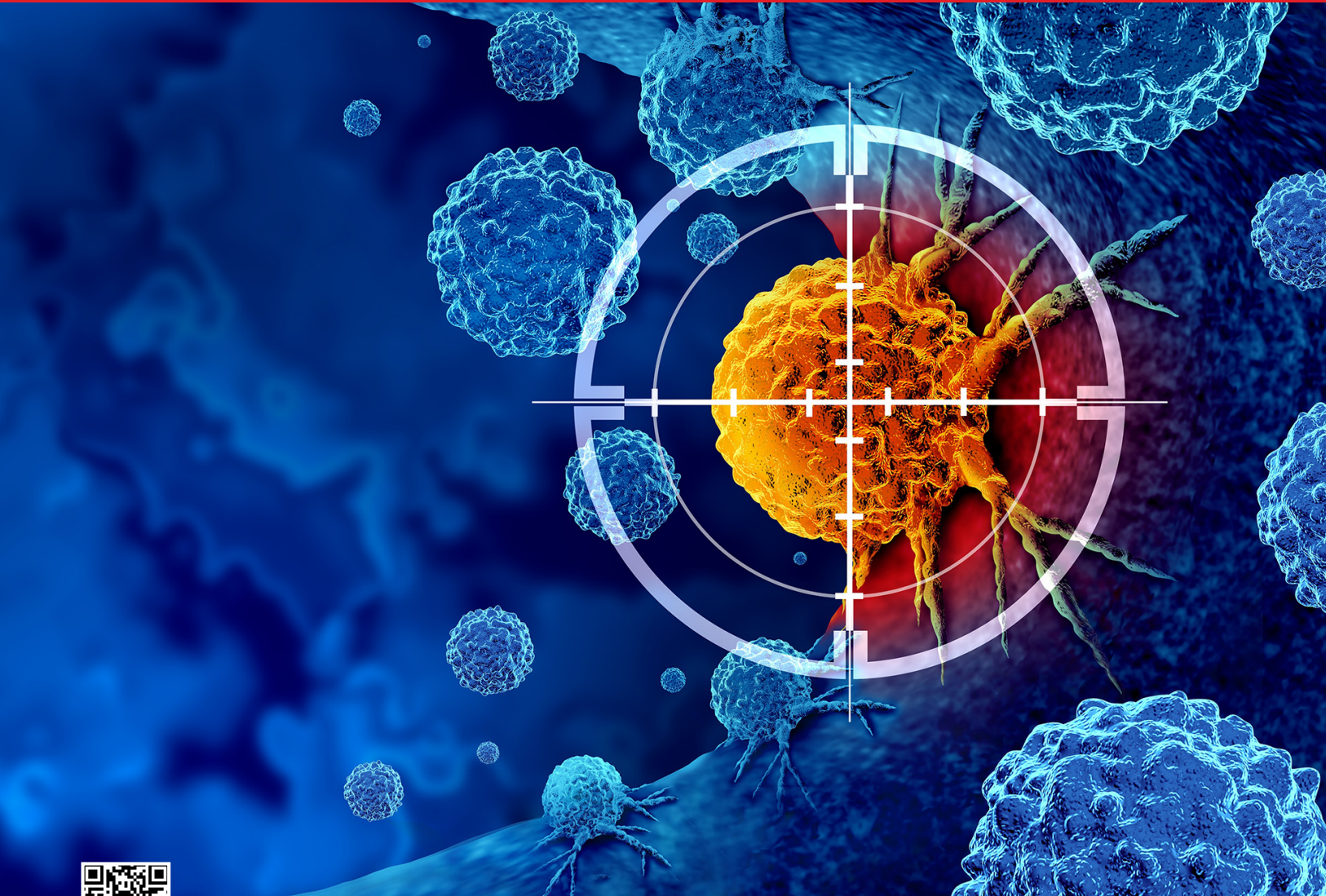


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Predictive Factors for Residual High-grade Cervical Intraepithelial Lesions or Carcinoma on Final Pathology in Patients with Positive Surgical Margins after Cervical Conization and Endocervical Curettage

Yeşim ÖZKAYA UÇAR¹, Okan AYTEKİN¹, Arife Ebru TAŞCI¹, Ayşe BURAN¹, Mehmet ÜNSAL¹, Mustafa ŞAHİN², Zehra ÖZTÜRK BAŞARIR¹, Fatih KILIÇ¹

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ABSTRACT

Objective: To evaluate the presence of residual preinvasive or invasive cervical disease after re-conization or hysterectomy in women with positive surgical margins following excisional treatment for high-grade squamous intraepithelial lesions (HSIL), and to identify pathological factors that may be related to residual disease.

Material and Methods: This retrospective cohort included 48 women with positive ectocervical margins, endocervical margins, or endocervical curettage (ECC) margins after initial excisional procedures (cold-knife conization, loop electrosurgical excision procedure, or needle excision of the transformation zone). All patients underwent either re-conization or hysterectomy. Cone volume was calculated using the semi-ellipsoid formula ($\pi/6 \times \text{anteroposterior} \times \text{transverse} \times \text{length}$). Categorical variables, including margin type, ECC results, human papillomavirus status, and excisional technique, were compared using the chi-square test or Fisher's exact test.

Results: Residual HSIL or carcinoma was identified in 45.9% of patients undergoing repeat excision, whereas 54.1% had only low-grade intraepithelial lesions or no preinvasive lesion. Ectocervical margin positivity showed limited predictive value for residual high-grade pathology. Endocervical margin positivity and ECC positivity demonstrated similar distributions, with residual HSIL or carcinoma present in approximately half of these patients. Residual pathology occurred following both cold-knife conization and electrosurgical excisional techniques. Residual disease occurred more frequently after cold-knife conization than after electrosurgical techniques; however, this difference was not statistically significant ($p=0.051$).

Conclusion: Residual high-grade disease was detected in nearly half of the patients undergoing repeat surgery after a margin-positive conization. Although certain variables such as excisional technique and endocervical margin involvement showed numerical differences, none reached statistical significance. These findings suggest that no single clinical or pathological factor reliably predicts residual disease, and margin status alone may be insufficient to guide management decisions.

Keywords: Cervical intraepithelial neoplasia; conization; margin; HSIL; LEEP; NETZ

INTRODUCTION

Cervical high-grade squamous intraepithelial lesions (HSIL) represent a clinically significant stage within the spectrum of human papillomavirus (HPV)-associated cervical disease.

These lesions constitute the primary target of excisional treatment, aimed at preventing progression to invasive cervical cancer while providing definitive histopathological diagnosis.¹ HSIL can be effectively treated with excisional procedures such as cold knife conization (CKC), loop

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electrosurgical excision procedure (LEEP), and needle excision of the transformation zone (NETZ). However, surgical margins may be positive, creating significant clinical uncertainty for subsequent treatment.²

Excisional treatment is the standard of care for histologically confirmed cervical intraepithelial neoplasia (CIN) 2 and CIN 3 according to American Society for Colposcopy and Cervical Pathology (ASCCP) and European Society of Gynaecological Oncology (ESGO) guidelines, which emphasize achieving clear margins to minimize residual or recurrent disease.^{3,4} Positive surgical margins are reported in approximately 12-44% of excisional procedures. Higher rates (23-44%) have been reported after LEEP, while lower rates (12-29%) have been reported after CKC.^{5,6} Despite this, surgical margin status alone does not reliably predict persistent or residual HSIL. Many women with positive surgical margins do not demonstrate residual disease at follow-up or on repeat excision. In a large systematic review including 29 studies, residual CIN2+ was detected in 32.3% of margin-positive patients.⁷ Similarly, residual CIN2+ has been reported in approximately 34.3% of women with positive margins.⁸ These findings indicate that positive margins may be associated with a persistently high-risk of disease, but they also show that most patients have no residual lesions. Therefore, the margin status should be interpreted as a risk factor rather than a definitive indicator, and careful patient selection is important when considering re-excision. Several clinical and pathological features have been reported as potential predictors of residual disease after conization, including endocervical margin involvement, endocervical curettage (ECC) positivity, higher CIN grade, HPV 16/18 infection, and insufficient cone depth or volume. However, the predictive performance of these factors remains inconsistent across studies, and none has shown adequate accuracy to guide management in isolation.^{9,10}

The present study aims to investigate residual disease in women who underwent a repeat excision or hysterectomy following a margin-positive conization. Additionally, the clinical and pathological parameters that predict residual HSIL or cancer were evaluated.

MATERIAL AND METHODS

This retrospective cohort study included 48 women who had positive surgical margins for HSIL in the ectocervix, the endocervix, or both, and/or a positive ECC following an initial excisional procedure performed in patients with preinvasive cervical disease. All patients were managed at a single tertiary referral center between September 2019 and November 2024, and complete clinical and pathological records were available for review. Women who did not undergo a second surgical

procedure, who had incomplete pathology reports, or who were followed outside the institution were excluded. Initial excisional techniques consisted of CKC, LEEP conization, or NETZ, selected according to surgeon preference. Excisional procedures (CKC, NETZ, or LEEP) were performed in the operating room under general or regional anesthesia. As part of the standard surgical protocol to minimize bleeding and facilitate manipulation, a No. 0 polyglactin suture was placed at the 3- and 9-o'clock positions of the cervix. CKC was then carried out using a scalpel to remove the entire transformation zone. NETZ was performed with a 10.2-cm needle electrode (E-Z Clean Electrosurgical Tips; Megadyne Medical Products™, Draper, UT), whereas LEEP conization was performed with a 15-20-mm loop electrode. For both NETZ and LEEP conization, the electrosurgical generator was typically set to a 40-W pure-cut mode. ECC was routinely performed immediately after excision. Following tissue removal, hemostasis was achieved using a ball electrode with a ≥40-W coagulation. For proper specimen orientation, a suture was placed at the 12 o'clock margin of the cone, and both the conization specimen and the ECC sample were submitted to pathology in separate containers, each containing 10% neutral buffered formalin. Surgical margins were considered positive when the lesion was located at the ectocervical and/or endocervical margins.

Residual disease was defined as the presence of HSIL (CIN 2-3), microinvasive carcinoma, or invasive carcinoma in the repeat conization or hysterectomy specimen. Cone volume was calculated using the semi-ellipsoid formula based on the anteroposterior (AP), transverse (T), and longitudinal (L) dimensions provided in the pathology report of the initial conization: $\text{Volume} = (\pi/6) \times \text{AP} \times \text{T} \times \text{L}$.

Following the initial procedure, each patient underwent either repeat conization or hysterectomy based on a clinical evaluation that considered age, reproductive period, availability of sufficient cervical tissue, and comorbidities. Pathological outcomes from the second surgical procedure were categorized into two clinical groups. The low-risk group consisted of women with no preinvasive lesions or low-grade intraepithelial lesions (LSIL), whereas the high-risk group comprised women with HSIL (CIN 2 or CIN 3), microinvasive carcinoma, or invasive squamous cell carcinoma. All clinical variables, operative details, and pathological findings were extracted from electronic medical records using a standardized data collection form to ensure accuracy and internal consistency. The study was approved by the Ankara Bilkent City Hospital Institutional Ethics Committee (approval number: TABED 1-25-1885, date: 19.11.2025), and all procedures were conducted in accordance with Declaration of Helsinki and with strict adherence to patient confidentiality.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26. Associations between categorical variables, including margin characteristics, ECC results, HPV type, conization technique, and final pathological outcome, were evaluated using the chi-square test; Fisher's exact test was used when expected cell counts were insufficient. Continuous variables such as age, cone volume, and the interval between the initial conization and the final surgical procedure were categorized based on their median values. All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant.

RESULTS

The mean age of the patients was 46.6 ± 11.53 years (range, 24-72 years). The median cone volume was 2.64 cm^3 (range, $0.31\text{--}18.90 \text{ cm}^3$). The median interval between the initial conization and the subsequent surgical procedure was 84 days (range 25-387 days). When surgical margin patterns were examined, endocervical margin positivity, ECC positivity, or both were

notably frequent. Thirty-six (75%) patients had a positive endocervical margin and/or a positive ECC, with or without a positive ectocervical margin. However, 16 (33.3%) patients had positive ectocervical margins, with or without positive endocervical margins or positive ECC. Isolated endocervical margin positivity was present in 29.2% of patients, isolated ECC positivity in 4.2%, and combined endocervical margin positivity with ECC involvement in 33.3%. In contrast, only 25% of patients had isolated ectocervical margin positivity. Electrosurgical methods, such as NETZ and LEEP, constituted the majority of conization techniques. The NETZ applied to 52.1% of patients and LEEP applied to 8.3% of patients, whereas CKC was performed in 39.6% of patients. Most initial conization pathology results demonstrated HSIL, predominantly CIN 3, with 38 patients (79.2%) diagnosed with CIN 3. HPV testing was available for 38 patients, of whom 79.2% were HPV-positive; among these, 66.7% had HPV 16 and/or 18, and 10.4% had other high-risk types. The baseline clinical and pathological characteristics of the cohort are summarized in Table 1.

TABLE 1: General features before final surgical procedure.

Features		Mean $\pm$ SD	Median (range)
Age (years)		46.6 $\pm$ 11.53	46 (24-72)
Conization material's volume (cm ³)		4.2 $\pm$ 3.90	2.64 (0.31-18.90)
Time between conization and final surgical procedure (day)		101.6 $\pm$ 64.87	84 (25-387)
		n	%
Type of positivity after conization and ECC	Only ectocervical margin positivity	12	25
	Only endocervical margin positivity	14	29.2
	Only ECC positivity	2	4.2
	Ectocervical margin and endocervical margin positivity	3	6.3
	Ectocervical margin and ECC positivity	-	-
	Endocervical margin and ECC positivity	16	33.3
	Ectocervical margin and endocervical margin and ECC positivity	1	2.1
Conization procedure	Cold conization	19	39.6
	NETZ	25	52.1
	LEEP conization	4	8.3
Pathologic result of conization and ECC	HSIL (CIN 2)	9	18.8
	HSIL (CIN 3)	38	79.2
	HSIL (undetermined)	1	2.1
Cervical smear	Normal cytology	6	12.5
	ASCUS	16	33.3
	LSIL	6	12.5
	ASC-H	5	10.4
	HSIL	12	25
	Not reported	3	6.3

TABLE 1: Continued.

Features		Mean $\pm$ SD	Median (range)
HPV result	Negative	2	4.2
	Positive	38	79.2
	Unknown	8	16.7
HPV type ¹	16 and/or 18	32	66.7
	Others	5	10.4
	Unknown	1	2.1
Colposcopy	Performed	43	89.6
	Not performed	5	10.4
Colposcopic biopsy result ²	No preinvasive lesion	4	9.3
	LSIL	4	9.3
	HSIL (CIN 2)	8	18.6
	HSIL (CIN 3)	21	48.8
	HSIL (undetermined)	6	14

ECC: Endocervical curettage; LEEP: Loop electrosurgical excision procedure; NETZ: Needle excision of the transformation zone; CIN: Cervical intraepithelial neoplasia; ASCUS: Atypical squamous cell of undetermined significance; ASC-H: Atypical squamous cell cannot rule out high grade intraepithelial lesion; LSIL: Low grade intraepithelial lesion; HSIL: High grade intraepithelial lesion; SD: Standard deviation; HPV: Human papillomavirus; ¹: n=38 patients with HPV positivity; ²: n=43 patients who underwent colposcopic procedure.

Management strategies were distributed relatively evenly: conization was performed in 52.1% of cases and hysterectomy in 47.9%. Twenty-two patients (45.9%) had residual high-grade disease (HSIL, microinvasive carcinoma, or cancer), while the remaining 26 patients (54.1%) had only LSIL or no preinvasive lesions. The final pathology revealed CIN 3 in 15 patients (31.2%), CIN 2 in 2 patients (4.2%), microinvasive carcinoma in 1 patient (2.1%), and squamous cell carcinoma in 1 patient (2.1%). The pathological outcomes following the second surgical procedure are summarized in Table 2.

Of the two patients diagnosed with carcinoma, one had microinvasive squamous cell carcinoma, and the other had invasive squamous cell carcinoma. The patient diagnosed with microinvasive carcinoma was 72 years old. HPV testing was negative, cervical cytology showed HSIL, and colposcopic

biopsy revealed HSIL. CKC was performed. Pathological examination showed HSIL, with positive endocervical surgical margins and positive ECC samples. The cone volume was 3.67 cm³. Due to insufficient remaining cervical tissue, a hysterectomy was performed, and microinvasive squamous cell carcinoma was diagnosed in the hysterectomy specimen. The second patient was 61 years old. Cervical cytology revealed Atypical squamous cell of undetermined significance with HPV-16 positivity. Colposcopic biopsy demonstrated HSIL. The NETZ procedure was performed. The conization specimen showed HSIL with positive ectocervical margins. Due to insufficient remaining cervical tissue, a hysterectomy was performed, and invasive squamous cell carcinoma was diagnosed.

TABLE 2: Surgical procedure of patients with surgical margin and/or endocervical curettage positivity and final pathologic results of surgical materials.

Features		n	%
Surgical procedure after conization and ECC	Re-conization	25	52.1
	Hysterectomy	23	47.9
Final pathologic result	No preinvasive lesion	19	39.5
	LSIL	7	14.6
	HSIL (CIN 2)	2	4.2
	HSIL (CIN 3)	15	31.2
	HSIL (undetermined)	3	6.3
	Microinvasive SCC	1	2.1
	SCC	1	2.1

ECC: Endocervical curettage; LSIL: Low grade intraepithelial lesion; HSIL: High grade intraepithelial lesion; CIN: Cervical intraepithelial neoplasia; SCC: Cervical squamous cell carcinoma.

When the relationship between residual high-grade disease and clinicopathological variables was examined, no statistically significant differences were identified. However, several variables demonstrated numerical differences without reaching statistical significance. The most notable numerical difference was observed in the association between conization technique and residual disease. Residual HSIL or carcinoma was identified in 63.2% of women who underwent cold-knife conization (n=12/19), compared with 34.5% (n=10/29) among those treated with NETZ or LEEP, but this difference did not reach statistical significance (p=0.051). Similarly, when evaluating the interval between conization and the final surgical procedure, the residual disease rate was 59.1% (n=13/22) in women who underwent the second surgery more than 84 days later, compared with 34.8% (n=8/23) in those with a shorter interval (p=0.102). The relationship between clinical and pathological factors and residual disease was presented in Table 3.

DISCUSSION

In our cohort, 45.9% of women undergoing repeat surgery were found to have residual HSIL or cancer. This result is consistent with previous reports showing that approximately half of margin-positive women harbor clinically significant residual disease at re-excision.¹¹ However, in our study, none of the evaluated clinical or pathological variables demonstrated a statistically significant association with residual high-grade disease. A numerical difference was observed across types of initial excisional procedure, although it did not reach statistical significance. Residual high-grade disease was numerically less frequent after electrosurgical excision than after cold-knife conization. Previous studies have reported clinically significant residual disease following repeat surgery in a substantial proportion of women with positive surgical margins after conization.^{12,13} In addition, several studies have suggested an association between the type of initial excisional

TABLE 3: Factors affecting residual lesions (HSIL or cancer) in final surgical procedure in patient with margin positivity after conization and endocervical curettage.

Factors		Residue lesion in final surgical procedure after conization and endocervical curettage		p-value
		No preinvasive lesion or LSIL	HSIL or cancer	
		n (%)	n (%)	
Age ¹	≤46 years	14 (58.3)	10 (41.7)	0.562
	>46 years	12 (50)	12 (50)	
Conization material's volume ^{1,2}	≤2.64 cm ³	11 (50)	11 (50)	0.763
	>2.64 cm ³	12 (54.5)	10 (45.5)	
Time between conization and final surgical procedure ^{1,3}	≤84 days	15 (65.2)	8 (34.8)	0.102
	>84 days	9 (40.9)	13 (59.1)	
Conization procedure	Cold conization	7 (36.8)	12 (63.2)	0.051
	NETZ or LEEP conization	19 (65.5)	10 (34.5)	
Ectocervical margin	Negative	16 (50)	16 (50)	0.413
	Positive	10 (62.5)	6 (37.5)	
Endocervical margin and/or ECC	Negative	8 (66.7)	4 (33.3)	0.316
	Positive	18 (50)	18 (50)	
Surgical procedure after conization and ECC	Re-conization	16 (64)	9 (36)	0.154
	Hysterectomy	10 (43.5)	13 (56.5)	
HPV type ⁴	16 and/or 18	19 (59.4)	13 (40.6)	0.377
	Others	4 (80)	1 (20)	
Colposcopic biopsy result ⁵	No preinvasive lesion or LSIL	5 (62.5)	3 (37.5)	0.477
	HSIL or cancer	17 (48.6)	18 (51.4)	
Colposcopic biopsy and conization and ECC result ⁶	HSIL (CIN 2)	5 (71.4)	2 (28.6)	0.353
	HSIL (CIN 3)	21 (52.5)	19 (47.5)	

¹: Median value; ²: n=44 patients, four patients excluded because of the diameter of the conization specimen was not reported; ³: n=45 patients, Time between conization and final surgical procedure wasn't known in three patients; ⁴: n=37 patients, HPV genotype was unknown in 11 patients; ⁵: n=43 patients, who underwent colposcopic procedure; ⁶: n=47 patients, the grade of CIN couldn't be determined in one patient; HPV: Human papillomavirus; ECC: Endocervical curettage; CIN: Cervical intraepithelial neoplasia; LSIL: Low grade intraepithelial lesion; HSIL: High grade intraepithelial lesion; LEEP: Loop electrosurgical excision procedure; NETZ: Needle excision of the transformation zone

procedure and the risk of residual high-grade lesions, with electrosurgical techniques generally associated with lower residual disease rates compared with cold knife conization.¹⁴ This difference has been attributed to variations in excision geometry, thermal effects, and the ability to achieve adequate lateral margins.^{15,16} Our findings are consistent with these observations, as residual high-grade lesions were observed less frequently after electrosurgical procedures than after cold knife conization, although the association reached only borderline statistical significance.

The literature consistently identifies endocervical margin involvement as a stronger predictor of residual CIN 2+ than ectocervical margin involvement. Across multiple series evaluating repeat surgical specimens following conization, residual high-grade disease has been reported in approximately 30-50% of patients with endocervical margin positivity, whereas the corresponding rate for isolated ectocervical margin involvement is substantially lower and generally remains below 10%.^{5,17,18} Residual high-grade lesions have been reported in approximately 30-55% of patients with positive surgical margins after LEEP, in 20-40% after cold-knife conization, and in 15-30% after larger excisional procedures such as NETZ. Nevertheless, across all these techniques, endocervical margin involvement remains the most consistent predictor of residual disease.^{5,11,12,17} This pattern is thought to reflect the anatomical trajectory of high-risk HPV-associated neoplasia, which frequently extends proximally into the endocervical canal. Our findings are consistent with this distinction. Isolated ectocervical margin positivity was rarely associated with high-grade residual pathology, whereas endocervical margin involvement and ECC positivity showed nearly equal proportions of high- and low-risk pathological outcomes. Despite this association, the predictive value of these markers remained insufficient for guiding clinical management in isolation echoing the conclusions of ASCCP and ESGO recommendations, which caution against reliance on margin status alone.^{3,19}

ECC positivity has been proposed as an additional risk factor for residual CIN 2+, with some studies reporting strong associations, particularly among women with concomitant endocervical margin involvement.²⁰ However, its sensitivity varies widely, especially after LEEP, where thermal artifact and tissue fragmentation may obscure histological interpretation.²¹ The results of our cohort reflect this variability. While ECC positivity was more common among women with high-risk outcomes, nearly half of ECC positive patients did not harbor clinically relevant pathology at repeat excision.^{17,20}

Cone volume has recently been investigated as a potential parameter reflecting excision depth and lesion burden.^{19,22}

In our cohort, cone volume did not demonstrate a statistically significant association with residual disease. Therefore, cone volume alone may have limited value as a predictive marker for residual high-grade pathology. Similar findings have been reported in external studies, where cone volume demonstrated statistical correlation but lacked sufficient discriminatory performance for clinical application.²³

The distribution of residual disease across excisional techniques in our study mirrors prior comparative analyses.²⁴ While CKC generally achieves clearer margins and deeper tissue sampling, our data did not reveal an advantage for CKC over LEEP/NETZ in preventing residual high-grade pathology. This observation aligns with multiple studies showing that procedural choice alone does not adequately stratify the risk of persistence or recurrence and must be interpreted alongside lesion characteristics and margin location.^{25,26}

The clinical management of margin-positive HSIL remains a complex balancing act between avoiding overtreatment and preventing undertreatment. Repeat excision may expose patients, particularly those of reproductive age, to adverse outcomes such as cervical stenosis, infertility, cervical insufficiency, and preterm birth. Large meta-analyses have confirmed increased preterm birth risk following deeper or repeated cervical excisions.²⁷ Conversely, under-treatment risks missing persistent HSIL or microinvasion, especially in cases where initial sampling may have been incomplete or affected by fragmentation.¹⁶ The identification of reliable predictors for residual disease thus remains a pressing clinical need.

Our findings support a multifactorial approach integrating endocervical margin status, ECC results, cone volume, and index pathology, rather than relying on any single parameter. Such approaches align with evolving risk-based models that incorporate virologic, cytological, and pathological features to refine prediction of CIN 2+ persistence.²⁸ While HPV genotype and viral load could further enhance prediction accuracy, these data were not available for all patients in our cohort and represent an important area for future study.

Study Limitations

This study underscores the limitations of relying solely on surgical margin assessment and emphasizes the value of composite clinical and pathological evaluation. Given that nearly half of women with positive margins did not have residual high-grade disease, individualized management strategies, particularly for women desiring fertility preservation, are warranted. Conversely, the substantial proportion of women with residual HSIL or carcinoma underscores the need for continued vigilance and risk-stratified indications for secondary excision.

CONCLUSION

Residual HSIL or carcinoma was detected in nearly half of the women undergoing repeat excision after a margin-positive conization. Residual disease was numerically lower among patients treated with electrosurgical techniques than among those who underwent cold-knife conization; however, this difference did not reach statistical significance. Overall, no single clinical or pathological parameter reliably predicted residual disease. This study is limited by its retrospective single-center design and modest sample size, which may affect generalizability. Therefore, larger prospective studies are needed to validate these observations.

