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Ki-67 Proliferation Index in Lymphoma Subtypes: Immunohistochemical Analysis With Clinicopathological Correlations and Prognostic Implications

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Faris M. Elmahdi [1], Leen N. Almohammadi [2], Yusra O. Zakaria [2], Danah A. Alraddadi [2], Ajwan M. Alahmadi [2], Sara AlTom [1]

1. Basic Sciences, Al-Rayan National College of Medicine, Madinah, SAU 2. Medicine and Surgery, Al-Rayan National College of Medicine, Madinah, SAU

Corresponding author: Faris M. Elmahdi, alfaris-sust@hotmail.com

Abstract

Background: The Ki-67 proliferation index (PI) is a well-established marker of tumor growth activity in lymphomas. Despite its diagnostic utility, standardized cutoff values and subtype-specific reference ranges remain poorly defined. This study evaluated Ki-67 expression across major lymphoma subtypes and its ability to discriminate between lymphoma groups of different biological behavior.

Methods: A retrospective cross-sectional study was conducted on 87 confirmed lymphoma cases retrieved from the pathology archives of hospitals in Al-Madinah Al-Munawwarah, Saudi Arabia. Immunohistochemistry using the MIB-1 monoclonal antibody was performed on formalin-fixed paraffin-embedded (FFPE) tissue. Ki-67 PI was assessed by manual counting of at least 500 tumor cells in hot-spot areas. Statistical analyses included the Kruskal-Wallis test, Mann-Whitney U test, chi-square test, and receiver operating characteristic (ROC) curve analysis.

Results: The cohort comprised 87 cases: diffuse large B-cell lymphoma (DLBCL) (n = 27, 31.0%), follicular lymphoma (FL) (n = 16, 18.4%), Hodgkin lymphoma (HL) (n = 13, 14.9%), mantle cell lymphoma (MCL) (n = 10, 11.5%), T-cell lymphomas (TCL) (n = 9, 10.3%), marginal zone/other indolent B-cell lymphomas (MZL) (n = 7, 8.0%), and Burkitt lymphoma (BL) (n = 5, 5.7%). Mean Ki-67 PI differed significantly across subtypes ($p<0.001$): BL (92.4 ± 4.2%), TCL (71.8 ± 18.3%), DLBCL (68.5 ± 16.7%), HL (52.3 ± 14.6%), MCL (38.6 ± 22.4%), FL (28.7 ± 15.8%), and MZL (22.3 ± 11.6%). For ROC analysis, HL was analyzed as a separate intermediate-behavior group, with the aggressive group comprising DLBCL, BL, and TCL (n = 41) and the indolent group comprising FL, MCL, and MZL (n = 33). A Ki-67 cutoff of 40% showed good discriminatory ability (area under the ROC curve (AUC) = 0.87, sensitivity = 92.7%, specificity = 75.8%).

Conclusion: Ki-67 PI demonstrates meaningful variation across lymphoma subtypes. A cohort-derived cutoff of 40% showed good discrimination between the predefined aggressive and indolent non-Hodgkin lymphoma (NHL) groups, although it should be regarded as exploratory. Standardized Ki-67 assessment may support routine lymphoma diagnostics, with application of subtype-specific thresholds once diagnosis is established, pending external validation and wider methodological harmonization across laboratories.

Categories: Pathology, Oncology, Internal Medicine
Keywords: immunohistochemistry, ki-67, lymphoma, mib-1, prognosis, proliferation index

Introduction

Lymphomas are a heterogeneous group of hematological malignancies arising from lymphoid tissues, collectively accounting for approximately 3-4% of all cancers and ranking as the seventh most common cancer globally [1]. Non-Hodgkin lymphoma (NHL) alone contributes to an estimated 544,000 new diagnoses and 260,000 deaths annually worldwide [2]. In Saudi Arabia, lymphomas represent a significant proportion of hematological malignancies, with distinct epidemiological features including a relatively younger age at diagnosis and a higher proportion of aggressive subtypes compared to Western populations [3,4]. With more than 90 distinct entities recognized by the World Health Organization (WHO), lymphomas demand precise diagnostic and prognostic tools to guide treatment decisions and predict clinical outcomes [5].

