AHOT | Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2026;59(2):156-162

Original Article

DOI: 10.4274/ahot.galenos.2026.2026-4-1

Are the Neutrophil-to-lymphocyte Ratio and Lymphocyte-to-monocyte Ratio Prognostic Markers in Elderly Patients with Diffuse Large B-cell Lymphoma?

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ABSTRACT

Aim: Neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR) are inflammatory markers that have been studied as prognostic factors in diffuse large B-cell lymphoma (DLBCL). However, the prognostic value of these markers in elderly patients is unclear.

Methods: A retrospective analysis was conducted on 82 *de novo* DLBCL patients aged ≥65 years who received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) or R-CHOP-like treatment. Kaplan-Meier and Cox regression analyses were used to evaluate overall survival (OS) and progression-free survival (PFS). The discriminatory power of NLR and LMR was examined using receiver operating characteristic (ROC) analysis. Restricted cubic spline analysis was used to evaluate possible nonlinear associations between these biomarkers and survival.

Results: The median follow-up time was 35.5 months. Analyses based on median values showed no significant association between NLR and LMR and OS and PFS. In ROC analysis, both NLR [area under the curve (AUC): 0.575, p=0.289] and LMR (AUC: 0.517, p=0.815) showed low discriminatory power. Multivariate analysis revealed that NLR was an independent prognostic factor for OS (hazard ratio: 1.11, 95% confidence interval: 1.01-1.22, p=0.032), whereas LMR was not significant. The restricted cubic spline analysis did not reveal a significant nonlinear association or a threshold effect between NLR and survival outcomes.

Conclusion: Although NLR was statistically associated with OS when analyzed as a continuous variable, no distinct threshold value was identified. These results show that, in older DLBCL patients, NLR may function as a weak, continuous biomarker rather than as a clinically significant risk indicator.

Keywords: Aged, lymphoma, lymphocyte-to-monocyte ratio, neutrophil-to-lymphocyte ratio, prognosis

Introduction

Diffuse large B-cell lymphoma (DLBCL) is responsible for one-third of all non-Hodgkin lymphomas [1]. The International Prognostic Index (IPI) [lactate dehydrogenase (LDH), stage, extranodal region, >60 years, Eastern Cooperative Oncology Group (ECOG)]; Revised-IPI (R-IPI) (stage, ECOG, LDH); and National Comprehensive Cancer Network (NCCN) IPI are risk scores used to estimate patient prognosis.

Prognosis in DLBCL also varies by subtype and biological characteristics. Due to difficulties in standardization, a comprehensive risk scoring system incorporating these factors is not yet available, thereby limiting clinicians' ability to accurately assess patient prognosis and tailor treatment strategies. There is still a need for simple scoring systems in daily practice [2]. Furthermore, the prognosis of DLBCL is poorer in elderly patients than in younger ones. Their tumor microenvironment, tumor immunology, and genetic mutations are thought to differ, which may contribute to the observed differences in prognosis and treatment response between elderly and younger patients.

Recently, several studies have suggested that the neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR), which are frequently measured in clinical practice, may

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Received: 23.04.2026 **Accepted:** 01.08.2026 **Publication Date:** 21.08.2026

Cite this article as: Tonyalı BT, Arıca D, Koca E, Karakuş S, Akı ŞZ. Are the neutrophil-to-lymphocyte ratio and lymphocyte-to-monocyte ratio prognostic markers in elderly patients with diffuse large B-cell lymphoma? Acta Haematol Oncol Turc. 2026;59(2):156-162

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be prognostic markers in DLBCL [3-6]. However, because the prognosis of elderly lymphoma patients differs, the effects of these ratios in this population remain unknown. This study aimed to characterize elderly patients with DLBCL and to evaluate whether LMR and NLR have prognostic significance in this population.

