Majalah Kedokteran Sriwijaya
Th.56 Nomor 3, July 2024

KI67 PROLIFERATION INDEX AND ITS ASSOCIATION WITH DIFFUSE LARGE B-CELL LYMPHOMA STAGE

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ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is the most common invasive subtype of NHL, with an increasing incidence of 150,000 new cases worldwide each year. The Ann Arbor staging system categorizes DLBCL into four stages. Ki67 immunohistochemical examination is a proliferation marker and is used as a prognostic and predictive marker in various organs. This study was conducted to determine the relationship between the Ki67 index, clinicopathological features, and DLBCL stage.This study is an observational analytic study with a cross-sectional retrospective study design using 40 samples of slide archives of DLBCL cases. The sampling method was purposive sampling of DLBCL specimens from January 2019 to December 2021. Staging data is obtained through medical records and based on Ann Arbor staging system assessments. The Ki67 index was assessed on tumor hot spots and averaged in percentage. Categorized as low and high, with a cut-off point value of 85%. The relationship between the Ki67 index and stage was analyzed using Fisher's exact test.Fisher's exact test showed a significant association between the Ki67 index and the DLBCL stage, with a p-value of 0.002. There was no significant association between age, gender, location, and subtype, with p-values of 0.201, 0.607, 0.132, and 0.105, respectively.There is a significant correlation between the Ki67 index (>85%) and the DLBCL stage.

Keywords: *Diffuse large B-cell lymphoma*; *Ki67 index*; *stage; Hans algorithm; proliverative index*

1. INTRODUCTION

The most prevalent non-Hodgkin's lymphoma (NHL) in the world is diffuse large B-cell lymphoma (DLBCL).[1] High-grade DLBCL can be asymptomatic until a late stage of the illness, and symptoms are primarily dependent on the involved location. It is common for DLBCL to affect any organ system.[2] Prognostic indicators are crucial in this situation to identify high-risk individuals who may benefit from more aggressive therapy or the introduction of novel therapeutic agents.[3]

As stand-in markers for gene profiling, immunohistochemical (IHC) staining of CD10, B-cell lymphoma 6 (Bcl-6), and multiple myeloma oncogene 1 (MUM1) is used. One method that is frequently used to subtype DLBCL into GCB and non-GCB phenotypes is the Hans IHC algorithm.[4] WHO acknowledged that although molecular-based gene profiling remains the gold standard for definitive classification, IHC-based categorization serves as a suitable stand-in as gene profiling is not always available. One indicator of cell proliferation is Ki67 immunohistochemistry.[5]

The diagnostic and prognostic value of the Ki67 index in human malignancies has been validated by several studies.[6] In many cases, a high proliferative index also indicates a better response to treatment. On the one hand, a high Ki67 index indicates an aggressive character of a tumor and hence a negative prognosis.[7] Several studies have assessed the proliferative index of DLBCL subtypes in our population.[8] This study aims to evaluate the relationship between the Ki67 index, clinicopathological features, and DLBCL stage.

2. METHODS

Study design and patients

This was a cross-sectional analytic retrospective laboratory-based study. This was conducted at the Department of Anatomic Pathology, Faculty of Medicine University of Sriwijaya, Dr. Mohammad Hoesin Hospital, Palembang, Indonesia. This study included a review of 286 specimens of patients who were diagnosed with DLBCL between January 2019 and December 2021, over three years. Medical record and immunohistochemistry investigation request forms were used to extract the required clinical stage data and histological results. Only cases with histologically and immunohistochemistry confirmed diagnosis of DLBCL were enrolled in the present study.

For further DLBCL subcategorization, IHC stains for CD10, Bcl-6, and MUM1 were used. The Hans algorithm was applied for the subtyping. Cases categorized as GCB subtype DLBCL were those with more than 30% CD10 expression or more than 30% Bcl-6 expression without MUM1 expression (in the absence of CD10 expression). DLBCL is the term for all other immunophenotypes that are not GCB subtypes. The Ki67 index was calculated as an average percentage and used to assess the tumor's hot regions. Data analysis was performed using the Statistical Programme for Social Sciences, version 29.0 (IBM Corps., Armonk, NY).

