Original Article

Ki-67 and Subtype as Prognostic and Predictive Markers of Diffuse Large B-Cell Lymphoma

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Abstract

Introduction: Since patients with similar International Prognostic Index (IPI) scores have varied outcomes, molecular signatures including Ki-67 overexpression have been studied to prognosticate diffuse large B-cell lymphoma (DLBCL), which have shown varied outcomes. **Objective:** To correlate Ki-67 expression with survival in two biologic subgroups of DLBCL. **Materials and Methods:** One hundred and twelve adults with DLBCL between 2008 and 2012 were identified. Ki-67 overexpression was determined using immunohistochemistry. **Results:** A total of 112 patients of DLBCL were identified and included in the study. The median age was 54 years (18–78 years), with a male/female ratio of 1.8:1. Median survival was greater in patients with low Ki-67 (n = 32) as compared to high Ki-67 (n = 44) (32 m vs. 21.5 m, P = 0.033). In the germinal center B-cell (GCB) subtype, low Ki-67 had a better survival as compared to high Ki-67 (35 m vs. 28 m, P = 0.044), whereas in the non-GCB (NGCB) subtype, the results were same but statistically insignificant (26.5 m vs. 18 m, P = 0.7). In the high IPI arm, low Ki-67 had a better survival (26.5 m vs. 17 m, P = 0.02), whereas in low IPI arm, the results were similar but statistically insignificant (39 m vs. 38 m, P = 0.837). Survival analysis was done in each treatment arm (CHOP and R-CHOP) based on Ki-67 expression (high or low) in GCB and NGCB arms. No statistically significant difference was noted in any of the four arms; 27.5 m versus 34 m (P = 0.738) in high versus low Ki-67 in CHOP-GCB arm, 15 m versus 22 m (P = 0.443) in high versus low Ki-67 in CHOP-NGCB arm, 27 m versus 44 m (P = 0.104) in high versus low Ki-67 in R-CHOP-GCB arm, and 31 m versus 35 m (P = 0.861) in high versus low Ki-67 in R-CHOP-NGCB arm. **Conclusions:** Ki-67 although an indicator of poor outcome, its use to predict outcomes alone in the absence of study of expression of concomitant markers such as myc/BCL6 would cause a bias in results. Furthermore, its relevance in the rituximab era needs further validation.

Keywords: *CHOP, diffuse large B-cell lymphoma, germinal center B-cell, Ki-67, nongerminal center B-cell, R-CHOP*

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease that displays a highly variable clinical outcome. International Prognostic Index (IPI) has been used to prognosticate and risk stratify the patients with DLBCL.[1] Furthermore, the favorable prognosis of germinal center B-cell-like (GCB) subtype has been confirmed in some but not other studies.[2-4] Ki-67 is a proliferation marker expressed in proliferating cells throughout the cell cycle (G1, S, G2, M).[5] Few studies have shown a positive correlation between Ki-67 and activated B-cell-like DLBCL, whereas others have shown with GCB subtype. Prognostic role of Ki-67 has, however, remained unclear owing to contradictory results in various studies.

As compared to the low cure rate of DLBCL in prerituximab era (30%–40%), addition of rituximab to standard chemotherapy has delivered improved results.[6-8] Although there was a controversial prognostic impact of Ki-67 in the prerituximab era, it was found to be a predictor of adverse prognosis in rituximab-treated patients in one study.[9-11] The studies correlating Ki-67 expression and clinical outcomes in DLBCL based on cell of origin are very few in the rituximab era.

Here, we have investigated the prognostic role of Ki-67 in the context of GCB/non-GCB (NGCB) subtypes and compared the responses achieved with and without

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addition of rituximab to the standard chemotherapy regimens.