Ethics

Ethics Committee Approval: The study was approved by the Ankara Bilkent City Hospital Institutional Ethics Committee (approval number: TABED 1-25-1885, date: 19.11.2025), and all procedures were conducted in accordance with Declaration of Helsinki and with strict adherence to patient confidentiality.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.Ö.U., Concept: Y.Ö.U., Design: Y.Ö.U., O.A., M.Ü., F.K., Data Collection or Processing: Y.Ö.U., O.A., M.Ü., F.K., Analysis or Interpretation: A.E.K., A.B., M.Ş., Z.Ö.B., Literature Search: Y.Ö.U., A.E.K., M.Ş., Writing: Y.Ö.U., A.B., Z.Ö.B.

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Trends in Overall Survival and Associated Factors in Metastatic Rectal and Rectosigmoid Cancer: A SEER-based Cohort Study (2000-2022)

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ABSTRACT

Objective: Metastatic rectal and rectosigmoid cancers remain associated with poor prognosis despite advances in systemic therapy. Although outcomes in metastatic colorectal cancer have improved overall, population-level survival trends specific to rectal cancer and their evolution across calendar time remain incompletely characterized.

Material and Methods: This population-based retrospective cohort study used data from the surveillance, epidemiology, and end results program. Adults diagnosed with metastatic rectal or rectosigmoid adenocarcinoma between 2000 and 2022 were included and categorized into prespecified diagnosis eras (2000-2007, 2008-2017, and 2018-2022). Overall survival (OS) was estimated by the Kaplan-Meier method and evaluated with multivariable Cox proportional hazards models adjusted for demographic and treatment-related factors. Adjusted survival curves were generated using regression standardization, and restricted mean survival time (RMST) was calculated over 60 months. Effect modification by age was assessed using stratified analyses and testing for interaction; proportional hazards assumptions were formally evaluated.

Results: A total of 33,277 patients with metastatic rectal or rectosigmoid cancer were included in the study. Median OS for the overall cohort was 16.2 months. Survival improved among patients diagnosed during 2008-2017 compared with those diagnosed during 2000-2007, with higher observed and adjusted survival estimates and longer RMST. In contrast, no statistically significant improvement in OS was observed in the most recent era (2018-2022). Older age was independently associated with worse OS, whereas receipt of chemotherapy and surgery to the primary tumor were associated with improved OS. Age-by-era interaction analyses did not demonstrate significant effect modification.

Conclusion: In this large population-based analysis, OS among patients with metastatic rectal and rectosigmoid cancer improved during the mid-diagnosis era, but appeared to plateau in more recent years. These findings indicate that survival gains have not occurred uniformly over time and underscore the importance of interpreting population-level outcomes within specific calendar eras and disease subtypes.

Keywords: Metastatic rectal cancer; overall survival; SEER; population-based study; temporal trends

INTRODUCTION

Colorectal cancer (CRC) remains a leading cause of cancer-related morbidity and mortality worldwide. According to the GLOBOCAN 2020 estimates, CRC is the third most commonly diagnosed malignancy and the second leading cause of

cancer-related death globally.¹ Rectal cancer accounts for approximately one-third of all CRC cases, and a substantial proportion of patients present with distant metastatic disease at the time of diagnosis.² Despite advances in multimodal treatment, the prognosis of metastatic rectal cancer remains poor, underscoring the ongoing clinical burden of this disease.

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Over the past two decades, the management of metastatic colorectal and rectal cancer has evolved substantially. The introduction of combination chemotherapy regimens, improvements in radiotherapy techniques, advances in surgical strategies, and wider adoption of multidisciplinary care have contributed to incremental survival gains in selected patient populations.³⁻⁶ However, survival outcomes in metastatic rectal cancer remain heterogeneous, and improvements have not been uniform across patient subgroups or calendar periods, highlighting the need for population-level evaluations of long-term outcomes.⁵⁻⁷

Several demographic and treatment-related factors, including age, sex, and receipt of systemic therapy, radiotherapy, or surgery, have been shown to influence survival in metastatic disease.^{3,8,9} Nevertheless, much of the existing evidence is derived from clinical trials or single-institution cohorts, which may not fully reflect real-world treatment patterns or long-term temporal trends.^{10,11} Population-based cancer registries, such as the surveillance, epidemiology, and end results (SEER) Program, provide a unique opportunity to evaluate survival outcomes across large, unselected cohorts over extended periods.^{8,11} However, relatively few studies have focused specifically on long-term survival trends in metastatic rectal and rectosigmoid cancer across distinct treatment eras using both observed and adjusted survival measures.^{5,8}

This study aimed, using SEER data, to characterize temporal trends in overall survival (OS) among patients with metastatic rectal and rectosigmoid cancer diagnosed between 2000 and 2022. Specifically, we evaluated changes in observed and adjusted survival across prespecified diagnostic eras and identified clinical and treatment-related factors independently associated with OS at the population level.

MATERIAL AND METHODS

Study Design and Data Source

This retrospective cohort study used data from the SEER Program of the National Cancer Institute (NCI), a population-based cancer registry system covering multiple U.S. geographic areas. Case listing and data extraction were performed using SEER*Stat software (Surveillance Research Program, NCI; SEER*Stat version 9.0.42.2). The study used deidentified, publicly available data; therefore, institutional review board approval and informed consent were not required.

Study Population

Eligible patients were adults (≥ 18 years) with microscopically confirmed, malignant rectal or rectosigmoid adenocarcinoma identified using ICD-O-3 primary site codes C20.9 (Rectum,

NOS) and C19.9 (Rectosigmoid junction). Metastatic disease at diagnosis was defined using SEER Summary Stage coded as "Distant". Cases identified solely from death certificates or autopsy reports, patients alive with no recorded survival time, and individuals with unknown age were excluded. Only malignant tumors were included. For patients with multiple primary tumors, one tumor per life page was retained in accordance with SEER multiple primary rules.

For temporal comparisons, the primary analysis categorized patients into prespecified diagnosis eras: 2000-2007, 2008-2017, and 2018-2022. A prespecified sensitivity analysis used an alternative calendar-time dichotomization (2000-2010 vs. 2011-2021).

Variables and Outcome Definition

Covariates included age at diagnosis (continuous), sex, and treatment-related variables derived from SEER recode fields: chemotherapy, radiation therapy, and surgery to the primary tumor. Because treatment fields may contain underreported or unknown values in registry data, "no" and "unknown" categories were combined as no/unknown for primary analyses.

The primary endpoint was OS, defined as the time from diagnosis to death from any cause or last follow-up, measured in months.

Ethical Considerations

This study was conducted using deidentified, publicly available data from the SEER. The use of SEER data does not involve direct patient contact and does not include identifiable private information. Therefore, institutional review board approval and informed consent were not required. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical Analysis

All statistical analyses were conducted using R version 4.5.2 (R Foundation for Statistical Computing, Vienna, Austria), with supplementary data processing and figure preparation performed in Python version 3.11.

Baseline characteristics were summarized overall and by diagnosis era. Kaplan-Meier methods were used to estimate observed OS, and survival distributions were compared using the log-rank test. Associations between covariates and OS were evaluated using Cox proportional hazards regression. Univariable Cox models were fitted for all covariates and reported in the supplementary materials. The primary multivariable Cox proportional hazards model included prespecified covariates selected a priori based on clinical relevance and prior literature, rather than solely on

univariable statistical significance. The covariates included age (continuous), sex, chemotherapy (yes vs. no/unknown), radiation therapy (yes vs. no/unknown), surgery to the primary tumor (any vs. none/unknown), and diagnosis era (2008-2017 vs. 2000-2007). The most recent diagnosis era (2018-2022) was not included in the primary multivariable model because it was not associated with OS in univariable analyses and had limited follow-up duration. Effect estimates are presented as hazard ratios (HRs) with 95% confidence intervals (CIs).

Adjusted survival curves by diagnosis era were estimated from the fitted multivariable Cox model using regression standardization. For each era, standardized survival probabilities were computed by applying the model coefficients to the full cohort's observed covariate distribution and averaging the predicted survival functions across individuals. Restricted mean survival time (RMST) was calculated as the area under the standardized survival curve from 0 to 60 months.

Effect modification by age was assessed using 2 complementary approaches: (1) age-stratified multivariable Cox models within prespecified age groups (<50, 50-59, 60-69, 70-79, and ≥ 80 years) adjusting for sex and treatment variables; and (2) inclusion of multiplicative interaction terms between mean-centered age (continuous) and diagnosis era in the multivariable model, with joint significance assessed using a Wald χ^2 test.

The proportional hazards assumption was evaluated using scaled Schoenfeld residuals for key covariates (including age and diagnosis era), with visual inspection for time trends. As a sensitivity check, a global time-interaction analysis was performed by interacting diagnosis-era and treatment variables with log (time). No material violations were identified.

Model discrimination for explanatory purposes was summarized using Harrell's concordance index (C-index). Internal validation was performed using bootstrap resampling (100 resamples) to estimate optimism and obtain an optimism-corrected C-index. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Study Population and Baseline Characteristics

A total of 33 277 patients with metastatic rectal or rectosigmoid cancer diagnosed between 2000 and 2022 were included in the analysis. Of these, 13 100 patients (39.4%) were diagnosed in 2000-2007, 19 351 (58.1%) were diagnosed in 2008-2017, and 826 (2.5%) were diagnosed in 2018-2022 (Table 1).

The median age of the overall cohort was 62 years [interquartile range (IQR): 52-71]. Patients diagnosed in 2000-2007 were older (median, 67 years; IQR, 52-77) than those diagnosed in 2008-2017 (median, 62 years; IQR, 52-72) and those diagnosed in 2018-2022 (median, 63 years; IQR, 54-71). Overall, 13,559 patients (40.8%) were female and 19 718 (59.2%) were male. Chemotherapy was administered to 20 463 patients (61.5%), radiation therapy to 10,927 patients (32.8%), and surgery to the primary tumor to 9 842 patients (29.6%) (Table 1).

Overall Survival by Diagnosis Era

During follow-up, 29 532 deaths were observed. The median OS for the entire cohort was 16.2 months (IQR: 6.1-38.7) (Table 1).

Median OS was 14.0 months (IQR: 4.0-31.0) in 2000-2007, 17.0 months (IQR: 6.0-38.0) in 2008-2017, and 14.4 months (IQR: 5.7-29.6) in 2018-2022. Observed OS rates at 12 months were 52.9%, 60.0%, and 54.5%, respectively; the corresponding 60-month OS rates were 10.8%, 15.0%, and 13.0% (Table 2). Kaplan-Meier curves demonstrated significant differences in unadjusted OS across diagnostic eras (log-rank $p < 0.001$) (Figure 1).

Cox Proportional Hazards Analyses

In univariable analyses, older age (HR per year: 1.02; 95% CI: 1.01-1.03), absence of chemotherapy (HR: 2.44; 95% CI: 2.38-2.50), and absence of surgery to the primary tumor (HR: 2.08; 95% CI: 2.04-2.12) were associated with worse OS (Supplementary Table 1).

In the multivariable Cox model adjusted for age, sex, chemotherapy, radiation therapy, and surgery of the primary tumor, a diagnosis in 2008-2017 was associated with improved OS compared with 2000-2007 (HR: 0.83; 95% CI: 0.81-0.85; $p < 0.001$). Chemotherapy (HR: 0.49; 95% CI: 0.47-0.50) and surgery to the primary tumor (HR: 0.46; 95% CI: 0.45-0.47) were independently associated with OS (Table 3).

Adjusted Survival, RMST, and Age-stratified Analyses

Standardized survival estimates derived from the multivariable Cox model showed an increase in adjusted OS across diagnostic eras. The RMST over 60 months was 20.9 months for patients diagnosed in 2000-2007, 23.8 months for those diagnosed in 2008-2017, and 24.7 months for those diagnosed in 2018-2022; this corresponds to an absolute difference of approximately 3.8 months between the earliest and most recent eras.

Age-stratified multivariable analyses demonstrated that the association between later diagnosis era and OS was generally

TABLE 1: Baseline characteristics of patients with metastatic rectal or rectosigmoid cancer by diagnosis era (SEER, 2000-2022).

Characteristics	Overall cohort (n=33,277) (%)	2000-2007 (n=13,100) (%)	2008-2017 (n=19,351) (%)	2018-2022 (n=826) (%)
Age, median (IQR), y	62 (52-71)	67 (52-77)	62 (52-72)	63 (54-71)
Survival months, median (IQR)	16.2 (6.1-38.7)	14.0 (4.0-31.0)	17.0 (6.0-38.0)	14.4 (5.7-29.6)
Age group, no. (%)				
<50 y	4 982 (15.0)	1 930 (14.7)	2 936 (15.2)	116 (14.0)
50-59 y	7 231 (21.7)	2 597 (19.8)	4 461 (23.1)	173 (20.9)
60-69 y	9 846 (29.6)	3 288 (25.1)	6 306 (32.6)	252 (30.5)
70-79 y	7 562 (22.7)	3 472 (26.5)	3 905 (20.2)	185 (22.4)
≥80 y	3 656 (11.0)	1 813 (13.8)	1 743 (9.0)	100 (12.1)
Sex, no. (%)				
Female	13 559 (40.8)	5 578 (42.6)	7 663 (39.6)	318 (38.5)
Male	19 718 (59.2)	7 522 (57.4)	11 688 (60.4)	508 (61.5)
Race (SEER recode), no. (%)				
White	25 694 (77.2)	10 487 (80.0)	14 594 (75.4)	613 (74.2)
Black	4 012 (12.1)	1 461 (11.2)	2 440 (12.6)	111 (13.4)
American Indian/Alaska Native	214 (0.6)	88 (0.7)	120 (0.6)	6 (0.7)
Asian or Pacific Islander	3 028 (9.1)	933 (7.1)	2 009 (10.4)	86 (10.4)
Unknown	329 (1.0)	131 (1.0)	188 (1.0)	10 (1.2)
Hispanic origin (NHIA), no. (%)				
Non-Hispanic	26 987 (81.1)	10 862 (82.9)	15 467 (79.9)	658 (79.7)
Hispanic	5 841 (17.6)	2 018 (15.4)	3 669 (19.0)	154 (18.6)
Unknown	449 (1.3)	220 (1.7)	215 (1.1)	14 (1.7)
Treatment characteristics, no. (%)				
Chemotherapy, yes	20 463 (61.5)	6 876 (52.5)	13 094 (67.7)	493 (59.7)
Chemotherapy, no/unknown	12 814 (38.5)	6 224 (47.5)	6 257 (32.3)	333 (40.3)
Radiation therapy, yes	10 927 (32.8)	3 901 (29.8)	6 768 (35.0)	258 (31.2)
Radiation therapy, no/unknown	22 350 (67.2)	9 199 (70.2)	12 583 (65.0)	568 (68.8)
Surgery to primary tumor, no surgery	21 614 (65.0)	8 608 (65.7)	12 481 (64.5)	525 (63.6)
Surgery to primary tumor, any surgery	9 842 (29.6)	3 828 (29.2)	5 769 (29.8)	245 (29.7)
Surgery to primary tumor, unknown	1 821 (5.5)	664 (5.1)	1 101 (5.7)	56 (6.8)

IQR: Interquartile range; NHIA: North American Hispanic Identification Algorithm; SEER: Surveillance, epidemiology, and end results. Data are presented as numbers (percentages) unless otherwise indicated. Percentages may not total 100% because of rounding. Treatment variables were derived from SEER recode fields and included categories for no treatment and unknown treatment status; the latter may reflect underreporting in registry data. Survival time is reported in months from diagnosis to death or last follow-up.

consistent across age groups, with attenuated estimates among patients aged 80 years or older (Supplementary Table 2). Formal testing for interaction showed no statistically significant interaction between age and diagnosis era (joint Wald test, $p=0.313$).

Sensitivity Analyses and Model Assumptions

In sensitivity analyses using an alternative calendar-time dichotomization (2000-2010 vs. 2011-2021), a diagnosis during 2011-2021 remained associated with improved OS (HR: 0.82; 95% CI: 0.79-0.85; $p<0.001$) (Supplementary Table 3).

No material violations of the proportional hazards assumption were observed based on visual inspection of scaled Schoenfeld residuals for age and diagnosis era (Figure 2). Model discrimination for explanatory analyses was stable (Harrell C-index: 0.69; optimism-corrected: 0.67).

DISCUSSION

In this large, population-based analysis of patients with metastatic rectal and rectosigmoid cancer, we observed substantial temporal changes in OS across more than two decades. Analysis of SEER data from 2000 through 2022 showed that OS improved notably during the mid-diagnosis

era (2008-2017) compared with the early era (2000-2007), with gains evident in both observed and adjusted survival metrics. These improvements were reflected not only in median OS but also in absolute survival measures, including RMST. In contrast, no statistically significant survival improvement was observed in the most recent era. Importantly, survival estimates for this period should be interpreted cautiously, given the limited follow-up duration and reduced statistical precision, which constrain definitive inference regarding

long-term outcomes. Collectively, these findings provide contemporary population-level evidence that survival gains in metastatic rectal and rectosigmoid cancer have not occurred uniformly over time and underscore the importance of interpreting long-term outcomes within specific calendar eras.

A particularly notable observation is that survival gains were not linear across calendar time. While patients diagnosed in the mid-era experienced a clear improvement in OS compared with earlier cohorts, this advantage did not extend into the most recent period. Several factors may contribute to this apparent attenuation. Real-world adoption of newer systemic therapies often lags behind evidence generated in randomized clinical trials and may be influenced by patient fitness, comorbidity burden, and access to care—factors that are incompletely captured in trial populations and registry-based analyses.^{12,13} In parallel, contemporary cohorts increasingly include older and more clinically heterogeneous patients, including individuals with biologically aggressive disease who were underrepresented in earlier treatment eras or clinical trials.^{9,14} Although advances in combination chemotherapy, targeted agents, and biologic therapies have improved outcomes for selected patient subgroups, these benefits may not translate uniformly at the population level, particularly in rectal cancer, where treatment sequencing, local control strategies, and metastatic behavior differ from those of colon cancer.³⁻⁷ The absence of a significant age-era interaction in our analyses further suggests that the observed attenuation of survival gains is unlikely to be explained solely

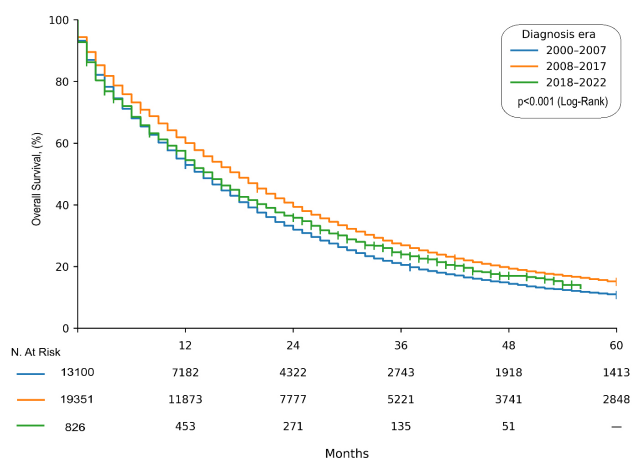


FIGURE 1: Kaplan-Meier curves for overall survival by diagnosis era.

Kaplan-Meier curves depict observed overall survival stratified by diagnosis era (2000-2007, 2008-2017, and 2018-2022). Time is presented in months, measured from diagnosis to death or last follow-up. Numbers at risk are displayed below the X-axis. Differences between survival curves were assessed using the log-rank test.

TABLE 2: Observed overall survival and median survival by diagnosis era.

Diagnosis era	n	Deaths	Median OS (months)	OS 12m	OS 24m	OS 36m	OS 48m	OS 60m
2000-2007	13,100	12,566	14.0 (4.0-31.0)	52.9%	32.0%	20.5%	14.4%	10.8%
2008-2017	19,351	17,341	17.0 (6.0-38.0)	60.0%	39.4%	26.9%	19.3%	15.0%
2018-2022	826	625	14.4 (5.7-29.6)	54.5%	35.8%	23.9%	17.0%	13.0%

IQR: Interquartile range; OS: Overall survival; median overall survival and observed survival rates at prespecified time points (12, 24, 36, 48, and 60 months) were estimated using the Kaplan-Meier method. Survival rates represent observed, unadjusted estimates by diagnosis era. Median survival is reported with interquartile ranges.

TABLE 3: Multivariable Cox proportional hazards analysis for overall survival.

Variable	HR	95% CI	p-value
Age (per 1 year)	1.01	1.01-1.01	<0.001
Female sex (vs. male)	0.93	0.91-0.95	<0.001
Chemotherapy (yes vs. no/unk)	0.49	0.47-0.50	<0.001
Radiation (yes vs. no/unk)	0.83	0.81-0.85	<0.001
Surgery to primary (any vs. none/unk)	0.46	0.45-0.47	<0.001
Era 2008-2017 (vs. 2000-2007)	0.83	0.81-0.85	<0.001

CI: Confidence interval; HR: Hazard ratio; hazard ratios were estimated using a multivariable Cox proportional hazards model adjusted for age, sex, chemotherapy, radiation therapy, surgery to the primary tumor, and diagnosis era. The reference categories were: male sex; no/unknown chemotherapy; no/unknown radiation therapy; no/unknown surgery to the primary tumor; and the diagnosis era 2000-2007.

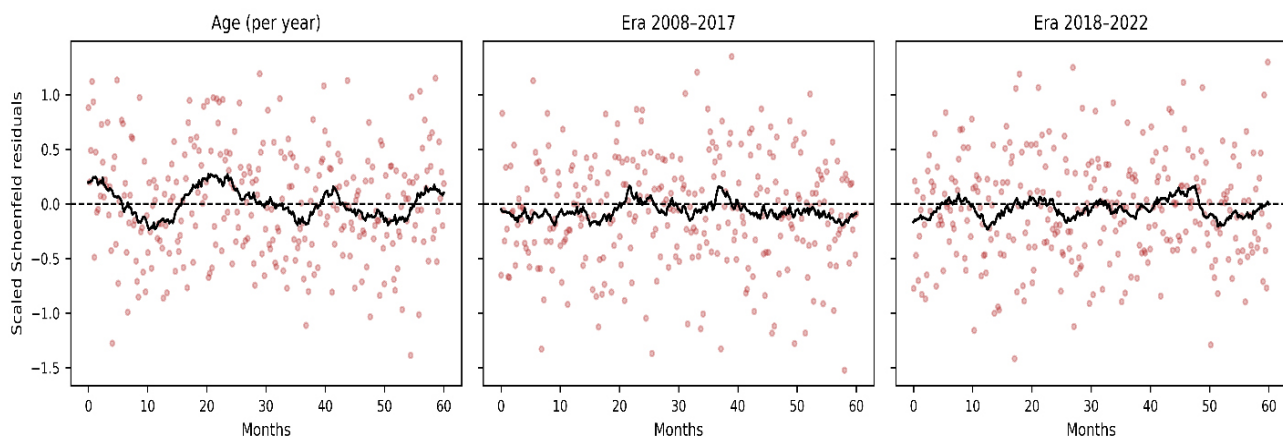


FIGURE 2: Scaled schoenfeld residuals for assessment of the proportional hazards assumption.

Scaled Schoenfeld residuals are shown for age (modeled as a continuous variable) and diagnosis era (2008-2017 and 2018-2022, with 2000-2007 as the reference category). The solid line represents a smoothed fit of the residuals over time, and the dashed horizontal line indicates zero. Visual inspection revealed no systematic time-dependent patterns, indicating no material violation of the proportional hazards assumption for the included covariates.

by age-related treatment selection. Rather, these findings highlight the complexity of translating therapeutic innovation into sustained population-level benefit and reinforce the need to interpret survival trends within the context of real-world practice.¹¹⁻¹³

Previous population-based and registry-based studies have generally reported improvements in survival among patients with metastatic CRC over time, attributing these gains to advances in systemic therapy and supportive care.³⁻⁶ At the same time, an expanding literature has emphasized that survival benefits demonstrated in clinical trials are not always replicated to the same extent in routine clinical practice, reflecting differences in patient selection, treatment delivery, and health system-related factors.^{12,13,15} Within this context, the present study provides a rectal cancer-specific, era-stratified assessment of OS in a large, unselected population-based cohort. The increasing incorporation of molecular stratification into treatment decision-making has led to meaningful survival improvements in selected subgroups; however, these benefits may be heterogeneously distributed at the population level. Survival advantages observed in carefully selected clinical trial populations may therefore be attenuated in real-world settings that increasingly include older patients, individuals with greater comorbidity burden, and those with biologically aggressive disease features.^{7,9,12,14} Viewed in this light, our findings align with prior population-level observations highlighting the gap between therapeutic efficacy and real-world effectiveness and underscore the importance of disease-specific and era-specific analyses when interpreting long-term survival trends in metastatic rectal and rectosigmoid cancer.¹⁵⁻¹⁸

From a clinical and population-level perspective, these findings suggest that improvements in OS cannot be assumed to occur uniformly over time, despite ongoing therapeutic advances. The attenuation of survival gains observed in the most recent era indicates that population-level outcomes may be shaped by a broader constellation of factors beyond the availability of new systemic therapies alone. These include patient vulnerability, treatment sequencing, multidisciplinary care integration, and health system-level constraints, which are not directly captured in registry data but may meaningfully influence real-world effectiveness.^{5,9,12,14} In metastatic rectal and rectosigmoid cancer—where treatment pathways and metastatic patterns differ from those of colon cancer—extrapolation from aggregated CRC cohorts may obscure clinically relevant heterogeneity.¹¹ Taken together, these observations support cautious interpretation of survival trends and emphasize the value of contextual, era-specific evaluation when assessing temporal changes in population-based outcomes.^{5,12,13}

Study Limitations

This study has several strengths. The use of a large, population-based dataset provides substantial statistical power and enhances generalizability across real-world clinical settings. The extended study period enabled robust evaluation of temporal survival patterns across diagnostic eras, and the focus on rectal and rectosigmoid cancers addresses an important gap in prior CRC-wide analyses. Methodologically, the use of both observed and adjusted survival estimates, including RMST, together with formal assessment of proportional hazards assumptions, enhances

the robustness of the findings. Several limitations should be acknowledged. The SEER database lacks detailed molecular, genomic, and inflammatory biomarkers, limiting adjustment for key biological factors that may influence prognosis and treatment selection. Treatment variables derived from SEER recode fields are subject to underreporting, and unknown treatment status is grouped with no treatment, potentially biasing associations toward the null. In addition, comorbidity burden, performance status, frailty, and socioeconomic factors are not systematically captured. Cause-specific mortality data were unavailable, precluding competing risk analyses. In addition, the follow-up duration for patients diagnosed in the most recent era (2018-2022) was limited, reducing statistical precision and constraining inferences regarding long-term survival patterns. Finally, given the retrospective, registry-based design, residual confounding cannot be fully excluded, and external validation in independent cohorts will be important to confirm generalizability.

