# Relationship Between Clinical Factors of lymphoma DLBCL GCB and Non-GCB Subtype with Ki-67 Proliferation Index in Siloam Karawaci Hospital 2014 - 2018

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**Abstract**

**Background:** Non-Hodgkin large cell B-lymphoma (DLBCL: Diffuse Large B-cell Lymphoma) were classified into two subtypes of Germinal Center B-Cell Like (GCB) and non-Germinal Center B-Cell Like (non-GCB), which GCB has a better prognosis. The Ki-67 antigen is one of the most reliable markers of cell proliferation. This research aims to establish the relationship between proliferation index Ki-67 with DLBCL subtypes and to evaluate the utility of Ki-67 proliferative index as a predictive marker for predicting stages of lymphoma.

**Methods:**
We obtained 60 cases of patient samples DLBCL in Siloam Hospital Lippo Karawaci in 2014-2018. Clinical and pathological data were obtained from medical records. Chi-square methods were used to analyze data.

**Result:** There were more ≤60 years compared to >60 years. In this study, there were more male patients than female patients. lymphoma provided in extranodal were higher than lymphoma in nodal form.

**Conclusions:** There is a relationship between Ki-67 proliferation index with lymphoma GCB and non-GCB subtypes and stage of lymphoma, which can be used as a predictive factor in predicting the stage of lymphoma.

## Introduction

Lymphoma is one of many types of hematological malignancy. Compared with the incidence of leukemia, lymphoma has a higher incidence rate. Lymphomas occur due to malignant changes in lymphoid tissue, usually local but can spread systemically.[1] Histologically, lymphoma is divided into Hodgkin's Lymphoma and Non-Hodgkin Lymphoma (NHL). Epidemiologically, Hodgkin's disease is relatively rare compared to NHL in Asia.[2] Evidence shows that NHL has an incidence rate of 90% of lymphoma that occurs.[3]

Diffuse Large B-cell Lymphoma (DLBCL) is the most common type of Non-Hodgkin Lymphoma and is highly heterogeneous, both clinically and morphologically. DLBCL is divided into two subtypes i.e. Germinal Center B-Cell Like (GCB) and non-Germinal Center B-Cell Like (non-GCB), where GCB has a better prognosis.[4]

DLBCL is the most common malignant tumor of lymphoid tissue originating from B cells. Based on DLBCL

location, DLBCL is divided into nodal and extranodal, where nodal is found in lymph nodes, while extranodal is found in abdomen, skin, brain, and bone and often metastasizes to bone marrow.[4]

Ki-67 is one of the most reliable cell proliferative markers. Ki-67, a nuclear non-histone protein, is synthesized early in cell proliferation. Ki-67 expressions have been widely used in clinical practice as an index for evaluating activity of lymphoma proliferation. High Ki-67 expression is strongly associated with worse overall survival (OS) for NHL. Nevertheless, the relationship between Ki-67 and results with DLBCL lymphoma are still contradictory and inconclusive in various studies. Ki-67 expression is related to differentiation degree in most tumors. Few studies show Ki-67 expression is highest in poorly differentiated carcinoma.

Based on the data above, this study aims to see the relationship between clinical factors of lymphoma DLBCL subtype GCB and non-GCB with a Ki-67 proliferative index at Siloam Hospital in 2014-2018 and to evaluate the utility of Ki-67 proliferative index as a predictive marker for predicting stages of lymphoma.

## Study Methods

### *Population and Sample Research*

This study is a cross-sectional retrospective analytical study. All data were taken from the medical records of DLBCL lymphoma patients at General Hospital Siloam Lippo Karawaci, Tangerang from 2014-2018. Samples with incomplete clinical data, as well as patients who did not undergo the Ki-67 examination were excluded. The sampling technique was carried out sequentially.

### *Staging and Ki-67*

Ann Arbor Staging System was used to classify the stage of lymphoma in this study. High Ki-67 proliferative index is defined by > 70 proliferative index, while low Ki-67 proliferative index is defined by < 70 proliferation index.