Materials and Methods

Adult patients of DLBCL diagnosed between 2008 and 2012 were identified from the tumor registry of a tertiary level hospital in south India. Patients fulfilled the following criterion to be included in the study: (1) histologically proven diagnosis of DLBCL, according to the WHO classification of tumors of hematopoietic and lymphoid tissues,[12] (2) availability of adequate amount of paraffin-embedded biopsy material, (3) age >18 years, (4) no previous treatment, (5) no previous neoplasm and no second primary malignancy, (6) no severe coincident diseases, and (7) did not have primary central nervous system lymphoma or posttransplant lymphoproliferative disorder or transformed lymphoma. All patients were staged using the Ann Arbor staging system and evaluated using the IPI. Hans algorithm was used to categorize into GCB and NGCB subtype.[13]

Formalin-fixed paraffin-embedded sections were utilized for immunohistochemistry (IHC). These tissues were stained with conventional hematoxylin and eosin and immunostaining to demonstrate Ki-67, CD10, bcl-6, and MUM-I. Evaluation of the immunostaining was performed within 7 days to avoid antigen degradation. Ki-67 expression was observed in the nucleus of the tumor cells, and percentage of expression was calculated as a ratio of the cells positive for Ki-67 to the total number of malignant cells.

Overall survival (OS) was analyzed from the date of initial diagnosis to the date of death of any cause or last follow-up visit. Chi-square test was used to compare the categorical data. Survival analysis was performed using the Kaplan–Meier method, and log-rank test was used for comparing the variables. $P < 0.05$ was considered to be statistically significant. Statistical analysis was done using the R software.

The standard CHOP regimen consisted of cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, and vincristine 1.4 mg/m^2 (maximum dose 2 mg). Rituximab was administered at the standard dose of 375 mg/m^2 on day 1 with CHOP regimen on day 2. The assessment of treatment response was done in accordance with International Working group Recommendations for Response Criterion for non-Hodgkin's lymphoma (NHL).[14,15]

Results

A total of 112 patients of DLBCL were identified and included in the study. The median age was 54 years (18–78 years), with a male/female ratio of 1.8:1. Bone marrow involvement was seen in 15 patients (13%) and B symptoms in 48 patients (43%). Disease was localized (Stage I/II) in 73 (65%) patients and advanced (Stage III/IV) in 39 (35%) patients. Mean follow-up was 60 months.

Subtype analysis into GCB type and NGCB was done in all the patients, and 64 were recognized as GCB and 48 as NGCB subtype. No significant correlation was found among the groups with regard to age, B symptoms, bulky disease, stage, and extranodal involvement. Median OS in GCB subtype was 34 months and in NGCB was 22 months ($P = 0.043$) [Figure 1].

Various Ki-67 cutoffs were evaluated but the most significant differences in OS occurred at 70% cutoff. Hence, cutoff of 70% or more was used to classify into high and low and was available for 76 patients. Forty-four were identified with high and 32 with low Ki-67. Relationship between baseline clinical features and Ki-67 was done using Chi-square test. No significant association was detected with age, B symptoms, bulky disease, stage, extranodal involvement, and treatment received (CHOP or R-CHOP). Median survival was greater in patients with low Ki-67 ($n = 32$) as compared to high Ki-67 ($n = 44$) (32 m vs. 21.5 m, $P = 0.033$) [Figure 2]. Further, subgroup analysis in Ki-67 arm was done based on subtype (GCB or NGCB) and IPI risk (high or low). In the GCB subtype, low Ki-67 had a better survival as compared to high Ki-67 (35 m vs. 28 m, $P = 0.044$) [Figure 3], whereas in the NGCB subtype, the results were same but statistically insignificant (26.5 m vs. 18 m, $P = 0.7$). In the high IPI arm, low Ki-67 had a better survival (26.5 m vs. 17 m, $P = 0.02$) [Figure 4], whereas in low IPI arm, the results were similar but statistically insignificant (39 m vs. 38 m, $P = 0.837$). Table 1 summarizes the impact of immunohistochemical markers on survival as discussed above.