Ki-67 is a nuclear protein encoded by the MKI67 gene on chromosome 10q26 and is one of the most widely used markers of cellular proliferation in diagnostic pathology [6]. Recent work has refined our understanding of Ki-67 as a graded rather than a binary marker, correlating more precisely with cell cycle dynamics and tumor proliferative capacity [7]. Immunohistochemical detection using the MIB-1 monoclonal antibody on formalin-fixed paraffin-embedded (FFPE) tissue is standard practice [8]. The Ki-67 proliferation

index (PI), defined as the percentage of Ki-67-positive tumor cells, has been extensively investigated as a prognostic marker across multiple malignancies, including lymphomas [9]. The biological basis for its prognostic value rests on the well-established principle that rapidly proliferating tumors tend to exhibit more aggressive behavior, higher metastatic potential, and less predictable responses to conventional therapies [10].

In lymphoma diagnostics, Ki-67 PI shows subtype-specific expression patterns reflecting the underlying biological heterogeneity of these diseases. In diffuse large B-cell lymphoma (DLBCL), the most common aggressive NHL, reported mean Ki-67 values range from 60% to 80%, with proposed prognostic cutoffs varying from 70% to 90% [11-14]. Follicular lymphoma (FL), an indolent B-cell malignancy, typically exhibits lower Ki-67 values (mean 20-35%), with higher proliferation rates associated with histological transformation and adverse outcomes [15,16]. Mantle cell lymphoma (MCL) demonstrates wide variability, ranging from below 10% in indolent classical variants to above 80% in blastoid forms, and a Ki-67 cutoff of 30% is an established independent prognostic factor [17-19]. Hodgkin lymphoma (HL), a distinct lymphoma entity with unique biology and treatment approach, shows intermediate Ki-67 expression (40-60% in Reed-Sternberg cells), assessed separately from reactive background lymphocytes [20]. Burkitt lymphoma (BL), characterized by MYC translocation and extraordinarily rapid cell division, consistently demonstrates Ki-67 approaching 95-100%, which serves as a diagnostic hallmark [21,22]. T-cell lymphomas (TCL) show variable Ki-67 expression depending on subtype [23,24].

Despite an extensive body of published data, several important gaps remain. Significant methodological heterogeneity in Ki-67 assessment reduces inter-laboratory reproducibility; methods vary between manual counting and digital image analysis and between hot-spot evaluation and whole-section scanning, each yielding different results for the same specimen [25,26]. Proposed prognostic cutoff values vary from 30% to 90% across studies, with no universally accepted thresholds [27]. Most published studies focus on individual lymphoma subtypes, limiting cross-subtype comparisons [28]. Data from Saudi Arabia and the broader Arabian Peninsula remain sparse [29]. Integration of Ki-67 PI with established clinical prognostic tools such as the International Prognostic Index (IPI) and Ann Arbor staging remains incompletely characterized [30].

This study was designed to address some of these gaps through immunohistochemical analysis of Ki-67 across major lymphoma subtypes in a retrospective 87-case cohort from hospitals in Al-Madinah Al-Munawwarah, Saudi Arabia. The primary objectives were: (i) to evaluate Ki-67 PI distribution across seven lymphoma subtypes; and (ii) to assess the ability of Ki-67 PI to distinguish between predefined lymphoma groups according to biological behavior, identifying an exploratory cutoff for discriminating aggressive NHL from indolent disease, with HL analyzed as a separate intermediate entity. Survival and other outcome data were not assessed.

Materials And Methods

Study design and setting

This retrospective cross-sectional study was based on archived pathology material and existing medical records from multiple hospitals in Al-Madinah Al-Munawwarah, Saudi Arabia. Cases were identified from departmental pathology archives and electronic medical record systems.