CONCLUSION

In this population-based analysis of metastatic rectal and rectosigmoid cancers, OS improved during the mid-diagnosis era, but did not continue to increase in the most recent period. These findings indicate that survival gains have not occurred uniformly over time and highlight the importance of interpreting long-term outcomes within specific calendar eras and disease subtypes. Continued improvement in real-world outcomes may require attention to factors beyond therapeutic innovation alone, including patient heterogeneity and health system-level influences.

Ethics

Ethics Committee Approval: The study was approved by the institutional ethics committee (approval number: TABED 1-25-1885, date: 19.11.2025), and all procedures were conducted in accordance with Declaration of Helsinki and with strict adherence to patient confidentiality.

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.Ö.U., Concept: Y.Ö.U., Design: Y.Ö.U., O.A., M.Ü., F.K., Data Collection or Processing: Y.Ö.U., O.A., M.Ü., F.K., Analysis or Interpretation: A.E.K., A.B., M.Ş., Z.Ö.B., Literature Search: Y.Ö.U., A.E.K., M.Ş., Writing: Y.Ö.U., A.B., Z.Ö.B.

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

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Supplementary Tables 1-3: <https://d2v96fxpocvxx.cloudfront.net/bd1986e1-0bc1-4f4d-af66-3a184850a065/content-images/31415aa4-dfbc-438c-a410-63d60fb19120.pdf>

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Impact of Post-chemotherapy Radiotherapy on Survival Outcomes in Inoperable Malignant Mesothelioma: A Single-center Retrospective Analysis

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ABSTRACT

Objective: Malignant pleural mesothelioma (MPM) is a rare and aggressive form of cancer with limited treatment options and a grim prognosis. While platinum-based chemotherapy remains the primary treatment for inoperable or metastatic disease, the role of radiotherapy (RT) in current treatment approaches remains incompletely defined. This study aimed to assess the effect of RT after first-line systemic chemotherapy on survival outcomes in patients with metastatic or inoperable malignant mesothelioma.

Material and Methods: We conducted a retrospective review of the medical records of 58 patients with unresectable or metastatic MPM who were treated from January 2022 through December 2024 at a tertiary oncology center. All patients received platinum-pemetrexed-based first-line chemotherapy followed by pemetrexed maintenance. Based on post-treatment management, patients were categorized into RT and non-RT groups. Kaplan-Meier analyses were conducted to assess progression-free survival (PFS) and overall survival (OS), and statistical significance was assessed using the log-rank test.

Results: RT was administered to 41.4% of patients after initial chemotherapy. The RT group showed a significantly longer median PFS (14.1 months) compared with the non-RT group (9.7 months) [hazard ratio (HR): 0.34; 95% confidence interval (CI): 0.16-0.73; $p<0.045$]. Similarly, median OS was notably longer in the RT group (24.6 months vs. 14.5 months; HR: 0.55; 95% CI: 0.31-0.72; $p<0.003$). Moreover, a greater proportion of RT patients received second-line immunotherapy. No grade ≥ 3 RT-related toxicities were observed.

Conclusion: RT after first-line chemotherapy may provide a survival advantage in certain patients with metastatic or inoperable mesothelioma, particularly when incorporated into sequential treatment plans that include immunotherapy. Further prospective studies are necessary to validate these results and improve patient selection.

Keywords: Malignant pleural mesothelioma; chemotherapy; radiotherapy; survival; multimodal treatment

INTRODUCTION

Malignant mesothelioma is a rare, very aggressive cancer that arises from the mesothelial surfaces of the pleura, pericardium, peritoneum, or tunica vaginalis. Malignant pleural mesothelioma (MPM) is the most prevalent subtype, accounting for about 80% of cases. The disease is strongly associated with occupational or environmental exposure to asbestos fibers, with a latency period that can last more than 30-40 years.^{1,2} Although asbestos has been banned

or regulated in many regions, it still poses health risks to countries. The global burden of mesothelioma remains high, especially in regions with ongoing or previous industrial exposure.^{3,4}

MPM has an insidious onset and often presents with non-specific symptoms such as shortness of breath, chest pain, or pleural effusion, which can delay diagnosis and lead to poor clinical outcomes. Most patients are diagnosed when the disease is already at an advanced stage and the median

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overall survival (OS) remains poor, typically ranging from 12 to 18 months, depending on histologic subtype and performance status.^{5,6} Epithelioid histology is associated with a better prognosis than that of the biphasic or sarcomatoid subtypes.⁷

The current standard of care for unresectable or metastatic MPM involves first-line platinum-based chemotherapy combined with pemetrexed, which has shown modest survival benefits since the pivotal phase III trial by Vogelzang et al.⁸ in 2003. More recently, the addition of immune checkpoint inhibitors, such as nivolumab and ipilimumab, has demonstrated improved outcomes in selected patient populations, especially in the CheckMate 743 trial, where dual immunotherapy showed a significant survival advantage over chemotherapy in unresectable MPM.⁵ Despite these advancements, therapeutic progress has been limited and prognosis remains poor.

Traditionally, radiotherapy (RT) has had a limited role in managing MPM, mainly due to concerns about lung toxicity and minimal survival benefits in the pre-modern RT era.⁹ Historically, its role has been confined to the palliation of localized symptoms, such as pain, chest wall invasion, or dyspnea. However, as modern RT methods such as intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT) become more common, interest in adding RT to multimodal treatment protocols has grown.¹⁰⁻¹² These advanced techniques enable safer delivery of higher doses with improved organ sparing, potentially offering local control without unacceptable toxicity.

Recent retrospective and prospective studies suggest that consolidative RT after systemic treatment may enhance local control and survival in certain patients, especially those with good performance status and limited disease burden.¹³⁻¹⁵ Nevertheless, there is a paucity of high-level evidence supporting the routine use of RT for metastatic or inoperable MPM, and clinical guidelines remain inconclusive regarding its definitive role.

This study aims to explore whether RT offers a survival advantage after initial systemic chemotherapy in patients with metastatic or inoperable malignant mesothelioma. Given the limited literature in this context, our study seeks to provide further insight into optimizing treatment strategies and guiding the integration of RT into the therapeutic landscape of advanced MPM.

MATERIAL AND METHODS

Patients diagnosed with metastatic and/or inoperable mesothelioma were retrospectively analyzed between January 2022 and December 2024 in the Medical Oncology

Department of University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital. Of 72 patients diagnosed with mesothelioma, 58 were considered inoperable and received first-line metastatic treatment and maintenance therapy. Patients who received initial treatment for metastatic disease are included in this study. However, patients under 18 years old who had operable mesothelioma and those with secondary malignancies were excluded from the study.

Patients' medical records, including age at diagnosis, gender, Eastern Cooperative Oncology Group Performance Status (ECOG PS), histology, first-line metastatic therapy, response to initial therapy, and second-line metastatic treatments were analyzed. Patients were categorized into two groups: those who received RT after first-line metastatic therapy and those who did not. This study aims to assess the effect of RT on metastatic or inoperable mesothelioma.

Statistical Analysis

The Mann-Whitney U test is used to analyze quantitative variables, whereas chi-square analysis or Fisher's exact test is used for qualitative variables. Kaplan-Meier curves and log-rank tests are used to compare survival between groups. Progression-free survival (PFS) measures the time from the start of first-line metastatic treatment to disease progression. OS is the duration from the beginning of first-line treatment to death caused by cancer or to the last follow-up. A 95% confidence interval (CI) was used to assess statistical significance; p-values below 0.05 were considered significant.

All analyses were conducted using SPSS software (version 27.0, SPSS Inc., Chicago, IL).

This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital's Ethics/Institutional Review Board (date: 30.12.2025, no: 2025/010.99/23/33).

RESULTS

Medical records of 58 patients diagnosed with unresectable mesothelioma from 2021 to 2025 were analyzed. Twenty-four patients (41.4%) were female and thirty-four (58.6%) were male; the median age was 58 (range 28-84). Among mesothelioma tumors, 86.2% were epithelioid and 13.8% were sarcomatoid. In the first-line metastatic setting, therapy distribution included 33 patients (56.9%) who received cisplatin and pemetrexed, and 25 patients (43.1%) who received carboplatin and pemetrexed. Additionally, if a response such as stable disease (SD), partial response (PR), or complete response (CR) was observed after the initial evaluation, pemetrexed maintenance therapy was continued.

Consequently, all patients in the study received maintenance pemetrexed therapy. Based on the first assessment, response rates were 12.1% for the CR group, 79.3% for the PR group, and 8.6% for the SD group. The median number of pemetrexed maintenance cycles was 8 (range 3-35). No drug discontinuation or interruption due to toxicity was observed. If disease progression was confirmed and the patient's performance status was deemed eligible, second-line metastatic therapies were planned. Follow-up treatments included gemcitabine (20.7%), a platinum-based combination with pemetrexed rechallenge (13.8%), nivolumab combined with ipilimumab (15.5%), nivolumab alone (17.2%), and no treatment (32.8%). Following initial therapy, patients were divided into two groups based on whether they received RT. The RT group accounted for 41.4% of participants, while the not receiving RT (non-RT) group accounted for 58.6%. Table 1 displays the baseline characteristics of the patients.

Efficacy

Patients are divided into two groups: those receiving RT and those non-RT. Response rates for patients with RT were 25%

CR and 75% PR; no SD was observed. In the non-RT group, response rates were 2.9% CR, 82.4% PR, and 14.7% SD.

The RT group experienced significantly higher survival rates. The median PFS was 12.1 months [RT group: 14.1 vs. non-RT group: 9.7; hazard ratio (HR): 0.34; 95% CI: 0.16-0.73; $p<0.045$] (Figure 1); the median OS was 20.5 months (RT group: 24.6 vs. non-RT group: 14.5; HR: 0.55; 95% CI: 0.31-0.72; $p<0.003$) (Figure 2).

As shown in Table 2, in multivariate Cox proportional hazards analysis, RT remained independently associated with OS (HR: 2.36; 95% CI: 1.07-5.21; $p=0.032$). ECOG PS was also identified as a strong, independent prognostic factor (HR: 5.15; 95% CI: 2.25-11.78; $p<0.001$). In contrast, age (HR: 0.99; 95% CI: 0.96-1.03; $p=0.592$), histology (HR: 1.27; 95% CI: 0.69-2.34; $p=0.426$), and gender (HR: 0.87; 95% CI: 0.40-1.89; $p=0.698$) were not significantly associated with OS.

TABLE 1: Patients' characteristics.

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	All	RT group	Non-RT group	p-value
Gender				
Male	58.6%	58.3%	41.2%	0.060 0.062
Female	41.4%	41.7%	58.8%	
Median age	58			
Histology				
Epiteloid	86.2%	79.2%	91.2%	0.068
Sarcomatoid	13.8%	20.8%	8.8%	
ECOG PS				
0	51.7%	70.8%	38.2%	0.020
1	48.3%	29.2%	61.8%	
First line metastatic setting therapy				
Carboplatin + pemetrexed	43.1%	12.5%	14.7%	0.064
Cisplatin + pemetrexed	56.9%	87.5%	85.3%	
Response rates of initial therapy				
CR	12.1%	25%	2.9%	0.072
PR	79.3%	75%	82.4%	
SD	8.6%		14.7%	
Metastatic second line therapies				
None	32.8%	20.8%	41.2%	0.030
Gemcitabine	20.7%	8.3%	29.4%	
Nivolumab	17.2%	37.5%	23.5%	
Nivolumab + ipilumab	15.5%	33.3%	5.9%	
RT: Radiotherapy; ECOG PS: Eastern Cooperative Oncology Group performance status; CR: Complete response; PR: Partial response; SD: Stable disease.				

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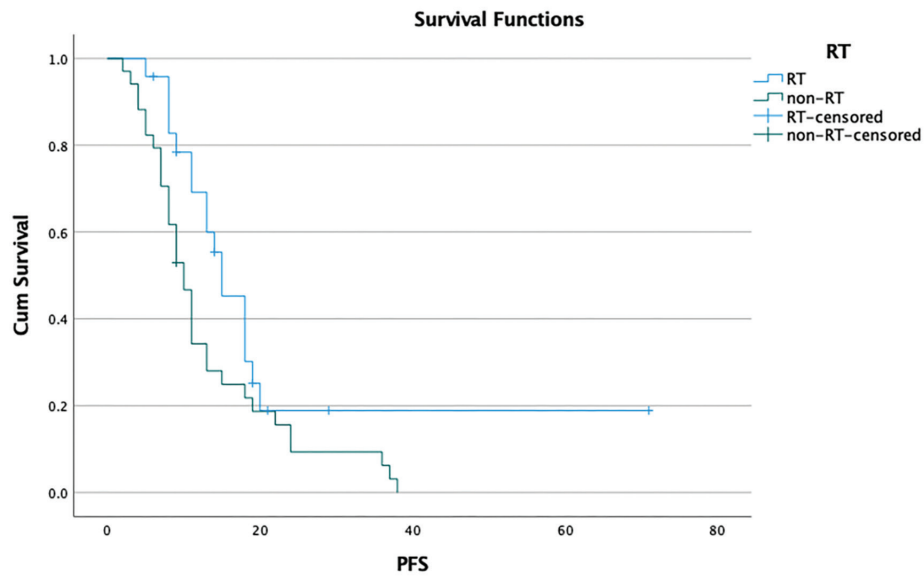


FIGURE 1: Kaplan-Meier curve of PFS between RT versus non-RT.

PFS: Progression-free survival; RT: Radiotherapy

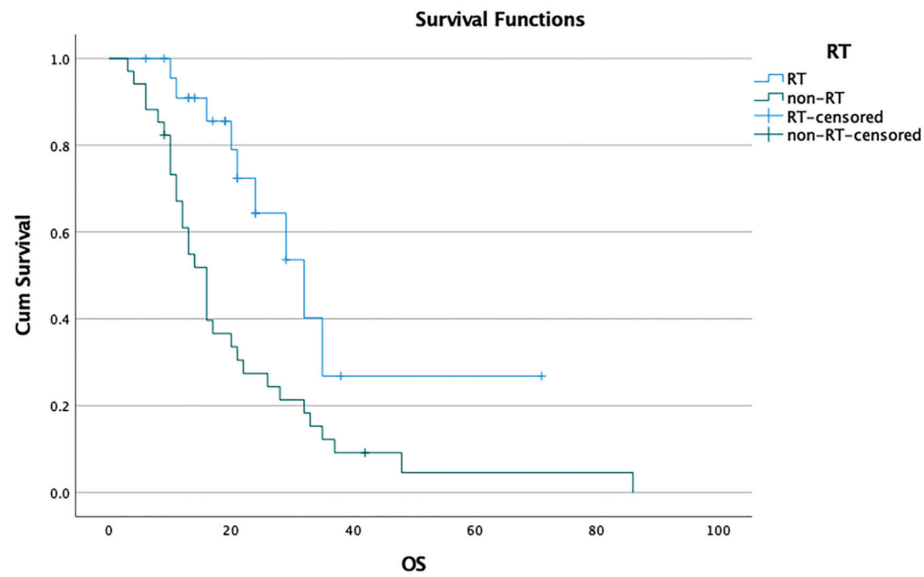


FIGURE 2: Kaplan-Meier curve of OS between RT group versus non-RT group.

RT: Radiotherapy; OS: Overall survival

TABLE 2: Multivariate cox regression analysis for overall survival.

Variable	HR (95% CI)	p-value
ECOG PS	5.15 (2.25-11.78)	<0.001
Age	0.99 (0.96-1.03)	0.592
Radiotherapy	2.36 (1.07-5.21)	0.032
Histology	1.27 (0.69-2.34)	0.426
Gender	0.87 (0.40-1.89)	0.698

HR: Hazard ratio; ECOG PS: Eastern Cooperative Oncology Group performance status; CI: Confidence interval.

DISCUSSION

In this retrospective cohort, incorporating RT following first-line systemic therapy was associated with notable gains in both PFS and OS for patients with metastatic or inoperable malignant mesothelioma. Specifically, the RT group showed a median PFS of 14.1 months and a median OS of 24.6 months, which were notably better than those in the non-RT group: 9.7 and 14.5 months, respectively. These results emphasize a potential role for RT as a consolidative or disease-modifying treatment in advanced mesothelioma - a concept that remains underexplored in modern therapeutic approaches.

The potential mechanisms behind the survival benefit observed in the RT group are complex. First, RT may enhance local disease control, particularly in cases of residual or bulky thoracic disease following systemic therapy. Improved locoregional control has been associated with longer survival in various solid tumors and may similarly benefit mesothelioma when employing curative-intent or high-precision techniques such as IMRT or VMAT.^{16,17}

Secondly, the immune-modulating effects of RT may have played a synergistic role in patients who later received immune checkpoint inhibitors as part of second-line therapy. RT can alter the tumor microenvironment and promote antigen presentation through immunogenic cell death, thereby boosting systemic immune responses—a phenomenon known as the abscopal effect. In our cohort, a larger proportion of patients in the RT group received second-line immunotherapy, particularly programmed cell death protein 1/programmed death-ligand 1 or cytotoxic T-lymphocyte associated protein 4 blockade. This sequence may have enhanced durable responses, as suggested by recent trials studying the combination of RT and immunotherapy in thoracic cancers.¹⁸⁻²¹

Patient selection may have influenced outcomes. The RT group had a higher proportion of patients with an ECOG PS of 0, possibly reflecting better baseline functional status and treatment tolerance. While this introduces selection bias, the survival benefit persisted even after adjusting for these differences, suggesting that RT may contribute intrinsically to disease control.

Notably, in multivariate analysis, RT remained an independent predictor of improved OS despite baseline imbalances between groups. This finding indicates that the observed survival benefit is not solely due to selection bias or differences in baseline characteristics, although residual confounding cannot be entirely ruled out given the retrospective design.

The role of RT in mesothelioma has historically been limited due to concerns regarding pulmonary toxicity and the diffuse, often unresectable nature of the disease. However, accumulating evidence supports its reconsideration within multimodal treatment algorithms. A prospective phase II

study (IMPRINT trial)²² demonstrated the safety and efficacy of hemithoracic IMRT after chemotherapy, showing promising local control and tolerability. Similarly, retrospective series have suggested a role for consolidative RT in prolonging survival when integrated into sequential strategies.²³⁻²⁵

Although some data suggest that hemithoracic IMRT after pleural decortication or extrapleural pneumonectomy may not confer additional survival benefit, our findings support the view that RT should not be entirely excluded from the treatment paradigm. In appropriately selected patients—particularly in the current era, where systemic therapies are increasingly prioritized—RT may still be a valuable component of multimodal management.²⁶

Study Limitations

This study has some limitations. One limitation is the lack of detailed RT planning information. Due to limited access to archived RT planning records in this retrospective dataset, specific treatment parameters such as radiation dose, fractionation schedules, target volumes, and treatment techniques could not be systematically obtained for all patients. Additionally, the intent of RT (consolidative versus palliative) could not be uniformly categorized. As a result, the potential impact of different RT approaches on clinical outcomes could not be assessed.

Taken together, these findings support the integration of RT as a feasible and potentially synergistic treatment option for selected patients with advanced mesothelioma, particularly when used in combination with or following immunotherapy. However, further randomized prospective studies are necessary to verify these findings and define optimal timing, dosage, and patient selection criteria for RT in this setting.

CONCLUSION

Mesothelioma generally has a low incidence rate and limited treatment options. Although surgery has been phased out as a primary treatment option in recent years and immunotherapy has yielded effective results and increased survival rates, rapid progress is not yet evident. The role of RT in mesothelioma remains controversial. RT combined with immunotherapy is known to be more effective than expected. We observe this particularly in studies of locally advanced lung cancer.

Our results indicate that RT following first-line chemotherapy may offer a survival benefit for patients with metastatic or inoperable mesothelioma. While our findings are encouraging, this study has a small sample size and a retrospective design; further prospective studies are necessary to determine the optimal timing, appropriate patient selection, and the best approach for integrating RT into standard treatment protocols for mesothelioma.

Ethics

Ethics Committee Approval: This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by University of Health Sciences Türkiye, Kartal Dr. Lütfi Kırdar City Hospital's Ethics/Institutional Review Board (date: 30.12.2025, no: 2025/010.99/23/33).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: S.Ö., S.A., Design: S.Ö., S.A., Data Collection or Processing: S.A., U.Ö., Analysis or Interpretation: S.Ö., S.A., Literature Search: S.A., U.Ö., Writing: S.Ö.

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

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Long-term Outcomes and Prognostic Factors in Chordoma: A 20-year Single-center Experience

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ABSTRACT

Objective: Chordoma is a rare, locally invasive malignant bone neoplasm originating from notochordal vestiges. However, long-term outcome data remain limited. Radical surgical excision and radiation therapy constitute the primary therapeutic approaches, whereas systemic treatments have demonstrated only marginal efficacy. This investigation sought to examine the clinical attributes, therapeutic approaches, and survival determinants in chordoma cases managed across two decades at a tertiary medical facility in Türkiye.

Material and Methods: A retrospective review was conducted of 18 patients diagnosed with chordoma from 2003 through 2024. Essential data, encompassing demographic, histological, and intervention particulars, were retrieved from the hospital archives. Survival metrics—such as overall survival (OS), progression-free survival (PFS1-3), local recurrence-free survival (LRFS), and metastasis-free survival (MFS)—were estimated using the Kaplan-Meier method. The influence of therapeutic and clinical parameters was evaluated using univariate Cox proportional hazards analyses.

Results: The median participant age stood at 53.5 years, with males constituting 72.2% of the cohort. The median observation duration was 96.2 months. Primary surgery was performed in 15 patients (83.3%), while 80% of patients received supplementary radiotherapy. Median OS (mOS) and PFS1 intervals were 45.3 months and 19.5 months, respectively. Subjects undergoing radiation exhibited significantly longer OS and PFS than non-irradiated counterparts ($p < 0.05$). Complete (R0) excision correlated with a substantially extended LRFS (27.7 versus 3.6 months, $p = 0.002$). Systemic treatment was associated with a marginal improvement in post-relapse survival ($p = 0.064$). Lesion dimensions, patient age, and sex lacked a meaningful association with survival outcomes.

Conclusion: This two-decade institutional survey highlights the pivotal importance of thorough surgical resection and adjuvant radiation therapy in improving prognosis for chordoma patients. Noteworthy survival advantages emerged even with standard photon-based radiotherapy. Timely detection, extensive surgical debulking, and combined-modality strategies remain fundamental to optimizing prognosis in this malignancy. Expanded collaborative research initiatives are essential to further delineate therapeutic protocols and elucidate the utility of systemic agents.

Keywords: Cancer diagnosis and treatments; chemotherapy; medical oncology; oncology

INTRODUCTION

Chordoma represents an uncommon osseous neoplasm arising from vestigial notochordal tissues. The annual occurrence sits around 0.08 per 100,000 individuals, comprising 1-4% of primary bone cancers.¹ These tumors predominantly affect the axial skeleton, most commonly appearing in the skull base (clivus) region (38-39%), followed by the sacral area (34%) and spinal segments (27%).²

Typically emerging during middle to late adulthood, incidence peaks between the 75-84 year bracket, with a slight male predominance observed at roughly 1.5:1.²

Histopathologically classified, these lesions are of notochordal origin, demonstrating slow but locally invasive growth. Although their expansion rate is relatively slow, chordomas exhibit infiltrative behaviour. They often reach large dimensions and compress critical features like

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vascular and neural pathways.¹ Clinical presentations shows regional variability: cranial lesions often manifest as cranial neuropathies or cephalalgia, whereas spinal or sacral masses may produce chronic discomfort, neurological impairment, or mass effect. While metastatic spread does occur, the disease trajectory predominantly hinges on local recurrence and progression.^{3,4} Thus, repeated surgeries may be necessary. Prognosis worsens, particularly with multiple recurrences; the literature reports that 5-year overall survival (OS) after the first local recurrence is 50%, but after the second recurrence, it drops below 20%.⁴

Diagnostic confirmation relies upon both radiographic assessment and microscopic evaluation. Pathognomonic vacuolated cells in biopsy specimens, coupled with immunohistochemical brachyury expression, establish conclusive identification.¹ The classic histological pattern predominates, though rarer chondroid and aggressive dedifferentiated variants exist. The exact prognostic significance of these histological variants remains uncertain given their scarcity.

Therapeutic strategies prioritize local intervention due to the tumor's infiltrative nature. Optimal management hinges on achieving maximum feasible surgical excision, with R0 resection representing the treatment standard. Considerable evidence confirms superior local control and survival among patients attaining margin-negative resections.¹ Indeed, large series show that local control and survival are significantly improved in patients who can achieve complete resection.^{3,4} However, anatomical constraints often limit radical excision. This is particularly relevant to skull-base, cervical, and sacral lesions, which are frequently large at diagnosis. Salvage procedures often become necessary as recurrence develops, with survival rates dropping precipitously after multiple recurrences, literature indicating five-year survival plunging from 50% after initial recurrence to under 20% following subsequent episodes.¹

Radiotherapy (RT) is a fundamental component of treatment for cases in which surgical excision remains incomplete or the risk of recurrence is elevated. Although chordomas exhibit notable resistance to traditional photon-based RT, more refined, high-dose fractionated techniques have demonstrated greater efficacy.⁵ Particle therapies, notably proton-beam and carbon-ion RT, enhance local tumor control by employing particles with linear energy transfer superior to that of photons. Similarly, innovative RT approaches—such as proton, carbon-ion, or stereotactic high-dose irradiation—have shown promising outcomes in managing residual disease, with select studies reporting results on par with surgical intervention.⁶ Typically, RT is advised post-surgery, particularly when surgical margins appear positive;

administering elevated doses might then prove advantageous in curbing local relapse.

