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Original Article

Epstein-Barr virus RNA percentage and its impact on prognosis in patients with diffuse large B-cell lymphoma

Fatma Göçlü[1], Hatice Terzi[2], Neşe Yeldir[3], Mehmet Şencan[2]

[1]Department of Internal Medicine, Zara State Hospital, Kahramanmaraş, Turkiye
[2]Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkiye
[3]Department of Medical Pathology, Bağcılar Medipol Mega University Hospital, İstanbul, Turkiye

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ABSTRACT

Aims: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphomas. This study aimed to investigate the impact of Epstein-Barr virus RNA (EBER) positivity on the prognosis of patients diagnosed with DLBCL.

Methods: This retrospective study was conducted on 105 patients diagnosed with DLBCL at Sivas Cumhuriyet University Hospital between June 1, 2009, and May 31, 2022.

Results: Of the 105 patients, 55.2% were male, and the mean age was 56.89 years. EBER positivity was detected in 4.8% of the patients. While 71% of EBER-negative patients achieved a complete response, overall survival and progression-free survival were shorter in EBER-positive patients. However, no significant relationship was observed between EBER positivity and prognostic indices (IPI, R-IPI, NCCN-IPI).

Conclusion: The findings of this study indicate that EBER positivity could act as a prognostic indicator, given its association with unfavorable survival rates and diminished response to treatment in individuals with DLBCL.

Keywords: Diffuse large B-cell lymphoma, EBER, prognosis

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most commonly observed subtype of non-Hodgkin lymphomas (NHL), accounting for approximately 30-40% of all NHL cases.[1] Its etiology includes genetic mutations, immune deficiencies, and viral infections. Epstein-Barr virus (EBV) targets B cells, establishing latent infections and, in some cases, exhibiting oncogenic effects.[2,3]

EBV positivity is associated with poor prognosis and clinical heterogeneity in DLBCL patients. Studies have reported that the prevalence of EBV in DLBCL ranges between 3% and 16.9%.[4,5] While EBV-positive DLBCL was initially thought to affect elderly patients predominantly, recent findings indicate that it can also occur in younger individuals.[6] EBV positivity is recognized as an independent prognostic factor affecting overall survival (OS) in DLBCL patients, along with variables such as older age, advanced disease stage, and activated B-cell-like characteristics.[5] The presence of EBV in DLBCL cases is typically detected using in situ hybridization techniques for EBV-encoded RNA (EBER).[6] Further research is needed to understand EBV's impact on DLBCL prognosis fully and to develop tailored treatment strategies for this subgroup of patients.[4] The role of EBV in DLBCL remains a topic of interest both diagnostically and prognostically. This study evaluates the relationship between EBV RNA positivity and the prognosis of DLBCL.

METHODS

Ethics

In this retrospective study, 105 patients diagnosed with DLBCL at the Hematology Clinic of Sivas Cumhuriyet University Health Services Application and Research Hospital between June 1, 2009, and May 31, 2022, were evaluated. The study was carried out with the permission of the Sivas Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date: 25.05.2022, Decision No: 2022-05/27). The Sivas Cumhuriyet University Scientific Research Projects (CÜBAP) unit supported the study with project number T-2022-977. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Data Collection and Analysis

The patients included in the study were between 18 and 90 years old, with sufficient pathology tissue samples and accessible treatment data. A reactive lymph node section previously considered EBER-positive was used as a positive control.

Patient information was obtained from e-Nabız, the Hospital Information Management System, the Death Notification System, the MEDULLA Physician Application, discharge summaries, outpatient clinic notes, pathology reports, and imaging reports. Clinical parameters such as Eastern Cooperative Oncology Group (ECOG) performance scores,

Corresponding Author: Hatice Terzi, dr.terzi@hotmail.com

Ann-Arbor staging, extranodal involvement, and B symptoms were recorded. The scores for the International Prognostic Index (IPI), Revised International Prognostic Index (R-IPI), and National Comprehensive Cancer Network-International Prognostic Index (NCCN-IPI) were determined.

Treatment response was evaluated according to the Lugano criteria, and the duration of OS and progression-free survival (PFS) were calculated.

Histopathological Evaluation and In Situ Hybridization

Hematoxylin-eosin-stained slides were re-examined, and suitable tissues were selected for in situ hybridization. The presence of EBV was assessed using the chromogenic in situ hybridization (CISH) method. Blue reactions in target cells confirmed positivity, graded on a scale from 1 to 3 based on intensity (**Figure 1**).