Study participants

A total of 87 confirmed lymphoma cases were included. Cases were identified through a systematic review of the pathology database using ICD-O codes for lymphoid neoplasms [31]. All archived cases meeting the eligibility criteria at the participating hospitals were included. Inclusion criteria were: (i) histologically confirmed lymphoma per WHO classification [5,22]; (ii) adequate FFPE tissue with at least 70% tumor cellularity; (iii) complete immunohistochemical panel available; (iv) complete clinical records available; and (v) treatment-naive status at time of biopsy. Cases were excluded if tissue was inadequate, if patients had received prior chemotherapy or radiotherapy, if composite or HIV-associated lymphomas were present, if post-transplant lymphoproliferative disorder was diagnosed, or if clinicopathological data were incomplete.

Histopathological classification

All cases were reviewed by two hematopathologists. Classification followed the revised fourth edition WHO classification (2017), updated with fifth edition criteria (2022) where applicable [5,22]. A comprehensive immunohistochemical panel was applied including CD20, CD3, CD5, CD10, CD23, BCL2, BCL6, MUM1/IRF4, cyclin D1, CD30, CD15, and PAX5. DLBCL cases were classified into germinal center B-cell (GCB) and non-GCB subtypes using the Hans algorithm [32]. FL was graded per WHO criteria [22]. HL subtypes were classified by morphology and immunophenotype and treated as a distinct clinical entity separate from NHL [33].

Immunohistochemical protocol

Ki-67 immunohistochemistry was performed on 4-µm sections from archived FFPE blocks. Following deparaffinization and rehydration, heat-induced epitope retrieval used citrate buffer (pH 6.0) at 120°C for 20

minutes [34]. Endogenous peroxidase was blocked with 3% hydrogen peroxide. The primary antibody was mouse monoclonal anti-Ki-67 (clone MIB-1; Dako/Agilent, Santa Clara, USA) at a 1:100 dilution for 60 minutes at room temperature [8]. Detection used an avidin-biotin-peroxidase complex (EnVision FLEX; Dako/Agilent, Santa Clara, USA) with 3,3'-diaminobenzidine (DAB) as chromogen. Mayer's hematoxylin served as counterstain. Tonsil tissue was used as a positive control; omission of primary antibody served as a negative control [35].

Ki-67 scoring methodology

Ki-67 PI was assessed using a standardized hot-spot method. Hot-spot regions were identified at low magnification (×40, ×100), and at least 500 tumor cells were counted at high magnification (×400) across multiple non-overlapping high-power fields [35]. Only unequivocal nuclear staining was scored positive. Interobserver reproducibility was assessed in 23 cases (26.4%) by a blinded second pathologist, yielding excellent agreement (intraclass correlation coefficient (ICC) = 0.91, 95% confidence interval (CI): 0.84-0.96) [36]. Digital image analysis using QuPath v0.3.2 (Queen's University Belfast, Northern Ireland, UK) was performed in 18 representative cases to validate manual results, confirming strong correlation (Pearson $r = 0.88$, $p<0.001$) [37].

Clinical data

Clinical information was obtained from existing medical records. Clinical staging followed the Ann Arbor system [38]. B symptoms were defined as unexplained fever >38°C, drenching night sweats, or unintentional weight loss >10% of body weight within six months. Elevated lactate dehydrogenase (LDH) was defined as values above the institutional reference range (>250 U/L). IPI was calculated for DLBCL cases only [39], reflecting its validated application in aggressive NHL; Mantle Cell Lymphoma International Prognostic Index (MIPI) was calculated for MCL cases [40].

Statistical analysis

Analyses were performed using IBM SPSS Statistics v26.0 (IBM Corp., Armonk, USA) and GraphPad Prism v9.0 (GraphPad Software, Boston, USA). The Kruskal-Wallis H test with Bonferroni-corrected post-hoc Mann-Whitney U comparisons was used across subtypes [41]. Receiver operating characteristic (ROC) curve analysis with the Youden index identified the optimal Ki-67 cutoff for discriminating aggressive NHL (DLBCL, BL, TCL) from indolent lymphomas (follicular lymphoma (FL), MCL, marginal zone/other indolent B-cell lymphomas (MZL)); HL was analyzed separately given its distinct clinical behavior [42]. For this exploratory ROC analysis, lymphoma entities were operationally categorized into predefined aggressive and indolent groups. This grouping was used solely for the binary ROC analysis and does not imply that these entities are biologically uniform. In particular, MCL and TCL are recognized as heterogeneous diseases with variable clinical and proliferative behavior. The ROC curve was constructed using Ki-67 PI as a continuous variable, and the area under the ROC curve (AUC) with its 95% CI was estimated nonparametrically. The optimal cutoff was defined as the value maximizing the Youden index (sensitivity + specificity - 1). The chi-square test was used to compare categorical variables between groups. Statistical significance was set at $p<0.05$ (two-tailed).