Methods

Patients newly diagnosed with DLBCL between 2012 and 2022 at the university hematology outpatient clinic or inpatient ward who received at least 4 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) or R-CHOP-like chemotherapy and had complete clinical data were included in the study. Patients who had previously received radiotherapy or chemotherapy were excluded. Before beginning any treatment, lymphocyte, neutrophil, and monocyte counts were measured using standard CBC testing. The absolute neutrophil count was divided by the absolute lymphocyte count to determine the NLR. The LMR was determined by dividing the absolute lymphocyte count by the absolute monocyte count. The study was designed retrospectively. This study was approved by the Ethics Committee of the Başkent University Medical and Health Sciences Research Board (approval no: KA23/260, date: 19.07.2023).

Statistical Analysis

Overall survival (OS) was defined as the time from diagnosis to death or last follow-up, and progression-free survival (PFS) was defined as the time from diagnosis to disease progression, death from any cause, or last follow-up. The associations of OS and PFS with NLR and LMR were evaluated using Kaplan-Meier curves and compared using the log-rank test. Univariate Cox regression analyses, followed by multivariate Cox proportional hazards regression analyses, were performed to assess the prognostic significance of the variables.

The cut-off values of NLR and LMR were determined based on their median values. Continuous variables were primarily analyzed without categorization to avoid loss of information.

In addition, restricted cubic spline functions were applied to Cox regression models adjusted for IPI to explore potential non-linear relationships between NLR/LMR and survival outcomes.

A two-sided p value of less than 0.05 was considered statistically significant, and all analyses included 95% confidence interval (CI). R software (version 4.5.3) and IBM SPSS Statistics 24.0 (IBM Corporation, Armonk, NY, USA) were used for statistical analyses.

Results

Between 2012 and 2022, 111 patients over the age of 65 were diagnosed with DLBCL. Thirteen patients were lost to follow-up, nine died before receiving therapy, and seven patients were excluded from the study because they refused treatment. The study included a total of 82 patients. The median age was 73.5 years, and 53.7% were female. 22% of patients were aged 80 years or older. 78% of patients had an ECOG performance status of 0-1. Extranodal involvement was detected in 69.5% (n=57) of the cases, while central nervous system (CNS) involvement was present in 4.9% (n=4). Bone marrow involvement was present in only 4.9% (n=4) of the patients. C-myc positivity was found in 25.5% (n=12), BCL6 positivity in 75.4% (n=52), and BCL2 positivity in 58.2% (n=39). 6 (7%) patients had a double hit, and 3 (3.6%) had a triple hit. The mean Ki-67 proliferation index was 75.44±14.67 (median: 80.00; minimum-maximum: 20.00-100.00) (Table 1).

Bulky masses were present in 12.2% (n=10) of patients. According to the IPI score, 18.5% were in the high-risk group [4,5]. According to the NCCN-IPI, 29.6% were in the high-risk group [6-8].

The median follow-up time was 35.53 months (0.13-237.07). At the end of the follow-up, the recurrence rate was 15.9% (n=13), and 29.3% (n=24) of patients had died.

The discriminative ability of NLR and LMR to predict mortality was evaluated using receiver operating characteristic (ROC) analysis. The analysis showed that the area under the curve (AUC) for NLR was 0.575 (95% CI: 0.422-0.727), which was not statistically significant (p=0.289). For the NLR ≥3.07 cut-off point, the sensitivity was calculated as 54.2% and the specificity as 55.2%. Similarly, for LMR, the AUC was 0.517 (95% CI: 0.360-0.673), indicating that LMR also lacked significant discriminative power in predicting mortality (p=0.815). For the LMR ≤2.45 cut-off point, the sensitivity was 54.2% and the specificity was 55.3%. The cut-off values of NLR and LMR were determined based on their median values.

As shown in Table 2, the overall median OS (months) was not reached. Median OS (months) differed significantly across ECOG (p=0.021) and extranodal involvement (p=0.013) groups. The median NLR and LMR values were not significant for OS.