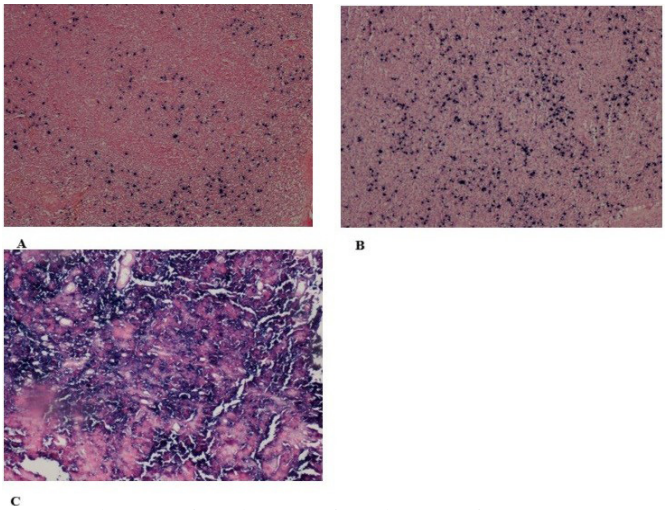

Figure 1. A) EBV grade 1, B) EBV grade 2, C) EBV grade 3

EBV: Epstein-Barr virus

Statistical Analysis

Data analysis was conducted using SPSS 26.0 software, with a 95% confidence interval. Categorical variables are presented as frequencies and percentages, while numerical variables are reported as mean, standard deviation, minimum, maximum, and median values.

Independent T tests, Mann-Whitney U tests, one-way ANOVA, and Kruskal-Wallis tests were utilized for group comparisons, while chi-square tests were applied to analyze categorical variables; Pearson and Spearman correlation tests were applied to assess relationships between numerical variables. Dependent T tests, Wilcoxon signed-rank test, and McNemar tests were conducted for repeated measurements. The Kaplan-Meier method was used for survival analyses, while ROC analysis assessed the predictive capability of measurements for exitus status.

RESULTS

Analysis of Clinical and Pathological Findings

In this study, 55.2% of the DLBCL patients were male, and 47% were female. Pathological examination revealed that 44.9% of the patients had germinal center B-cell (GCB) type, 100% were Ki67 positive, 80.2% were CD5 positive, 34.1% were CD10 positive, 100% were CD20 positive, 66.3% were Bcl-6 positive, 70.5% were Mum-1 positive, and 79.3% were Bcl-2 positive. Additionally, 4.8% of the patients tested positive for EBER.

The patients' interim and post-treatment positron emission tomography/computed tomography (PET/CT) imaging were evaluated according to the Lugano response criteria, with 61% showing a complete response at interim imaging and 68.6% showing a complete response at post-treatment imaging. At the time of diagnosis, 47.6% of the patients were in stage 4, 39% had extranodal involvement, 88.6% had lymph node involvement, 28.6% had bone marrow involvement, 61% had organ involvement, and 46.7% had B symptoms. Of the patients, 67.6% had an ECOG score of 1, 50.5% were in the low-medium risk group based on the IPI score, 46.7% were in the good-risk group according to the R-IPI score, and 39.0% were in the low-medium risk group based on the NCCN-IPI score (**Figure 2**).

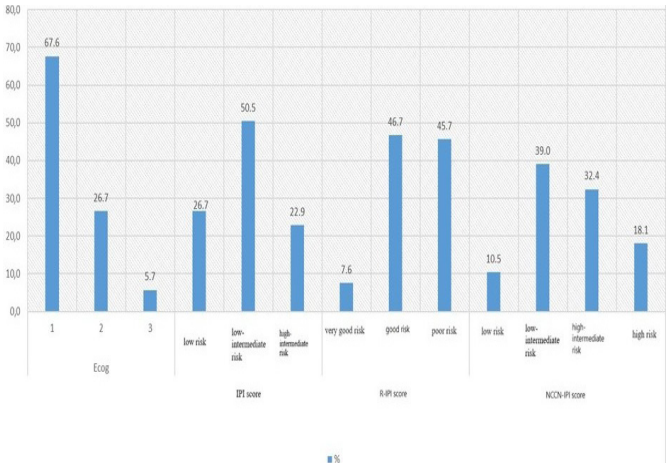

Figure 2. Distribution of patients according to ECOG, IPI, R-IPI, and NCCN-IPI scores

ECOG: Eastern Cooperative Oncology Group, IPI: International Prognostic Index, R-IPI: Revised International Prognostic Index, NCCN: National Comprehensive Cancer Network

Treatment and Survival Analysis

As first-line treatment, 79% of patients received R-CHOP (Rituximab-Cyclophosphamide-Doxorubicin-Vincristine-Prednisone), 9.5% received second-line R-DHAP (Rituximab-Dexamethasone-Cisplatin-Cytarabine), 2.9% received third-line R-GIFOX (Rituximab-Gemcitabine-Ifosfamide-Oxaliplatin), and 1% received fourth-line Pixantron. Autologous stem cell transplantation was performed in 9.5% of patients, radiotherapy in 17.1%, and central nervous system (CNS) prophylaxis in 38.1%. No statistically significant relationship was found between the patients' pathological classification as GCB or non-GCB lymphoma and their ECOG, IPI, R-IPI, or NCCN-IPI scores ($p>0.05$). The average OS time was determined to be 41.49 months, while the PFS period averaged 36.99 months.