Ethical considerations

Given the retrospective nature of this study, the requirement for individual informed consent was waived by the Research Ethics Committee of Al-Rayan Medical Colleges (AMC) (approval number: HA-03-M-122-099).

Results

The study population consisted of 87 patients with a median age at diagnosis of 52 years (range, 18-79 years; mean ± SD, 51.6 ± 16.4 years). Male patients accounted for 50 cases (57.5%) and female patients for 37 (42.5%), resulting in a male-to-female ratio of 1.4:1. By age group, 32 patients (36.8%) were aged ⩽45 years, 36 (41.4%) were 46-65 years, and 19 (21.8%) were >65 years.

DLBCL was the most common subtype (n = 27, 31.0%), followed by FL (n = 16, 18.4%), HL (n = 13, 14.9%), MCL (n = 10, 11.5%), TCL (n = 9, 10.3%), MZL (n = 7, 8.0%), and BL (n = 5, 5.7%). Among DLBCL cases, 15 (55.6%) were classified as GCB subtype and 12 (44.4%) as non-GCB subtype. FL was graded as grade 1 in six cases (37.5%), grade 2 in seven cases (43.8%), and grade 3 in three cases (18.8%). HL subtypes included nodular sclerosis (n = 8, 61.5%), mixed cellularity (n = 4, 30.8%), and lymphocyte-rich (n = 1, 7.7%).

Regarding clinicopathological characteristics, 20 patients (23.0%) presented with early-stage disease (Ann Arbor stages I-II), whereas 67 (77.0%) had advanced-stage disease (Ann Arbor stages III-IV). B symptoms were present in 49 patients (56.3%), and elevated serum LDH (>250 U/L) was recorded in 59 patients (67.8%). Among patients with DLBCL (n = 27), the IPI risk distribution was as follows: low risk, seven cases (25.9%); low-intermediate risk, eight cases (29.6%); high-intermediate risk, seven cases (25.9%); and high risk, five cases (18.5%). The demographic and clinicopathological characteristics of the study population are summarized in Table *1*.

Characteristic	Value
Age at diagnosis, mean ± SD (years)	51.6 ± 16.4
Median age, years (range)	52 (18-79)
≤45 years, n (%)	32 (36.8)
46-65 years, n (%)	36 (41.4)
>65 years, n (%)	19 (21.8)
Male, n (%)	50 (57.5)
Female, n (%)	37 (42.5)
Male:female ratio	1.4:1
Diffuse large B-cell lymphoma (DLBCL), n (%)	27 (31.0)
Follicular lymphoma (FL), n (%)	16 (18.4)
Hodgkin lymphoma (HL), n (%)	13 (14.9)
Mantle cell lymphoma (MCL), n (%)	10 (11.5)
T-cell lymphomas (TCL), n (%)	9 (10.3)
Marginal zone/other indolent B-cell lymphomas (MZL), n (%)	7 (8.0)
Burkitt lymphoma (BL), n (%)	5 (5.7)
Ann Arbor stage I-II, n (%)	20 (23.0)
Ann Arbor stage III-IV, n (%)	67 (77.0)
B symptoms present, n (%)	49 (56.3)
Elevated LDH (>250 U/L), n (%)	59 (67.8)
IPI low risk (DLBCL only, n=27), n (%)	7 (25.9)
IPI low-intermediate risk, n (%)	8 (29.6)
IPI high-intermediate risk, n (%)	7 (25.9)
IPI high risk, n (%)	5 (18.5)

TABLE 1: Demographic and clinical characteristics of the study (N = 87)

Statistical significance was predefined at p<0.05.

LDH: lactate dehydrogenase; IPI: International Prognostic Index; SD: standard deviation.