Regarding the numerical variables of the Ki67 index, we converted them into binomial categorical variables by using a cut-off value. p-values < 0.05 were considered as significant. Ethical issues were thoroughly addressed by relevant guidelines and regulations, while the study protocol was approved by the Ethics Committee ("Ethical Exemption" ID. No.DP.04.03/D.XVIII.6.8/ETIK/078/2024.

Immunohistochemical staining of Ki67 antibody

The DLBCL specimens for the immunohistochemical (IHC) procedure were sectioned at the thickness of 4 μm by using a microtome. The parts were dewaxed by heating them to 60°C for thirty minutes on a hot plate. Subsequently, the tissue slices were dipped in ethanol concentrations ranging from 100% to 70% to hydrate them. Each portion received two drops of a 3% hydrogen peroxide solution, which blocked endogenous peroxidase and prevented background staining for 15 minutes. After that, the slides were rinsed under running tap water. A pH 8.0 heat antigen retrieval solution containing 100X EDTA citrate buffer was employed. After heating the antigen retrieval solution in a pressure cooker until it began to boil, the slides were submerged in it. The pressure cooker's lid was then closed, and the slides were taken out two minutes after the pressure reached its maximum. The slides were then submerged in tap water to stop them from drying out.

3. RESULTS

A total of 40 DLBCL patients were observed in this study. The majority of patients were more than 60 years old (72.5%), and <60 years old (27.5%). Females were slightly more than males and they consisted of 52.5% (21/40). Also, 70% (28/40) of patients were extranodal disease and 30% (12/40) were nodal disease. A total of 82.5% (33/40) of DLBCL were non-GCB subtypes; while 17.5% (7/40) were GCB subtypes (Table 1).

According to the Ann Arbor staging system and its Cotswolds' modification, patients were divided into four groups, stage I, II, III, and IV with 7 (17.5%), 9 (22.5%), 15 (37.5%), and 9 (22.5%), respectively.[9] The Ki67 index was higher (57.5%) than the low Ki67 index (42.5%), based on the ROC curve with a cut-off point of 85%.

Table 1. Clinicopathological characteristics of DLBCL (n=40)

Clinicopathological characteristics	Frequency (n)	Percentage (%)
Age		
<60 y	29	72.5
≥60 y	11	27.5
Gender		
Male	19	47.5
Female	21	52.5
Location		
Nodal	12	30
Extranodal	28	70
Subtype		
GCB	7	17.5
Non GCB	33	82.5
Stage		
I	7	17.5
II	9	22.5
III	15	37.5
IV	9	22.5
Ki67 index		
High (>*cut off point*)	23	57.5
Low (<*cut off point*)	17	42.5

The median score of the Ki67 index was used as a cut-off value to classify the cases of DLBCL into low expression (<85%) and high frequency of the Ki67 index (≥85%). A total of 65.2% of patients < 60 years old had a higher ki67 index than 34.8% of patients ≥60 years old (Table 2) with p value 0.201.

Table 2. Correlation of characteristic DLBCL with Ki67 index

Clinicopathological characteristics	Ki67 index		p-value
	High Frequency (%)	Low Frequency (%)	
Age			
<60 y	15 (65.2)	14 (82.4)	0.201
≥60 y	8 (34.8)	3 (17.6)	
Gender			
Male	11 (47.8)	8 (47.1)	0.607
Female	12 (52.2)	9 (52.9)	
Location			
Nodal	9 (39.1)	3 (17.6)	0.132
Extranodal	14 (60.9)	14 (82.4)	
Subtype			
GCB	6 (26.1)	1 (5.9)	0.105
Non GCB	17 (73.9)	16 (94.1)	
Stage			
I	0 (0)	7 (41.2)	**0.002***
II	4 (17.4)	5 (29.4)	
III	11 (47.8)	4 (23.5)	
IV	8 (34.8)	1 (5.9)	

Fisher's exact test, *p value significant as <0.05

In female patients, the Ki67 index was high in 52.2% of cases, whereas the index was low in 52.9% of cases. In males, a higher Ki6 index was found in 47.8% and 47.1% had a lower Ki67 index.