### *Study Analysis*

This study was conducted with a total of 60 subjects consisting of GCB and Non-GCB DLBCL patients at Siloam Hospital, Karawaci from 2014 to 2018. The statistical data obtained were analyzed using the Chi Square test. Relationship between Ki-67 proliferative index and stage of the lymphoma used Chi Square Test for Independence or Cross Tabulation analysis. All analyses were done in SPSS 23.0.

## Result

### *Subject Characteristic*

**Table 1.** Frequency and Percentage Based on Characteristic of Study Subjects lymphoma Patients at Siloam Hospitals Karawaci in 2014-2018

| Subject characteristic (n=60) | | Frequency | Persentage (%) |
|---|---|---|---|
| **Age** | ≤60 | 40 | 66.7 |
| | >60 | 20 | 33.3 |
| **Sex** | Male | 32 | 53.3 |
| | Female | 28 | 46.7 |
| **Location** | Extranodal | 34 | 56.7 |
| | Nodal | 26 | 43.3 |
| **Stage** | I | 3 | 5.0 |
| | II | 9 | 15.0 |
| | III | 24 | 40.0 |
| | IV | 24 | 40.0 |

Table 1. shows characteristics of participants enrolled in the study. Age, sex, location and stage were shown in the table above

**Table 2.** Distribution of descriptions of age, sex, location, stadium with subtypes of lymphoma of DLBCL and Ki-67

| | | GCB | Non-GCB | Ki-67 high | Ki-67 low |
|---|---|---|---|---|---|
| **Age** | ≤60 | 22 (55.0%) | 18 (45.0%) | 29 (72,5%) | 11 (27,5%) |
| | >60 | 12 (12 (60.0%) | 8 (40%) | 18 (90.0%) | 2 (10.0%) |
| **Sex** | Male | 18 (56.2%) | 14 (43.6%) | 22 (68.8%) | 10 (31.2%) |
| | Female | 16 (57.1%) | 12 (42.9%) | 25 (89.3%) | 3 (10.7%) |
| **Location** | Extra nodal | 21 (61.8%) | 13 (38.2%) | 26 (76.5%) | 8 (23.5%) |
| | Nodal | 13 (50.0%) | 13 (50.0%) | 21 (80.0%) | 5 (19.2%) |
| **Stage** | I | 3 (100%) | 0 (0.0%) | 0 (0.0%) | 3 (100%) |
| | II | 9 (100%) | 0 (0.0%) | 0 (0.0%) | 9 (100%) |
| | III | 20 (83.3%) | 4 (16.7%) | 23 (95.8%) | 1 (4.2%) |
| | IV | 2 (8.3%) | 22 (91.7%) | 24 (100%) | 0 (0.0%) |

GCB: Germinal Center B-Cell Like, Non-GCB: Non-Germinal Center B-Cell Like

Table 2. shows the distribution of lymphoma subtypes (GCB and non-GCB) and Ki-67 proliferative index level among the characteristics of participants enrolled in the study.

### *Statistical Test Results*

**Table 3.** Results of Analysis of Age, Gender, Location, Stadium with Ki-67

| Subject characteristic (n=60) | | Ki-67 | | P-Value |
|---|---|---|---|---|
| | | High | Low | |
| **Age** | ≤60 | 29 (72.5%) | 11 (27.5%) | 0.186 |
| | >60 | 18 (905) | 2 (10%) | |
| **Sex** | Male | 22 (68.8%) | 10 (31.2%) | 0.066 |
| | Female | 25 (89.3%) | 3 (10.7%) | |
| **Location** | Extra nodal | 26 (76.5%) | 8 (23.5%) | 0.760 |
| | Nodal | 21 (80.0%) | 5 (19.2%) | |
| **Stage** | I | 0 (0.0%) | 3 (100%) | 0.000 |
| | II | 0 (0.0%) | 9 (100%) | |
| | III | 23 (95.8%) | 1 (4.2%) | |
| | IV | 24 (100%) | 0 (0.0%) | |

Table above shows those with stage I 0 (0%) subjects have a high Ki-67 proliferation index and 3 (100%) subjects had a low Ki-67 proliferative index. In stage II, all 9 subjects have a high Ki-67 proliferative index. Stage III included 23 (98.5%) subjects who had a high Ki-67 proliferation index value. Subjects who have stage IV include 24 (100%) subjects who have a high Ki-67 proliferation index value and 0 (0%) subjects who have a low Ki-67 proliferation index value. Through these data, a p-value of 0.00 can be obtained, indicating a significant relationship between the proliferation of Ki-67 with the stage.