Relationships with EBER Positivity

A statistically significant correlation was observed between EBER positivity and germinal center versus non-germinal center B-cell status ($p=0.037$), with all EBER-positive cases classified as germinal center B-cells. However, no significant associations were identified between EBER positivity and the expression of Ki67, CD5, CD10, CD20, Bcl-6, Mum-1, or Bcl-2 ($p>0.05$) (**Table 1**). A significant relationship was observed between EBER positivity and post-treatment imaging status

(p=0.041), with a higher complete response rate in EBER-negative patients (71%). No statistically significant association was found between EBER positivity and ECOG, IPI, R-IPI, or NCCN-IPI score levels (p>0.05) (Table 2).

Table 1. Relationship between EBER positivity and germinal center, Ki67, CD, Bcl-6, Mum-1, Bcl-2

		EBER		
		Positive	Negative	p
Germ cell	Germinal	4 (100)	31 (41.9)	0.037*
	Non-germinal	0 (0)	43 (58.1)	
Ki67	Positive	5 (100)	100 (100)	x
	Negative	0 (0)	0 (0)	
CD5	Positive	4 (100)	69 (79.3)	0.581
	Negative	0 (0)	18 (20.7)	
CD10	Positive	3 (75)	27 (32.1)	0.113
	Negative	1 (25)	57 (67.9)	
CD20	Positive	5 (100)	100 (100)	x
	Negative	0 (0)	0 (0)	
Bcl-6	Positive	4 (100)	51 (64.6)	0.295
	Negative	0 (0)	28 (35.4)	
Mum-1	Positive	1 (25)	54 (73)	0.074
	Negative	3 (75)	20 (27)	
Bcl-2	Positive	2 (50)	63 (80.8)	0.188
	Negative	2 (50)	15 (19.2)	

*A p-value of <0.05 indicates a significant relationship, while a p-value of >0.05 indicates no considerable relationship; the chi-square test, EBER: Epstein-Barr virus RNA

Table 2. Association between EBER positivity and interim and post-treatment imaging outcomes

		EBER		
		Positive	Negative	p
Interim PET/CT	Stable disease	0 (0)	2 (2)	0.356
	Partial response	1 (20)	22 (22)	
	Complete response	2 (40)	62 (62)	
	Progression	0 (0)	2 (2)	
	No imaging	2 (40)	12 (12)	
Post-treatment PET/CT	Stable disease	0 (0)	0 (0)	0.041*
	Partial response	1 (20)	5 (5)	
	Complete response	1 (20)	71 (71)	
	Progression	1 (20)	9 (9)	
	No imaging	2 (40)	15 (15)	

*A p-value of <0.05 indicates a significant relationship, while a p-value of >0.05 indicates no considerable relationship; the chi-square test, EBER: Epstein-Barr virus RNA, PET/CT: Positron emission tomography/computed tomography

Survival Durations and Factors

Overall survival (p=0.015) and PFS (p=0.031) showed a significant difference between patients who were EBER-positive and those who were EBER-negative. EBER-positive patients had shorter OS (14.38 months) and PFS (12.74 months) than EBER-negative patients (Table 3, Figure 3, 4). However, no significant differences in OS or PFS durations were noted based on gender or germinal/non-germinal B-cell status (p>0.05).