Systemic treatment options occupy a restricted niche in chordoma management. Conventional chemotherapy is minimally effective, and there is no established protocol. Given the frequent overexpression of platelet-derived growth factor receptors (PDGFR) and epidermal GFR (EGFR) in chordomas, tyrosine kinase inhibitors, such as imatinib, have been evaluated. A phase II trial using imatinib reported disease stabilization in 70% of advanced cases, alongside a median progression-free survival (PFS) of 9 months and OS of 34 months.⁷ In a retrospective study, the best response under imatinib was stable disease in 74% of patients, and the median PFS was 10 months.⁷ Although partial response or long-term stabilization have been reported in small case series with EGFR inhibitors (erlotinib, gefitinib, etc.) and angiogenesis-targeted agents (sorafenib, pazopanib, etc.), they do not have a standardized level of evidence and cure with systemic treatment alone is rare.^{1,8} Presently, an integrated approach—primarily extensive resection combined with RT—dominates chordoma care, while evolving targeted and immunotherapeutic strategies remain confined to investigative settings for refractory disease.

Existing literature is predominantly derived from retrospective, fragmented cohorts with inconsistent follow-up. Consequently, deficiencies persist in robust real-world evidence and extended survival data. Additionally, no comprehensive national dataset exists for chordomas in Türkiye. This investigation seeks to address such omissions by detailing the clinicopathological and oncological trajectories of chordoma patients monitored over two decades at a specialized Turkish institution, thereby refining therapeutic paradigms for these infrequent malignancies.

MATERIAL AND METHODS

Patient Selection

The study was retrospective. Twenty-two patients with histopathologically confirmed chordoma, diagnosed at University of Health Sciences Türkiye, Ankara Bilkent City Hospital between 2003 and 2024, were analyzed using the hospital automation system. Patients with missing or incomplete clinical data in the hospital records were excluded. A total of four patients were excluded for this reason. A total of 18 patients were included in the study. Clinical, pathological, treatment, and survival data were collected from the electronic patient record system. Age, gender, size, resection margin status (R0, R1/R2), primary surgery, whether adjuvant or definitive RT was administered, systemic treatment history, systemic treatments and number of stages received,

surgery in case of recurrence, recurrence RT status, time to development of local recurrence during follow-up, time to distant metastasis, times to progression, date of last follow-up or exitus, tumor location (thoracic-cervical and lumbar-sacral), histological variant features, Ki-67 proliferation index, and EGFR mutation status were recorded. RT was delivered using conventional photon-based techniques. The total dose typically ranged from 60 to 74 Gy, administered in daily fractions of 1.8-2 Gy. RT was delivered postoperatively in most cases, particularly in patients with positive or close surgical margins or as definitive treatment in inoperable cases. Surgical margins were evaluated based on histopathological examination and classified as R0 (microscopically margin-negative) or R1/R2 (microscopically or macroscopically margin-positive). Information on the surgical technique (*en bloc* versus intralesional resection) was not consistently available because the study was retrospective.

Ethics Approval

This study was conducted with the approval of the University of Health Sciences Türkiye, Ankara Bilkent City Hospital Medical Research Scientific and Ethical Evaluation Board and in accordance with the Declaration of Helsinki (approval number: TABED 1-25-1840, date: 05.11.2025).

Statistical Analysis

For descriptive data analysis, continuous variables are presented as mean $\pm$ standard deviation or median and interquartile range (IQR), and categorical variables are presented as numbers and percentages. Follow-up duration was calculated using the Reverse Kaplan-Meier method. Kaplan-Meier methods were used for survival analyses, and differences between groups were assessed using the log-rank test. Patients were monitored with imaging studies (magnetic resonance imaging or computed tomography) at approximately 6- to 12-months intervals, or earlier when

clinically indicated. Disease progression was determined based on radiological evidence of local recurrence or distant metastasis, as assessed by the treating physicians. OS (survival), PFS1-3, local recurrence-free survival (LRFS), time from diagnosis to metastasis, survival after first recurrence, survival after metastasis, and metastasis-free survival (MFS) were analyzed as time-to-event outcomes, with surviving patients censored. OS was defined as the time from diagnosis to death from any cause or to last follow-up. PFS1-3 was defined as the time from the initiation of each treatment line to disease progression or death, whichever occurred first. LRFS was defined as the time from surgery to the occurrence of local recurrence. MFS was defined as the time from diagnosis to the development of distant metastasis. The effects of clinical variables (age, sex, tumor size, resection status, RT status, systemic therapy) on survival were analyzed using a univariate Cox proportional hazards model; results are reported as hazard ratio (HR) and 95% confidence intervals (CIs). Statistical significance was set at $p < 0.05$. Analyses were performed using SPSS Statistics version 25.1.

RESULTS

Descriptive Statistics

Descriptive statistics are presented in Table 1. The majority of the 18 patients were male, and the median age was 53.5 years (Table 1). The median tumor size was 36 mm, the median follow-up time was 96.2 months, and the rate of primary surgery was 83.3%.

Survival Statistics

The median OS (mOS) was 45.3 months (IQR: 19.43-93.9), and mPFS1 was 19.5 months (IQR: 8.67-66.7). Two-year OS was 72.2%, and five-year OS was 44.4% (Table 2). At the end of follow-up, 7 patients were still alive. During follow-up, 5/18 (27.8%) patients were progression-free at five years after diagnosis. The two-year PFS rate was 50% (9/18).

TABLE 1: Descriptive statistics.

Variable	Value
Number of patients	18
Gender	5 females (27.8%), 13 males (72.2%)
Median age (IQR)	53.5 years of age (IQR: 39.0-66.3)
Median tumor size (IQR)	36 mm (IQR: 25.5-75.5)
Median follow-up time (reverse Kaplan-Meier)	96.2 months (95% CI: 58.8-133.6)
Number of patients undergoing primary surgery	15/18 (83.3%)
Resection margin status	R0: 8/15 (53.3%); R1-R2: 7/15 (46.7%)
Tumor localisation	Lower spine/upper spine 13/5
Adjuvant RT	12/15 (80%)
Systemic treatment	9/18 (50%)
IQR: Interquartile range; R1-R2: Microscopic/macroscopic positive surgical margin; CI: Confidence interval; RT: Radiotherapy.	

During follow-up, 83.3% (15/18) of patients experienced recurrence. While the first recurrence in 11 patients was a local recurrence, the first recurrence in 4 patients was a distant metastasis. Additionally, 2 patients developed distant metastases following local recurrence.

The mOS after the first recurrence was approximately 40.1 months, and the mOS after distant metastasis was 20.1 months. The 5-year LRFS was 44.4% (8/18).

According to survival analyses, no significant differences were found in OS or PFS between gender groups or age groups. While female patients appeared to have longer OS and PFS than male patients, this difference was not statistically significant (OS: $p=0.420$; PFS: $p=0.932$). Similarly, when

comparing the <53.5 and ≥ 53.5 age groups, no significant difference was found in OS ($p=0.800$) and PFS ($p=0.257$).

In contrast, those who received adjuvant or inoperable definitive RT after surgery were associated with significant increases in PFS and OS compared to those who did not receive RT (mPFS 22.4 months vs. 5.97 months, $p<0.001$; mOS 47.9 months vs. 7.7 months, $p=0.042$) (Table 3). No significant effect on survival was observed in the classification based on tumor size (<36 mm vs. ≥ 36 mm). For tumors smaller than 36 mm, the mPFS was 19.5 months; for tumors larger than 36 mm, the mPFS was 10.7 months (log-rank $p=0.928$). The mOS values were 81.5 and 36.5 months, respectively (log-rank $p=0.928$). When tumor size was included in the Cox regression

TABLE 2: Survival analyses.

Endpoint	Median (months)	IQR/95% CI	2-year ratio (%)	5-year ratio (%)	Notes
OS	45.3	IQR: 19.43-93.9	72.2	44.4	7 patients alive at follow-up
PFS1	19.5	IQR: 8.67-66.7	50.0	-	5/18 patients progression-free at 5 years
PFS2	10.7	IQR: 4.1-21.5	-	-	Calculated in 7 patients
PFS3	14.7	IQR: 7.4-75.5	-	-	Calculated in 3 patients
LRFS	27.2	95% CI: 14.4-40.0	-	44.4	Significantly longer in the group receiving radiotherapy
MFS	8.8	95% CI: 8.1-9.4	-	72.2	Median could not be calculated in the group not receiving radiotherapy
Recurrence rate	-	-	-	83.3 (15/18)	11 local, 4 metastatic first recurrences
Median OS after first recurrence	40.1	-	-	-	
Median OS after metastasis	20.1	-	-	-	

IQR: Interquartile range; CI: Confidence interval; OS: Overall survival; PFS: Progression-free survival; MFS: Metastasis-free survival.

TABLE 3: Comparison of survival outcomes according to treatment modalities in chordoma patients.

Treatment group	Median OS (months)	Median PFS (months)	Median LRFS (months)	Median MFS (months)	Log-rank p	Notes
Radiotherapy (RT)	81.5	22.4	27.2	8.8	<0.001	Significantly longer OS and PFS compared to no RT
No RT	7.7	5.97	Not reached	6.0		
R0 resection	81.5	27.6	27.7	Not reached	0.316	No metastasis observed during follow-up
R1-R2 resection	19.3	8.3	3.6	8.3	0.727	
Systemic therapy	87.9	-	-	25.5	0.064	Trend toward longer post-relapse survival
No systemic therapy	43.2	-	-	12.4		
Primary surgery	-	-	27.2	-		
Non-surgical group	-	-	-	11.4		

R1-R2: Microscopic/macrosopic positive surgical margin; OS: Overall survival; PFS: Progression-free survival; LRFS: local recurrence-free survival; MFS: Metastasis-free survival; RT: Radiotherapy.

model as a continuous variable, no significant associations were found between tumor size and PFS ($p=0.434$) or OS ($p=0.180$).

Comparison between primary operable and inoperable patients was not possible because of the small number of inoperable patients (3/18). Time from diagnosis to metastasis could only be calculated for patients who received RT (median 10.1 months, IQR: 6.78-63.98). Analysis for the no-RT group was not possible because there was only one case.

Median LRFS was 27.2 months (95% CI: 14.4-40.0) in patients who received RT, while median survival in the no-RT group could not be calculated and was very short. The difference between the groups was statistically significant (log-rank $p=0.002$).

Among all patients, the median MFS was 8.8 months (95% CI: 8.1-9.4). The median MFS was 38.2 months in those who received RT and 6.0 months in those who did not. The difference between the two groups, although not statistically significant, was within the range of significance (log-rank $\chi^2=3.0$; $p=0.083$). LRFS was significantly longer in patients who underwent R0 resection than in those who underwent R1-R2 resection (median 27.7 months vs. 3.6 months, $p=0.002$). None of the eight patients who underwent R0 resection developed metastases during follow-up. While mPFS was 27.6 months after R0 resection, it was 8.3 months after R1-R2 resections ($p=0.316$). mOS was 81.5 months in the R0 group and 19.3 months in the R1-R2 group ($p=0.727$).

In analyses stratified by primary surgery status, the median LRFS was 27.2 months in patients who underwent primary surgery and developed local recurrence. The median MFS in non-operated patients was 11.4 months. Two patients progressed to metastasis after local recurrence. One of these patients underwent surgery for recurrence, did not receive RT, and had an RT-free interval between recurrence and metastasis of 0.76 months. The other patient received RT to the recurrent lesion without surgery for the recurrence and had an interval between recurrence and metastasis of 26.76 months.

The median post-relapse survival of the two patients who underwent surgery and RT after local recurrence was 37.17 months, whereas it was 25.5 months for patients who received RT alone. The median LRFS of those whose first progression was local recurrence was 81.5 months (95% CI: 18.2-144.7; log-rank $\chi^2=0.055$; $p=0.814$).

Among those who did not experience recurrence, the mOS was >100 months. The mOS of patients who developed recurrence was shorter, with a lower median; however, this difference did not reach statistical significance, likely because of the small number of patients (3/18) who did not develop recurrence ($\chi^2=2.389$; $p=0.122$). Univariate Cox regression analyses for OS revealed no significant associations between resection status (HR=1.266; 95% CI: 0.336-4.777; $p=0.728$), tumor size (≥ 36 mm vs. <36 mm) (HR=1.056; 95% CI: 0.321-3.479; $p=0.928$), and age (<60 vs. ≥ 60 years) (HR=1.175; 95% CI: 0.336-4.106; $p=0.800$), but baseline RT status (HR=0.198; 95% CI: 0.035-1.114; $p=0.066$) approaching statistical significance (Table 4).

Univariate Cox regression analyses for LRFS revealed no significant effect of tumor size (HR=1.059; 95% CI: 0.302-3.715; $p=0.929$), but a trend toward a protective effect of younger age (<60 vs. ≥ 60 years) was observed (HR=0.277; 95% CI: 0.054-1.420; $p=0.124$). Univariate Cox regression analyses for MFS revealed a protective effect of younger age (<60 vs. ≥ 60 years), which did not reach statistical significance (HR=0.277; 95% CI: 0.054-1.420; $p=0.124$), and tumor size was not observed (HR=0.408; 95% CI: 0.025-6.621; $p=0.529$) (Table 5).

The mean PFS was 8.77 months in thoracic-cervical cases and 22.40 months in lumbar-sacral cases ($p=0.137$). The mean PFS was 19.47 months in thoracic-cervical cases and 81.46 months in lumbar-sacral cases ($p=0.577$).

After the first progression, consecutive PFS times could be calculated for a smaller subset of patients. PFS2 was available for 7 patients; the median was 10.7 months (range, 3.8-27.6 months). PFS3 was available for only 3 patients, with

TABLE 4: Univariate Cox regression analysis for prognostic factors affecting survival in chordoma patients.

Variable	HR	95% CI	p-value	Interpretation
Resection status (R0 vs R1-R2)	1.266	0.336-4.777	0.728	Not significant
Tumor size (≥ 36 mm vs <36 mm)	1.056	0.321-3.479	0.928	Not significant
Age (<60 vs ≥ 60 years)	1.175	0.336-4.106	0.800	Not significant
Initial RT	0.198	0.035-1.114	0.066	Trend toward significance (protective effect)
Younger age (LRFS)	0.277	0.054-1.420	0.124	Trend toward better local control
Younger age (MFS)	0.277	0.054-1.420	0.124	Trend toward better metastasis-free survival
Tumor size (MFS)	0.408	0.025-6.621	0.529	Not significant

HR: Hazard ratio; CI: Confidence interval; RT: Radiotherapy; LRFS: Local recurrence-free survival; MFS: Metastasis-free survival.

TABLE 5: Patterns of recurrence and post-relapse treatments in chordoma patients.	
Recurrence feature	Value
Patients without recurrence	3/18 (16.7%)
Patients with recurrence	15/18 (83.3%)
First recurrence type	Local: 11 (73.3%); distant metastasis: 4 (26.7%)
Secondary metastasis after local relapse	2 patients
Salvage surgery + RT after recurrence	1 patient - median OS after relapse: 37.2 months
Salvage RT alone after recurrence	1 patient - median OS after relapse: 25.5 months
Systemic therapy after recurrence	9/18 patients
Median OS after recurrence	25.5 months
RT: Radiotherapy; OS: Overall survival	

a median of 14.7 months (range, 7.4-75.5 months) (Table 6). Median duration of 1st-line therapy: 7.6 months (range, 3.1-15.1). Median duration of 2nd-line therapy: 8.2 months (range, 3.4-14.0). Patients receiving ≥3 lines of therapy (n=3) had numerically longer OS. In most cases, clinical benefit (progressive disease/stable disease) occurred within the first 2 treatment lines. The longest total treatment exposure was in patient 5 (cumulative duration around 40+ months).

Nine (50%) of the 18 chordoma patients included in the study received systemic therapy. Imatinib (n=7) was the most commonly used first-line agent, while 2 patients were treated with internal mammary artery. Three of the 7 patients receiving imatinib were switched to sunitinib, sorafenib, or temozolomide as second-line therapy. In some cases, agents such as dasatinib, erlotinib, carboplatin, and temozolomide were used in third and subsequent lines of therapy. The best responses to first-line therapy were stable disease, partial response, or complete response in 44.4% (4/9) of the patients; progressive disease was observed in 55.6% (5/9). Although a tendency toward longer OS was observed in patients receiving advanced systemic therapies, statistical significance was not reached because of the small number of patients. In the subgroup of patients who developed recurrence, those who received systemic therapy had a longer OS than those who did not, although this difference was not statistically significant (87.9 months vs. 43.2 months, p=0.317). Post-relapse survival was 25.5 months in patients who received systemic therapy and 12.4 months in those who did not (p=0.064). Pathology reports included various histological and molecular findings. One patient had an EGFR mutation and was proficient mismatch repair. Additionally, histological variants were reported in two patients: one a chondroid chordoma and the other a myxoid variant. A limited number of patients in our cohort exhibited different histopathological or molecular features; due to insufficient numbers of patients, their impact on survival could not be assessed.

DISCUSSION

In our series, RT was associated with significantly longer OS and PFS (mOS 81.5 vs. 7.7 months; PFS, p<0.001) compared with surgery alone, underscoring the critical role of adjuvant RT in chordoma treatment. This finding is consistent with reports that high-dose postoperative RT improves local control and survival, particularly in cases of positive surgical margins. Advanced RT modalities such as proton or carbon-ion therapy have achieved tumor control and survival rates comparable to those achieved with surgical resection. These approaches offer an alternative for patients who are not surgical candidates, particularly due to advanced age or high operative risk.⁶ Previous studies have demonstrated favourable outcomes with carbon ion therapy in selected patients.⁶ The findings in our series also suggest that the addition of RT has a positive impact on the course of the disease. Notably, although only conventional photon RT was available in our center, its judicious high-dose use still conferred substantial benefit; the future introduction of proton/ion therapy may further improve outcomes. Some patients did not receive RT due to advanced age, comorbidities, or individualized treatment decisions based on clinical judgment.

In our study, patients who underwent *en bloc* R0 resection experienced markedly longer LRFS (median 27.7 vs. 3.6 months) and showed a trend toward improved OS, highlighting the importance of achieving clear margins. Also, none of the eight patients who had an R0 resection developed metastases during follow-up. Larger series have confirmed the value of wide excision. In one analysis of 130 patients, clear surgical margins were an independent predictor of local control (5-year LRFS 47% vs. lower with intralesional resection; p=0.003).⁶ That study also identified tumor size as an independent prognostic factor, with larger tumors associated with significantly worse outcomes. In our cohort (mean tumor diameter of ~5 cm), we observed a non-significant trend toward shorter PFS in patients with tumors

TABLE 6: Systemic therapy lines, treatment duration, and best responses in chordoma patients.

Pt. No.	1 st -line therapy (duration, mo)	2 nd -line (duration)	3 rd -line (duration)	4 th -line (duration)	5 th -line (duration)	≥6 th -line (duration)	Other agents	Best response (PR/SD/PD)	Notes
1	Imatinib (4.2 mo)	-	-	-	-	-	-	PD	Progression after short course
2	IMA (3.1 mo)	-	-	-	-	-	-	PD	Primary progression
3	Imatinib (6.4 mo)	-	-	-	-	-	-	SD	Disease stabilization
4	Imatinib (8.5 mo)	Sunitinib (14.0 mo)	Sorafenib (5.0 mo)	Dasatinib (5.4 mo)	Erlotinib (9.3 mo)	-	-	SD	Long OS, multiple lines
5	Imatinib (7.6 mo)	Sunitinib (8.2 mo)	Temozolomide (9.5 mo)	Imatinib rechallenge (3.6 mo)	Dasatinib (5.1 mo)	Erlotinib + carboplatin (6.8 mo)	Temozolomide	SD	Prolonged disease control
6	Imatinib (11.2 mo)	Sunitinib (6.1 mo)	-	-	-	-	-	SD	Stabilization in 2 nd line
7	Imatinib (9.8 mo)	Sorafenib (3.4 mo)	-	-	-	-	-	PD	Early progression
8	Imatinib (15.1 mo)	-	-	-	-	-	-	PR	Durable partial response
9	Imatinib (5.6 mo)	-	-	-	-	-	-	SD	Durable stabilization

Pt: Patient; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; OS: Overall survival; Mo: Months.

>36 mm compared to those with tumors 36 mm. This is in line with population-based data showing that greater tumor size at diagnosis confers a poorer prognosis.⁹ These findings underscore the value of early intervention.

Tumor location may also influence outcomes, although our sample size was too small to demonstrate a statistically significant effect. Some series suggest that chordomas of the mobile spine have better long-term survival than those of the sacrum or skull base.¹⁰ One single-center study reported approximately 80% 10-year OS in mobile spine chordoma patients versus around 50% in sacral chordomas (a difference that was not statistically significant).³ For example, while 5- and 10-year mOS values of patients with mobile vertebral chordomas were reported as around 80% in one series, these rates were reported as 78% in 5 years and 48% in 10 years in the same study for sacral chordomas, despite being statistically insignificant.³ Conversely, another analysis found similar survival between sacral and mobile spine disease, but a higher risk of distant metastasis in sacral tumors.^{11,12} These patterns likely reflect the challenges of treating chordomas in certain locations. Tumors in the skull base are adjacent to critical neurological structures, limiting the radiation dose that can be safely delivered.^{3,13} In fact, RT doses are limited in skull base chordomas due to their proximity to sensitive brain structures, making local control difficult.^{3,14} Sacral tumors often reach large sizes and require extensive resection with considerable morbidity, making complete removal difficult. In our predominantly lumbosacral series, this may explain the pronounced benefit of adjuvant high-dose RT in improving local tumor control and survival.

Patient age did not have a significant impact on outcomes in our cohort (median age 53). This may be due to the relatively small number of older patients in our study. By contrast, larger epidemiological studies have identified older age (especially ≥60 years) at diagnosis as an independent risk factor for worse survival, presumably reflecting factors such as poorer general health and more advanced disease at presentation.⁹ It remains important to consider that younger patients are often better candidates for aggressive multimodal therapy, which may lead to better outcomes.

Systemic therapies for chordoma remain controversial adjuncts. In our study, about half of the patients received targeted systemic treatments (mostly tyrosine kinase inhibitors such as imatinib), but we observed no clear survival advantage from these therapies (mOS 88 vs. 43 months with vs. without systemic treatment; $p=0.317$). Although patients receiving systemic therapy had numerically longer survival, this difference was not statistically significant. Although a trend toward improved survival was observed in patients receiving systemic therapy, the difference did not reach

statistical significance. Therefore, these findings should be interpreted with caution and considered hypothesis-generating. Small series have documented partial responses or prolonged stable disease with various targeted agents (e.g., imatinib, sunitinib, pazopanib, erlotinib), particularly in patients with PDGFR, KIT, or EGFR-positive tumors.^{1,7,15} However, without randomized trial data it remains uncertain whether such drugs significantly extend OS, as complete tumor responses are rare and disease control is usually temporary.⁷ Novel approaches, including brachyury-targeted vaccines and immune checkpoint inhibitors, have shown promise in isolated refractory cases (with a few partial responses reported).¹ Ongoing research and future clinical trials will be crucial in determining the definitive role of systemic therapies in advanced chordoma.

Study Limitations

This study has several limitations. First, due to its retrospective design, some patients were excluded because of incomplete or missing clinical data, which may have introduced selection bias. In addition, the small sample size limits the statistical power of the analyses and the generalizability of the findings. Treatment-related toxicities were not systematically recorded due to the retrospective design of the study, limiting the ability to assess the safety profile of the treatments.

CONCLUSION

The findings of our study confirm the critical importance of comprehensive surgical resection and RT in the treatment of chordoma. When extensive surgical resection is possible, local recurrence rates decrease and long-term survival increases. When resection is inadequate or patients are at high risk of recurrence, adjuvant high-dose RT significantly contributes to disease control, as emphasized in the literature. Various studies have demonstrated that, thanks to advances in RT technologies, success rates comparable to those achieved with surgery can be achieved even in RT-resistant tumors like chordoma. Our study demonstrates the positive impact of the effective use of existing RT on survival, even in settings where proton or carbon-ion therapy is not routinely available in our country. Furthermore, it reiterates the prognostic importance of factors such as tumor size and patient age, since early diagnosis and intervention may lead to better outcomes in younger and smaller patients. While systemic therapies are not the cornerstone, they may have a role in disease control in selected patients; further studies are needed to confirm their efficacy.

Larger case series are needed in this area, and the results of ongoing clinical trials are awaited. Achieving

optimal outcomes in patients with chordoma requires a multidisciplinary approach that integrates surgery and RT, is supplemented by systemic therapies when necessary, and is personalized to each patient. Comparing our experience with data from the literature will contribute to determining optimal treatment strategies for this rare tumor.

Ethics

Ethics Committee Approval: This study was conducted with the approval of the University of Health Sciences Türkiye, Ankara Bilkent City Hospital Medical Research Scientific and Ethical Evaluation Board and in accordance with the Declaration of Helsinki (approval number: TABED 1-25-1840, date: 05.11.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.A.E., Concept: D.U., Design: Ö.B., Data Collection or Processing: B.Y., Analysis or Interpretation: B.Y., E.A., Literature Search: B.Y., Writing: B.Y., D.U.

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Prognostic Significance of Systemic Immune Inflammation Index (SII) and Systemic Inflammation Response Index (SIRI) in Patients with Locally Advanced Gastric Cancer Treated with Perioperative FLOT Therapy

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ABSTRACT

Objective: In recent years, studies have demonstrated the prognostic importance of the systemic immune inflammation index (SII) and the systemic inflammation response index (SIRI) in gastric cancer. We aimed to determine the prognostic significance of SII and SIRI in patients with locally advanced gastric cancer who underwent perioperative fluorouracil, leucovorin, oxaliplatin, and docetaxel therapy at our center.

