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Evaluation of Ki-67 expression and large cell content as prognostic markers in MZL: a multicenter cohort study

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Marginal zone lymphoma (MZL) can have varied presentations and pathologic features, including high Ki-67 expression (> 20%) as well as increased numbers of large B cells (LC). However, there are limited data available demonstrating the prognostic significance of these variables in patients with MZL. In this multi-institutional retrospective cohort study of patients with MZL treated at 10 centers, we evaluated the association between the presence of Ki-67 expression and increased LCs on survival and risk of histologic transformation (HT). A total of 785 patients were included (60% with extranodal MZL, 20% with nodal MZL, and 20% with splenic MZL). Among the 440 patients with Ki-67 staining, 22% had high Ki-67 (Ki-67 >20%). The median progression-free survival (PFS) for patients with high Ki-67 was 5.4 years compared to 7.0 years for patients with low Ki-67 (HR = 1.45, 95%CI = 1.03–2.05). Ki-67 > 20% strongly correlated with high LDH level. The risk of HT was higher in patients with increased Ki-67 than those without (5-year risk, 9.8% vs 3.87%, $p = 0.01$). Twelve percent of patients had LC reported on biopsy with 6% having >10% LC. The presence of LC was associated with high Ki-67 ($p < 0.001$), but not associated with shorter PFS or overall survival (OS). The cumulative risk for HT was higher in patients with LC compared to those without LC (5-year risk, 9.4% vs 2.9%, $p = 0.04$). Receipt of anthracycline-based therapy did not impact PFS or OS in either group. Ki-67 staining >20% was a prognostic factor for worse survival and strongly correlated with elevated LDH. Novel therapies should be investigated for their potential ability to overcome the high-risk features in MZL. Our data reinforce the importance of obtaining biopsies at relapse or progression, particularly in patients with baseline high Ki-67 and increased LCs, given their increased risk for HT.

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INTRODUCTION

Marginal zone lymphoma (MZL) is a heterogeneous disease with three main subtypes including extranodal MZL of mucosa-associated lymphoid tissue (EMZL), representing most cases, splenic MZL (SMZL), and nodal MZL (NMZL) [1, 2]. MZL is generally characterized by slow growth and a good prognosis, with a median survival of >10 years [3, 4]. Some patients can initially be observed at diagnosis, while others are treated with radiation, rituximab monotherapy, or a combination of rituximab and chemotherapy [2]. However, some MZLs may behave more aggressively, and in 3-20% of cases, MZL can undergo histologic transformation (HT) to a higher grade lymphoma, most commonly diffuse large B cell lymphoma (DLBCL) [5].

Unlike follicular lymphoma which has a standard grading system, there are no pathologic grades associated with MZL. MZL is mostly composed of small to medium sized cells with low proliferation. Ki-67 is a marker of cell proliferation with low Ki-67 generally associated with indolent lymphomas while higher Ki-67 is found in aggressive lymphomas [6]. Occasionally, MZL shows a higher Ki-67 expression, but no specific cut-off value is used for diagnosing or assessing MZL. There are also cases of MZL where large B-cells (LC) are increased in the biopsy, occasionally constituting as much as 20% of cells or more [7]. The large B-cells are interspersed among the small and intermediate-sized cells but lack of sheets of large cells differentiates MZL with LC component from transformation to DLBCL. The prognostic significance of the presence of LC and higher Ki-67 in MZL

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remains uncertain. Additionally, there is therapeutic uncertainty regarding the use of standard MZL-directed therapy versus intensified therapy such as rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) in the presence of LC component or high Ki-67 expression [8]. Hence, we conducted a large multi-institutional study to evaluate the association between LC and Ki-67 expression at diagnosis on subsequent outcomes in patients with MZL.

PATIENTS AND METHODS

Study Design

This multicenter retrospective cohort study included adult patients (18 years or older) with MZL diagnosed on or after Jan 1, 2010, at 10 US medical centers. To be eligible for analysis, patients must have information on LC content or Ki-67 expression as determined by immunohistochemistry on the diagnostic biopsy by local pathology review. Patients with HT at or within 60 days after MZL diagnosis or those who did not receive any therapy for MZL were excluded.