Patients with extranodal site DLBCL had a higher Ki67 index (60.9%) than the nodal site (39.1%). In contrast, the low Ki67 index was 17.6% for nodal location and 82.4% for extranodal DLBCL patients. A higher proportion of non-GCB subtype DLBCL exhibited greater than 85% Ki67 index than GCB subtype DLBCL.

We found that patients with high scores of Ki67 index expressed in high stage DLBCL. A total of 15 responders with stage III DLBCL had high Ki67 categories in 11 samples (47.8%) and low Ki67 categories in 4 samples (23.5%). In stage IV, one sample (5.9%) had a low Ki67 index and eight samples (34.8%) had a high Ki67 index. Four samples (17.4%) had a high Ki67 index at stage II, whereas five samples (29.4%) had a low Ki67 index. In contrast, no individuals with a high Ki67 index were discovered, and all samples, or seven samples, had a low Ki67 index (41.2%).

The more advanced the stage, allowing the greater the Ki67 proliferation index. A significant association of the Ki67 index was noted with DLBCL stages, with a p-value of 0.002. On the other hand, no significant association was noted with age, gender, location, and subtype DLBCL (with p-values = 0.201, 0.607, 0.132, 0.105).

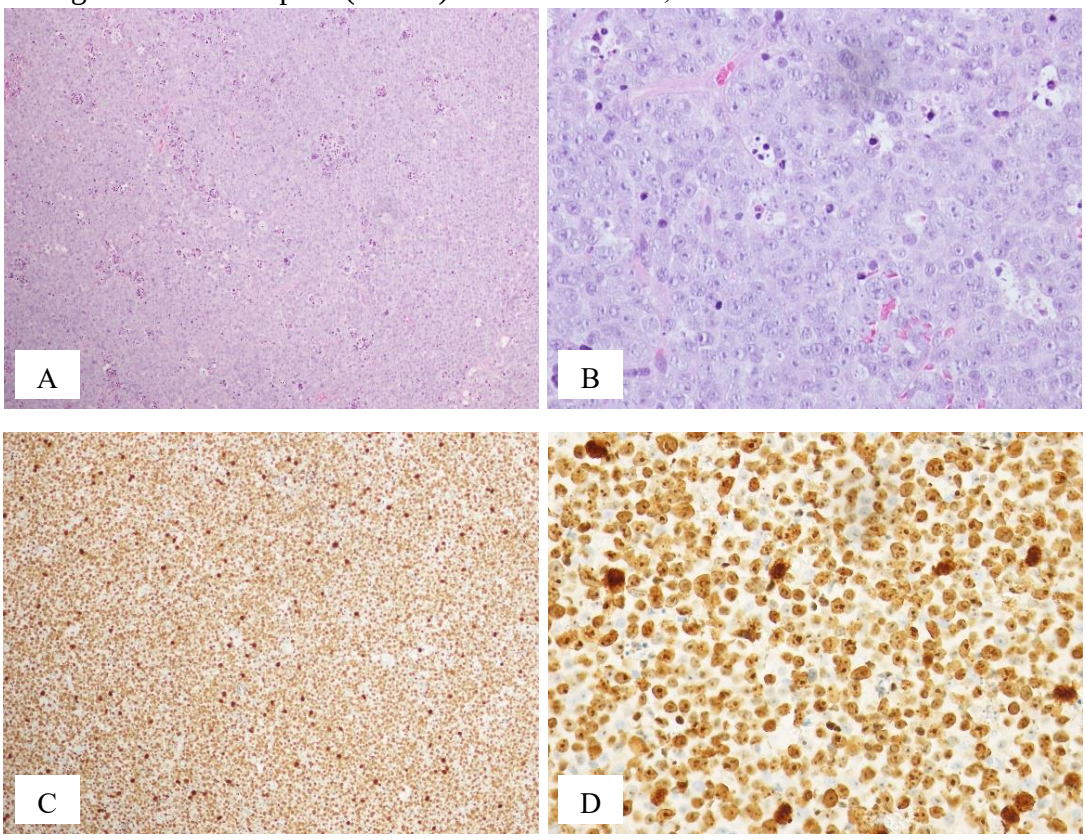

Figure 1. Non-germinal center subtype (Non-GCB) DLBCL. A. Hematoxylin and an eosin-stained section showing a sheet-like architecture (100x). B. Tumor cells are large-sized atypical lymphoid cells with scant cytoplasm (400x). C. Ki67 IHC stain depicting 95% proliferative index in tumor cell (100x). D. (400x).

4. DISCUSSION

Diffuse large B-cell lymphoma is a heterogenic neoplasm that emphasizes the urgent need for the detection of novel prognostic parameters and the development of a personalized treatment strategy.[10,11]

Out of all the NHL subtypes, The most common subtype, accounting for around 40% of NHL cases, is DLBCL.[11] Some instances of DLBCL have a worse prognosis than others. Poor predictive accuracy of the IHC algorithms may be due to their fundamental binary character, which classifies events as either GCB or non-GCB.[3,12]

Although some studies found a negative correlation between the Ki67 index and clinical outcomes and others found a positive correlation between high Ki67 expression and the DLBCL outcome, Ki67 is still thought to be a valuable and reliable measure related to prognosis.[3] Therefore, more research is needed to distinguish between these variables and define the association between Ki67 and the prognosis for DLBCL to validate the earlier contradictory/confusing research.[5]