Material and Methods: Our study was a retrospective, single-center study that included 167 patients with locally advanced gastric cancer between June 2019 and December 2023.

Results: The SII parameter demonstrated very high discriminatory power [area under the curve (AUC)=0.963; 95% confidence interval (CI): 0.938-0.988]. For a cut-off value of ≥ 664.00 , the sensitivity was 87.1% and the specificity was 87.8%, and the result was statistically significant ($p<0.001$). Similarly, the SIRI value exhibited high predictive performance (AUC=0.952; 95% CI: 0.922-0.982). For a cut-off value of ≥ 1.27 , the sensitivity was 88.2%, and the specificity was 89.2%, establishing this parameter as a strong predictor of mortality ($p<0.001$). In patients with SII <664.00 , the 2-year and 5-year overall survival (OS) rates were 94.7% and 83.7%, respectively, while in the SII ≥ 664.00 group, the 2-year OS dropped to 13.9%, and the 5-year OS could not be calculated ($p<0.001$). A similar trend was observed in terms of SIRI; In patients with SIRI <1.27 , the 2-year OS was 88.1% and the 5-year OS was 85.3%, while in the SIRI ≥ 1.27 group, the 2-year OS decreased to 19.4% ($p<0.001$). According to the multivariate Cox regression analysis results, having an SII ≥ 664.00 increased the risk of death 8.49 times [Hazard ratio (HR): 8.49; 95% CI: 4.35-16.57; $p<0.001$], and having a SIRI ≥ 1.27 increased the risk of death 7.88 times (HR: 7.88; 95% CI: 3.99-15.60; $p<0.001$). Similarly, having an SII ≥ 664.00 increased the risk of progression 5.13 times (HR: 5.13; 95% CI: 3.14-8.39; $p<0.001$) and having a SIRI ≥ 1.27 increased the risk of progression 3.89 times (HR: 3.89; 95% CI: 2.39-6.31; $p<0.001$).

Conclusion: There is a need for biomarkers that can be used clinically for prognostic and predictive purposes in gastric cancer patients. Lower SII and SIRI levels are significantly associated with improved OS and disease-free survival in patients with gastric cancer. More comprehensive analyses that combine it with other markers have been and continue to be conducted.

Keywords: Systemic immune inflammation index; systemic inflammation response index; prognostic significance; locally advanced gastric cancer; perioperative FLOT therapy

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INTRODUCTION

Gastric cancer ranks highly among malignancies in terms of both incidence and mortality. It is the 5th most common malignancy worldwide and the 4th leading cause of cancer-related mortality.¹ Although surgical resection is the primary treatment for stomach cancer, half of the patients die within 5 years after radical surgery.²

Because symptoms appear late and there is usually no pathognomonic sign, patients are often diagnosed at an advanced stage. Therefore, the importance of neoadjuvant therapy in gastric cancer has increased over the years. Neoadjuvant therapy has been shown to improve pathological and radiological response.^{3,4} According to guidelines, the recommended approach to the medical treatment of locally advanced gastric cancer is perioperative therapy, consisting of neoadjuvant and adjuvant chemotherapy. Perioperative systemic therapy utilizes the fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT) regimen.⁵ Recent studies have demonstrated a survival benefit of perioperative durvalumab treatment combined with the FLOT regimen in certain patients, and the guidelines have been revised.⁶

Systemic inflammation is a predictor of cancer prognosis, playing a critical role in proliferation, migration, invasion, and metastasis. Many studies have investigated the prognostic significance of systemic inflammation markers in patients with malignancy.⁷⁻⁹ In recent years, the literature contains studies demonstrating the prognostic importance of the systemic immune inflammation index (SII) and the systemic inflammation response index (SIRI) in gastric cancer.^{10,11}

In our study, we aimed to determine the prognostic significance of SII and SIRI in patients with locally advanced gastric cancer who received perioperative FLOT therapy at our center.

MATERIAL AND METHODS

Our study was conducted retrospectively and as a single-center study, and included 167 patients with locally advanced gastric cancer between June 2019 and December 2023. Pathological staging was done using the 7th edition of the tumour, node, and metastasis staging system.¹² Patients with incomplete data, those with metastatic disease, those diagnosed with more than one primary malignancy, and individuals under 18 years of age were excluded from the analysis.

For all patients, tumor localization, tumor grade, histopathology, tumor type (diffuse or intestinal), T and N stages, and counts of neutrophils, lymphocytes, monocytes, and platelets were recorded from their medical records at the

time of diagnosis. SII and SIRI were calculated for all patients at the time of diagnosis. Staging of all patients was performed using thoracic, abdominal, and pelvic computed tomography with intravenous and oral contrast, followed by laparoscopic staging. Additionally, radiological and pathological responses to neoadjuvant therapy, development of recurrence or distant metastasis during treatment or follow-up, overall survival (OS), and disease-free survival (DFS) were assessed.

Radiological response evaluation after 4 cycles of neoadjuvant FLOT was performed using the Response Evaluation Criteria in Solid Tumors.¹³ All patients received a total of 8 cycles of FLOT treatment, consisting of 4 neoadjuvant cycles and 4 adjuvant cycles. All patients included in the study received full doses of neoadjuvant and adjuvant therapy. Patients who underwent dose reduction or were unable to complete adjuvant therapy due to toxicity were excluded from our study. OS was defined as the time from diagnosis to death or to the date of the last follow-up. DFS was defined as the time from the last adjuvant chemotherapy to the detection of recurrence/metastasis. Pathological response to neoadjuvant therapy was assessed using the College of American Pathologists (CAP) score, which measures the tumor regression grade after surgery.¹⁴ All patients included in our study were microsatellite-stable, human epidermal growth factor receptor 2-negative, and had a histopathologic diagnosis of adenocarcinoma with a tumor grade of 2-3. The SII was calculated using the formula: platelet count $\times$ neutrophil count/lymphocyte count. The SIRI was calculated using the formula: monocyte count $\times$ neutrophil count/lymphocyte count. The parameters included in these indices were derived from peripheral blood samples.

All patients underwent routine follow-ups every 3 months for the first 2 years after completion of adjuvant chemotherapy and every 6 months between the 2nd and 5th years. During these follow-ups, thoracic, abdominal, and pelvic computed tomography scans with intravenous and oral contrast were performed, and carcinoembryonic antigen (CEA) and CA19-9 levels were recorded.

The study was conducted according to the principles outlined in the Declaration of Helsinki and was approved by the University of Health Sciences Türkiye, Prof. Dr. Cemil Taşcıoğlu City Hospital Ethics Committee (approval number: 388, date: 21.10.2025).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (Statistical Package for the Social Sciences; IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as n and % for categorical variables, and mean $\pm$ standard deviation and median (minimum-maximum) for continuous variables. Receiver operating characteristic

(ROC) curve analysis was used to predict mortality using various indices. Optimal cut-off values were determined using the Youden index, which identifies the threshold that maximizes the sum of sensitivity and specificity. The Kaplan-Meier method was used to compare OS and DFS between clinical groups. Finally, multivariate Cox regression results for mortality and progression risk associated with various clinical variables are presented; $p < 0.05$ was considered statistically significant.

RESULTS

A total of 167 patients were included in the study. The mean age of the patients was 58.50 ± 11.02 years, with a median age of 61 (26-78). Regarding gender distribution, 70.1% of the patients were male, and 29.9% were female. Tumor localization was most frequent in the cardia (35.9%), followed by the antrum (29.9%) and the corpus (19.2%). The distributions of patients' socio-demographic and clinical variables are shown in Table 1. The mean SIRI was 1.60 ± 1.05 (median, 1.35), and the mean SII was 826.58 ± 531.96 (median, 685.00). Median SII values were 646.0 (257.0-1233.0) in stage 2 patients and 717.0 (342.0-3681.0) in stage 3 patients. The median SIRI values were 1.15 (0.45-2.90) in stage 2 patients and 1.47 (1.11-6.80) in stage 3 patients. On radiological response evaluation, a complete response was observed in 16.2% of patients, a partial response in 57.5%, and stable disease in 26.3%. The mean follow-up period was 29.83 ± 17.50 months, with a median follow-up period of 24 months. During follow-up, 67.1% of patients experienced recurrence, 55.7% died, and 44.3% survived.

TABLE 1: Distributions of socio-demographic and clinical variables.

Variables	n	%
Age		
Mean $\pm$ SD	58.50 ± 11.02	
Median (min-max)	61.0 (26-78)	
<60	76	45.5
>60	91	54.5
Gender		
Male	117	70.1
Female	50	29.9
Tumor localization		
Cardia	60	35.9
Corpus	32	19.2
Antrum	50	29.9
Diffuse	9	5.4
Tumor type		
Diffuse	42	25.1
Intestinal	125	74.9

TABLE 1: Continued.

Variables	n	%
Clinical T stage		
T2	27	16.2
T3	81	48.5
T4	59	35.3
Clinical N stage		
N0	12	7.2
N1	56	33.5
N2	56	33.5
N3	43	25.7
CA19-9		
Mean ± SD	443.48±4322.22	
Median (min-max)	9.90 (0.30-55730.0)	
CEA		
Mean ± SD	7.24±13.34	
Median (min-max)	3.00 (0.33-79.00)	
SIRI		
Mean ± SD	1.60±1.05	
Median (min-max)	1.35 (0.18-6.80)	
SII		
Mean ± SD	826.58±531.96	
Median (min-max)	685.00 (121.00-3681.00)	
Radiological response		
Complete response	27	16.2
Partial response	96	57.5
Stable disease	44	26.3
CAP score		
0	21	12.6
1	39	23.4
2	58	34.7
3	49	29.3
Recurrence		
No	55	32.9
Yes	112	67,1
Mortality		
Alive	74	44.3
Dead	93	55.7
Follow-up time (months)		
Mean ± SD	29.83±17.50	
Median (min-max)	24.00 (6.00-66.00)	
SD: Standard deviation; CEA: Carcinoembryonic antigen; SII: Systemic immune inflammation index; SIRI: Systemic inflammation response index; CAP: College of American Pathologists.		

SD: Standard deviation; CEA: Carcinoembryonic antigen; SII: Systemic immune inflammation index; SIRI: Systemic inflammation response index; CAP: College of American Pathologists.

The performance of various biochemical and inflammatory parameters in predicting mortality in patients was evaluated by ROC curve analyses (Table 2). The area under the curve (AUC) for CA19-9 was 0.535 [95% confidence interval (CI): 0.447-0.623], indicating low predictive ability. With a cut-off value of ≤ 9.95 , the sensitivity was 54.8% and, the specificity was 55.4%, and the result was not statistically significant ($p=0.436$). Similarly, for CEA, the AUC was 0.535 (95% CI: 0.446-0.624), with a cut-off of ≥ 2.99 , sensitivity of 57.0%, and specificity of 56.8%, showing no significant predictive power ($p=0.438$). When inflammatory indices were analyzed, the SII demonstrated excellent discriminatory power (AUC=0.963; 95% CI: 0.938-0.988). For a cut-off value of ≥ 664.00 , the sensitivity was 87.1% and, the specificity was 87.8%, and the result was statistically significant ($p<0.001$). Similarly, the SIRI value also exhibited high predictive performance (AUC=0.952; 95% CI: 0.922-0.982). For a cut-off value of ≥ 1.27 , the sensitivity was 88.2%, and the specificity was 89.2%, establishing this parameter as a strong predictor of mortality ($p<0.001$). These findings demonstrate that inflammatory markers SII and SIRI are the most powerful predictors of mortality, whereas CA19-9 and CEA show limited discriminatory ability.

Table 3 shows that, when the relationships between inflammatory indices and radiological response, and between

inflammatory indices and CAP treatment response scores were evaluated in patients, significant differences were observed between the SII and SIRI groups (all $p<0.001$). The radiological complete response rate was 33.8% in patients with SII <664.0 , while this rate was only 1.1% in the group with SII ≥ 664.0 . Similarly, the radiological complete response rate was 33.8% in the SIRI <1.27 group, while it decreased to 1.1% in the group with SIRI ≥ 1.27 . High SII and SIRI levels were associated with significant increases in partial response rates and, in particular, stable disease rates. When evaluated according to the CAP score, the complete response rate was determined to be 26.0% in the SII <664.0 group and 1.1% in the SII ≥ 664.0 group. Similarly, for SIRI, a complete response was observed in 26.0% at low SIRI levels, whereas it decreased to 1.1% at high SIRI levels. Conversely, the rate of poor responses increased significantly in groups with a high inflammatory index. Poor response was recorded at 51.2% in the SII ≥ 664.0 group, and at 50.0% in the SIRI ≥ 1.27 group. These findings indicate that high SII and SIRI values are associated with a significantly lower treatment response as measured by both radiological responses and CAP scores, suggesting that an increased inflammatory load can negatively affect treatment efficacy.

TABLE 2: Analysis of the predictive value of various clinical parameters in differentiating mortality in patients.

Variables	AUC	95% CI	Cut-off	Sensitivity (%)	Specificity (%)	p
CA19-9	0.535	0.447-0.623	≤ 9.95	54.8	55.4	0.436
CEA	0.535	0.446-0.624	≥ 2.99	57.0	56.8	0.438
SII	0.963	0.938-0.988	≥ 664.00	87.1	87.8	<0.001
SIRI	0.952	0.922-0.982	≥ 1.27	88.2	89.2	<0.001

AUC: Area under the curve; CI: Confidence interval; CEA: Carcinoembryonic antigen.

TABLE 3: Comparison of radiological response and CAP score with SII and SIRI groups.

Variables	SII			SIRI		
	<664.0 (n=77)	≥664.0 (n=90)	p-value	<1.27 (n=77)	≥1.27 (n=90)	p-value
Radiological response						
Complete response	26 (33.8)	1 (1.1)	<0.001	26 (33.8)	1 (1.1)	<0.001
Partial response	39 (50.6)	57 (63.3)		41 (53.2)	55 (61.1)	
Stable disease	12 (15.6)	32 (35.6)		10 (13.0)	34 (37.8)	
CAP score						
0	20 (26.0)	1 (1.1)	<0.001	20 (26.0)	1 (1.1)	<0.001
1	35 (45.5)	4 (4.4)		31 (40.3)	8 (8.9)	
2	19 (24.7)	39 (43.3)		22 (28.6)	36 (40.0)	
3	3 (3.8)	46 (51.2)		4 (5.1)	45 (50.0)	

Pearson's chi-square test, $p<0.05$ statistically significant.

SII: Systemic immune inflammation index; SIRI: Systemic inflammation response index; CAP: College of American Pathologists.

As shown in Table 4, the 2-year and 5-year OS rates were 51.1% and 43.5%, respectively, with a median OS of 27 months (95% CI: 19.98-34.02). The most significant survival differences emerged in subgroup analyses based on inflammatory indices. In patients with SII <664.00, the 2-year and 5-year OS rates were 94.7% and 83.7%, respectively, while in the SII ≥664.00 group, the 2-year OS dropped to 13.9%, and the 5-year OS could not be calculated ($p<0.001$). Similarly, the median OS was not reached in the low SII group, whereas the median OS was 15 months in the high SII group. A similar trend was observed in terms of SIRI; In patients with SIRI <1.27, the 2-year OS was 88.1% and the 5-year OS was 85.3%, while in the SIRI ≥1.27 group, the 2-year OS decreased to 19.4% ($p<0.001$). The median OS was not reached in the

low SIRI group, whereas it was 15 months in the high SIRI group. Figures 1 and 2 show the Kaplan-Meier curves for OS according to SII and SIRI levels, respectively.

As shown in Table 5, when all study patients were evaluated, the 2-year DFS rate was 33.5% and the 5-year DFS rate was 32.9%. The median DFS was calculated as 12 months (95% CI: 9.69-14.30). Inflammatory indices were found to be the strongest predictors of DFS. In patients with an SII value <664.00, the 2-year and 5-year DFS rates were 66.2% and 64.9%, respectively, while in the SII ≥664.00 group, the 2-year DFS was only 5.6%, and the 5-year DFS could not be calculated ($p<0.001$). While the median DFS was not reached in the low SII group, it was only 9 months in the high SII group. Similarly, the 2-year DFS rate was 64.9% in patients with SIRI <1.27,

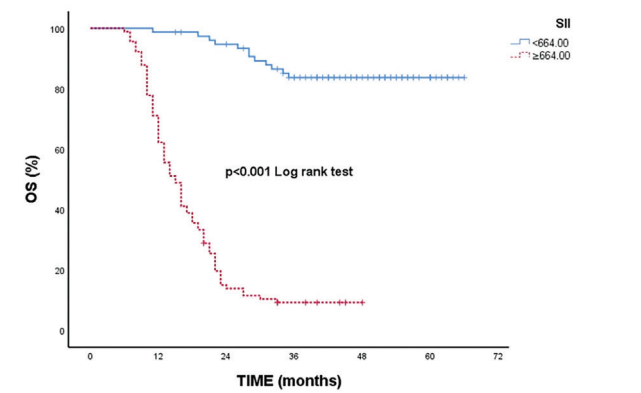


FIGURE 1: The Kaplan-Meier curves for OS according to SII levels.
OS: Overall survival; SII: Systemic immune inflammation index

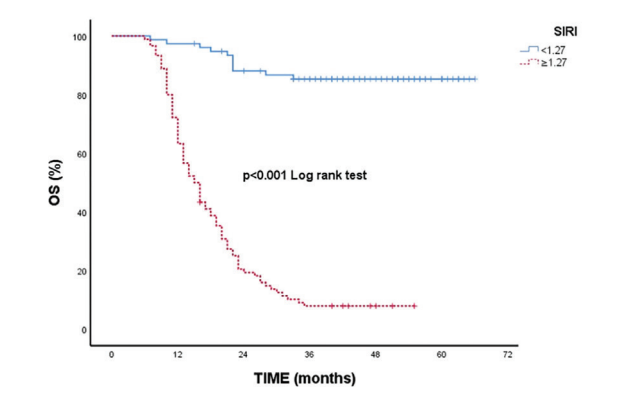


FIGURE 2: The Kaplan-Meier curves for OS according to SIRI levels.
OS: Overall survival; SIRI: Systemic inflammation response index

TABLE 4: Patient OS comparisons.				
Variables	2 years %	5 years %	Median (months) (95% CI)	p-value
General	51.1	43.5	27.00 (19.98-34.02)	
Age				
<60	56.2	47.7	30.00 (-)	0.377
>60	46.9	39.9	23.00 (16.85-29.4)	
Gender				
Male	48.5	38.8	23.00 (17.04-28.95)	0.055
Female	57.0	54.8	-	
SII				
<664.00	94.7	83.7	-	<0.001
≥664.00	13.9	-	15.00 (12.85-17.14)	
SIRI				
<1.27	88.1	85.3	-	<0.001
≥1.27	19.4	-	15.00 (12.67-17.32)	
Kaplan Meier, Log rank test, p<0.05 statistically significant. OS: Overall survival; SII: Systemic immune inflammation index; SIRI: Systemic inflammation response index; CI: Confidence interval.				

while it was only 6.7% in the SIRI ≥ 1.27 group ($p < 0.001$). The median DFS was 9 months in the high SIRI group, but was not reached in the low SIRI group. Figures 3 and 4 show the Kaplan-Meier curves for DFS according to SII and SIRI levels, respectively.

SII and SIRI were significant in univariate analyses. Variables found to be significant in univariate analyses were included in the multivariate Cox regression model. According to the model results, having an SII ≥ 664.00 increased the risk of

death 8.49 times [hazard ratio (HR): 8.49; 95% CI: 4.35-16.57; $p < 0.001$] and having a SIRI ≥ 1.27 increased the risk of death 7.88 times (HR: 7.88; 95% CI: 3.99-15.60; $p < 0.001$) (Table 6). Similarly, according to the results of the multivariate Cox regression analysis, having an SII ≥ 664.00 increased the risk of progression 5.13 times (HR: 5.13; 95% CI: 3.14-8.39; $p < 0.001$) and having a SIRI ≥ 1.27 increased the risk of progression 3.89 times (HR: 3.89; 95% CI: 2.39-6.31; $p < 0.001$) (Table 7).

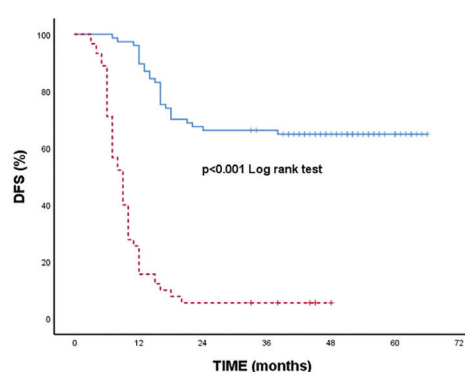


FIGURE 3: The Kaplan-Meier curves for DFS according to SII levels.
DFS: Disease-free survival; SII: Systemic immune inflammation index

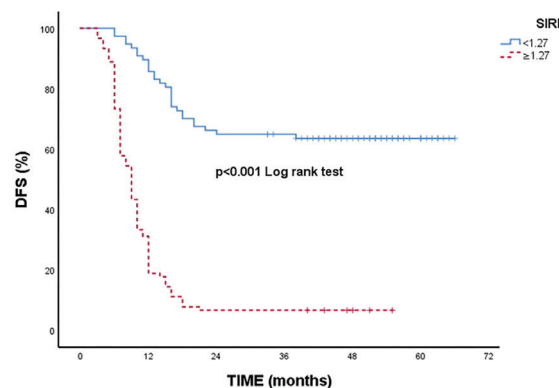


FIGURE 4: The Kaplan-Meier curves for DFS according to SIRI levels.
DFS: Disease-free survival; SIRI: Systemic inflammation response index

TABLE 5: Patient DFS comparisons.				
Variables	2 years %	5 years %	Median (months) (95% CI)	p-value
General	33.5	32.9	12.00 (9.69-14.30)	
Age				
<60	38.2	36.7	14.00 (10.94-17.05)	0.436
>60	29.7	29.7	12.00 (8.89-15.10)	
Gender				
Male	30.8	29.9	12.00 (9.88-14.11)	0.142
Female	40.0	40.0	16.00 (10.06-21.93)	
SII				
<664.00	66.2	64.9	-	<0.001
≥664.00	5.6	-	9.00 (7.78-10.21)	
SIRI				
<1.27	64.9	63.6	-	<0.001
≥1.27	6.7	-	9.00 (7.58-10.41)	
Kaplan Meier, Log rank test, p<0.05 statistically significant. DFS: Disease-free survival; SII: Systemic immune inflammation index; SIRI: Systemic inflammation response index; CI: Confidence interval.				

TABLE 6: Multivariate Cox regression results on the mortality risk of various clinical variables.		
Variables	HR (95% CI)	p-value
SII		
<664.00	ref	<0.001
≥664.00	8.49 (4.35-16.57)	
SIRI		
<1.27	ref	<0.001
≥1.27	7.88 (3.99-15.60)	
HR: Hazard ratio; ref: Reference; SII: Systemic immune inflammation index; SIRI: Systemic inflammation response index; CI: Confidence interval.		

TABLE 7: Multivariate Cox regression results on the progression risk of various clinical variables.		
Variables	HR (95% CI)	p-value
SII		
<664.00	ref	<0.001
≥664.00	5.13 (3.14-8.39)	
SIRI		
<1.27	ref	<0.001
≥1.27	3.89 (2.39-6.31)	
HR: Hazard ratio; ref: Reference; SII: Systemic immune inflammation index; SIRI: Systemic inflammation response index; CI: Confidence interval.		

DISCUSSION

The medical treatment of locally advanced gastric cancer has long been the perioperative FLOT regimen. Some studies published in the last two years have investigated the benefit of adding immunotherapies to this conventional treatment regimen, and current guidelines have shown that adding durvalumab to the FLOT regimen, as well as neoadjuvant/perioperative immunotherapies in microsatellite instability-high patients, contributes to survival.¹⁵⁻¹⁷

On the other hand, the effect of inflammation on cancer development and progression has been studied for years, and many inflammatory parameters and scores are associated with malignancy. The common conclusion from these studies is that inflammatory scores are predictive in cancer patients and closely related to survival.¹⁸⁻²⁰ While data show a link between inflammation and many types of cancer, the literature has also examined the relationship between SIRI and SII and disease recurrence, OS, and DFS in gastric cancer.²¹⁻²³ Our study, which included a similar number of patients, demonstrated that low SIRI levels significantly contributed to 3-year and 5-year survival.

A meta-analysis of 7 studies conducted between 2017 and 2023 showed that high SIRI values were associated with poorer OS and DFS in patients with gastric cancer. The accepted cut-off values for SIRI in these studies ranged from 0.58 to 1.35. Our study is similar to this meta-analysis in that the cut-off value calculated using a ROC curve for SIRI is 1.27.²⁴ In a study with a similar number of patients to our research, it was shown that low SIRI levels contributed statistically significantly to 3-year and 5-year survival.²⁵ In another study of 107 patients receiving neoadjuvant chemotherapy, SIRI levels were shown to be predictive of OS and DFS.²⁶ Our study is similar to the studies mentioned above and consistent with the literature. We also found that low SIRI levels had a statistically significant positive effect on both OS and DFS; this positive effect persisted in multivariate Cox regression analyses of mortality and progression. Our study showed that high SIRI values predicted mortality, with sensitivity of 88.2% and specificity of 89.2%, which are among the highest rates reported in the literature.

The significance of SII, similar to that of SIRI, in patients with gastric cancer has been examined in numerous recent studies. In another study that included patients who received perioperative FLOT therapy and were similar to those in our study, high SII values were statistically significant predictors of poor OS and DFS. The number of patients and the SII cut-off value in that study were nearly identical to those in our study.²⁷ A meta-analysis of data from 30 studies again found a close association between high SII values and worse OS and DFS.²⁸ As in patients receiving perioperative chemotherapy, in patients with stage 1-2 resectable gastric cancer, lower SII levels were found to be significantly associated with higher 5-year survival.²⁹ Another study, which examined data from 5995 gastric cancer patients across 16 studies, showed that high SII levels were statistically significantly associated with worse OS.³⁰ In our study, consistent with the literature, we found that high SII levels were significantly associated with worse OS and DFS, and that SII had a sensitivity of 87.1% and a specificity of 87.8% for predicting mortality.