Ki-67 PI differed significantly among lymphoma subtypes (Kruskal-Wallis H = 59.4, p<0.001). BL demonstrated the highest proliferative activity (mean Ki-67 PI, 92.4 ± 4.2%), followed by TCL (71.8 ± 18.3%) and DLBCL (68.5 ± 16.7%). HL showed intermediate proliferative activity (52.3 ± 14.6%), whereas indolent lymphomas exhibited substantially lower Ki-67 values, including MCL (38.6 ± 22.4%), FL (28.7 ± 15.8%), and MZL (22.3 ± 11.6%). Detailed Ki-67 distributions are presented in Table *2*.

Subtype	n	Mean ± SD (%)	Median (%)	Range (%)	IQR (%)
Burkitt lymphoma (BL)	5	92.4 ± 4.2	93	85-98	90-95
T-cell lymphomas (TCL)	9	71.8 ± 18.3	75	42-92	58-85
Diffuse large B-cell lymphoma (DLBCL)	27	68.5 ± 16.7	70	35-92	58-82
Hodgkin lymphoma (HL)*	13	52.3 ± 14.6	53	28-78	42-63
Mantle cell lymphoma (MCL)	10	38.6 ± 22.4	32	8-82	22-52
Follicular lymphoma (FL)	16	28.7 ± 15.8	25	10-62	18-35
Marginal zone/other indolent B-cell lymphomas (MZL)	7	22.3 ± 11.6	20	8-42	15-28

TABLE 2: Ki-67 proliferation index across lymphoma subtypes (N = 87)

Kruskal-Wallis test: H = 59.4, df = 6, p<0.001. Statistical significance was predefined at p<0.05.

*HL was analyzed as a separate intermediate biological entity and was excluded from the aggressive versus indolent ROC analysis; Ki-67 was assessed in Hodgkin/Reed-Sternberg cells only.

IQR: interquartile range; SD: standard deviation.

For comparative analysis, HL was analyzed separately because of its distinct biological behavior and therapeutic characteristics. The aggressive NHL group comprised DLBCL, BL, and TCL (n = 41, 47.1%), whereas the indolent group included FL, MCL, and MZL (n = 33, 37.9%). HL cases (n = 13, 14.9%) were evaluated independently.

Aggressive NHL demonstrated significantly higher Ki-67 PI than indolent lymphomas (72.1 ± 17.6% vs. 30.3 ± 17.9%; Mann-Whitney U test, p<0.001), with median values of 70% (IQR, 58-84%) and 25% (IQR, 18-38%), respectively. HL exhibited intermediate Ki-67 values (52.3 ± 14.6%), significantly higher than those of the indolent group (p = 0.001) but lower than those of aggressive NHL (p = 0.018).

Among aggressive NHL cases, 38 of 41 patients (92.7%) demonstrated Ki-67 expression ⩾40%, compared with only eight of 33 patients (24.2%) in the indolent group (χ^2 = 46.8, p<0.001). Nine of 13 HL cases (69.2%) also demonstrated Ki-67 expression ⩾40%. Comparative findings are summarized in Table *3*.

Parameter	Aggressive NHL (n = 41)	HL (n = 13)	Indolent (n = 33)	p-value
Mean Ki-67 PI ± SD (%)	72.1 ± 17.6	52.3 ± 14.6	30.3 ± 17.9	<0.001
Median Ki-67 PI (%)	70	53	25	<0.001
Range (%)	35-98	28-78	8-82	-
IQR (%)	58-84	42-63	18-38	-
Ki-67 ≥40%, n (%)	38 (92.7)	9 (69.2)	8 (24.2)	<0.001
Ki-67 <40%, n (%)	3 (7.3)	4 (30.8)	25 (75.8)	<0.001

TABLE 3: Comparison of Ki-67 proliferation index according to biological behavior

Pairwise comparisons were performed using the Mann-Whitney U test with Bonferroni correction. Overall group comparison was performed using the Kruskal-Wallis test and chi-square test (χ^2 = 46.8, df = 2, p<0.001). Statistical significance was predefined at p<0.05.

HL: Hodgkin lymphoma; NHL: non-Hodgkin lymphoma; IQR: interquartile range; SD: standard deviation; PI: proliferation index.