In this study, we evaluated the Ki67 index in DLBCL subtypes. We observed that the mean Ki-67 index of non-GCB DLBCL was greater than that of GCB-type DLBCL.[5] Nuclear non-histone protein Ki67, which is exclusively linked to cell proliferation, is commonly used to track a variety of cancers, such as neuroendocrine tumors and lymphomas.[13,14]

A previous retrospective study found that individuals with stage 1 DLBCL or the DLBCL community as a whole had worse survival outcomes from extranodal disease than from nodal disease. Extranodal stage I DLBCL had a worse outcome than nodal stage 1 DLBCL.[15] The nodal DLBCL patient population composition has not changed, whereas the extranodal DLBCL patient cohort makeup has changed, with most patients having advanced (Stages III and IV) disease.[16] The prognostic significance of extranodal disease in patients with Ann Arbor stage 2 DLBCL or individuals with illness affecting the same side of the diaphragm, however, has not been well documented in investigations. In DLBCL on the same side of the diaphragm, there were no differences in survival between individuals with solitary extranodal disease (Ann Arbor stage 2 extranodal) and those with nodal disease (Ann Arbor stage 2).[17]

The correlation between a high Ki67 proliferation index and a worse overall survival rate has been validated by several recent studies.[18] Our results were consistent with other studies that reported a high Ki67 index in DLBCL that was not GCB-type.[19]

The limitation of this study is that patient follow-up was not available to assess differences in survival between DLBCL subtypes. Large-scale clinical trials are thus advised to evaluate the follow-up in DLBCL subgroups according to the Ki67 index. Furthermore, gene rearrangements were not assessed by molecular research.

5. CONCLUSION

The Ki67 index has predictive and prognostic value as it is a measure of tumor-cell proliferation. There is a significant correlation between the Ki67 index (>85%) and the DLBCL stage.

ACKNOWLEDGEMENTS

Not applicable

FUNDING

Not applicable

CONFLICT OF INTERESTS

The authors reported no potential conflicts of interest

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