We collected variables known to be significantly associated with survival outcomes in all subtypes of MZL [9, 10]. Values of laboratory tests (albumin, hemoglobin, serum lactate dehydrogenase [LDH], and beta-2-microglobulin [B2M]) were harmonized according to the upper or lower limits of normal at each institution. All staging procedures (e.g., bone marrow evaluations) and treatment evaluations were conducted according to local practice.

Ethics approval and consent to participate

The study was approved by the institutional review board at Ohio State University and at all participating sites and was conducted in compliance with the Declaration of Helsinki. As this was a retrospective study, informed consent was waived.

Study objectives and definitions

The percentage of cells stained with Ki-67 or described as large was extracted by investigators from the pathology reports. If a range of Ki-67 expression was reported in various areas of the tumor, the higher bound was used for analysis. The presence of large cells or Ki-67 expression in non-tumor structures (e.g. germinal centers) was not considered.

The study cohort was divided into those with high ($>20\%$) or low ($\leq 20\%$) Ki-67 expression, and according to the presence or absence of LC at diagnosis. The Ki-67 cut-off of 20% was chosen based on concordance statistics in the univariate analysis of an association with PFS; the evaluation of LC cutoff would require a more thorough inter-institutional harmonization with central review, so we arbitrarily evaluated groups with "any reported LC" or ">10% LC" (Figure S1). The primary endpoint evaluated was progression-free survival (PFS), defined as the time from the start of first-line therapy until lymphoma relapse, progression, or death from any cause. Patients who remained alive on the date of the last clinical assessment were censored. The secondary objectives included the evaluation of overall survival (OS), defined as the time from diagnosis until death from any cause or censoring at the last clinical assessment, and the cumulative incidence of HT.

Statistical analysis

Demographic and disease characteristics were summarized using medians and ranges for continuous variables, and frequencies and percentages for categorical variables. They were compared among study groups using the Wilcoxon rank sum test or Fisher's exact test, respectively. To mitigate bias related to data missing at random (including performance status missing in 11%, B symptoms in 3%, LDH in 14%, albumin in 11%, and bone marrow assessment in 32%), we used multiple imputation using chained equations to create 50 imputed datasets in which all model-based analyses were conducted. Coefficients and standard errors were averaged using Rubin's rules. The imputation models included all analytic variables, including the cumulative hazard of death.

PFS was estimated using the Kaplan-Meier method and compared between groups using the log-rank test. Cox proportional hazard regression models were used to estimate the hazard ratios (HR) for risk of progression or death. The multivariable models included variables selected based on expert knowledge of their potential prognostic significance in MZL: age (modeled as a restricted cubic spline), sex, stage, MZL subtype, performance status, hemoglobin, serum albumin, LDH, and presence of a monoclonal paraprotein. The proportional hazard assumption was checked using Schoenfeld residuals after fitting Cox models. The cumulative incidence of relapse and cumulative incidence of transformation was calculated treating death as a competing risk and compared between groups using Gray's test and competing regression models. OS was calculated from diagnosis and compared using the log-rank test. Analyses were performed using Stata version 17 (StataCorp, College Station, Texas), and all statistical tests were two-sided with a type-1 error of <0.05 indicating statistical significance. All estimates were reported with 95% confidence intervals (95%CI).

RESULTS

Patient Characteristics and Outcomes – Entire Study Cohort

A total of 785 patients with MZL were included in the analysis, including 473 (60%) with EMZL, 158 (20%) with NMZL and 154 (20%) with SMZL. Key clinical characteristics are included in Table S1. The median age was 63 years and 53% of patients were female. Forty-six percent of patients had stage 1 or 2 MZL at diagnosis, whereas 54% had advanced stage (3 or 4) disease. One third of patients did not receive systemic therapy, including 160 (20.4%) treated with first-line radiation therapy, 60 (7.6%) with surgical excision, and 38 (4.8%) with other modalities (e.g. antibiotic therapy). Patients treated with systemic therapy most frequently received rituximab (58%) followed by rituximab plus bendamustine (BR, 32%), R-CHOP (7%) and rituximab plus cyclophosphamide, vincristine, prednisone (R-CVP, 3%). The median PFS from diagnosis was 7.4 years (95% CI = 6.3–8.6) and median OS was not reached (NR). A total of 32 transformation events occurred in the study cohort. The risk of HT was 3.7% at 5 years (95% CI 2.4–5.3%) and 9.0% at 10 years (95% CI 5.4–13.7%).