Further subgroup analysis was done based on treatment received. Treatment was received by 109 patients, CHOP in 66 and R-CHOP in 43 patients. R-CHOP arm had a significant survival advantage over the CHOP arm (38 m vs. 24 m; $P < 0.05$) [Figure 5]. The two treatment arms were first analyzed independently based on cell type (GCB/NGCB) and Ki-67 (high/low). In the CHOP group,

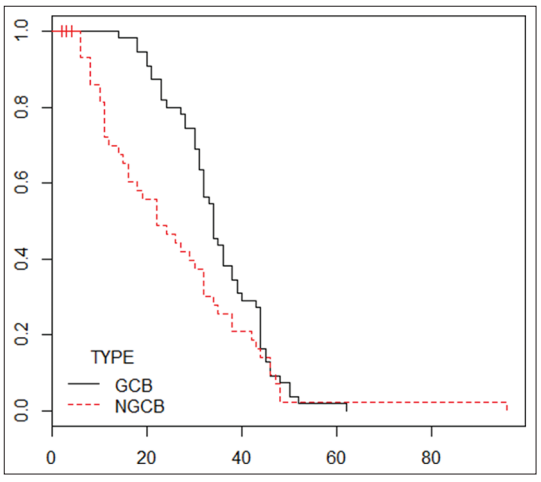

Figure 1: GCB vs NGCB

GCB had a survival advantage over NGCB (32 m vs. 14 m; $P < 0.05$) [Figure 6], whereas in the R-CHOP group, no significant difference was seen (44 m 34.5 m; $P = 0.76$). In R-CHOP group, low Ki-67 had a survival advantage over high Ki-67 (43.5 m vs. 30 m; $P < 0.05$) [Figure 7], whereas no significant difference was seen in CHOP group (26.5 m vs. 24.5 m; $P = 0.6$). Later, analysis was done in each treatment arm (CHOP and R-CHOP) based on Ki-67 expression (high or low) in GCB and NGCB arms. No statistically significant difference was noted in any of the four arms; 27.5 m versus 34 m ($P = 0.738$) in high versus low Ki-67 in CHOP-GCB arm, 15 m versus 22 m ($P = 0.443$) in high versus low Ki-67 in CHOP-NGCB arm, 27 m versus 44 m ($P = 0.104$) in high versus low Ki-67 in R-CHOP-GCB arm, and 31 m versus 35 m ($P = 0.861$) in high versus low Ki-67 in R-CHOP-NGCB arm. Table 2 summarizes the impact of subtype and Ki-67 in CHOP and R-CHOP arms.

To summarize, GCB and low Ki-67 subtypes had a survival advantage independently. However, on subgroup analysis, low Ki-67 had a significant survival advantage in only GCB and high IPI arms. In two treatment arms, GCB had a survival advantage in CHOP arm and low Ki-67 in R-CHOP arm. However, on further subgroup analysis based on Ki-67 (high/low) in GCB/NGCB arms in CHOP/R-CHOP groups, no significant differences were noticed. Therefore, the independent prognostic significance of Ki-67 is nullified when grouping is done on the basis of cell type (GCB/NGCB). Furthermore, addition of rituximab nullifies the poor prognostic effect of NGCB subtype.

Discussion

Cell proliferation rate indicated by Ki-67 PI could be of diagnostic as well as prognostic significance in DLBCL. Ki-67 monoclonal antibody is a large nuclear protein doublet expressed in all phases of cell cycle (except G0) in proliferating cells, first generated by Scholzen and Gerdes.[16] As MAb Ki-67 is normally not immunoreactive in formalin-fixed, paraffin-embedded tissue sections, MIB-1 was raised which had an immunoreactive pattern similar to that of MAb Ki-67 in tissue sections.[17] Nowadays, latter is the most popular approach for measuring the growth