As shown in Table 3, the overall median PFS could not be reached. Median PFS (months) differed significantly between gender groups (p=0.030). Median NLR and LMR values were not significantly associated with PFS.

ECOG and extranodal involvement were found to be significant in the univariate analysis, as shown in Table 2. The multivariate Cox regression model contained these variables, which were determined to be significant in univariate analysis. Extranodal involvement was found to be an independent negative prognostic factor for OS in the multivariate Cox regression analysis including LMR [hazard ratio (HR): 4, 95% CI: 1.16-14.29, p=0.028]. However, LMR was not found to be significant when included as a continuous variable in the model (p=0.209) (Table 4).

In multivariate analysis including NLR, NLR was determined to be an independent prognostic factor for OS (HR: 1.11, 95% CI: 1.01-1.22, p=0.032). Extranodal involvement approached statistical significance in this model (p=0.051) (Table 5).

Restricted cubic spline analyses, adjusted for IPI, did not demonstrate a clear non-linear relationship between baseline NLR and survival outcomes. Although a gradual trend was observed, no distinct threshold effect was identified, and CIs were wide, particularly at higher NLR values (Figures 1 and 2).

Table 1. Distribution of sociodemographic and clinical variables		
Variables	**N**	**%**
Age		
Mean ± SD	74.72±7.42	
Median (min-max)	73.5 (65-95)	
Gender		
Female	44	53.7
Male	38	46.3
ECOG		
0	30	36.6
1	34	41.5
2	15	18.3
3	3	3.7
NLR		
Mean ± SD	3.87±3.20	
Median (min-max)	2.91 (0.10-16.57)	
LMR		
Mean ± SD	3.29±5.13	
Median (min-max)	2.58 (0.39-46.40)	
Stage		
1	22	27.5
2	9	11.3
3	12	15.0
4	37	46.3
Extranodal involvement		
Absent	25	30.5
Present	57	69.5
CNS involvement		
Absent	78	95.1
Present	4	4.9
Bone marrow involvement		
Absent	78	95.1
Present	4	4.9
c-MYC		
Negative	35	74.5
Positive	12	25.5
BCL6		
Negative	17	24.6
Positive	52	75.4
BCL2		
Negative	28	41.8
Positive	39	58.2
Ki67		
Mean ± SD	75.44±14.67	
Median (min-max)	80.00 (20.00-100.00)	
Bulky disease		
Negative	72	87.7
Positive	10	12.2
IPI score		
1	22	27.2
2	22	27.2
3	22	27.2
4	8	9.9
5	7	8.6
R-IPI		
1	3	75.0
2	1	25.0
NCCN-IPI		
1	2	2.5
2	7	8.6
3	16	19.8
4	14	17.3
5	18	22.2
6	14	17.3
7	6	7.4
8	4	4.9
Treatment response		
Complete response	60	73.2
Partial response	6	7.3
Refractory	4	4.9
Not evaluable	12	14.7
Relapse		
Absent	69	84.1
Present	13	15.9
Mortality		
Alive	58	70.7
Deceased	24	29.3
Follow-up time (months)		
Mean ± SD	53.02±51.24	
Median (min-max)	35.53 (0.13-237.07)	

ECOG: Eastern Cooperative Oncology Group, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, CNS: Central nervous system, NCCN-IPI: National Comprehensive Cancer Network IPI

Discussion

The most popular clinical forecasting models for DLBCL patients receiving R-CHOP are IPI, R-IPI, and NCCN-IPI. Even though IPI has been developed across cohorts that include all age groups, it does not fully address factors particularly significant to older patients, and it does not account for the increased age-related heterogeneity among individuals over 60, including comorbidities and the effects of polypharmacy on treatment outcomes. Accumulating evidence suggests that prognostic factors change with age, and the importance of non-lymphoma-related factors increases [7,8]. The change in