Large cells (LC)

Patient Characteristics. Of the 785 patients included, 91 (12%) had LC reported in the biopsy, with 44 (6%) having >10% LC recorded. The presence of LC was associated with a high Ki-67 ($p < 0.001$) but not with other clinical characteristics, including MZL subtype (Table 1). Patients with LC-containing MZL received R-CHOP more frequently as first-line treatment than those without LC (18% vs. 5%, respectively; $p = 0.004$). Among the 219 patients who received frontline rituximab with chemotherapy, those with LC more frequently received R-CHOP (33%, compared with 12% of patients without LC; $p = 0.007$).

Survival Outcomes. The median PFS for patients with and without presence of LC was 5.7 years (95%CI = 3.7-NR) and 7.6 years (95%CI = 6.7-8.7), respectively. Presence of LC was not significantly associated with a shorter PFS from diagnosis, either in a univariate analysis (HR = 1.31, 95% CI = 0.94–1.84) or in a multivariate model (aHR = 1.47, 95% CI = 0.0–1.87; Fig. 1A**)**. The results were similar when PFS was examined in the 3 subtypes of MZL separately (Fig. 1B–D).

The median OS from diagnosis was NR for patients both with and without presence of LC, and the presence of LC was not associated with OS in aggregate MZL or in any MZL subtype (Fig. 1E–H). Additionally, we did not observe a significant difference in PFS or OS from start of systemic therapy according to LC presence ($n = 522$; Figure S2), or from time of radiation or surgery in p–atients with stage 1 MALT lymphoma ($n = 168$; Figure S3).

We then analyzed the survival outcomes based on the type of first-line systemic immunochemotherapy. Among patients with LC, there was no significant difference in PFS (aHR = 2.46, 95% CI 0.86–7.09, Figure S4A) or OS (HR = 3.56, 95% CI 0.59–21.4, Figure S4B) according to type of first line therapy (R-CHOP vs BR/R-CVP), although the number of patients was too small ($n = 33$) in this group to derive a precise estimate.

Table 1. Baseline characteristics of patients with marginal zone lymphoma stratified by presence or absence of large cells.

Factor	No Large Cells $n = 694$ (%)	Large Cells Present $n = 91$ (%)	*p*-value
Median age in years, (IQR)	63 (55–72)	64 (57–72)	0.44
Sex			0.58
Male	329 (47.4%)	40 (44.0%)	
Female	365 (52.6%)	51 (56.0%)	
MZL Subtype			0.12
NMZL	133 (19.2%)	25 (27.5%)	
SMZL	141 (20.3%)	13 (14.3%)	
EMZL	420 (60.5%)	53 (58.2%)	
Stage			0.99
1/2	321 (46.3%)	42 (46.2%)	
3/4	373 (53.7%)	49 (53.8%)	
ECOG PS			0.99
0-1	575 (92.1%)	72 (92.3%)	
2-4	49 (7.9%)	6 (7.7%)	
Bone Marrow Involvement			0.16
No	251 (52.3%)	35 (62.5%)	
Yes	229 (47.7%)	21 (37.5%)	
B Symptoms			0.54
No	566 (84.6%)	73 (82.0%)	
Yes	103 (15.4%)	16 (18.0%)	
LDH			0.89
Normal	460 (77.4%)	60 (76.9%)	
Elevated	134 (22.6%)	18 (23.1%)	
Ki-67%,			<0.001
≤20%	313 (81.3%)	29 (52.7%)	
>20%	72 (18.7%)	26 (47.3%)	
First Line Therapy			**0.004**
Rituximab	279 (60.0%)	29 (46.8%)	
R-CHOP	23 (4.9%)	11 (17.7%)	
BR	149 (32.0%)	20 (32.3%)	
R-CVP	14 (3.0%)	2 (3.2%)	

BR bendamustine, rituximab; *ECOG* Eastern Cooperative Oncology Group; *PS* performance status; *EMZL* extranodal marginal zone lymphoma; *IQR* interquartile range; *LDH* lactate dehydrogenase; *MZL* marginal zone lymphoma; *NMZL* nodal marginal zone lymphoma; *R-CHOP* rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; *R-CVP* rituximab, cyclophosphamide, vincristine, prednisone; *SMZL* splenic marginal zone lymphoma.