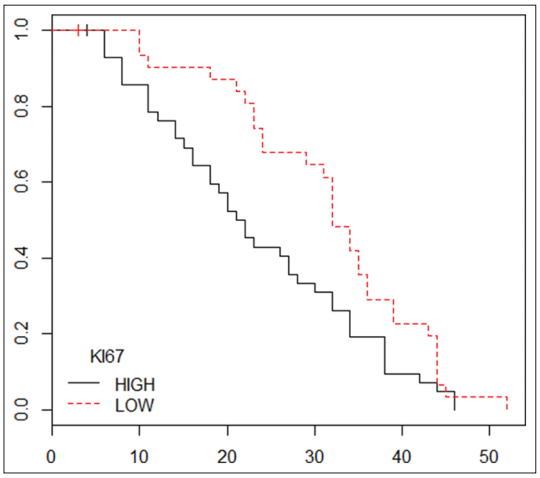

Figure 2: HIGH vs LOW Ki67

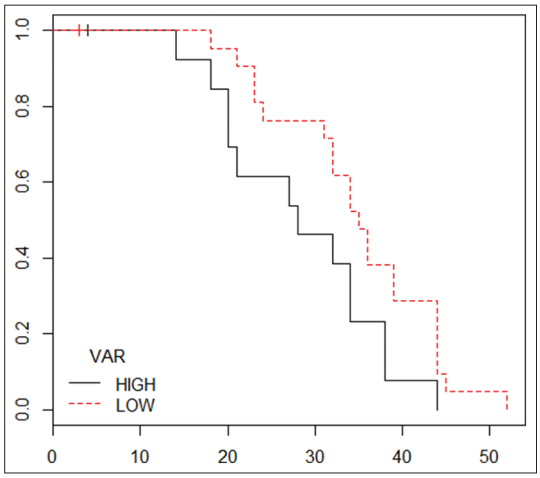

Figure 3: GCB TYPE – HIGH vs LOW Ki67

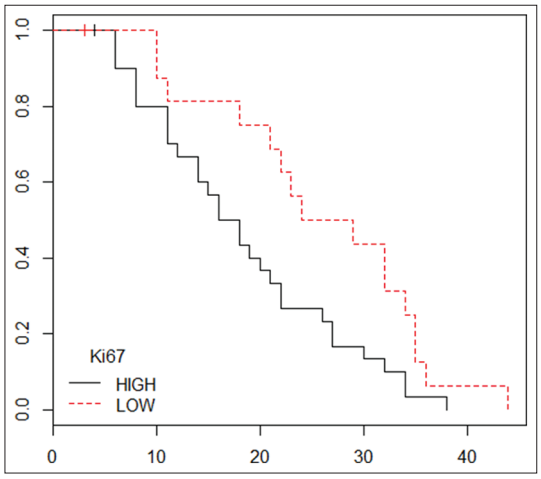

Figure 4: HIGH IPI – HIGH vs LOW Ki67

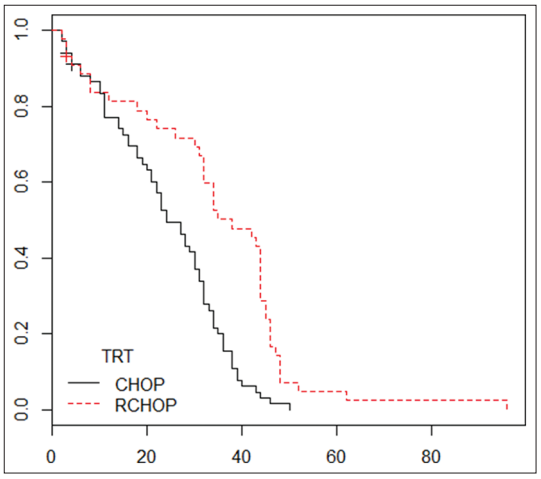

Figure 5: CHOP vs RCHOP

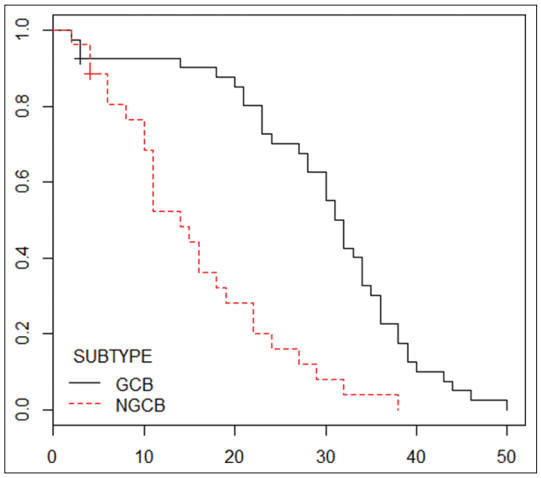

Figure 6: CHOP – GCB vs NGCB

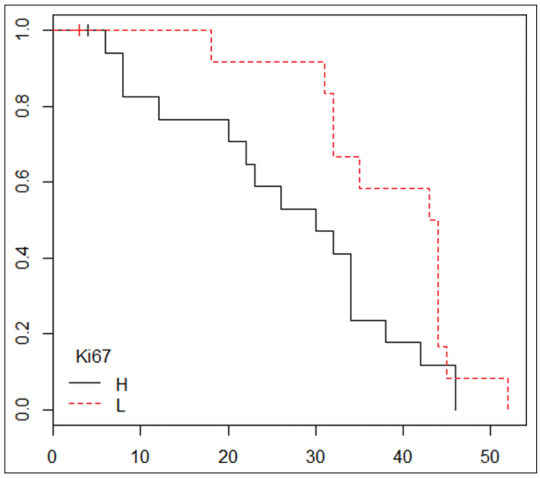

Figure 7: RCHOP – HIGH vs LOW Ki67

fraction of cell populations, which could be correlated with histological grade, clinical behavior, and outcomes in various tumor types.[18-21]

Earlier studies have used proliferation markers to reliably distinguish between high- and low-grade NHL. In working formulation,[22] mean MIB-1 was 29.7% in low-grade, 53.1% in intermediate-grade, and 75.1% in high-grade lymphomas. In Kiel classification,[23] 39.5% for low-grade and 75.7% for high-grade lymphomas were the mean proliferation indices (PIs). Prognostic significance of Ki-67 has been examined previously in various studies, and a wide range of expression has been noticed. In one study,[24] cutoff of 70% distinguished the patients of DLBCL with good and poor prognosis and had a survival implication. Moreover, Ki-67 significantly added to the prognosis of the patients of DLBCL in GCB and high IPI arms.[24]

Jerkeman *et al*. conducted the largest study in this regard, in which Ki-67 was evaluated in 185 cases.[25] Compared to either moderate or high Ki-67, low Ki-67 was associated with low failure-free survival. Moreover, patients with either low or high Ki-67 showed a trend toward OS than those with moderate expression. In a similar study by Grogan *et al*.,[26] high Ki-67 (>60%) was a strong and independent predictor of poor survival. Miller *et al*. also demonstrated the independent effect of cell proliferation on survival.[27] Broyde *et al*. demonstrated the prognostic significance of Ki-67 in low IPI and bulky disease.[24] On the other hand, few studies had shown an opposite effect. Hall *et al*. showed that in cases with PI >80%, patients who achieved a good response to therapy were less likely to relapse.[28] Hasselblom *et al*. demonstrated that low rather than high Ki-67 is an adverse prognostic factor in respect to PFS and OS.[11] The International Lunenburg Lymphoma Biomarker Consortium examined the variation in scoring of different molecular prognostic markers by IHC in DLBCL.[29] They noted poor reproducibility between laboratories for Ki-67 staining and concluded that it should be used only in the context of clinical trials.