Table 2. Patient OS comparisons

Variables	2 year survival %	5 year survival %	Median (95% CI)	p
Overall survival	76.2	73.6	- (-)	
Gender				
Female	76.5	76.5	- (-)	0.452
Male	75.9	68.3	94.23 (29.51-158.95)	
ECOG				
0	83.1	83.1	- (-)	0.021
1	81.8	75.9	- (-)	
2	57.8	57.8	66.20 (0.00-174.54)	
3	33.3	33.3	2.33 (0.00-4.74)	
Stage				
1	72.7	72.7	- (-)	0.096
2	88.9	88.9	- (-)	
3	100.0	100.0	- (-)	
4	66.6	60.6	94.23 (-)	
Extranodal involvement				
Absence	91.7	91.7	- (-)	0.013
Present	69.6	65.7	94.23 (-)	
CNS involvement				
Absence	60.7	60.7	- (-)	0.630
Present	78.0	71.5	94.23 (25.31-163.14)	
Bone marrow involvement				
Absence	77.5	74.9	- (-)	0.102
Present	50.0	-	4.96 (-)	
c-MYC				
Negative	86.9	86.9	- (-)	0.098
Positive	66.7	66.7	- (-)	
BCL6				
Negative	81.4	81.4	- (-)	0.830
Positive	78.3	78.3	134.00 (-)	
BCL2				
Negative	78.6	78.6	134.00 (-)	0.487
Positive	83.1	83.1	- (-)	
Bulky disease				
Absence	75.9	73.1	- (-)	0.775
Present	78.8	78.8	- (-)	
IPI score				
Low risk (0-2)	77.3	77.3	- (-)	0.483
Intermediate-high risk (3-5)	74.5	69.5	134.00 (44.69-223.31)	
NCCN-IPI				
Low risk (0-3)	88.0	88.0	- (-)	0.114
Intermediate-high risk (4-8)	70.6	67.2	134.00 (-)	
NLR				
>2.91	79.4	79.4	- (-)	0.305
≤2.91	63.2	55.3	- (-)	
LMR				
<2.58	73.6	69	- (-)	0.430
≥2.58	79.2	72.6	- (-)	

Kaplan-Meier: Log-rank test: $p<0.05$ statistically significant
ECOG: Eastern Cooperative Oncology Group, CNS: Central nervous system, R-IPI: Revised International Prognostic Index, NCCN-IPI: National Comprehensive Cancer Network IPI, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, CI: Confidence interval

Table 3. Patient PFS comparisons

Variables	2 year survival %	5 year survival %	Median (95% CI)	p
Overall	85.5	82.4	- (-)	
Gender				
Female	95.0	90.2	- (-)	0.030
Male	74.0	74.0	94.23 (-)	
ECOG				
0	82.3	82.3	94.23 (-)	0.735
1	83.3	83.3	- (-)	
2-3	76.9	76.9	- (-)	
Extranodal involvement				
Absence	90.2	90.2	- (-)	0.082
Present	82.8	77.9	- (-)	
CNS involvement				
Absence	76.5	76.5	- (-)	0.302
Present	89.0	81.6	- (-)	
Bone marrow involvement				
Absence	86.5	83.4	- (-)	0.177
Present	50.0	-	14.90 (-)	
c-MYC				
Negative	84.6	84.6	- (-)	0.673
Positive	75.8	75.8	- (-)	
BCL6				
Negative	93.8	93.8	- (-)	0.218
Positive	81.2	75.4	- (-)	
BCL2				
Negative	91.8	91.8	- (-)	0.190
Positive	79.9	72.6	- (-)	
Bulky disease				
Absence	85.2	81.9	- (-)	0.790
Present	88.9	88.9	- (-)	
IPI score				
Low risk (0-2)	86.6	86.6	- (-)	0.205
Intermediate-high risk (3-5)	83.6	77.2	- (-)	
NCCN-IPI				
Low risk (0-3)	85.9	85.9	- (-)	0.393
Intermediate-high risk (4-8)	84.8	80.4	- (-)	
NLR				
>2.91	79.5	75.1	- (-)	0.227
≤2.91	63.2	54.1	- (-)	
LMR				
<2.58	72.5	66.9	- (-)	0.423
≥2.58	79.7	73	- (-)	