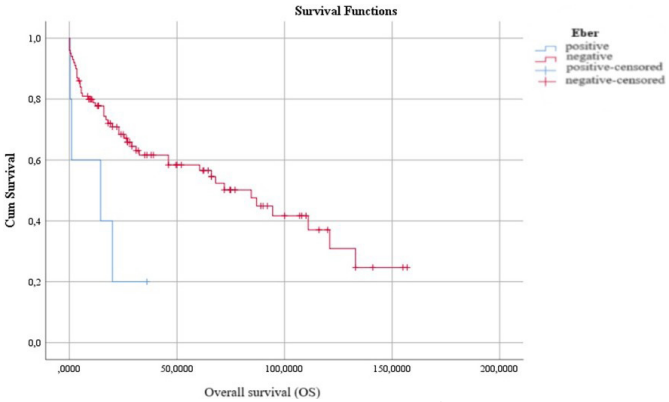

Figure 3. Association Between EBER Positivity and OS

EBER: Epstein-Barr virus RNA, OS: Overall survival

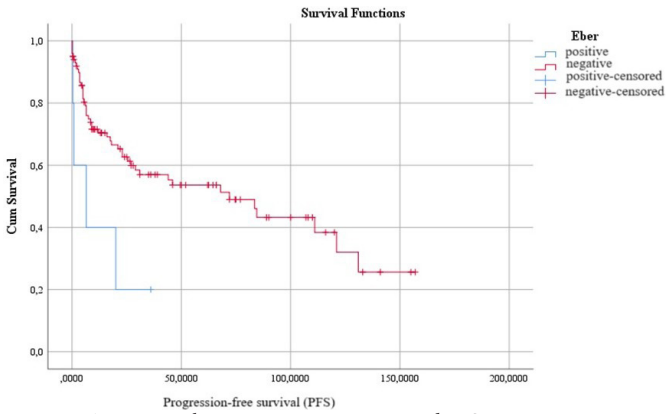

Figure 4. Association between EBER Positivity and PFS

EBER: Epstein-Barr virus RNA, PFS: Progression-free survival

DISCUSSION

DLBCL represents approximately one-third of all non-Hodgkin lymphoma cases, ranking as one of the most common subtypes. The role of EBV in the biological and clinical characteristics of DLBCL has not been fully clarified.[7] In this context, many studies have examined the prognostic impact of EBV infection. Specifically, identifying prognostic factors during diagnosis could be crucial in determining the need for early and intensive treatment in DLBCL patients. Some studies have indicated that EBER positivity holds significant prognostic value in DLBCL, while others have not supported these findings.[5,8-16] Numerous studies have reported variability in the frequency of EBER positivity among patients with DLBCL. For instance, in Asian countries (Korea and Japan), the frequency of EBER positivity ranges from 8-9%, while in Western countries, this rate is 1-3%.[8,11,17,18] In a study by Park et al.[14] involving 380 DLBCL patients, 9% were EBER positive.[19] Furthermore, in a meta-analysis of 13 studies, including 4111 DLBCL patients, the frequency of EBER positivity was reported to range from 1.4% to 14.9%. In our study, 4.8% of patients diagnosed with DLBCL were found to be EBER positive. Among the 5 EBER-positive patients, 1% were classified as grade 1, 1.9% as grade 2, and 1.9% as grade 3 based on staining intensity.

Table 3. Relationship between EBER Positivity and overall survival and progression-free survival

		Overall survival				Progression-free survival			
Factor	Group	n (%)	Mean	95% CI Mean	p	n (%)	Mean	95% CI Mean	p
EBER	Positive	1 (20)	14.380	2.795-25.965	0.015*	1 (20)	12.746	0.812-24.68	0.031*
	Negative	53 (53)	78.732	64.299-93.164		53 (53)	75.717	60.643-90.791	

*p<0.05 indicates a significant effect, p>0.05 indicates no significant effect; Kaplan-Meier test, EBER: Epstein-Barr virus RNA, CI: Confidence interval

Many studies have reported a negative association between EBER positivity and OS durations.[5,10,13] Tracy and colleagues, through their research, revealed that neither EBV positivity nor immunosuppression status significantly influences the treatment approaches or survival rates in patients diagnosed with diffuse large B-cell lymphoma (DLBCL).[12] Hong and colleagues highlighted that serum survivin positivity serves as an independent adverse prognostic factor influencing disease progression in DLBCL patients treated with R-CHOP. However, they noted that the expression of EBER in tumor tissues does not have a significant impact on clinical outcomes.[16] Jarrett et al.[20] conducted a study with 437 classical Hodgkin lymphoma patients, identifying EBER positivity as an independent factor influencing survival. Similarly, our study found that EBER-positive patients had shorter OS and PFS durations compared to EBER-negative patients, with this difference being statistically significant. Most research has indicated that EBER-positive patients have higher IPI scores, with a statistically significant relationship.[11] However, in another study involving 89 patients, no significant difference was found between EBER positivity and IPI scores.[5] Contrary to the findings in the literature, our study did not detect a statistically significant relationship between EBER positivity and IPI scores. A study conducted with 362 patients in North America found a significant association between EBER positivity and GCB and non-GCB subtypes of DLBCL.[12] However, some studies did not find this association statistically significant.[13,15] Our study identified a statistically significant relationship between EBER positivity and GCB and non-GCB DLBCL subtypes.

The prognostic and biological effects of EBER positivity on DLBCL have been reported with varying results in the literature. Our study has contributed to the existing knowledge in this field and confirmed the negative impact of EBER positivity on survival durations. Additionally, the relationships between EBER positivity, IPI score, and GCB subtypes suggest that further studies are needed to better understand these associations.

CONCLUSION

Our study found a statistically significant relationship between EBER positivity and OS and PFS durations. We concluded that EBER-positive patients had lower OS and PFS than EBER-negative patients. EBV-positive patients should be closely monitored during treatment planning. In future studies, a more detailed investigation of the molecular mechanisms of EBV may contribute to developing new strategies for managing DLBCL.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Sivas Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date: 25.05.2022, Decision No: 2022-05/27).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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