Table 1: Summarizing the impact of immunohistochemistry on survival irrespective of treatment received

	n	Median OS	*P*
Ki-67			
High	44	21.5	0.022
Low	32	32	
GCB type			
High Ki-67	15	28	0.044
Low Ki-67	22	35	
NGCB type			
High Ki-67	29	18	0.705
Low Ki-67	10	26.5	
High IPI			
High Ki-67	31	17	0.020
Low Ki-67	17	26.5	
Low IPI			
High Ki-67	15	38	0.837
Low Ki-67	13	39	

OS: Overall survival, NGCB: Nongerminal center B-cell, IPI: International Prognostic Index

Therefore, Ki-67 appears to have a predictive value for OS in DLBCL as an independent prognostic factor and in those with GCB type and high IPI.

Addition of rituximab to standard chemotherapy has remarkably improved the response and survival in DLBCL, and R-CHOP is the standard treatment for DLBCL.[6-8] For the antitumor activity of rituximab, several mechanisms such as antibody-dependent cellular cytotoxicity, apoptosis, and complement-dependent cytotoxicity have been implicated.[30,31]

Addition of rituximab has greatly improved the survival in NGCB DLBCL, which was significantly worse in the prerituximab era.[32,33] This might be due to inhibition of nuclear factor-κβ, which is responsible for proliferation and survival of NGCB cell lines.[34,35] In our study also, the poor prognostic impact of the NGCB subtype was nullified by the addition of rituximab to the CHOP regimen.

Table 2: Summarizing the impact of subtype and Ki-67 in CHOP and R-CHOP group

	n	Median OS	*P*
CHOP	66	24	<0.05
R-CHOP	43	38	
CHOP group			
GCB	40	32	<0.05
NGCB	25	14	
High Ki-67	24	24.5	0.6
Low Ki-67	18	26.5	
GCB			
High Ki-67	11	27.5	0.738
Low Ki-67	12	34	
NGCB			
High Ki-67	15	15	0.443
Low Ki-67	7	22	
R-CHOP group			
GCB	20	44	0.76
NGCB	22	34.5	
High Ki-67	18	30	<0.05
Low Ki-67	13	43.5	
GCB			
High Ki-67	4	27	0.104
Low Ki-67	10	44	
NGCB			
High Ki-67	14	31	0.861
Low Ki-67	3	35	

NGCB: Nongerminal center B-cell, OS: Overall survival

In the present study, we studied and compared the prognostic impact of Ki-67 in patients who received CHOP and R-CHOP as treatment. Ki-67 had a prognostic relevance to survival in the R-CHOP arm, with high Ki-67 associated with shorter OS. This is in accordance with various studies in the postrituximab era, identifying high Ki-67 as a predictive marker of poor survival. However, before the rituximab era, most of the controversial results were obtained. The Nordic Lymphoma Group Study and various other studies did not show a significant difference in DLBCL patients categorized on the basis of Ki-67 expression.[25,36,37] This is in accordance to our study, in which Ki-67 had no survival implication in the CHOP arm.

Later, analysis was done in each treatment arm (CHOP and R-CHOP) based on Ki-67 expression (high or low) in GCB and NGCB arms. No statistically significant difference was noted in any of the four arms, but the maximum was in the R-CHOP-GCB arm. Therefore, the independent prognostic significance of Ki-67 was nullified when grouping was done on the basis of cell type (GCB/NGCB). Another study by Li *et al*. demonstrated poor survival with high Ki-67 expression in NGCB arm in patients receiving R-CHOP chemotherapy.[38]

Conclusions

Ki-67 appears to have a predictive value for OS in DLBCL as an independent prognostic factor and in those with GCB type and high IPI. Addition of rituximab significantly improves the survival in the NGCB arm. Although Ki-67 has an effect on survival in R-CHOP arm irrespective of the subtype (GCB/NGCB), the prognostic significance is nullified when grouped according to subtype (GCB/NGCB). Although Ki-67 showed a trend toward significance in the R-CHOP-GCB arm, more studies are needed in this regard to reach a particular conclusion.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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