In addition to its role as a prognostic indicator, studies in the literature also investigate the diagnostic role of SII³¹ and its importance in evaluating the response to neoadjuvant therapy. There are limited studies demonstrating a relationship between the CAP score, which is particularly used to assess pathological response to neoadjuvant therapy, and SII.³² In this respect, our study will make a significant contribution to the literature.

Study Limitations

Limitations of our study include its single-center, retrospective design and a relatively small number of patients.

CONCLUSION

In conclusion, there is a need for biomarkers that can be used clinically for prognostic and predictive purposes in gastric cancer patients. These indices have both advantages and disadvantages. Although both indices (SII and SIRI) are easy to use and inexpensive, the parameters included in these indices can also be affected by non-malignant factors. More comprehensive analyses have been and continue to be conducted by combining it with other markers.

Ethics

Ethics Committee Approval: The study was conducted according to the principles outlined in the Declaration of Helsinki and was approved by the University of Health Sciences Türkiye, Prof. Dr. Cemil Taşcıoğlu City Hospital Ethics Committee (approval number: 388, date: 21.10.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

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

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

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Protective Effect of Black Mulberry (*Morus nigra*) Fruit Extract on Methotrexate-related Intestinal Damage in Rats

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ABSTRACT

Objective: In this study, we aimed to evaluate the potential protective effects of black mulberry (*Morus nigra*, MN) extract, known for its antioxidant properties, against gastrointestinal mucositis induced by high-dose methotrexate (MTX) treatment, assessed using histological and biochemical parameters.

Material and Methods: Male Wistar albino rats (200-250 g) were randomly assigned to four groups of 14 animals each:

- Group 1 received 30 mg/kg MTX intraperitoneally on day 1 to induce intestinal damage.
- Group 2 received 30 mg/kg MTX intraperitoneally on day 1, followed by 500 mg/kg of MN extract administered by gavage. One subgroup received MN for 4 days; the other subgroup received MN for 6 days.
- Group 3 received intragastric saline only (0.001 mL/kg-2.5 mL/kg).
- Group 4 received 500 mg/kg MN extract by oral gavage for 4 or 6 days, depending on the subgroup.

The animals were sacrificed on either day 4 or day 6. Blood samples were analyzed for total oxidant status (TOS) and total antioxidant status (TAS). Tissue samples from the duodenum, jejunum, and ileum were examined for levels of Ki-67, myeloperoxidase (MPO), malon dialdehyde (MDA), tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β), TOS, and TAS.

Results: In the group treated with both MTX and MN extract, levels of MDA, MPO, IL-1 β , and TNF- α were significantly lower than those in the MTX-only group. Ki-67 levels were higher in intestinal tissues, indicating enhanced epithelial regeneration.

Conclusion: Our findings suggest that MN extract protects against MTX-induced mucosal damage and promotes intestinal epithelial healing. These promising results highlight the potential of MN as a supportive treatment in preventing MTX-related toxicity; however, further large-scale and clinical studies are needed to confirm its efficacy.

Keywords: Childhood cancers; intestinal mucositis; methotrexate; *Morus nigra*; rat

INTRODUCTION

Despite the significant increase in life expectancy among cancer patients in recent years, attributable in part to the advent of effective treatment approaches, nutritional

problems resulting from oral and gastrointestinal mucositis, which develop during treatment, remain a major cause of morbidity and mortality.¹ Approximately 400,000 new oral mucositis are diagnosed each year as a result of chemotherapy

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(CT) and radiotherapy.² Methotrexate (MTX), a folic acid antimetabolite and antineoplastic chemotherapeutic agent, is widely used in the treatment of leukemia and various solid tumors. MTX can cause diarrhea, anorexia, malabsorption, malnutrition, and dehydration in patients. It also inhibits epithelial proliferation and enterocyte function, disrupting the intestinal mucosal barrier and causing mucositis. MTX-induced mucositis occurs on average between days 3 and 7 before the development of neutropenia and thrombocytopenia.³ Intestinal mucositis is the most common cause of drug-related dose reductions, leading to malnutrition, growth retardation, and impaired quality of life.^{4,5} Mucositis is not only an epithelial change but also a process in which reactive oxygen radicals, transcription factor activation, apoptosis, and cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and IL-6 are increased.⁵

New antioxidant agents are currently being tested to assess their efficacy in preventing or reducing CT-associated mucositis, a form of systemic oxidative damage. Antioxidant molecules, including but not limited to n-acetylcysteine, glutamine, L-carnitine, taurine, and melatonin, have demonstrated significant potential in reducing oxidative damage to the small intestine, liver, and kidneys.⁶⁻⁹ Many agents are being investigated in both animal and clinical studies for the prevention and treatment of mucositis.

Mulberry is a fruit species that grows in temperate, tropical, and subtropical climates. *Morus alba* (white mulberry), *Morus nigra* (MN) (black mulberry), and *Morus rubra* (purple mulberry) are the most common species.¹⁰ Mulberries have been demonstrated to be a source of numerous natural antioxidants, including flavonoids, phenolic acids, and vitamins C and E. Of these, mulberry phenolics are best known for their antioxidant properties.^{11,12}

In this study, we investigated whether MN, which is known to have antioxidant properties, exerts beneficial effects on histopathological alterations and plasma oxidative stress parameters in MTX-induced mucositis of the rat small intestine. Biomarkers such as TNF- α , IL-1 β , Ki-67, malon dialdehyde (MDA), and myeloperoxidase (MPO) were assessed immunohistochemically to evaluate the effect of MN. This

experimental study is the first to investigate the effect of MN on MTX-induced oxidative damage.

MATERIAL AND METHODS

The present study was conducted on a group of 56 adult male Wistar albino rats, with a mean weight of 250-300 grams, which were housed in appropriate cages at a temperature of 24 ± 1 °C on a 12 hours light/dark cycle and fed a standard laboratory diet. The experimental animals were divided into four groups, of 14 animals each. The subjects were divided into two subgroups, corresponding to fourth-day and sixth-day treatments, with seven subjects in each subgroup.

The animals used in this study were obtained from the Manisa Celal Bayar University Experimental Animals Application and Research Center. The Manisa Celal Bayar University Local Ethics Committee for Animal Experiments granted ethical approval (approval number: 77.637.435-64, date: 08.11.2016). Since this is an animal experiment, consent is not required.

The first group received a single intraperitoneal administration of 30 mg/kg MTX (Koçak Farma Pharmaceuticals, İstanbul, Türkiye) on the first day. The second group received a single intraperitoneal dose of 30 mg/kg MTX on the first day, followed by oral gavage of 500 mg/kg MN extract commencing after the MTX administration and continuing for four days in the first subgroup and six days in the second subgroup. The third group received an intragastric saline solution at doses ranging from 0.001 mL/kg to 2.5 mL/kg for four days in the first subgroup and six days in the second subgroup (control group). The fourth group was given an oral gavage of MN extract at a dose of 500 mg/kg for four days in the first subgroup and six days in the second subgroup (Table 1). The subjects were sacrificed under general anesthesia on either the fourth or sixth day, depending on the study group, and approximately 3 mL of cardiac blood was collected from each animal under sterile conditions. Intestinal tissues were also removed.

Paraffin Tissue Tracking

After fixation, all samples were kept under running water overnight. They were then kept in 60%, 70%, and 80% alcohol solutions for 30 minutes each, and dehydrated in

TABLE 1: Formation of the experimental groups.

Groups	Application	Day 4	Medicine day	Day 6	Medicine day
Group 1	30 mg/kg MTX	7	1 day	7	1 day
Group 2	30 mg/kg MTX + 500mg/kg MN extract	7	1 day MTX + 4 day MN	7	1 day MTX + 6 days MN
Group 3	0.001 mL/kg-2.5 mL/kg intragastric saline solution (control)	7	4 days	7	6 days
Group 4	500mg/kg MN extract	7	4 days	7	6 days

MTX: Methotrexate; MN: *Morus nigra*.

100% alcohol for 1 hour with two changes. After being kept in xylene-alcohol solution for 30 minutes, they were transferred to xylene and kept for 1 hour with two changes for transparency. All samples were kept in an xylene-paraffin mixture for 30 minutes and embedded in paraffin.

Histochemical Staining

The samples taken from the animals were sectioned at 5 microns and kept overnight at 60 °C for paraffinization. The next day, the sections were placed in xylene for 1 hour, washed in running water for 5 minutes, and then transferred through a series of alcohols (96%, 80%, 70%, and 60%) for 2 minutes each. The sections were washed again for 5 minutes and kept in hematoxylin (Haematoxylin Harris, RRSP67/E) for 3 minutes. Then they were washed in running water for 5 minutes, immersed in 1% acid-alcohol solution, and removed. It was washed again in running water for 5 minutes and stained with eosin (Surgipath, Unno1989, 01601E) for 1 minute. After washing in running water for 5 minutes, the sections were soaked in 80% and 96% alcohol, each for 1 minute, then dried and immersed in xylene for 1 hour. All stained slides were covered with Entellan (Spring Bioscience, DMM-125) and examined under a light microscope (Olympus, BX43).

Immunohistochemistry

For immunohistochemical staining, the sections were incubated in an oven at 60 °C overnight, followed by clearing in two changes of xylene, 30 minutes each. They were then rehydrated through a graded series of alcohols ranging from 95% to 60% and then kept in distilled water for 10 minutes. The samples were outlined with an immunohistochemistry pen (IM3580, Immunotech, Marseille, France) and incubated in 0.5% trypsin solution (EK001-10K, Biogenex, San Ramon, USA) for 15 min at room temperature. Sections were washed three times for 5 minutes each with phosphate buffer (PBS). Three percent H_2O_2 was applied for 5 minutes to inhibit endogenous tissue peroxidase. The sections were washed with PBS 3 times for 5 minutes each and then treated with blocking solution (85-9043, Histostainplus kit, Invitrogen) for 10 minutes. After the blocking solution was removed from the tissue, the sections were incubated overnight with primary antibodies at a 1/100 dilution: anti-MPO (D3ZGE2), anti-Ki-67 (ABM40064, Abbkine), anti-TNF- α (ABP52624, Abbkine), anti-IL-1 β (Abp51611, Abbkine), and anti-MDA (ab6463, Abcam). The next day, the sections were washed 3 times with PBS and stained with anti-mouse/anti-rabbit biotin-streptavidin hydrogen peroxidase secondary antibody (85-9043, Histostainplus kit, Invitrogen) for 30 minutes per staining step. The sections were washed three times with PBS for 5 minutes each between steps, and stained with

diaminobenzidine (catalog nos. 71897 and 71898; Millipore) for 5 minutes to visualize the immunohistochemical reaction. After background staining with Mayer's hematoxylin (02274390059, J.T.Barker, Deventer, Holland) for 2 minutes, the sections were washed with distilled water for 10 minutes and then covered with a closing medium (K002, DBS, California, USA).

Immunohistochemical Assessments

Histochemical and immunohistochemical staining (for MPO, MDA, IL-1 β , Ki-67, and TNF- α antibodies) was performed on the small intestine. A semiquantitative method was used for immunohistochemical evaluation; the histologic score (H-score) was determined based on the distribution area and staining intensity of the cells. Histopathological and immunohistochemical evaluations were performed by investigators blinded to group allocation. The mean proportion of stained cells was graded as follows: 0 for <1% of the stained area, 1 for 1% to 25%, 2 for 26% to 50%, 3 for 51% to 75%, and 4 for >75%. Staining intensity was graded as follows: 0, negative staining; 1, weak staining; 2, moderate staining; 3, strong staining. The H-score for each specimen was calculated as follows:

H-score = degree of cell area-stained X average staining intensity

A total score of 0 to 12 was calculated and rated as negative (-, score: 0), weak (+, score: 1 to 4), moderate (++, score: 5 to 8) or strong (++++, score: 9 to 12).¹³

Biochemical Analysis

Blood samples from the rats were collected intracardially into lithium-heparinized tubes. Blood samples were centrifuged at 3500 rpm for 10 minutes, and plasma was separated and stored at -80 °C until analyzed in bulk. Plasma samples were analyzed for total oxidant status (TOS) and total antioxidant status (TAS) using a spectrophotometric method with commercial kits (RelAssay, Gaziantep, Türkiye). In the TOS analysis, total antioxidant molecules in the sample were measured spectrophotometrically, and the kit was calibrated using hydrogen peroxide. Results were given as micromol hydrogen peroxide equivalent/liter ($\mu\text{mol } H_2O_2 \text{ Eq/L}$).¹⁴ TAS was analyzed via the antioxidant effect of ABST (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation, and the results were given as micromol Trolox equivalent/L.¹⁵ The oxidative stress index (OSI) is an indicator of oxidative stress.

The TOS/TAS ratio is calculated using the following formula.¹⁶

$$\text{OSI (arbitraryunit)} = [\text{TOS } (\mu\text{mol } H_2O_2 \text{ Eq/L}) / \text{TAS } (\mu\text{mol Trolox Eq/L})] \times 100.$$

Obtaining *Morus nigra*

At the Ege University Faculty of Pharmacy, Department of Pharmacognosy Research Laboratory, the fruits were brought to room temperature and homogenized using a blender. They were macerated in 70% aqueous ethanol (10k to 1k fruit homogenate) for 24 hours on a shaker, and then filtered through filter paper. The filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. The extract was stored in the refrigerator.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean $\pm$ standard deviation for continuous variables and as number (n) and percentage (%) for categorical variables. The normality of data distribution was assessed using the Shapiro-Wilk test. For comparisons involving multiple groups and time points (days 4 and 6), a two-way mixed-design analysis of variance was applied to evaluate the effects of treatment groups, time, and their interaction. When significant differences were detected, post hoc pairwise comparisons were performed using the Bonferroni correction method. For non-normally distributed data, appropriate non-parametric tests were applied (Kruskal-Wallis test for multiple group comparisons and Mann-Whitney U test for pairwise comparisons). Categorical variables were compared using the chi-square test where applicable. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Macroscopic Findings

All subjects tolerated MTX. After sacrifice, intestinal tissues were removed, and macroscopic observations were made. In groups 1 and 2, swelling (edema) and mucositis were observed in the intestine and anus; foci of hyperemic hemorrhage were observed in Group 1. In Groups 3 and 4, no macroscopic changes were observed in any segment of the intestine (Figure 1). In addition, no pathologically enlarged mesenteric lymph nodes were found in any of the four groups.

Light Microscopic Findings

Light-microscopic examination revealed epithelial irregularities, polymorphonuclear leukocytes (PNL), and bleeding foci in the lamina propria of samples from the duodenum, jejunum, and ileum in Group 1. In Group 2, epithelial irregularities decreased; PNL infiltration was observed, but erythrocytes were absent from the lamina propria. Single-layered prismatic epithelium, loose connective tissue, and a normal lamina propria were observed in all



FIGURE 1: Macroscopic appearance of intestinal tissues after sacrifice (A: Normal intestinal tissue; B: congested and edematous intestinal wall).

intestinal samples from Groups 3 and 4. No histologic changes were observed in the muscular and adventitial layers in any of the intestinal specimens examined.

Immunohistochemical Findings

Immunohistochemical Evaluations

IL-1 β , Ki-67, TNF- α , MDA, and MPO were analyzed immunohistochemically in samples from the duodenum, jejunum, and ileum. A semiquantitative method was used for immunohistochemical evaluation, and the H-Score was determined based on the distribution area and staining intensity of the stained cells.

IL-1 β

Analysis of Table 2 showed that IL-1 β measurements taken on days 4 and 6 exhibited statistically significant differences between groups and between regions. On day 4, IL-1 β measurements from the ileum region in the MTX + MN group were statistically higher than those from the duodenum and jejunum regions ($p < 0.05$). On day 6, in the MTX + MN group, IL-1 β measurements from the duodenum were higher than those from other regions, while measurements from the jejunum were significantly lower than those from other regions ($p < 0.05$). On days 4 and 6, IL-1 β measurements in the MTX group were significantly higher than those in the other groups across all regions ($p < 0.05$). IL-1 β measurements in the duodenum and jejunum of the MTX + MN group on day 4 were significantly lower than the measurements taken on day 6 ($p < 0.05$). The IL-1 β measurements taken on day 4 in the jejunum of the MTX group, the ileum of the MTX + MN group, and the duodenum of the MN group were significantly higher than the measurements taken on day 6 ($p < 0.05$) (Table 2, Figure 2).

TABLE 2: Immunohistochemical evaluation of IL-1 β measurement between regions and groups.

Group	Regions	IL-1 β		Test statistics [‡]
		Day 4	Day 6	
MTX	Duodenum	11.25 $\pm$ 1.39 ^A	10.88 $\pm$ 1.55 ^A	p=0.598, η^2 =0.04
	Jejunum	11.63 $\pm$ 1.06 ^A	8.75 $\pm$ 0.46 ^B	p<0.001, η^2=0.93
	Ileum	11.25 $\pm$ 1.39 ^A	11.63 $\pm$ 1.06 ^A	p=0.351, η^2 =0.13
MTX+MN	Duodenum	3.38 $\pm$ 0.52 ^D	7.00 $\pm$ 1.85 ^C	p<0.001, η^2=0.84
	Jejunum	3.88 $\pm$ 0.35 ^D	2.13 $\pm$ 0.35 ^E	p<0.001, η^2=0.94
	Ileum	8.50 $\pm$ 0.53 ^B	4.75 $\pm$ 1.04 ^D	p<0.001, η^2=0.88
Control	Duodenum	1.00 $\pm$ 0.53 ^E	0.88 $\pm$ 0.35 ^E	p=0.685, η^2 =0.03
	Jejunum	0.50 $\pm$ 0.53 ^E	0.63 $\pm$ 0.52 ^E	p=0.598, η^2 =0.04
	Ileum	1.50 $\pm$ 0.53 ^E	1.25 $\pm$ 0.46 ^E	p=0.351, η^2 =0.13
MN	Duodenum	3.75 $\pm$ 0.46 ^D	2.00 $\pm$ 0.53 ^E	p<0.001, η^2=0.82
	Jejunum	1.00 $\pm$ 0.00 ^E	1.00 $\pm$ 0.00 ^E	p=0.999, η^2 =0.00
	Ileum	1.13 $\pm$ 0.35 ^E	1.25 $\pm$ 0.46 ^E	p=0.598, η^2 =0.04
Test statistics [†]		p<0.001, η^2=0.97	p<0.001, η^2=0.96	

Effect size (η^2); ^{*}: within-group comparison; [†]: between-group comparison. Data are presented as mean $\pm$ standard deviation ($\bar{X} \pm SD$). Bold values indicate statistical significance (p<0.05). Values with different superscript letters (A-E) within the same row or column differ significantly according to the Bonferroni post-hoc test (p<0.05).

IL: Interleukin; MTX: Methotrexate; MN: *Morus nigra*.

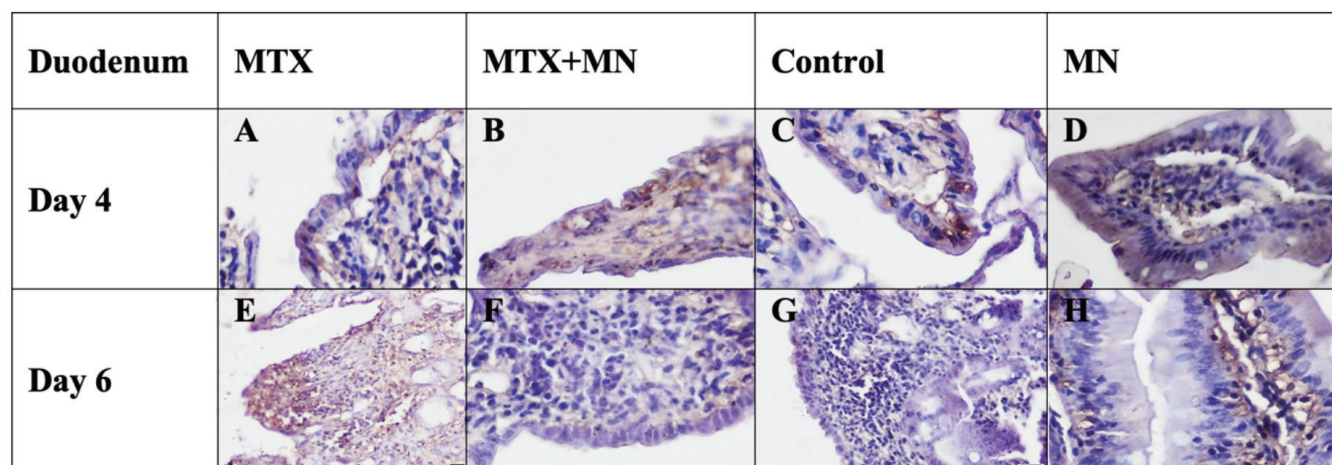


FIGURE 2: Representative immunohistochemical staining images of IL-1 β in the duodenum in the MTX, MTX + MN, control, and MN groups on days 4 and 6. (A-D) day 4; (E-H) day 6. (A, E) MTX; (B, F) MTX + MN; (C, G) control; (D, H) MN groups. Images were captured at $\times 200$ and $\times 400$ magnifications (scale bars: 20 μ m and 10 μ m, respectively).

MTX: Methotrexate; MN: *Morus nigra*

Ki-67

Thorough examination of Table 3 revealed statistically significant differences in Ki-67 measurements on days 4 and 6 among groups and regions. On day 4, the ileum of the MTX + MN group exhibited significantly lower Ki-67 measurements than the duodenum and jejunum (p<0.05). On the fourth day, the Ki-67 measurements from the duodenum and jejunum in the MTX + MN group, and from the ileum in the Control and

MN groups, were statistically higher than those in the other groups (p<0.05). On day 6, Ki-67 levels in the duodenum of the MTX + MN group were higher than in other regions, whereas levels in the ileum were statistically lower than in other regions (p<0.05). On day 6, the Ki-67 measurements in the duodenum and jejunum regions were significantly higher than those in the MTX + MN group (p<0.05). Furthermore, the Ki-67 measurements taken on day 4 in the jejunal regions of the MTX + MN group, in the ileal and jejunal regions of the

TABLE 3: Immunohistochemical evaluation of Ki-67 measurement between regions and groups.

Group	Regions	Ki-67		Test statistics [‡]
		Day 4	Day 6	
MTX	Duodenum	1.00±0.00 ^F	1.13±0.35 ^{EF}	p=0.351, $\eta^2=0.13$
	Jejunum	0.63±0.52 ^F	1.00±0.00 ^F	p=0.080, $\eta^2=0.38$
	Ileum	1.00±0.00 ^F	1.13±0.35 ^{EF}	p=0.351, $\eta^2=0.13$
MTX+MN	Duodenum	6.75±1.04 ^A	7.00±1.07 ^A	p=0.732, $\eta^2=0.02$
	Jejunum	6.00±0.00 ^A	4.50±0.93 ^B	p=0.003, $\eta^2=0.75$
	Ileum	1.75±0.46 ^D	1.63±0.52 ^D	p=0.598, $\eta^2=0.04$
Control	Duodenum	2.88±0.35 ^D	2.75±0.46 ^D	p=0.598, $\eta^2=0.04$
	Jejunum	2.25±0.46 ^D	1.00±0.00 ^F	p<0.001, $\eta^2=0.89$
	Ileum	3.63±0.52 ^C	2.25±0.46 ^D	p=0.004, $\eta^2=0.72$
MN	Duodenum	4.00±0.00 ^C	3.00±0.00 ^D	p<0.001, $\eta^2=0.99$
	Jejunum	1.00±0.00 ^F	1.50±0.53 ^E	p=0.033, $\eta^2=0.50$
	Ileum	3.63±0.52 ^C	2.63±0.74 ^D	p=0.033, $\eta^2=0.50$
Test statistics [†]		p<0.001, $\eta^2=0.95$	p<0.001, $\eta^2=0.91$	

Effect size (η^2); ϕ : within-group comparison; [†]: between-group comparison. Data are presented as mean $\pm$ standard deviation ($\bar{X} \pm SD$). Bold values indicate statistical significance ($p<0.05$). Values with different superscript letters (A-F) within the same row or column differ significantly according to the Bonferroni post-hoc test ($p<0.05$).

MTX: Methotrexate; MN: *Morus nigra*.

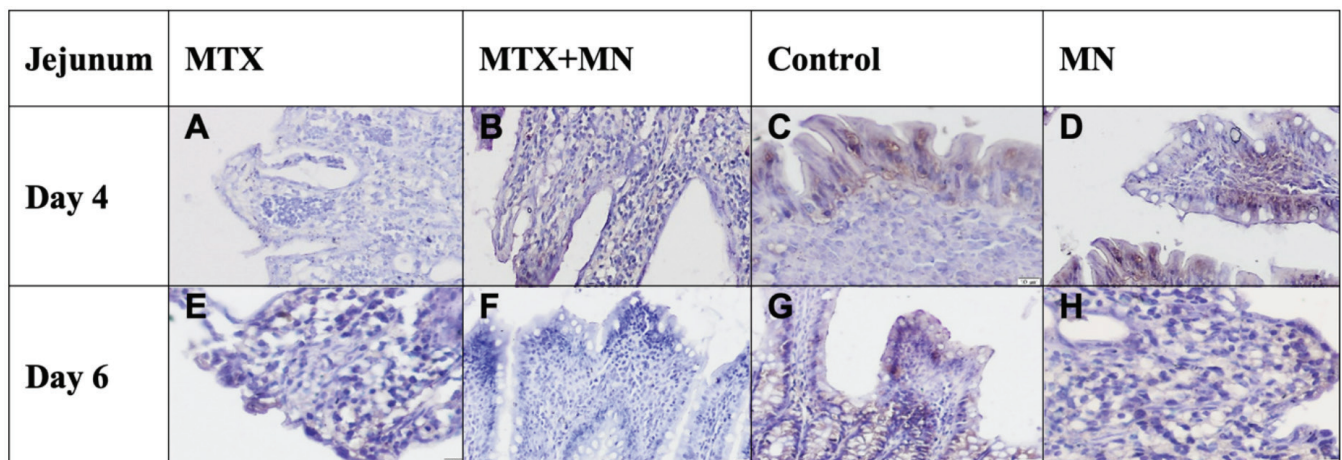


FIGURE 3: Representative immunohistochemical staining images of Ki-67 in the duodenum in the MTX, MTX + MN, control, and MN groups on days 4 and 6. (A-D) day 4; (E-H) day 6. (A, E) MTX; (B, F) MTX + MN; (C, G) control; (D, H) MN groups. Images were captured at $\times 200$ and $\times 400$ magnifications (scale bars: 20 μ m and 10 μ m, respectively).

