inguinal, axillary and submandibular which can be single or multiple. Based on staging using Ann Arbor Staging, DLBCL patients in

Table 1. Clinical and pathological characteristics DLBCL

Parameters	n=36
Mean age of diagnosis	51.83 ± 14.08 (22 – 77 y.o)
Age of diagnosis category	
- < 50 y.o	17 (47.2%)
- > 50 y.o	19 (52.8%)
Location	
- Nodal	9 (25%)
- Extra-node	27 (75%)
Stage	
- Early (I & II)	22 (61.1%)
- Advanced (III & IV)	14 (38.9%)
Cell of origin subtype	
- GCB	18 (50%)
- Non GCB	18 (50%)
3-year Overall survival	
- Survive	21 (58.3%)
- Death	15 (41.7%)
BCL2	
- Positive	20 (55.6%)
- Negative	16 (44.4%)
c-MYC	
- Positive	24 (66.7%)
- Negative	12 (33.3%)
DEL	
- DEL	15 (41.7%)
- Non-DEL	21 (58.3%)

Abbreviation; double expressor lymphoma (DEL); germinal center B-cell-like (GCB); c-myelocytomatosis (C-MYC); B-cell lymphoma 2 (BCL2); double large B-cell lymphoma (DLBCL)

Table 2. Clinical features based on cell of origin subtype DLBCL

Parameters	GCB	Non-GCB	p
Age of diagnosis			0.738
- < 50 years old	8 (44.5%)	9 (50%)	
- > 50 years old	10 (55.5%)	9 (50%)	
Location			0.700
- Nodal	5 (27.8%)	4 (22.2%)	
- Extranodal	13 (72.2%)	14 (77.8%)	
Stage			0.171
- Early	13 (72.2%)	9 (50%)	
- Advanced	5 (27.8%)	9 (50%)	

this study were mostly diagnosed at an early stage (61.1%) (Table 1).

Based on cell of origin subtypes DLBCL, 18 patients (50%) had GCB subtype and 18 patients (50%) had non-GCB subtype. After a follow-up in the first 3 years after diagnosis and receiving

R-CHOP chemotherapy treatment, 15 patients (41.7%) died within three years (Table 1). Pathological microscopic findings of DLBCL can be seen in Figures 1 and 2.

DLBCL patients with GCB subtype in this study, 55.5% were diagnosed at the

Table 3. Clinical features based on DEL subtype DLBCL

Parameters	DEL	Non-DEL	p
Age of diagnosis			0.463
- < 50 years old	6 (40%)	11 (52.4%)	
- > 50 years old	9 (60%)	10 (47.6%)	
Location			0.845
- Nodal	4 (26.7%)	5 (23.8%)	
- Extranodal	11(73.3%)	16 (76.2%)	
Stage			0.028
- Early	6 (40%)	16 (76.2%)	
- Advanced	9 (60%)	5 (23.8%)	

Table 4. Association between DEL subtype and cell of origin subtype

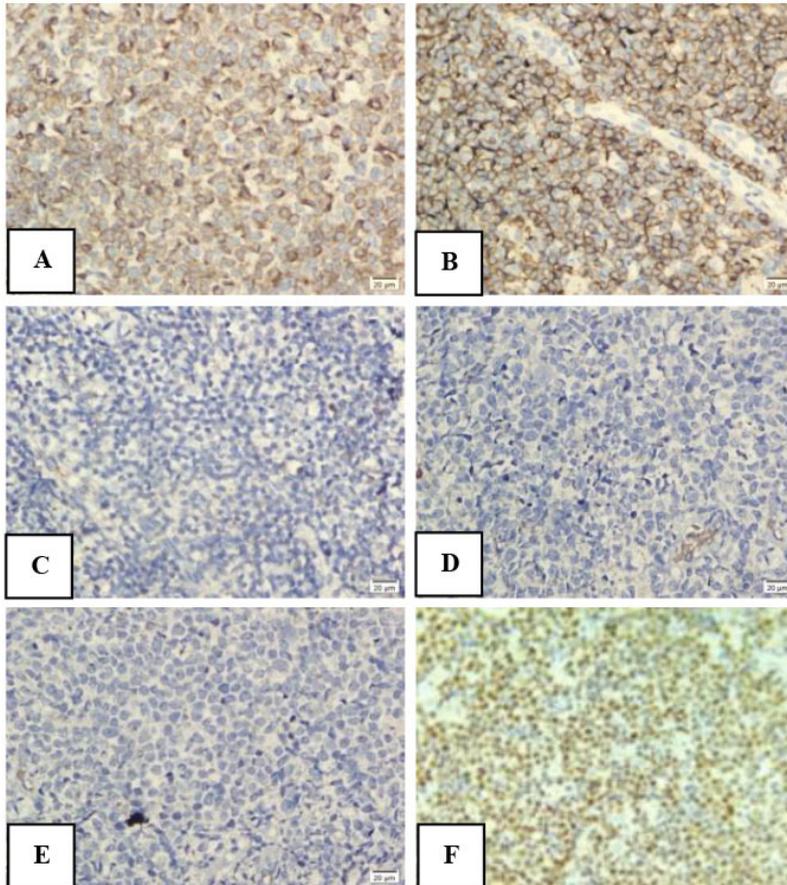|	GCB	Non-GCB	p
DEL Subtype			0.310
- DEL	6 (33.3%)	9 (50%)	
- Non-DEL	12 (66.7%)	9 (50%)	