Kaplan-Meier: Log-rank test: p<0.05 statistically significant
ECOG: Eastern Cooperative Oncology Group, CNS: Central nervous system, R-IPI: Revised International Prognostic Index, NCCN-IPI: National Comprehensive Cancer Network, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, CI: Confidence interval

Table 4. Multivariate Cox regression results on the mortality risk of various clinical variables in LMR patients

Variables	HR (95% CI)	p
ECOG		0.224
0	ref	
1	0.39 (0.14-1.14)	0.086
2 and 3	0.43 (0.16-1.22)	0.113
Extranodal involvement		
Absence	ref	0.028
Present	4.00 (1.16-14.29)	
LMR (continuous)	1.03 (0.98-1.08)	0.209

-2 Log log likelihood:179:467: p=0.025
ECOG: Eastern Cooperative Oncology Group, LMR: Lymphocyte-to-monocyte ratio, HR: Hazard ratio, CI: Confidence interval

Table 5. Multivariate Cox regression results on the mortality risk of NLR and various clinical variables in patients

Variables	HR (95% CI)	p
ECOG		0.117
0	ref	
1	0.43 (0.14-1.33)	0.964
2 and 3	0.42 (0.14-1.23)	0.251
Extranodal involvement		
Absence	ref	0.051
Present	3.57 (0.99-12.50)	
NLR (continuous)	1.11 (1.01-1.22)	0.032

-2 Log log likelihood: 172.371, p=0.022
ECOG: Eastern Cooperative Oncology Group, NLR: neutrophil-to-lymphocyte ratio, HR: Hazard ratio, CI: Confidence interval

tumor microenvironment and immunogenicity with aging has led to the search for immunological markers for prognostic factors in elderly lymphoma patients [9]. NLR and LMR have previously been studied as potential prognostic markers in several lymphoma subtypes. However, there is a lack of research specifically focused on older patients with DLBCL. This study adds to the existing research by being one of the few to examine the prognostic value of NLR and LMR in elderly patients with DLBCL.

Previous studies have suggested that the normal range for the NLR is between 2.1 and 5.54 [10-12]. For LMR, the values span from 2.2 to 3 [13-15]. Koh et al. [15] reported that an LMR of 2.36 or lower was a significant predictor of OS and PFS in older patients with DLBCL. However, no specific studies have examined the relationship between the NLR and DLBCL in elderly patients. A universally accepted cut-off value for these ratios has not yet been established.

In our study, no significant cut-off value could be determined using ROC analysis. Analyses based on median values did not demonstrate significant prognostic effects of NLR and LMR on OS and PFS. However, in multivariate analysis, NLR was identified as a statistically significant continuous predictor. Furthermore, restricted cubic spline analyses did not demonstrate a clear non-linear relationship or threshold effect

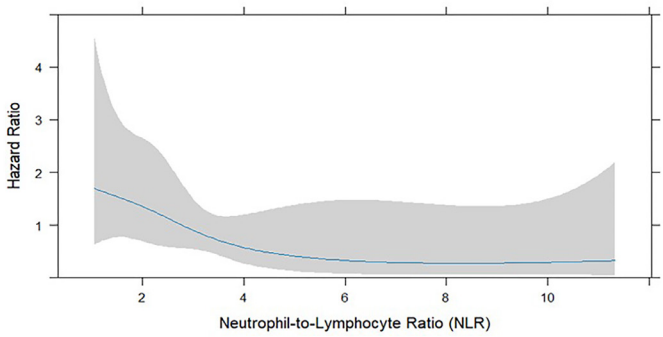


Figure 1. Restricted cubic spline for NLR and PFS Restricted cubic spline curve showing the association between baseline neutrophil-to-lymphocyte ratio and PFS adjusted for IPI. Shaded areas represent 95% confidence intervals
NLR: Neutrophil-to-lymphocyte ratio, IPI: International Prognostic Index, PFS: Progression-free survival