MTX: Methotrexate; MN: *Morus nigra*

control group, and in the duodenal and ileal regions of the MN group were significantly higher than those taken on day 6 ($p<0.05$) (Table 3, Figure 3).

TNF- α

A subsequent analysis of Table 4 revealed statistically significant differences in TNF- α measurements among the groups and regions. On day 4, TNF- α levels in the ileum of the MTX + MN group were significantly higher than those in

the duodenum and jejunum ($p<0.05$). Similarly, on day 4, the TNF- α measurement in the Mtx group was significantly higher compared with the other groups across all regions ($p<0.05$). On day 6, no statistically significant differences in TNF- α measurements were observed among the regions within the MTX + MN group ($p>0.05$). However, TNF- α measurements on day 4 in all regions of the MTX group and in the ileum region of the MTX + MN group were statistically higher than measurements taken on day 6 ($p<0.05$) (Table 4, Figure 4).

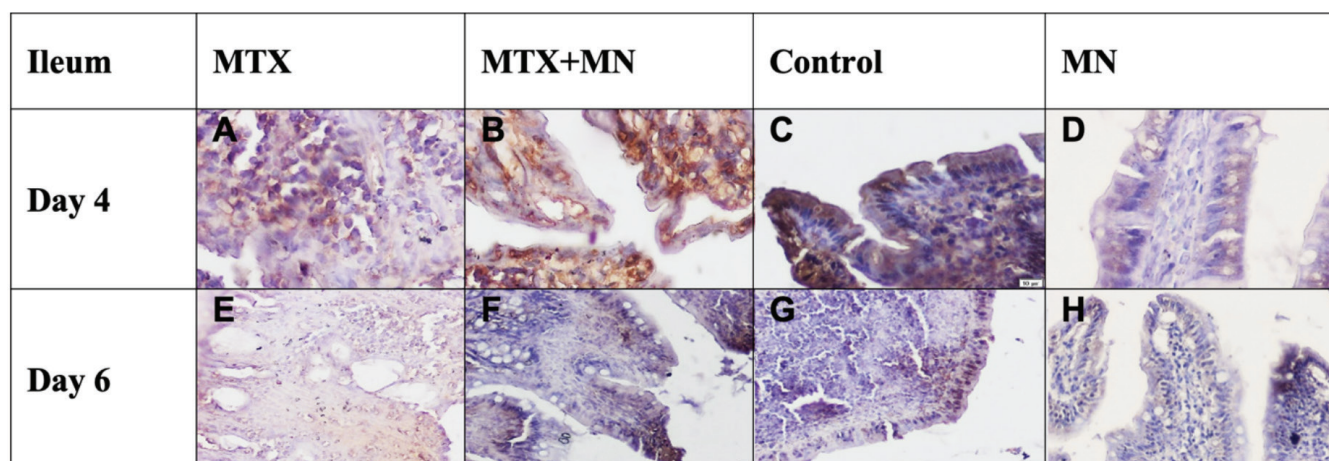


FIGURE 4: Representative immunohistochemical staining images of TNF-α in the duodenum in the MTX, MTX+MN, control, and MN groups on days 4 and 6. (A-D) day 4; (E-H) day 6. (A, E) MTX; (B, F) MTX + MN; (C, G) control; (D, H) MN groups. Images were captured at $\times 200$ and $\times 400$ magnifications (scale bars: 20 μm and 10 μm , respectively).

TNF-α: Tumor necrosis factor-α; MTX: Methotrexate; MN: *Morus nigra*

TABLE 4: Immunohistochemical evaluation of TNF-α measurement between regions and groups.

Group	Regions	TNF-α		Test statistics ^φ
		Day 4	Day 6	
MTX	Duodenum	8.75±0.46 ^B	4.50±0.93 ^D	p<0.001, $\eta^2=0.91$
	Jejunum	11.63±1.06 ^A	8.88±0.35 ^B	p<0.001, $\eta^2=0.86$
	Ileum	10.88±1.55 ^A	6.25±0.71 ^C	p<0.001, $\eta^2=0.92$
MTX+MN	Duodenum	3.25±0.46 ^D	3.25±0.46 ^D	p=0.999, $\eta^2=0.00$
	Jejunum	4.00±0.00 ^D	4.00±0.00 ^D	p=0.999, $\eta^2=0.00$
	Ileum	8.75±0.46 ^B	3.13±0.35 ^D	p<0.001, $\eta^2=0.99$
Control	Duodenum	3.38±1.06 ^D	3.25±0.46 ^D	p=0.785, $\eta^2=0.01$
	Jejunum	1.13±0.35 ^E	1.25±0.46 ^E	p=0.598, $\eta^2=0.04$
	Ileum	2.75±0.46 ^D	2.75±0.46 ^D	p=0.999, $\eta^2=0.00$
MN	Duodenum	4.25±0.71 ^D	5.75±0.71 ^C	p=0.020, $\eta^2=0.56$
	Jejunum	0.50±0.53 ^E	0.75±0.46 ^E	p=0.451, $\eta^2=0.08$
	Ileum	1.75±0.46 ^E	2.75±0.46 ^D	p=0.007, $\eta^2=0.67$
Test statistics [†]		p<0.001, $\eta^2=0.97$	p<0.001, $\eta^2=0.95$	

Effect size (η^2); ϕ : within-group comparison; [†]: between-group comparison. Data are presented as mean $\pm$ standard deviation ($\bar{X} \pm \text{SD}$). Bold values indicate statistical significance (p<0.05). Values with different superscript letters (A-E) within the same row or column differ significantly according to the Bonferroni post-hoc test (p<0.05).
TNF-α: Tumor necrosis factor-α; MTX: Methotrexate; MN: *Morus nigra*.

MDA

Analysis of Table 5 revealed statistically significant differences in MDA measurements across groups and regions. On day 4, the MDA measurements from the jejunum region in the MTX + MN group were significantly higher than those in the duodenum and ileum regions (p<0.05). On Day 4, MDA measurements in the duodenum and ileum from the MTX group and in the jejunum from the MTX + MN group were statistically higher than those in the other groups (p<0.05). On day 6, the MDA measurement in the jejunum region in

the MTX + MN group was significantly higher than in the duodenum and ileum regions (p<0.05). On day 6, the MDA measurement in the MTX group was statistically significant compared with the other groups across all regions (p<0.05). Furthermore, the MDA measurements obtained on day 4 from the MTX + MN group in all regions were significantly higher than those obtained on day 6 (p<0.05) (Table 5, Figure 5).

MPO

Analysis of Table 6 showed that MPO measurements taken on days 4 and 6 differed significantly across groups and regions.

TABLE 5: Immunohistochemical evaluation of MDA measurement between regions and groups.				
Group	Regions	MDA		Test statistics [‡]
		Day 4	Day 6	
MTX	Duodenum	11.63±1.06 ^A	11.25±1.39 ^A	p=0.598, $\eta^2=0.04$
	Jejunum	9.00±0.00 ^B	9.00±0.00 ^B	p=0.999, $\eta^2=0.00$
	Ileum	11.63±1.06 ^A	11.25±1.39 ^A	p=0.598, $\eta^2=0.04$
MTX+MN	Duodenum	10.88±1.55 ^B	2.50±0.53 ^D	p<0.001, $\eta^2=0.96$
	Jejunum	12.00±0.00 ^A	9.00±0.00 ^B	p<0.001, $\eta^2=0.99$
	Ileum	8.63±0.52 ^B	5.50±0.93 ^C	p<0.001, $\eta^2=0.88$
Control	Duodenum	0.75±0.46 ^E	0.75±0.46 ^E	p=0.999, $\eta^2=0.00$
	Jejunum	1.00±0.00 ^E	1.00±0.00 ^E	p=0.999, $\eta^2=0.00$
	Ileum	2.13±0.64 ^D	2.13±0.83 ^D	p=0.999, $\eta^2=0.00$
MN	Duodenum	0.13±0.35 ^E	0.38±0.52 ^E	p=0.351, $\eta^2=0.13$
	Jejunum	2.00±0.00 ^D	2.00±0.00 ^D	p=0.999, $\eta^2=0.00$
	Ileum	2.25±0.71 ^D	2.13±0.35 ^D	p=0.685, $\eta^2=0.02$
Test statistics [†]		p<0.001, $\eta^2=0.98$	p<0.001, $\eta^2=0.97$	

Effect size (η^2); ϕ : within-group comparison; [†]: between-group comparison. Data are presented as mean $\pm$ standard deviation ($\bar{X} \pm SD$). Bold values indicate statistical significance ($p<0.05$). Values with different superscript letters (A-E) within the same row or column differ significantly according to the Bonferroni post-hoc test ($p<0.05$).
MDA: Malon dialdehyde; MTX: Methotrexate; MN: *Morus nigra*.

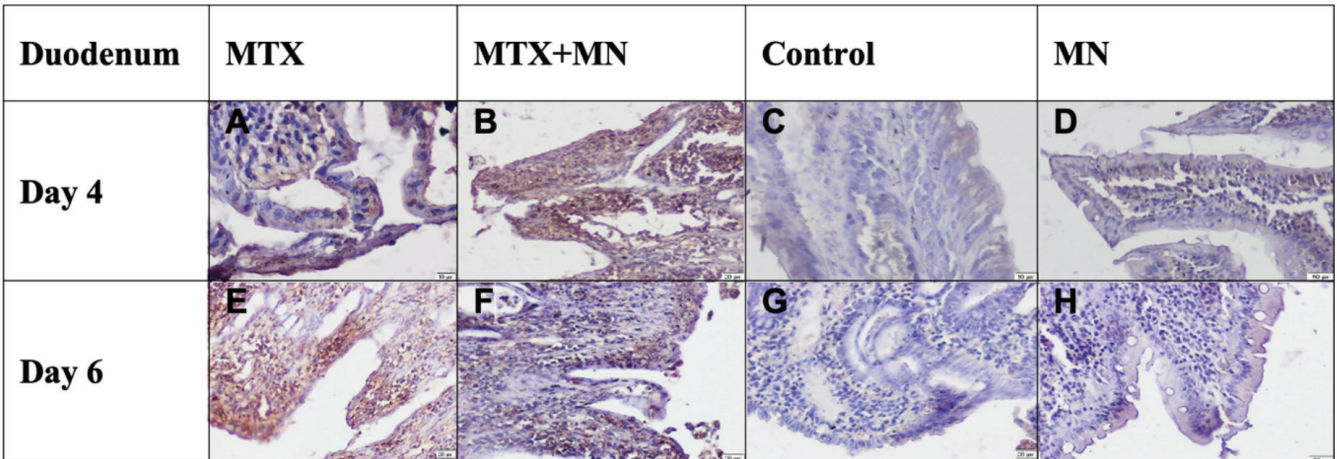


FIGURE 5: Representative immunohistochemical staining images of MDA in the duodenum in the MTX, MTX + MN, control, and MN groups on days 4 and 6. (A-D) day 4; (E-H) day 6. (A, E) MTX; (B, F) MTX + MN; (C, G) control; (D, H) MN groups. Images were captured at $\times 200$ and $\times 400$ magnifications (scale bars: 20 μm and 10 μm , respectively).
MDA: Malon dialdehyde; MTX: Methotrexate; MN: *Morus nigra*

On day 4, MPO measurement in the ileum region in the MTX + MN group was significantly higher than measurements in the duodenum and jejunum regions ($p<0.05$). On days 4 and 6, MPO measurements in the MTX group were significantly higher than those in the other groups across all regions ($p<0.05$) (Table 6, Figure 6).

Biochemical Findings

Evaluation of the TAS on day 6 revealed that the difference between Group 1 and Group 2 was not statistically significant

($p=1.000$). However, the increase in TAS value was statistically significant compared with Groups 3 and 4 ($p=0.020$, $p=0.003$, respectively). Compared with Group 2, Group 4 showed a statistically significant increase in TAS value ($p=0.010$). No statistically significant differences were observed between the groups on days 4 and 6 for TOS values (Table 7).

DISCUSSION

This study investigated the protective effect of MN on MTX-induced small intestinal mucositis in rats. Our

TABLE 6: Immunohistochemical evaluation of MPO measurement between regions and groups.

Group	Regions	MPO		Test statistics [‡]
		Day 4	Day 6	
MTX	Duodenum	11.63±1.06 ^A	11.25±1.39 ^A	p=0.598, $\eta^2=0.04$
	Jejunum	12.00±0.00 ^A	12.00±0.00 ^A	p=0.999, $\eta^2=0.00$
	Ileum	8.75±0.46 ^B	8.88±0.35 ^B	p=0.598, $\eta^2=0.04$
MTX+MN	Duodenum	1.13±0.35 ^E	1.25±0.46 ^E	p=0.598, $\eta^2=0.04$
	Jejunum	2.25±0.46 ^D	2.13±0.35 ^D	p=0.598, $\eta^2=0.04$
	Ileum	5.75±0.71 ^C	5.50±0.93 ^C	p=0.598, $\eta^2=0.04$
Control	Duodenum	0.50±0.53 ^E	0.38±0.52 ^E	p=0.685, $\eta^2=0.03$
	Jejunum	1.75±0.46 ^D	1.75±0.46 ^D	p=0.999, $\eta^2=0.00$
	Ileum	2.25±0.46 ^D	2.25±0.46 ^D	p=0.999, $\eta^2=0.00$
MN	Duodenum	2.00±0.53 ^D	1.88±0.83 ^D	p=0.763, $\eta^2=0.01$
	Jejunum	1.75±0.46 ^D	1.63±0.52 ^D	p=0.685, $\eta^2=0.03$
	Ileum	2.13±0.99 ^D	2.25±0.46 ^D	p=0.763, $\eta^2=0.01$
Test statistics [†]		p<0.001, $\eta^2=0.98$	p<0.001, $\eta^2=0.98$	

Effect size (η^2); ϕ : within-group comparison; $\dagger$: between-group comparison. Data are presented as mean $\pm$ standard deviation ($\bar{X} \pm SD$). Bold values indicate statistical significance ($p<0.05$). Values with different superscript letters (A-E) within the same row or column differ significantly according to the Bonferroni post-hoc test ($p<0.05$).

MPO: Myeloperoxidase; MTX: Methotrexate; MN: *Morus nigra*.

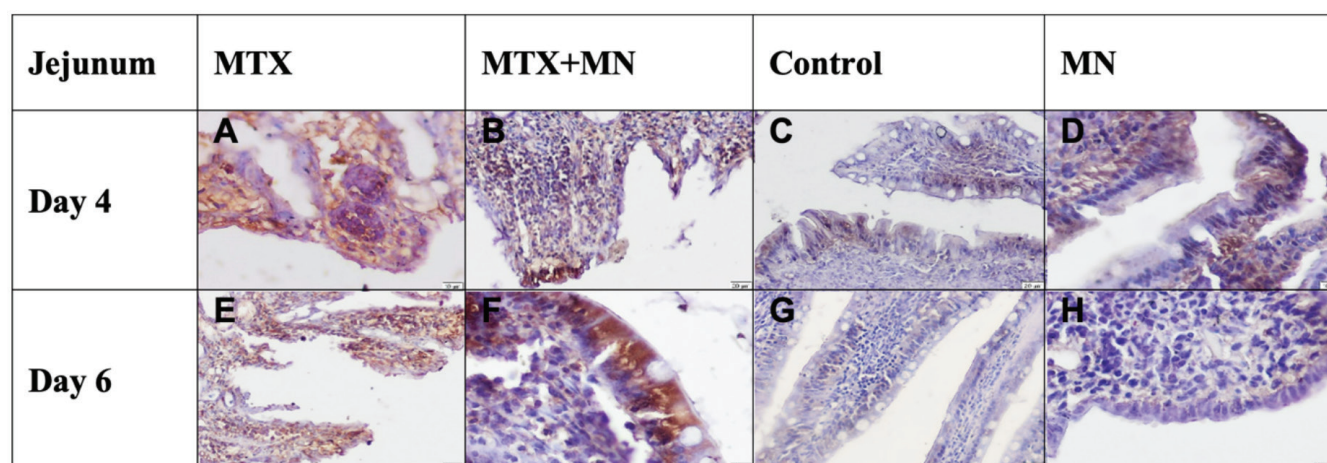


FIGURE 6: Representative immunohistochemical staining images of MPO in the duodenum in the MTX, MTX + MN, control, and MN groups on days 4 and 6. (A–D) day 4; (E–H) day 6. (A, E) MTX; (B, F) MTX + MN; (C, G) Control; (D, H) MN groups. Images were captured at $\times 200$ and $\times 400$ magnifications (scale bars: 20 μm and 10 μm , respectively).

MPO: Myeloperoxidase; MTX: Methotrexate; MN: *Morus nigra*

immunohistochemical results showed that MTX increased the levels of oxidative stress markers (MDA and MPO) and inflammatory markers (IL-1 β and TNF- α), decreased the level of Ki-67, a proliferation marker, in the duodenum, jejunum, and ileum of rats. When we compared the rats given MTX and 500 mg/kg MN extract together with the rats given MTX alone, MDA, MPO, IL-1 β , and TNF- α levels decreased in the MN-treated group, while Ki-67 levels increased in small intestinal tissues. Histopathological examination showed that 500 mg/kg MN extract could provide adequate protection of small intestinal

tissue against MTX-induced oxidative damage. Our biochemical results showed that MN had no TAS-enhancing effect.

MTX induced epithelial disorganization, PNL-cell infiltration, and bleeding foci in the small intestinal tissues of rats. Histopathologic examination after the addition of MN showed that MTX-induced epithelial disorganization and intestinal mucositis had resolved. Proliferation and self-renewal of small intestinal epithelial cells occur in crypts. Crypt cells, which can regenerate very rapidly under normal physiologic conditions, migrate to the ends of the villi and enable the gastrointestinal

TABLE 7: Statistical data of total antioxidant status (TAS) and total oxidant status (TOS) values.

Group	TAS (1.2-1.5 milimol/L)		TOS (4-6 micromol/L)	
	Day 4	Day 6	Day 4	Day 6
	Average $\pm$ SD	Median (min/max)	Median (min/max)	Average $\pm$ SD
1	1.88 $\pm$ 0.50	1.10 (0.95/1.42)	98.43 (39.13/221.69)	34.08 $\pm$ 11.42
2	1.52 $\pm$ 0.22	1.17 (1.05/1.25)	46.87 (22.58/158.31)	31.48 $\pm$ 12.60
3	1.61 $\pm$ 0.10	1.57 (1.46/2.10)	41.63 (16.92/104.79)	35.77 $\pm$ 18.87
4	1.46 $\pm$ 0.24	1.68 (1.35/1.79)	30.77 (23.58/86.16)	42.17 $\pm$ 24.79
p-value	0.093	<0.001	0.110	0.697
1 $\rightarrow$ 2	ad.	1.000	ad.	ad.
1 $\rightarrow$ 3	ad.	0.020	ad.	ad.
1 $\rightarrow$ 4	ad.	0.003	ad.	ad.
2 $\rightarrow$ 3	ad.	0.053	ad.	ad.
2 $\rightarrow$ 4	ad.	0.010	ad.	ad.
3 $\rightarrow$ 4	ad.	1.000	ad.	ad.

One-way ANOVA (Brown-Forsythe), Kruskal-Wallis test (Monte Carlo); Post-hoc test: Dunn's Test.
 ANOVA: Analysis of variance; SD: Standard deviation; Min: Minimum; Max: Maximum; TAS: Total antioxidant status; TOS: Total oxidative status; OSI: Oxidative stress index; 1st group: Methotrexate; 2nd group: Methotrexate + *Morus nigra*; 3rd group: Control; 4th group: *Morus nigra*.

epithelium to renew itself completely in approximately 2-3 days. Miyazono et al.¹⁷ showed that oxidative stress, especially neutrophil infiltration, plays a role in damage after MTX treatment. Taminiau et al.¹⁸ have shown that the most severe effects of MTX-induced histopathologic changes are crypt and villous ablation in the proximal small intestine. For this reason, we examined all parts of the small intestine. We observed that macroscopic changes and mucositis in the small intestine of sacrificed rats decreased from proximal to distal regions. The jejunal epithelium enters a highly proliferative phase 96 hours after MTX administration. Increased mitotic activity, deepened crypts, and crypt hyperplasia have been shown to be greatest on days 4 to 6 after MTX administration.¹⁹ Therefore, we collected duodenal, jejunal, and ileal tissues under deep anesthesia at 96 and 144 hours after a single intraperitoneal dose of MTX for histopathologic evaluation of small intestinal injury. Immunohistochemically, MTX-induced intestinal injury was observed on days 4 and 6. At the end of our study, immunohistochemical scores for oxidant and inflammatory parameters in the small intestine were lower in the MTX + MN group, whereas proliferative scores were higher in the MTX group. This finding suggests that the addition of MN to MTX reduces intestinal damage.

Previous studies have confirmed that MTX toxicity is associated with increased inflammatory cytokine production.^{20,21} Antioxidant activity and inhibition of IL-1 β may help prevent MTX-induced intestinal inflammation.²² de Araújo et al.²³ reported that in addition to oxidants, proinflammatory IL-1 β and TNF- α levels were significantly increased in damaged intestinal tissue. In our study, we showed that the levels of

the proinflammatory cytokines IL-1 β and TNF- α and the oxidant/antioxidant balance in small intestinal tissues shifted in favor of oxidants in Group 1. However, in Group 2, MN suppressed the increase in IL-1 β and TNF- α levels in small intestinal tissues by preventing a shift in the balance toward oxidants. Although MTX increased IL-1 β and TNF- α levels, simultaneous administration of MN prevented MTX-induced deleterious effects in all small intestinal segments. According to our results, MN showed anti-inflammatory properties by decreasing IL-1 β and TNF- α secretion.

Sugiyama et al.²⁴ reported that the rate of Ki-67 antigen-positive cells decreased significantly in those given MTX. In a similar study, Leitão et al.²⁵ found that the number of Ki-67 positive cells, a proliferation marker, decreased significantly on day 5 in the jejunum epithelium of rats given MTX. The absence of Ki-67 immunoreactivity in Group 1 supports that MTX inhibits epithelial proliferation; the observation of increases in Ki-67, especially in the duodenum and jejunum, as a result of MN administration in Group 2, supports that the extract triggers proliferation to prevent epithelial damage.

Active oxygen species are known to play a role in MTX-induced oxidative stress.²⁶ Odabasoglu et al.²⁷ reported increased MDA and decreased total glutathione in inflammatory tissue. Likewise, studies have confirmed that MTX directly increases MDA levels in small intestinal tissue and causes oxidative stress by decreasing total GH levels.²⁸ In light of this information, studies have turned to antioxidants to reduce the side effects of MTX.^{29,30} In the present study, MN effectively suppressed MTX-induced oxidation by

preventing the increase in MDA. This finding aligns with the results reported by Jahovic et al.³¹, who administered a single intraperitoneal dose of 20 mg/kg MTX to rats. Their research revealed a decrease in glutathione levels in the blood, liver, kidney, and small intestine tissues and a significant increase in MPO and MDA levels. These increased levels of MDA and MPO serve as indicators of inflammatory response. Consistent with the literature, our study found that MPO levels were elevated in the group receiving MTX compared to the other groups. Our findings demonstrated that while MDA and MPO immunoreactivities increased after MTX administration, they decreased in all three intestinal segments after extract administration, suggesting that extract administration reduces MDA and MPO in both early and late periods. Our findings demonstrated a significant decrease in oxidative stress parameters following MN administration across all treatment groups and intestinal segments.

Evaluation of TAS reveals that oxidative reactions during hepatic metabolism of MTX lead to toxic side effects, causing hepatotoxicity and damage to rapidly proliferating normal tissues such as bone marrow and intestine. Measurements of oxidative stress, antioxidant defense, oxidant-antioxidant balance, and factors affecting this balance (TOS, TAS, and OSI) are available, and the levels of these factors can be measured and calculated separately.^{32,33} In our study, statistical analysis of TOS and TAS levels measured to assess the antioxidant properties of MN revealed no significant results. This showed that MN had no TAS-enhancing effect. Despite the absence of significant changes in systemic TAS levels, the observed reduction in tissue oxidative stress markers suggests that the antioxidant effects of MN may be predominantly localized to intestinal tissue. Since mucositis primarily affects the gastrointestinal mucosa, MN may exert its protective effects locally without significantly altering systemic antioxidant capacity. Additionally, plasma TAS measurements may not be sufficiently sensitive to reflect regional oxidative changes in specific tissues. The duration of treatment and timing of sample collection may also have contributed to the lack of detectable systemic differences. Therefore, these findings suggest that MN exerts its protective effects mainly at the tissue level rather than through systemic antioxidant modulation.

Studies have shown that the mechanism of action of MN extract is related to its antioxidant effect, acting by disrupting the activation of the reactive form of MTX. This is due to an alkaloid, such as a flavonoid, contributing to the antioxidant effect of MN. Flavonoids had inhibitory effects on the death of normal cells induced by various oxidative stresses thanks to their strong antioxidant properties.³² The findings of this

study corroborated the literature and demonstrated that the most pronounced anti-inflammatory effect was observed in the MN group, in which inflammatory parameters declined.

To the best of our knowledge, no study has demonstrated a protective effect of MN against MTX-induced intestinal mucositis. In our study, we observed that MN was well tolerated in rats and produced the expected effect without causing abnormal changes in biochemical or immunohistochemical parameters. Morphometric and histopathologic findings from our study showed that MN exerted a protective effect against MTX-induced mucosal damage and promoted regeneration of the intestinal epithelium. Our findings are unique in that they provide the first evidence demonstrating the protective effect of MN against MTX-induced gastrointestinal toxicity at the tissue level. These results suggest that MN has the potential to protect against the adverse effects of MTX. However, further studies encompassing more extensive clinical applications are necessary to fully ascertain the efficacy and potential benefits of MN in this context.