Figure 1. (A) CD10 positive in DLBCL GCB type; (B) BCL6 positive in DLBCL GCB type; (C) MUM1 negative in DLBCL GCB type; (D) CD10 negative in DLBCL non-GCB type; (E) BCL6 negative in DLBCL non-GCB type; (F) MUM1 positive in DLBCL non-GCB type.

characteristics of age at diagnosis, location and stage in GCB and non-GCB subtypes DLBCL (Table 2).

Based on BCL2 and c-MYC protein expression, DLBCL can be classified into double expressor lymphoma (DEL) type. BCL2 protein expression was found in 55.6% of patients, while c-MYC protein expression was found in 66.7% of patients. From all samples, there were 15 patients (41.7%) who had co-expression of BCL2 and c-MYC or having DEL subtype.

DLBCL patients with DEL subtype in this study, 60% were diagnosed at the age of more than 50 years, 73.3% were located extranodal and 60% came at an advanced stage, while the DLBCL patients non-DEL subtype, 52.4% were diagnosed at the age less than 50 years, 76.2% located extranodal and 76.2% presented at an early stage. There was no significant relationship between age at diagnosis and location between DEL and non-DEL DLBCL, but there was a significant relationship between patient stage and DEL subtype in DLBCL patients, whereas DLBCL DEL subtype was associated with higher patient stage (Table 3).

In this study, 66.7% of patients with GCB subtype DLBCL had DEL subtype, while 50% of non-GCB DLBCL patients had DEL subtype. There was no significant relationship and differences between the original cell's molecular subtypes and the DEL subtypes of DLBCL in this study (Table 4).

In the survival analysis, it appears that DLBCL patients with non-GCB subtypes had a significantly lower overall survival (38.9%) than DLBCL GCB subtypes (77.8%) based on log-rank test, with the median survival of non-GCB DLBCL patients is 6 months. In the survival analysis, it appears that patients with DEL subtype DLBCL had a significantly lower overall survival (33.3%) than non-DEL subtype (76.2%) based on the log-rank test, with the median survival of DLBCL patients with DEL subtype was 5 months. It also appeared that patients with advanced DLBCL had a significantly lower overall survival (14.3%) than patients with early-stage DLBCL (86.4%) based on the log-rank test, with the median survival of DLBCL patients with advanced-stage being 5 months (Figure 3 and 4).
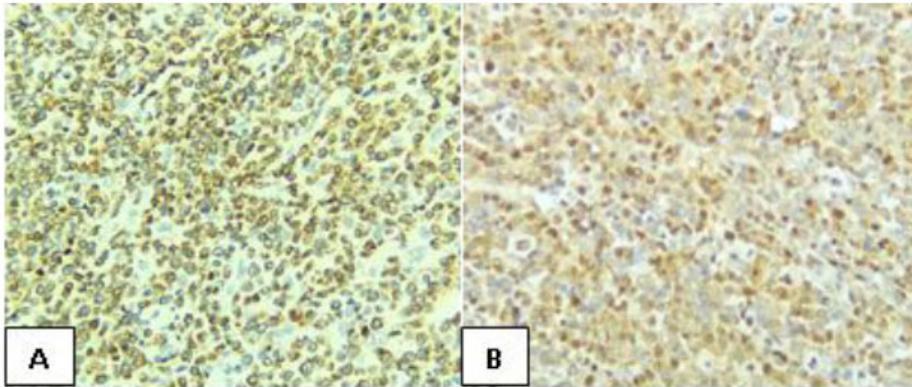
age of more than 50 years, 72.2% were located extranodal and 72.2% presented at an early stage, while the DLBCL patients non-GCB subtype, 50% were diagnosed at

the age of more than 50 years, 77.8% were located extranodal and 50% presented at an early stage. There were no significant differences and associations between the