Cumulative Incidence of HT. The cumulative risk for HT was higher in the LC group (5 year risk, 9.4%, 95%CI = 4.0-17.6) compared to the group without LC reported on pathology (5-year risk, 2.9%, 95%CI = 1.8-4.5; Gray's test p = 0.044) (Fig. 2A).

Patients with ≥ 10% LC content. We additionally examined outcomes among patients ($n = 44$) with ≥10% LCs reported (compared with all others; see Table S2 for the breakdown of patient characteristics**)**. The median PFS for patients with ≥10% versus 0-10% LCs was 5.7 years (95% CI = 2.8 to NR) and 7.6 years (95% CI = 6.7–8.7), respectively (log-rank $p = 0.38$, Figures S4A–D). The median OS was not reached for either group (log-rank $p = 0.87$; Figures S4E–H). We observed no significant difference in PFS or OS within any subgroup. The cumulative 5 year risk for HT in the >10% LC group was 7.6% (95%CI = 1.9–18.5) compared to 3.2% (95%CI = 2.0–4.8) for others but the difference was not statistically significant (Gray's test $p = 0.30$).

Ki-67%

Patient Characteristics. Ki-67 staining was performed on biopsies from 440 patients. Of those, 98 (22%) had high Ki-67 expression, defined as Ki-67 > 20% (Table 2). We determined a cut off of 20% to provide the best prognostic value for high Ki-67 in MZL (Figure S1). High Ki-67 correlated with increased LDH (34% versus 19%, $p = 0.005$), but not with other clinical parameters or with the use of R-CHOP as first line chemotherapy ($p = 0.07$).

Survival Outcomes. The median PFS for patients with high and low Ki-67 expression was 5.4 years (95%CI = 3.2–8.7) and 7.0 years (95%CI = 5.9–9.4), respectively. Patients with high Ki-67 had significantly shorter PFS from diagnosis in the univariate analysis (HR = 1.45, 95%CI = 1.03–2.05, Fig. 3A). As Ki-67 expression strongly correlated with high LDH, we analyzed a multivariate model excluding LDH and found that high Ki-67 expression was associated with significantly shorter PFS (aHR = 1.50, 95% CI = 1.01–2.24). Similar observations were made when measuring PFS from the start of the first-line systemic therapy (Figure S6A; aHR = 1.65, 95%CI = 1.05–2.60 in a model without LDH). In addition, high Ki-67 was associated with a shorter PFS from diagnosis in the univariate model for EMZL (HR = 1.64, 95% CI = 1.04–2.60; Fig. 3B), whereas there was no statistical significance in SMZL or NMZL (Fig. 3C, D). Similar observations were made for PFS analysis from the start of first-line systemic therapy (Figure S6B–D).

The median OS was not reached for patients with either high or low Ki-67 expression. High Ki-67 was associated with a statistically significant shorter OS from diagnosis in the univariate model (HR = 2.03, 95% CI = 1.16–3.53, Fig. 3E) and in the multivariate model that did not include LDH (aHR = 2.19, 95%CI = 1.08-4.45). While high Ki-67 was associated with shorter OS from diagnosis in SMZL in univariate analysis (HR = 4.53, 95% CI = 1.09–18.8, Fig. 3G), this association was not statistically significant in the multivariate model (aHR = 3.45, 95%CI = 0.19–63.3), and we observed no significant association in EMZL or NMZL (Fig. 3F, H). High Ki-67 was also associated with shorter OS from start of systemic therapy in the univariate model (HR = 2.33, 95% CI = 1.20–4.50, Figure S6E), but not in the multivariate model (aHR = 2.33, 95%CI = 0.93–5.86). The association with OS from the start of systemic therapy in the MZL subtypes was in line with that seen for OS from diagnosis (Figures S6F, H).

High Ki-67 was not prognostic for PFS or OS among patients treated with radiation or surgery for stage 1 EMZL (Figure S7). Furthermore, among patients with Ki-67 > 20%, there was no difference based on the type of first line therapy received (R-CHOP vs BR/R-CVP) in PFS (HR = 1.90, 95% CI 0.70–5.17, Figure S8A) or OS (HR = 2.12, 95% CI 0.51–8.88, Figure S8B).