ROC analysis was performed to distinguish aggressive NHL (DLBCL, BL, and TCL; n = 41) from indolent lymphomas (FL, MCL, and MZL; n = 33). HL was excluded from this binary classification because of its distinct biological characteristics. The ROC analysis yielded an AUC of 0.87 (95% CI, 0.79-0.95; p<0.001). The optimal Ki-67 cutoff identified by the Youden index was 40%, providing a sensitivity of 92.7% (38/41; 95%

CI, 80.6-97.5%), a specificity of 75.8% (25/33; 95% CI, 59.0-87.2%), a positive predictive value of 82.6%, a negative predictive value of 89.3%, a positive likelihood ratio of 3.82, and a negative likelihood ratio of 0.10. The diagnostic performance at this cutoff is summarized in Table *4*.

Cutoff (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)	Youden Index
40	92.7	75.8	82.6	89.3	0.87 (0.79-0.95)	0.685

TABLE 4: Diagnostic performance of the 40% Ki-67 cutoff for differentiating aggressive and indolent non-Hodgkin lymphomas (N = 74)

Values calculated from verified case counts: sensitivity 38/41, specificity 25/33. 95% CI for sensitivity: 80.6-97.5%; for specificity: 59.0-87.2%. Positive likelihood ratio = 3.82; negative likelihood ratio = 0.10. Overall ROC performance: AUC = 0.87 (95% CI: 0.79-0.95), p<0.001. The cutoff of 40% was determined using the Youden index. Statistical significance was predefined at p<0.05.

PPV: positive predictive value; NPV: negative predictive value; AUC: area under the ROC curve; ROC: receiver operating characteristic; CI: confidence interval.

Discussion

In this study conducted in the Al-Madinah cohort, BL showed the highest mean Ki-67 expression (92.4%), consistent with its biology as one of the most proliferative human tumors. Chong et al. reported a mean Ki-67 of 94.4% in BL using digital image analysis and that near-100% Ki-67 expression remains a diagnostic hallmark distinguishing BL from DLBCL with MYC rearrangement [21].

Our DLBCL cohort showed a mean Ki-67 expression of 68.5%, closely matching the findings of Hashmi et al. (66.4%) [11]. Previous studies have demonstrated that high Ki-67 expression is associated with poor prognosis and increased risk of relapse in DLBCL [12,13]. However, Hasselblom et al. reported a paradoxical association between low Ki-67 expression and adverse outcomes, highlighting the biological complexity of DLBCL and the potential influence of molecular subtype and treatment regimen on the prognostic significance of Ki-67 [43].

TCL exhibited high proliferative activity (mean Ki-67: 71.8%), whereas HL showed intermediate Ki-67 expression (mean: 52.3%), assessed exclusively in Reed-Sternberg cells. HL was analyzed as a separate entity due to its distinct pathobiology, treatment approach, and intermediate proliferative profile, which precludes its simple classification with either aggressive NHL or indolent lymphomas [20]. MCL demonstrated the widest Ki-67 range (8-82%), reflecting its biological spectrum from indolent to blastoid variants [17-19,44,45]. FL showed a clear grade-associated increase in Ki-67 expression, consistent with previous reports [15,16]. MZL and other indolent B-cell lymphomas showed the lowest Ki-67 values, consistent with their favorable clinical behavior.

ROC analysis identified a Ki-67 cutoff of 40% as the optimal threshold for distinguishing aggressive NHL from indolent lymphomas, with HL analyzed separately, showing good discriminatory performance (AUC = 0.87). This cutoff represents an intermediate threshold between the MCL-specific cutoff of 30% [18] and higher thresholds proposed for additional risk stratification within DLBCL (70-80%) [14]. Importantly, the grouping used for the ROC analysis should be interpreted as an analytical framework rather than a definitive biological classification. MCL, in particular, encompasses a spectrum of proliferative behavior, ranging from relatively indolent forms to highly aggressive blastoid or pleomorphic variants, and TCL are also biologically heterogeneous. Therefore, the 40% cutoff should be regarded as a cohort-derived exploratory discriminator between the predefined analytical groups rather than a universal threshold, and it should not replace subtype-specific interpretation of Ki-67 once the diagnosis is established. Previous studies have suggested different Ki-67 thresholds according to lymphoma subtype. Li et al. proposed 50% as a general NHL cutoff [26], while He et al. confirmed through systematic meta-analysis that optimal Ki-67 thresholds vary among lymphoma entities [27].