Figure 2. DEL subtype DLBCL; **(A)** BCL2 overexpressed ≥ 50%; **(B)** c-MYC overexpressed ≥ 40%.
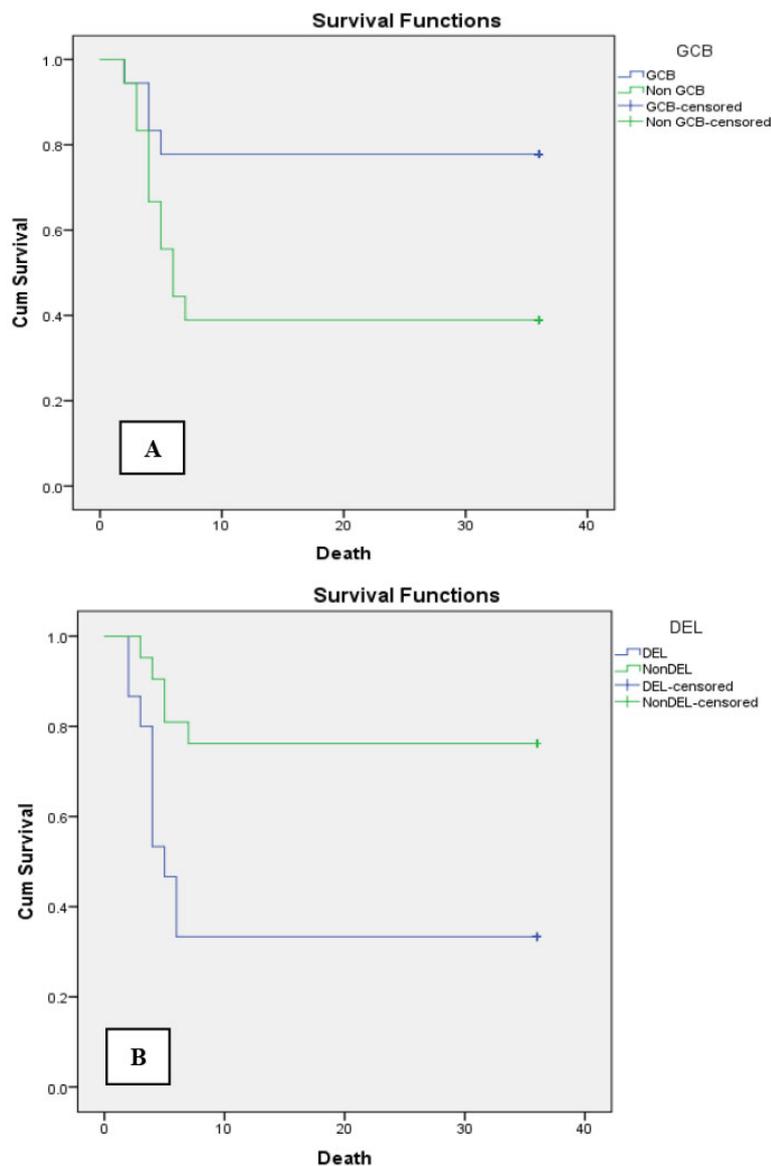

Figure 3. Overall survival DLBCL patients **A.** Based on cell of origin subtype (GCB vs. non-GCB). **B.** Based on DEL subtype (DEL vs. non-DEL).

DISCUSSION

Nearly 50% of DLBCL patients are diagnosed at stage I or II (early stage) without a PET/CT scan. If you add a PET / CT scan, the percentage of DLBCL stage I and II patients is reduced. About 64% of DLBCL patients are mostly found in stage III and IV, but in other studies in Asia, it was found that mostly DLBCL patients were found in stage I and II as many as 52 - 60% of patients.[13,14,15] In this study, DLBCL patients in Dr. Kariadi Hospital were 61.1% with stage III or IV based on the patient's CT scan results. 72.2% of the DLBCL GCB subtype in this study were diagnosed at stage I or II, while 50% of DLBCL non GCB were diagnosed at stage I or II and 50% at stage III or IV, so there was no significant difference and association between stage and cell of origin subtype DLBCL. Previous studies also stated that there was no significant difference between nodal/extranodal locations and molecular cell subtypes from DLBCL.[13,16-18]