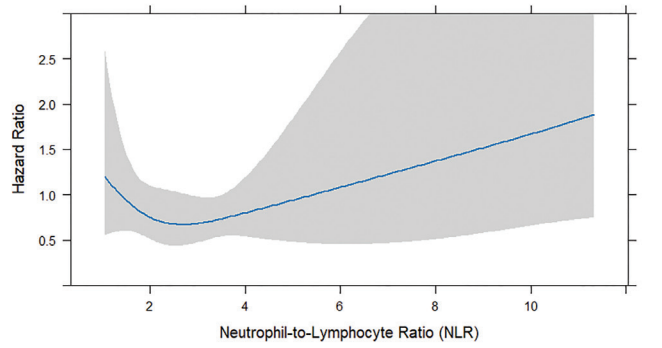


Figure 2. Restricted cubic spline for NLR and OS
Restricted cubic spline curve illustrating the association between baseline NLR and OS adjusted for IPI. Shaded areas represent 95% confidence intervals
NLR: Neutrophil-to-lymphocyte ratio, IPI: International Prognostic Index, OS: Overall survival

between NLR and survival outcomes. Although a gradual trend was observed, the absence of a distinct cut-off point supports the use of NLR as a continuous variable.

However, the low discriminative performance observed in ROC analyses suggests that, although NLR is statistically associated with survival, it provides limited clinical discrimination and functions as a relatively weak continuous biomarker.

The complete response rate (73.2%) and 5-year OS and PFS rates (73.6% and 82.4%, respectively) obtained in our study are consistent with the elderly DLBCL series reported in the literature [16,17]. A high ECOG performance score (p=0.021) and the presence of extranodal involvement (p=0.013) were associated with a statistically significant reduction in median OS, consistent with the existing literature. Results regarding the prognostic value of LMR are heterogeneous in the literature. While some studies show that low LMR is associated with OS and PFS, other studies show that LMR is not an independent prognostic marker [18,19]. Several factors may explain the lack of prognostic significance of LMR in our study. First, age-related alterations in immune function may impair the extent to which lymphocyte and monocyte counts accurately reflect tumor biology in elderly patients. Second, the statistical power to detect weaker relationships may have been compromised by the relatively small sample size and limited occurrences. Finally, the effect of LMR may be overshadowed by stronger clinical variables and other inflammatory markers, such as NLR, in multivariate models, thereby leading to an underestimation of its significance in predicting outcomes in elderly patients with cancer.

Study Limitations

The limitations of this study include a retrospective design, a single-center setting, a small sample size, a lack of immunotherapy-based medications, and incomplete molecular data.

Conclusion

NLR was associated with OS when analyzed as a continuous variable; however, it did not demonstrate meaningful discriminatory ability or a clinically useful threshold. These findings suggest that NLR functions as a weak, continuous biomarker rather than a robust risk- stratification tool in elderly patients with DLBCL. LMR was also not prognostically significant. To further understand the clinical role of inflammatory biomarkers in this cohort, larger prospective studies are required.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of the Başkent University Medical and Health Sciences Research Board (approval no: KA23/260, date: 19.07.2023).

Informed Consent: Because the study was designed retrospectively no written informed consent form was obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.T.T., D.A., E.K., S.K., Ş.Z.A., Concept: B.T.T., Ş.Z.A., Design: B.T.T., Ş.Z.A., Data Collection or Processing: B.T.T., Analysis or Interpretation: B.T.T., Literature Search: B.T.T., Ş.Z.A., Writing: B.T.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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