High Ki-67 expression was significantly associated with advanced stage (p = 0.003), elevated LDH (p = 0.001), B symptoms (p = 0.008), and high-risk IPI (p<0.001), consistent with previous reports [4,28,29]. These findings are biologically plausible, as highly proliferative tumors are associated with rapid tumor growth and dissemination, resulting in advanced disease presentation. The association between Ki-67 and LDH reflects increased cellular turnover and tumor burden [30]. Furthermore, the correlation between Ki-67 expression and IPI risk category in DLBCL supports the contribution of proliferative activity to disease aggressiveness and prognostic assessment.

Several methodological strengths support the reliability of our findings. The MIB-1 antibody clone is a widely validated reagent for Ki-67 assessment in FFPE tissue [8]. The hot-spot counting method, including

evaluation of at least 500 tumor cells in non-overlapping high-power fields, has been supported by consensus recommendations and demonstrates strong prognostic correlation [35]. Additionally, excellent interobserver agreement (ICC = 0.91) and concordance between manual counting and digital image analysis further enhance the reproducibility of Ki-67 assessment [36,37].

Several limitations should be acknowledged. First, although HL was analyzed separately to better reflect its biological behavior, this approach may limit direct comparison with studies that classified HL together with aggressive NHL. In addition, the aggressive and indolent groups included biologically heterogeneous entities; in particular, MCL spans indolent to blastoid variants and the TCL group combined different subtypes. Their assignment to these groups may have influenced the derived cutoff. Second, the retrospective multicenter design may introduce variability in tissue processing and clinical management. Third, the sample size was relatively small, particularly for BL (n = 5) and MZL (n = 7). The Ki-67 values reported for these subtypes should therefore be interpreted as descriptive, and no firm subtype-specific conclusions can be drawn from them. Fourth, the 40% cutoff was derived and evaluated in the same cohort without internal or external validation, which may overestimate its diagnostic performance; it therefore requires validation in independent, larger cohorts before any clinical application. Fifth, no survival, relapse, progression, or treatment-response data were available; therefore, the prognostic value of Ki-67 could not be directly assessed in this cohort, and our findings reflect associations with lymphoma subtype and biological behavior rather than demonstrated prognostic significance. Finally, molecular characterization, including gene-expression profiling and MYC/BCL2/BCL6 rearrangement status, was not available.

Conclusions

Ki-67 PI showed significant variability across lymphoma subtypes, and a cohort-derived cutoff of 40% showed good discrimination between the predefined aggressive and indolent NHL groups (AUC = 0.87). However, this threshold should be considered exploratory and should not replace subtype-specific interpretation of Ki-67. These findings suggest that standardized Ki-67 assessment may provide a practical adjunct to routine lymphoma diagnosis. As no outcome data were available, the prognostic value of Ki-67 could not be established in this study. External validation in larger prospective multicenter cohorts with survival and treatment-response data is required before the proposed cutoff can be recommended for clinical use.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Faris M. Elmahdi, Leen N. Almohammadi, Danah A. Alraddadi, Ajwan M. Alahmadi, Yusra O. Zakaria, Sara AlTom

Acquisition, analysis, or interpretation of data: Faris M. Elmahdi, Leen N. Almohammadi, Danah A. Alraddadi, Ajwan M. Alahmadi, Yusra O. Zakaria, Sara AlTom

Drafting of the manuscript: Faris M. Elmahdi, Leen N. Almohammadi, Danah A. Alraddadi, Ajwan M. Alahmadi, Yusra O. Zakaria, Sara AlTom

Critical review of the manuscript for important intellectual content: Faris M. Elmahdi, Leen N. Almohammadi, Danah A. Alraddadi, Ajwan M. Alahmadi, Yusra O. Zakaria, Sara AlTom

Supervision: Faris M. Elmahdi

Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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