In this study, DLBCL patients with advanced-stage were more often found in DEL subtype (60%) than non-DEL (23.8%), whereas DLBCL patients with early-stage were more often found in non-DEL subtype DLBCL (76.2 %) than DEL subtype (40%). Based on statistical tests, there were significant differences and associations between DEL subtype and advanced stage. Previous studies also mentioned difference between stage and DEL subtype, where patients with DEL subtype tended to have more advanced stages of patients than non-DEL, so they had a worse prognosis.[17,18,19]

In this study, patients with DLBCL subtype DEL was mostly GCB subtype (66.7%), while DLBCL patients with non-DEL subtypes had the same proportion between GCB and non-GCB subtypes (50%), but there was no significant difference and association between DEL subtypes and cell of origin subtype of DLBCL. These results are in line with previous studies which also stated that DLBCL patients with non-DEL subtypes were more common in GCB subtypes.[12,18,20] However, DLBCL patients with DEL subtypes, in previous studies it was stated that they were found more frequently in non-GCB subtypes.[12,18,20] the limited sample in this study can cause
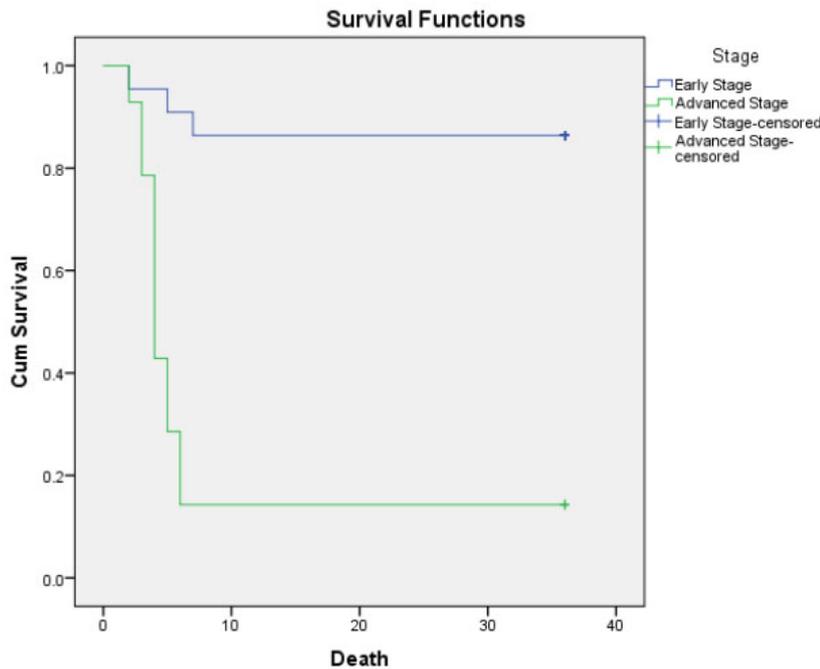
Figure 4. Overall survival DLBCL patients based on stage (Early-stage vs. Advanced stage).

the difference in this study's results with earlier studies in the DEL subtype.

In this study, DLBCL patients have the same proportion of GCB and non-GCB subtypes (50%), whereas in previous studies in Europe and America, DLBCL patients with GCB subtype had a slightly higher proportion of 60%, and non-GCB subtype 40%. The difference in frequency between GCB and non-GCB subtypes is highly dependent on geographic location, race, median age of the patient population, and the investigators' methodology; however, in general, it showed that the proportion of GCB subtype DLBCL patients in Asian countries tends to be lower. This difference in proportion is thought to be closely related to the characteristics of race and geographical location.[2,21,22] This is in line with this study which appears to be a lower proportion of GCB subtypes so that the proportion of GCB and non-GCB subtypes has the same proportion (50%).

Patients with GCB subtype DLCBL are also known to have a better prognosis than non-GCB, with higher overall survival and progression-free survival than non-GCB subtypes. DLBCL patients with GCB subtype had an overall survival of 1 year

90% and 2 years of 74%, while the non-GCB subtype had an overall survival of 1 year 61% and 2 years of 46%, which is lower than the GCB subtype.[2,13,18] This study also showed a similar result, where DLBCL patients with GCB subtype had a significantly better overall survival, which was 77.8% compared to the non-GCB subtype which was 38.9%. When compared with previous studies it appears that the overall survival of GCB subtype DLBCL patients in this study was lower than GCB subtype DLBCL patients in other studies (77.8% vs. 90%), this could be due to 4 GCB subtype patients who died in this study, all of them had advanced tumor stages, and in addition, of these 4 patients, 2 of them were GCB subtypes with MUM1 overexpression or triple positive CD10, BCL6 and MUM1. Based on previous studies, DLBCL patients with triple-positive CD10, BCL6, and MUM1 had lower overall survival than classic GCB subtype DLBCL (i.e. positive CD10, positive/negative BCL6 and negative MUM1.[23,24] In this study it also appeared that DLBCL patients with non-GCB subtype had lower overall survival than non-GCB subtype DLBCL patients in other studies (38.9% vs. 46%), this could

be due to 12 DLBCL patients with non-GCB subtype who died, 9 of them (75%) had an advanced tumor stage.