**Table 4.** Analysis lymphoma Subtype DLBCL with Proliferation Index Ki-67

| | Ki-67 | | P-Value |
|---|---|---|---|
| | **High** | **Low** | |
| **GCB** | 21 (61.8%) | 13 (38.2%) | 0.000 |
| **Non-GCB** | 26 (100%) | 0 (0.0%) | |

Table 4 above shows lymphoma of the GCB subtype which has a high Ki-67 proliferation index value as many as 21 subjects or equivalent to a percentage of 61.8% and those with a low Ki-67 proliferation index value as many as 13 subjects or equivalent to 38.2%. The Non-GCB Subtype lymphoma which had a high Ki-67 proliferation index value were 26 subjects or equivalent to a percentage of 0%. From these data, a p value of 0.000, indicates that there is a significant relationship between GCB and Non-GCB lymphoma subtypes with Ki-67 proliferation.

## Discussion

From the study analysis above, it indicates that the age group ≤60 years old has a higher percentage of lymphoma than the age group >60 years old. In another study, it was found that the average age of LNH patients in this study was 53 years. This result is the same as findings reported by Mozaheb (2012). This study reported that the average age of diagnosed LNH was 45-55 years old. Similarly, results presented by Yasmin, et al (2005) reported that the average age of diagnosed LNH patients was 50-55 years old.[6,7]

From the result of this study, there were more cases of lymphoma in males. Based on the theory that has been described in the literature review in accordance with the results of the analysis where men are more susceptible to lymphoma than women. Lifestyle or habits that can increase the risk of lymphoma, such as drinking alcohol and smoking are more common habits much favored by men.[5]

From the results of this study, it shows extranodal located lymphoma are more numerous than nodal located lymphoma, this result is in line with the research conducted by Megko S Kennedy, et al.[8]

From the results of this study, it indicates that the Non-GCB subtype lymphoma has a higher stage than the GCB subtype lymphoma, and has a significant relationship with the Ki-67

proliferation index (p value <0.05). The results of this study indicate that the Non-GCB subtype lymphoma has a Ki-67 proliferation index which is higher than the GCB subtype lymphoma. Research conducted by Youssef, et al also showed the same results. Proliferative index value limit of 70% is a value to distinguish bad prognosis and vice versa, so it can be concluded that Non-GCB subtype DLBCL lymphoma has a worse prognosis than GCB DLBCL lymphoma.[9]

The results of this study can be concluded that the p value of clinical factors of age, sex, location, has no relationship with the Ki-67 proliferation index. While the clinical factor stage has a p value <0.05, namely (p: 0.0000), it can be concluded that the stage has a relationship with the Ki-67 proliferation index. Based on a literature review, high Ki-67 is associated with the stage of lymphoma and can be a determinant factor of prognosis for DLBCL lymphoma patients. Other clinical factors such as age, gender, location did not have a significant relationship to Ki-67. According to Kramer, et al. the difference in results obtained was caused by several factors including differences in the size of the sample used, the way of interpreting Ki-67 differences, as well as errors in assessing the expression of Ki-67. In addition, there is no international agreement in determining how to assess the expression of Ki-67 and determine the limit value of Ki-67.[10]

This study was conducted in a cross-sectional manner, data were collected retrospectively and no follow-up was performed for the patients. Therefore, this study cannot be used to search for causality and the mechanism of the relationship between the Ki-67 proliferation index and the DLBCL subtype. Randomized clinical trials are needed to determine the effectiveness of the Ki-67 proliferation index with DLBCL subtype as a diagnostic tool.

## Conclusion

There is a relationship between Ki-67 proliferation index to the stage of lymphoma GCB and non-GCB subtypes. There was no significant relationship between age, gender, and tumor location with the Ki-67 proliferation index. As evidence shows high Ki-67 proliferative index correlates with higher lymphoma stage, therefore Ki-67 biomarker can be used as a predictive factor in predicting the stage of lymphoma.

## Acknowledgment

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