Cumulative Incidence of HT. The cumulative incidence of HT was significantly higher in the high Ki-67 group compared to the low Ki-67 group (5-year risk, 9.8% [95%CI = 4.5–17.4] vs 3.7% [95% CI = 1.9–6.6], Gray's test $p = 0.011$; Fig. 2B). When stratified by subtypes, this association was only statistically significant for MALT lymphoma, but the power in NMZL and SMZL was limited due to sample size (Fig. S9).

DISCUSSION

In this large retrospective cohort of patients with newly diagnosed MZL, Ki-67 staining >20% was a prognostic factor for worse PFS and OS and strongly correlated with elevated LDH. In contrast,

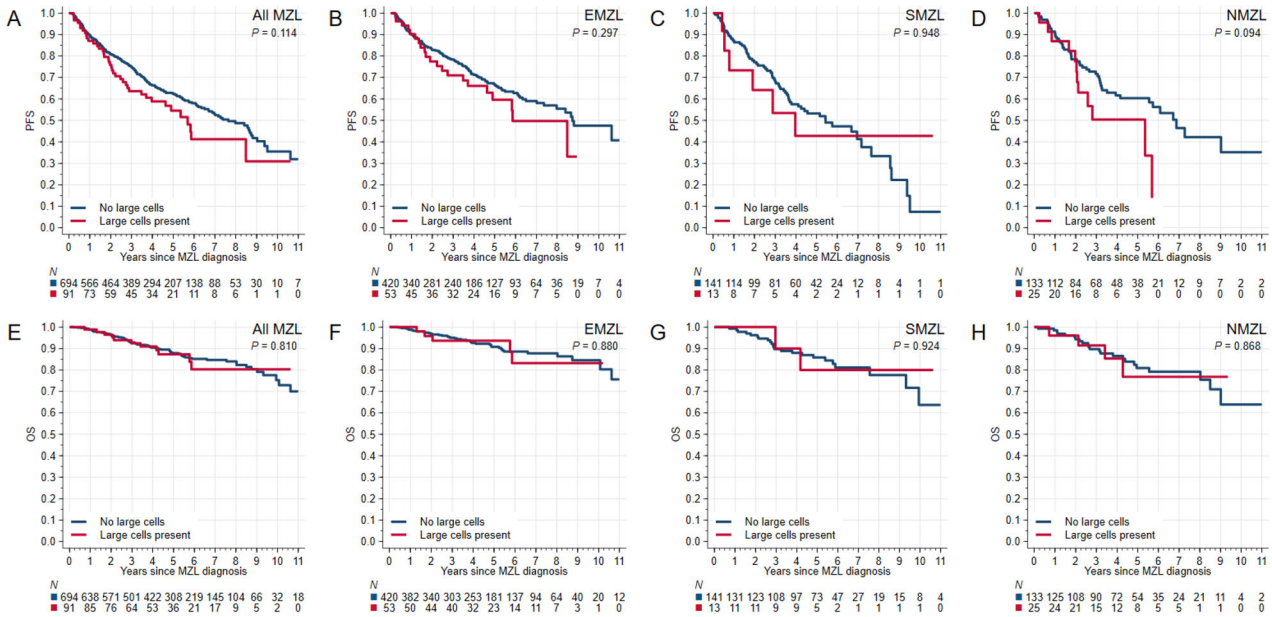

Fig. 1 PFS from diagnosis for all patients and by MZL subtype based on the presence of large cells. A–D PFS; **E–H** OS.