Patients with DEL subtype were significantly worse prognosis than non-DEL, with lower overall survival. DLBCL patients with DEL subtype had a 3-year overall survival of about 36%, while the non-DEL subtype had a 3-year overall survival of 80%, which was better than the DEL subtype.[2,12,25] This study also showed similar results, where DEL subtype DLBCL had a significantly worse overall survival, 33.3% in DEL subtype compared to 76.2% in non-DEL subtype. Compared with previous studies, it appears that the overall survival of DLBCL patients with DEL and non-DEL subtypes in this study did not differ significantly from those in other studies (33.3% vs. 36% in DEL subtypes and 76.2 vs. 80% in non-DEL subtype). There were 10 patients with DEL subtype who died in the first year of this study, 60% were patients with non-GCB subtype, and 90% had advanced tumor stage. This is consistent with previous studies which showed that patients with DEL and non-GCB subtypes had a worse prognosis with lower overall survival. It also appears in this study that the DLBCL patients who died in the first 1 year, 15 patients, regardless of cell of origin subtype, 80% of them had an advanced stage. Previous studies have also shown that DLBCL patients with stage III or IV have a significantly lower overall survival than stage I or II.[16,17,19,26]

DLBCL patients with DEL subtype are known to have a poorer prognosis and poor therapeutic response to R-CHOP. This is related to the rearrangement of the MYC gene rearrangement, an oncogene protein accompanied by breakpoint at BCL2 gene locus, an anti-apoptotic protein. Damage to these two tumor cell growth-regulating genes has a synergistic effect that makes tumor cells difficult to kill and continues to grow.[12,27,28]

DLBCL patients with non-GCB subtypes are known to have a worse prognosis. This is associated with a higher number of mutations in DLBCL non-GCB subtype than the number of mutations in the GCB subtype. In DLBCL non-GCB subtype mutations were found in at least 20 growth-regulating genes, namely the BCL6, inhibitor of CDK 4 (INK4),

positive regulatory domain containing 1 (PRDM1), tumor necrosis factor-alpha-induced proteins in malignant tumors 3 (TNFAIP3), SPIB, caspase recruitment domain-containing protein 11 (CARD11), myeloid differentiation primary response 88 (MYD88), MYC/BCL2, nuclear factor kappa-light-chain-enhancer of activated B cell (NFKB), CD79A, CD79B, CREBBP, E300, MLL2, MEF2B, TBL1XR1, NOTCH1, NOTCH2, BRAF and TP53, whereas in DLBCL GCB subtype mutations were found in at least 7 growth-regulating genes, namely genes BCL2, EZH2, CREBBP, TNFRSF14, GNA13, SGK1 and C-REL.[29–31]

CONCLUSION

DLBCL patients with non-GCB subtype had significantly lower overall survival than the GCB subtype (38.9% vs 77.8%) (p: 0.026). DLBCL patients with DEL subtype had lower overall survival than non-DEL subtype (33.3% vs. 76.2%) significantly (p: 0.006). DLBCL patients with advanced-stage had lower overall survival compared to the early stage (14.3% vs. 86.4%) significantly (p: 0.000).

ETHICAL APPROVAL

The study was approved by the Ethics Committee at Faculty of Medicine, Diponegoro University (Committee's authorization number: 106/EC/FK-RSDK/III/2018).

CONFLICTS OF INTEREST

The authors affirm no conflict of interest in this study.

FUNDING

AUTHOR CONTRIBUTIONS

Conceptualization, Hermawan Istiadi; methodology, Udadi Sadhana; software, Vega Karlowee; validation, Dik Puspasari; formal analysis, Ika Pawitra Miranti; investigation, Hermawan Istiadi; resources, Devia Eka Listiana; data curation, Hermawan Istiadi; writing—original draft preparation, Hermawan Istiadi; writing—review and editing, Hermawan Istiadi; visualization, Awal Prasetyo; supervision, Dik Puspasari; project administration, Vega Karlowee; funding acquisition, Udadi Sadhana.

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