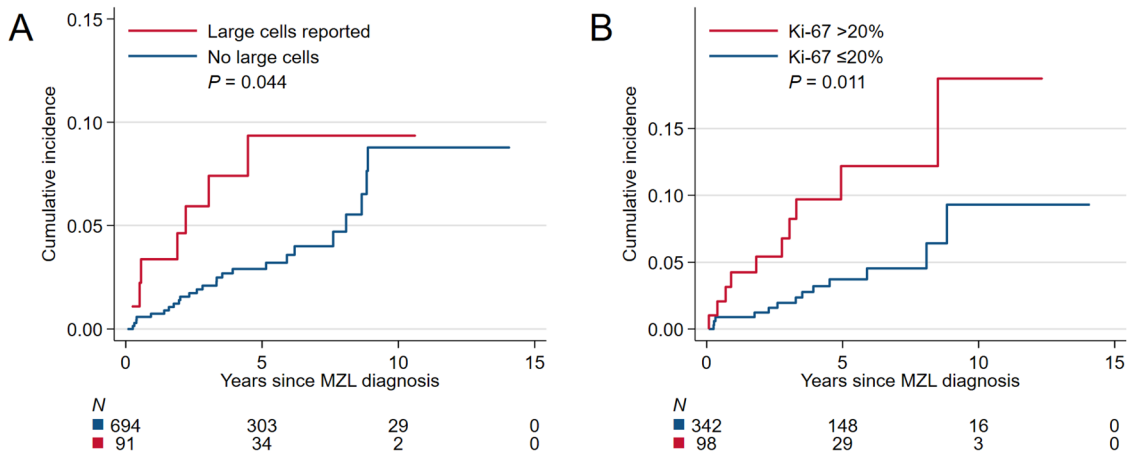

Fig. 2 Cumulative incidence of histologic transformation. A Based on the presence or absence of large cells; **B** based on Ki-67% >20% vs ≤20%.

presence of LC was not significantly associated with PFS or OS. Both high Ki-67 and presence of LC were associated with an increased risk of HT, although initial treatment with anthracycline-based chemotherapy did not appear to impact PFS or OS in the presence of high Ki-67 or LC.

Several clinicopathological prognostic factors have been recently reported in patients with MZL [11–13]. Our hypothesis-generating study is limited by reliance on local pathology assessments, and so would be best complemented by a study with a central expert review. However, our data reflect the current diagnostic practices in the US academic centers and information that clinicians receive and use to for clinical management. Prior studies characterizing LCs in MZL and exploring their prognostic significance are limited by sample sizes and treatment characteristics. For example, in one clinicopathologic study of 21 patients with NMZL, 57% of patients had >20% LCs [14]. The presence of increased LCs was associated with a high Ki-67, but not with PFS, and, in contrast to our cohort or current clinical practice, most patients were treated with anthracycline-based chemotherapy. In another study evaluating 161 cases with various subtypes of MZL, 33 (20%) had increased LCs defined as >10 LCs per high power field [15]. In line with our study, the presence of LC was associated with a high Ki-67 and increased risk of HT, but also with a shorter PFS, which we did not observe in a larger cohort. Of note, their LC cohort had a larger proportion of patients with NMZL (48%), which has been associated with worse survival [16], but similarly to our group, the most common treatment regimen used as first line was single agent rituximab. Compared to these studies, we found a lower rate of LC reported on the initial biopsy, which could be related to a more heterogeneous patient population. Based on our observations, the LC content in MZL, even when further classified as >10% LC content, does not appear to provide useful prognostic information, although it should always trigger concern for a possible presence of DLBCL component when the biopsy is inadequate or there are other concerning clinical or radiographic features. Moreover, a specific cutoff to distinguish "MZL with increased LC content" from overt HT has not been established. Because of our retrospective approach and uncertainty of the accuracy of LC quantification among participating institutions, we arbitrarily classified cases as "no reported LC", and 0–10% or >10%

Table 2. Baseline characteristics of patients with marginal zone lymphoma stratified by Ki-67 expression (≤20% vs > 20%).

Factor	Ki-67 ≤ 20% $n = 342$ (%)	Ki-67 > 20% $n = 98$ (%)	*p*-value
Median age in years, (IQR)	64 (54–72)	63 (55–72)	0.86
Sex			0.07
Male	160 (46.8%)	35 (35.7%)	
Female	182 (53.2%)	63 (64.3%)	
MZL Subtype			0.07
NMZL	73 (21.3%)	32 (32.7%)	
SMZL	62 (18.1%)	14 (14.3%)	
EMZL	207 (60.5%)	52 (53.1%)	
Stage			0.17
1/2	154 (45.0%)	36 (36.7%)	
3/4	188 (55.0%)	62 (63.3%)	
ECOG PS			0.99
0–1	290 (91.5%)	86 (92.5%)	
2–4	27 (8.5%)	7 (7.5%)	
Bone Marrow Involvement			0.48
No	129 (54.9%)	39 (60.0%)	
Yes	106 (45.1%)	26 (40.0%)	
B Symptoms			0.11
No	288 (86.2%)	76 (79.2%)	
Yes	46 (13.8%)	20 (20.8%)	
LDH			**0.005**
Normal	248 (81.0%)	56 (65.9%)	
Elevated	58 (19.0%)	29 (34.1%)	
First Line Therapy			0.07
Rituximab	130 (58.0%)	39 (51.3%)	
R-CHOP	11 (4.9%)	9 (11.8%)	
BR	77 (34.4%)	23 (30.3%)	
R-CVP	6 (2.7%)	5 (6.6%)	

BR bendamustine, rituximab, *ECOG* Eastern Cooperative Oncology Group, *PS* performance status, *EMZL* extranodal marginal zone lymphoma, *IQR* interquartile range, *LDH* lactate dehydrogenase; *MZL* marginal zone lymphoma; *NMZL* nodal marginal zone lymphoma; *R-CHOP* rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; *R-CVP* rituximab, cyclophosphamide, vincristine, prednisone; *SMZL* splenic marginal zone lymphoma.

reported LC, although a more specific assessment guided by standardized quantification might provide a deeper insight. As our study lacked central pathologic review, we cannot rule out the possibility that the poor prognostic value of LC could result from inconsistencies in its subjective assessment and reporting by the pathologists in routine practice. Furthermore, assessment of LC content might differ in specimens obtained from the bone marrow (in SMZL), nodal (NMZL) or extranodal tissues, which we were not able to study in depth.

We observed more significant associations between elevated Ki-67 expression (specifically, >20% based on empirically optimal cutoff) and survival outcomes in MZL. Several prior studies have attempted to evaluate the prognostic significance of Ki-67 expression in MZL but were limited in their approach. One study pooled 91 cases of MZL and lymphoplasmacytic lymphoma, finding that Ki-67 expression >20% was associated with worse OS [17]. The authors identified IRF4/MUM1 expression as an additional prognostic biomarker with median OS ranging from 9.8 years for patients who did not have either increased Ki-67 or IRF4 expression, to 3.6 years for those who had one or both markers. In another study, a cohort of 38 patients with NMZL was dichotomized using the observed median Ki-67 level of 9.1%, finding that higher Ki-67 was associated with presence of LC, higher MYC expression, and shorter PFS [18]. In a study of primary extra-gastric EMZL, Ki-67 ≥ 10% was associated with shorter PFS [19]. In addition, in genomic studies of MZL, Ki-67 overexpression was associated with the less favorable NNK subtype of SMZL [20] and with the PTPRD mutation in NMZL [21]. Our results from a large multi-center cohort support the suggestion that Ki-67 expression >20% is associated with a worse prognosis, but with the ability to deploy multivariable models, we have demonstrated that this association remains statistically significant upon adjustment for other known prognostic factors like age, performance status, and disease stage.

Recently, Arcaini et al. described a new prognostic score (MZL-IPI) applicable to all patients with MZL considered for systemic treatment, which stratified patients into 3 risk groups (low, intermediate, and high) based on 5 variables that were independently associated with inferior PFS in multivariable modeling [22]. These variables included LDH above the upper limit of normal, hemoglobin <12 g/dL, absolute lymphocyte count $<1 \times 10^9$/L, platelets $<100 \times 10^9$/L, and MZL subtype (nodal or disseminated). Ki-67% was not among the 14 variables that were evaluated in the model development—likely as it has not been historically consistently evaluated or reported in clinical practice. As outlined in the results and discussion, Ki-67% strongly correlated with elevated LDH, but it may also represent additional biologic aspects related to proliferative nature of MZL. Thus, Ki-67 should be considered for evaluation in future studies that examine prognosis or molecular features in MZL as a potential independent variable, and a standardized reporting of this biomarker in practice may be of practical and research value.

Acknowledging the uncertain treatment selection process in our retrospective cohort, we did not observe statistically significant differences in outcomes for patients with either high Ki-67 expression or increased LC content receiving non-anthracycline regimen when immunochemotherapy was selected for first-line treatment. Nevertheless, both histologic features (which are correlated) were associated with an increased risk of HT within the first 5 years of follow up, suggesting the biological relevance of high LC content or Ki-67 expression. Prior reports have recommended treatment of patients with MZL with >50% LCs with an anthracycline based regimen, but patients in that review were often reported to have sheets of LCs so they may be better characterized as transformed to DLBCL [23]. Further research is warranted to evaluate specific underpinning of these histologic phenotypes and to determine whether novel treatment approaches can avert the risk of transformation.

Our study has several limitations. As a retrospective study, there are likely biases related to treatment selection including the use of anthracycline based therapy. This retrospective analysis relied on institutional pathology review, as opposed to central review. Nevertheless, the management of patients in our cohort relied on information provided by the pathologists to the treating clinicians and thus reflects decision-making and outcomes with the current clinical practice in academic centers in the US. Because we did not have access to patient samples, we could not evaluate other potentially associated prognostic markers such as IRF4, *MYC*, or *TP53* expression, known cytogenetic markers like the t(11;18)(q21;q21) and genetic sequencing data. Lastly, some subgroups had small numbers of patients, making it difficult to draw conclusions, even though we attempted to minimize the information loss using multiple imputation for missing data. We presented analyses both "in aggregate" and in histologic subtypes

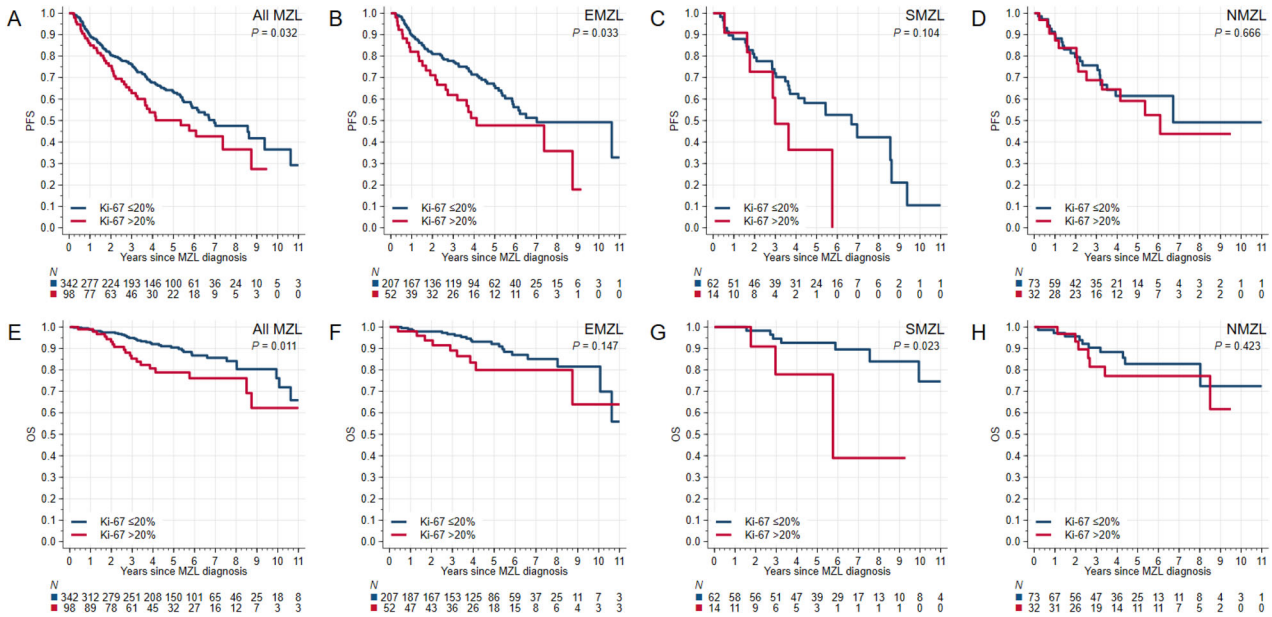

Fig. 3 PFS from diagnosis for all patients and by MZL subtype based on Ki67 >20%. A–D PFS; **E–H** OS.

of MZL, but we acknowledge limited power in subsets that preclude a conclusive analysis of interactions or subtype-specific effects.

In conclusion, our study provides a large-sample, multicenter characterization of the prognostic significance of Ki-67 and increased LCs in MZL in real-world clinical practice. Ki-67 staining >20% was a prognostic factor for worse survival and strongly correlated with elevated LDH. Larger prospective or retrospective analyses based on centralized histological review should be done to validate our findings. Novel therapies, including emerging targeted and immune-based treatments, should be investigated for their potential ability to overcome the high-risk features in MZL. In addition, our data reinforce the importance of obtaining biopsies at relapse or progression, particularly in MZL patients with baseline high Ki-67 and increased LCs, given their increased risk for HT.

DATA AVAILABILITY

Data is available upon request to the corresponding author as permitted by the IRB.

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