Study Limitations

This study has several limitations. The MN extract was not phytochemically characterized [e.g., by high-pressure liquid chromatography (LC) or LC/tandem mass spectrometry], which may affect the reproducibility and the mechanistic interpretation. Only male rats were included, precluding the assessment of sex-related differences. The use of a single, relatively high dose limits the evaluation of dose-response relationships. Only two time points were assessed, which may not fully capture the dynamic course of mucositis and may increase the risk of type I error due to multiple comparisons. In addition, no formal power analysis was performed to determine sample size. Finally, functional outcome measures (e.g., intestinal permeability, nutrient absorption, and clinical indices of mucositis severity) were not evaluated, which may limit the physiological interpretation of the results.

CONCLUSION

The results of this study provide macroscopic, microscopic, and immunohistochemical evidence that MN's strong antioxidant and anti-inflammatory properties prevent MTX-induced intestinal damage. To the best of our knowledge, no studies demonstrate the protective effect of MN at the tissue level against MTX-induced intestinal mucositis. The morphometric and histopathologic findings in our study show that MN has a protective effect against MTX-induced mucosal damage and promotes regeneration of the intestinal epithelium. MN emerges as a promising agent in the prevention and treatment of MTX-induced oral and gastrointestinal mucositis.

Ethics

Ethics Committee Approval: The Manisa Celal Bayar University Local Ethics Committee for Animal Experiments granted ethical approval (approval number: 77.637.435-64, date: 08.11.2016).

Informed Consent: Since this is an animal experiment, consent is not required.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: A.C.E., A.T.Y., S.V., İ.T., Concept: A.C.E., A.T.Y., H.G., Design: A.C.E., F.F., İ.T., H.G., Data Collection or Processing: A.C.E., A.T.Y., F.F., F.T., Analysis or Interpretation: F.F., S.V., İ.T., F.T., Literature Search: A.C.E., İ.T., H.G., Writing: A.C.E., A.T.Y., F.T.

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

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Baseline Serum Albumin-to-Creatinine Ratio as an Independent Prognostic Marker in *De Novo* Metastatic HER2-negative Gastric Cancer

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ABSTRACT

Objective: Systemic inflammation, nutritional status, and metabolic reserve are recognized determinants of survival in metastatic gastric cancer patients. The serum albumin-to-creatinine ratio (ACR) combines nutritional and renal/metabolic information, but its prognostic value in gastric cancer remains unclear. We aimed to investigate the association between baseline ACR and survival outcomes in patients with *de novo* metastatic human epidermal growth factor receptor 2 (HER2)-negative gastric cancer.

Material and Methods: This retrospective, single-center study included 112 adults with histologically confirmed *de novo* metastatic HER2-negative gastric cancer diagnosed between April 2011 and January 2024. Baseline serum albumin and creatinine levels obtained within 15 days prior to treatment initiation were used to calculate the ACR. Patients were categorized into low and high ACR groups according to the cohort median. Progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method and Cox proportional hazards models.

Results: The median follow-up duration was 40.6 months. The median PFS for the entire cohort was 6.8 months, and the median OS was 13.7 months. Patients with high ACR had significantly longer PFS (7.2 vs. 6.0 months; $p=0.005$) and OS (16.9 vs. 10.2 months; $p<0.001$) than those with low ACR. In multivariable analysis, high ACR remained independently associated with improved PFS [hazard ratio (HR): 0.65; 95% confidence interval (CI): 0.44-0.97; $p=0.034$] and OS (HR: 0.38; 95% CI: 0.21-0.67; $p<0.001$). Eastern Cooperative Oncology Group performance status ≥ 1 was also independently associated with worse survival outcomes.

Conclusion: Baseline serum ACR is an independent prognostic marker for both PFS and OS in patients with *de novo* metastatic HER2-negative gastric cancer. As a simple and routinely available laboratory parameter, ACR may contribute to risk stratification in advanced disease.

Keywords: Gastric cancer; metastatic disease; albumin-to-creatinine ratio; prognosis; survival

INTRODUCTION

Gastric cancer (GC) is one of the leading causes of cancer-related mortality worldwide, with a substantial proportion of patients presenting with advanced or metastatic disease at the time of diagnosis.¹ In *de novo* metastatic GC, curative treatment is not feasible, and systemic therapy is the main therapeutic approach. Although the incorporation of combination

chemotherapy regimens and, more recently, targeted agents and immune checkpoint inhibitors has modestly improved survival outcomes, the prognosis for metastatic disease remains poor and varies considerably among patients.²⁻⁵ This clinical variability underscores the need for readily available, reproducible prognostic biomarkers that may facilitate risk stratification, guide treatment decisions, and support individualized decision-making in clinical practice.

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Cancer-associated systemic inflammation and nutritional impairment are increasingly recognized as key determinants of tumor progression and survival across malignancies.^{6,7} In GC, several inflammation- and nutrition-based biomarkers derived from routine laboratory parameters, such as C-reactive protein (CRP), serum albumin, neutrophil-to-lymphocyte ratio, modified Glasgow prognostic score, and CRP-to-albumin ratio (CAR), have been associated with overall survival (OS).⁸⁻¹⁰ Serum albumin reflects both nutritional status and systemic inflammatory activity, and reduced levels have consistently been associated with poor oncologic outcomes.^{11,12} Renal function parameters, including serum creatinine and estimated glomerular filtration rate (eGFR), have also been associated with unfavorable outcomes in various malignancies; however, data specifically addressing their prognostic role in metastatic GC remain limited.^{13,14} These observations suggest that biomarkers integrating inflammatory, nutritional, and metabolic dimensions may provide complementary prognostic information for advanced disease.

The albumin-to-creatinine ratio (ACR) integrates two routinely measured laboratory parameters that reflect complementary aspects of the host condition, including nutritional status and renal function. By combining these variables into a single index, the ACR may provide a concise and clinically practical summary of systemic status. In recent years, low ACR levels have been associated with inferior survival across common solid tumors and have also been reported as a prognostic indicator in specific clinical settings, such as liposarcoma and patients treated with immune checkpoint inhibitors.¹⁵⁻¹⁷ However, the prognostic significance of ACR has not yet been examined in GC. Therefore, we aimed to investigate the association between baseline serum ACR and survival outcomes in patients with *de novo* metastatic human epidermal growth factor receptor 2 (HER2)-negative GC and to assess its potential utility as an accessible prognostic biomarker in this population.

MATERIAL AND METHODS

Patient Population and Data Collection

This retrospective, single-center study included adult patients (≥ 18 years) with histologically confirmed, *de novo* metastatic HER2-negative GC diagnosed between April 2011 and January 2024. All eligible patients received first-line platinum- and fluoropyrimidine-based systemic therapy.

As part of the inclusion criteria, patients were required to have baseline laboratory measurements, including serum albumin and creatinine levels, obtained within 15 days prior to the initiation of systemic therapy. Patients were excluded

if baseline laboratory data required for ACR calculation were unavailable, if clinical or radiological follow-up data were insufficient, or if a synchronous malignancy other than GC was present. Patients with an eGFR < 60 mL/min/1.73 m² at treatment initiation were excluded to minimize the potential confounding effects of impaired renal function on ACR measurements. In addition, patients meeting the Kidney Disease: Improving Global Outcomes criteria for acute kidney injury at treatment initiation were excluded.¹⁸ eGFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation.¹⁹

Demographic and clinical variables, including age, sex, Eastern Cooperative Oncology Group (ECOG) performance status, tumor location, and metastatic sites, were obtained from medical records. Baseline laboratory parameters included serum albumin, creatinine, lactate dehydrogenase (LDH), carcinoembryonic antigen (CEA), and hemoglobin levels. The ACR was calculated as the ratio of serum albumin (g/dL) to serum creatinine (mg/dL). As no established cut-off value has been defined for ACR, patients were categorized into low- and high-ACR groups based on the cohort median.

Definition of Survival Outcomes

Progression-free survival (PFS) was defined as the time from the initiation of first-line systemic therapy to radiologically confirmed disease progression or death from any cause, whichever occurred first. Patients without documented disease progression or death were censored on the date of their last follow-up visit.

OS was defined as the time from the initiation of first-line systemic therapy to death from any cause. Patients who were alive at the last follow-up were censored on the date of their last visit.

Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki and approved by the İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Ethics Committee (approval number: 1064826; date: 14.08.2024). Given the retrospective nature of this study, the requirement for informed consent was waived.

Statistical Analysis

All statistical analyses were conducted using the IBM SPSS Statistics software, version 27.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed variables were expressed as mean $\pm$ standard deviation, whereas non-normally distributed variables were presented as median with interquartile range (IQR). Comparisons between groups

were performed using the independent samples t-test or the Mann-Whitney U test, as appropriate. Categorical variables were summarized as frequencies and percentages and were compared using the chi-square test or Fisher's exact test when necessary.

PFS and OS were estimated using the Kaplan-Meier method, and differences between groups were evaluated using the log-rank test. Univariate Cox proportional hazards regression analyses were performed to assess the association between baseline clinical and laboratory variables and the survival outcomes. Variables with a p-value <0.10 in the univariate analyses were included in the multivariate Cox regression models. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated for each outcome. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

A total of 112 patients with *de novo* metastatic HER2-negative GC were included in the analysis. The median baseline ACR was 49.6 (IQR: 41.3-58.4). Patients were categorized into low- and high-ACR groups based on the cohort median (n=56 in each group).

The median age of the entire cohort was 58 years (IQR: 51-67.8 years). The age distribution was comparable between the low and high ACR groups; however, the sex distribution differed significantly, with a higher proportion of female patients in the high ACR group (p<0.001).

All other baseline demographic, clinical, and laboratory variables, including ECOG performance status, body mass index, tumor-related characteristics, metastatic burden, and key laboratory parameters, were similarly distributed between the two groups, suggesting a largely homogeneous baseline profile. The detailed demographic, clinical, and laboratory characteristics of the study population are summarized in Table 1.

All patients received fluoropyrimidine- and platinum-based first-line chemotherapy. The distribution of treatment regimens according to the ACR groups is shown in Table 2.

Survival Outcomes According to ACR

The median follow-up duration for the entire cohort was 40.6 months (95% CI: 30.6-50.7). During the follow-up period, 108 (96.4%) patients experienced disease progression, and 102 (91.1%) died.

The median PFS of the entire cohort was 6.8 months (95% CI: 6.0-7.5). When participants were stratified by ACR group, the

median PFS was 6.0 months (95% CI: 4.8-7.3) in the low ACR group and 7.2 months (95% CI: 5.7-8.7) in the high ACR group. The difference between the two groups was statistically significant (p=0.005) (Figure 1).

In the univariate Cox regression analysis for PFS, ECOG PS (≥ 1 vs. 0) was associated with worse PFS (HR: 1.88; 95% CI: 1.19-2.97; p=0.007), whereas high ACR was associated with improved PFS (HR: 0.58; 95% CI: 0.39-0.86; p=0.006). Primary tumor location [stomach vs. gastroesophageal junction (GEJ)] showed a borderline association with worse PFS (p=0.064). In the multivariate model, ECOG PS (HR: 1.71; 95% CI: 1.07-2.75; p=0.026) and primary tumor location (HR: 1.64; 95% CI: 1.03-2.61; p=0.038) were independently associated with worse PFS, while high ACR remained independently associated with improved PFS (HR: 0.65; 95% CI: 0.44-0.97; p=0.034). The results of the univariate and multivariate Cox regression analyses are shown in Table 3.

The median OS for the entire cohort was 13.7 months (95% CI: 10.7-16.7). When stratified according to ACR groups, the median OS was 10.2 months (95% CI: 8.8-11.6) in the low ACR group and 16.9 months (95% CI: 15.6-18.3) in the high ACR group. The difference between the two groups was statistically significant (p<0.001) (Figure 2).

In the univariate Cox regression analyses, sex, ECOG PS, LDH status, and ACR were significantly associated with OS. Tumor location (stomach vs. GEJ) and CEA status showed a borderline association. In the multivariable model, ECOG PS ≥ 1 was independently associated with worse OS (HR: 2.14; 95% CI: 1.18-3.85; p=0.012), whereas high ACR remained independently associated with improved OS (HR: 0.38; 95% CI: 0.21-0.67; p<0.001). The other variables were not statistically significant. Detailed results of the univariate and multivariate Cox regression analyses for OS are presented in Table 4.

DISCUSSION

To our knowledge, this is the first study to evaluate the prognostic significance of the baseline serum ACR in patients with *de novo* metastatic HER2-negative GC. Our findings demonstrated that lower ACR levels were significantly associated with poorer PFS and OS, and that ACR remained an independent prognostic factor after adjustment for relevant clinical and laboratory variables. These results suggest that ACR, a composite marker reflecting baseline metabolic and nutritional status, may serve as a clinically meaningful prognostic indicator in advanced HER2-negative GC.

Albumin has a well-established role in the prognostic assessment of GC and constitutes a central component of several inflammation- and nutrition-based scores. Indices

TABLE 1: Baseline demographic, clinical, and laboratory characteristics according to ACR group.

	All patients (n=112)	Low ACR (n=56)	High ACR (n=56)	p-value
Age (years), median (IQR)	58.0 (51-67.8)	60.5 (54.3-69.0)	55.5 (47.0-66.8)	0.065
Age category, n (%)				0.308
<65 years	77 (68.8)	36 (64.3)	41 (73.2)	
≥65 years	35 (31.3)	20 (35.7)	15 (26.8)	
Gender, n (%)				<0.001
Female	35 (31.3)	7 (12.5)	28 (50)	
Male	77 (68.8)	49 (87.5)	28 (50)	
ECOG PS, n (%)				0.340
0	28 (25.0)	11 (19.6)	17 (30.4)	
1	78 (69.6)	41 (73.2)	37 (66.1)	
2	6 (5.4)	4 (7.1)	2 (3.6)	
BMI (kg/m ²) median (IQR)	23.5 (20.8-26.1)	23.4 (21.7-26.9)	23.5 (20.0-26.0)	0.427
Primary tumor location, n (%)				0.663
GEJ	28 (25.0)	13 (23.2)	15 (26.8)	
Stomach	84 (75.0)	43 (76.8)	41 (73.2)	
Lauren classification, n (%)				0.718
Diffuse	59 (52.7)	27 (48.2)	32 (57.1)	
Intestinal	26 (23.2)	13 (23.2)	13 (23.2)	
Unknown	27 (24.1)	16 (28.6)	11 (19.6)	
Signet ring cell component, n (%)				0.918
Absent	52 (46.4)	26 (46.4)	26 (46.4)	
Present	49 (43.8)	24 (42.9)	25 (44.6)	
Unknown	11 (9.8)	6 (10.7)	5 (8.9)	
Number of metastatic sites, n (%)				1.000
≤2 sites	72 (64.3)	36 (64.3)	36 (64.3)	
>2 sites	40 (35.7)	20 (35.7)	20 (35.7)	
Metastatic sites, n (%)				0.331
Liver metastasis	43 (38.4)	24 (42.9)	19 (33.9)	
Lung metastasis	15 (13.4)	7 (12.5)	8 (14.3)	
Peritoneal metastasis	47 (42.0)	25 (44.6)	22 (39.3)	
CEA status				0.648
≤ULN	58 (51.8)	27 (48.2)	31 (55.3)	
>ULN	39 (34.8)	20 (35.7)	19 (33.9)	
Unknown	15 (13.4)	9 (16.1)	6 (10.7)	
LDH (U/L), median (IQR)	192 (154-272)	188.5 (152.5-257)	194 (154-305)	0.596
Hemoglobin (g/dL), mean ± SD	11.4±2.1	11.7±2.1	11.1±2.1	0.115
Albumin (g/dL), mean ± SD	3.90±0.50	3.86±0.48	3.93±0.52	0.464
Creatinine (mg/dL), mean ± SD	0.80±0.19	0.95±0.11	0.65±0.13	<0.001

ACR: Albumin-to-creatinine ratio; BMI: Body mass index; CEA: Carcinoembryonic antigen; ECOG: Eastern Cooperative Oncology Group; GEJ: Gastroesophageal junction; IQR: Interquartile range; LDH: Lactate dehydrogenase; SD: Standard deviation; ULN: Upper limit of normal.

TABLE 2: First-line treatment characteristics according to ACR group.

Treatment regimen	All patients (n=112)	Low ACR (n=56)	High ACR (n=56)
Oxaliplatin-based chemotherapy, n (%)	77 (68.8)	40 (71.4)	37 (66.1)
FOLFOX	67 (59.8)	35 (62.5)	32 (57.1)
CAPOX	8 (7.1)	4 (7.1)	4 (7.1)
FOLFOX + pembrolizumab	2 (1.8)	1 (1.8)	1 (1.8)
Cisplatin-based chemotherapy, n (%)	35 (31.2)	16 (28.6)	19 (33.9)
Cisplatin + 5-FU	24 (21.4)	13 (23.2)	11 (19.6)
DCF	5 (4.5)	1 (1.8)	4 (7.1)
Cisplatin + 5-FU + pembrolizumab	6 (5.4)	2 (3.6)	4 (7.1)

ACR: Albumin-to-creatinine ratio; FOLFOX: Fluorouracil, leucovorin, and oxaliplatin; CAPOX: Capecitabine and oxaliplatin; DCF: Docetaxel, cisplatin, and fluorouracil; 5-FU: 5-fluorouracil.

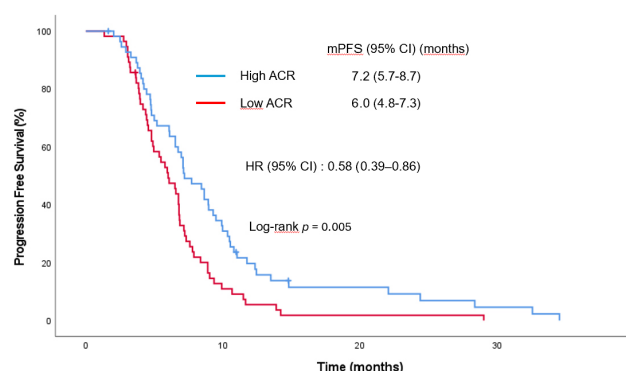


FIGURE 1: Kaplan-Meier curve for progression-free survival according to the ACR group in patients with *de novo* metastatic HER2-negative gastric cancer.

ACR: Albumin-to-creatinine ratio; CI: Confidence interval; HER2: Human epidermal growth factor receptor 2; PFS: Progression-free survival; HR: Hazard ratio

such as the CAR and the Prognostic Nutritional Index have been shown to correlate with survival outcomes in both early-stage and metastatic settings, underscoring the role of systemic inflammatory burden and nutritional reserve in disease progression.^{10,11,20} However, these scores primarily focus on inflammatory and immune-related parameters.

In contrast, creatinine has received limited attention as a prognostic marker in metastatic GC, and data directly evaluating its association with survival in this setting remain scarce. Tanaka et al.¹³ demonstrated in a surgically treated cohort of patients with locally advanced disease that preoperative eGFR was associated with postoperative complications and clinical outcomes. Although this population differs from *de novo* metastatic patients, the findings suggest that renal and metabolic reserves may influence oncologic outcomes.

In the metastatic setting, where cachexia and progressive muscle loss are common, parameters incorporating creatinine may capture an additional dimension of physiological

TABLE 3: Univariate and multivariate Cox regression analyses for progression-free survival.

Variable	Univariate analyses		Multivariate analyses	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (per 1-year increase)	1.01 (0.99-1.02)	0.252	1.71 (1.07-2.75)	0.026
Sex (male vs. female)	1.33 (0.87-2.02)	0.184		
ECOG PS (≥ 1 vs. 0)	1.88 (1.19-2.97)	0.007		
BMI (per 1 kg/m ² increase)	1.00 (0.95-1.04)	0.911	1.64 (1.03-2.61)	0.038
Tumor location (stomach vs. GEJ)	1.55 (0.98-2.45)	0.064		
Liver metastasis (present vs. absent)	1.20 (0.81-1.78)	0.365		
Lung metastasis (present vs. absent)	1.58 (0.91-2.75)	0.106	1.39 (0.79-2.45)	0.261
Peritoneal metastasis (present vs. absent)	0.79 (0.54-1.17)	0.244		
CEA status ($>ULN$ vs. $\leq ULN$)	1.15 (0.76-1.75)	0.512		
LDH status ($>ULN$ vs. $\leq ULN$)	1.35 (0.87-2.11)	0.187	0.65 (0.44-0.97)	0.034
ACR group (high vs. low)	0.58 (0.39-0.86)	0.006		

ACR: Albumin-to-creatinine ratio; BMI: Body mass index; CEA: Carcinoembryonic antigen; CI: Confidence interval; ECOG PS: Eastern Cooperative Oncology Group performance status; GEJ: Gastroesophageal junction; HR: Hazard ratio; LDH: Lactate dehydrogenase; ULN: Upper limit of normal.

vulnerability that is not reflected by inflammation-based markers.

Against this background, integrating albumin and creatinine into a composite index such as ACR may provide a broader assessment of host-related health status in advanced disease. The prognostic value of ACR has recently been explored in broader oncological settings. Zhao et al.¹⁵ demonstrated that lower ACR levels were independently associated with inferior OS in a large cohort of patients with solid tumors, including gastrointestinal malignancies. In a disease-specific analysis, Panotopoulos et al.¹⁶ reported that alterations in albumin and creatinine levels were associated with poor disease-specific survival in patients with liposarcoma. Similarly, Bas et al.¹⁷

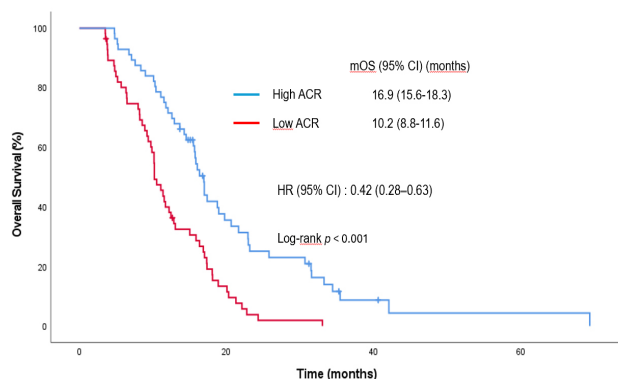


FIGURE 2: Kaplan-Meier curve for overall survival according to the ACR group in patients with *de novo* metastatic HER2-negative gastric cancer.

ACR: Albumin-to-creatinine ratio; CI: Confidence interval; HER2: Human epidermal growth factor receptor 2; OS: Overall survival; HR: Hazard ratio

showed that a lower serum ACR independently predicted shorter progression-free and OS in patients treated with immune checkpoint inhibitors.

Despite these findings, the ACR has not been previously examined in GC. By evaluating a homogeneous cohort of patients with *de novo* metastatic HER2-negative disease, our study adds disease-specific evidence to the existing literature. Notably, the association between ACR and OS appeared more pronounced than the association observed for PFS, which may suggest that ACR captures host-related determinants of long-term outcomes beyond early tumor response. This observation warrants further investigation in prospective studies.

In our cohort, the high ACR group included a higher proportion of female patients. However, sex did not retain independent prognostic significance in the multivariable analysis, suggesting that the survival differences associated with ACR were unlikely to be solely attributable to differences in sex distribution. Previous studies on metastatic GC have reported inconsistent findings regarding the prognostic impact of sex, and its role remains uncertain.^{21,22}

The prognostic significance of the primary tumor location in GC remains debatable. In a meta-analysis of non-metastatic GC, Petrelli et al.²³ reported that tumors located in the upper third of the stomach were associated with worse survival than more distal tumors. In contrast, Nakayama et al.²⁴ did not observe a significant difference in survival according to tumor localization among patients with advanced GC.

In our cohort, tumor localization was associated with PFS, whereas its association with OS was not statistically significant after adjusting for other clinical variables. These

TABLE 4: Univariate and multivariate Cox regression analyses for overall survival.

Variable	Univariate analyses		Multivariate analyses	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (per 1-year increase)	1.01 (0.99-1.02)	0.207		
Sex (male vs. female)	1.87 (1.19-2.95)	0.007	0.98 (0.53-1.82)	0.945
ECOG PS (≥ 1 vs. 0)	2.18 (1.33-3.55)	0.002	2.14 (1.18-3.85)	0.012
BMI (per 1 kg/m ² increase)	1.02 (0.97-1.08)	0.358		
Tumor location (stomach vs. GEJ)	1.56 (0.98-2.48)	0.062	1.53 (0.92-2.52)	0.099
Liver metastasis (present vs. absent)	1.27 (0.85-1.89)	0.247		
Lung metastasis (present vs. absent)	1.32 (0.74-2.33)	0.347		
Peritoneal metastasis (present vs. absent)	0.85 (0.56-1.27)	0.419		
CEA status (>ULN vs. $\leq$ ULN)	1.53 (0.99-2.36)	0.054	1.52 (0.96-2.41)	0.074
LDH status (>ULN vs. $\leq$ ULN)	1.61 (1.02-2.53)	0.041	1.67 (0.97-2.87)	0.065
ACR group (high vs. low)	0.42 (0.28-0.63)	<0.001	0.38 (0.21-0.67)	<0.001

ACR: Albumin-to-creatinine ratio; BMI: Body mass index; CEA: Carcinoembryonic antigen; CI: Confidence interval; ECOG PS: Eastern Cooperative Oncology Group performance status; GEJ: Gastroesophageal junction; HR: Hazard ratio; LDH: Lactate dehydrogenase; ULN: Upper limit of normal.

findings suggest that the prognostic relevance of the anatomical subsite in metastatic GC is inconsistent across endpoints and may be modified by concomitant clinical and pathological characteristics. Therefore, tumor location alone may provide limited prognostic information when evaluated independently of other established risk factors.

Several aspects of this study merit further consideration. First, we evaluated a relatively homogeneous cohort of patients with *de novo* metastatic HER2-negative GC who all received first-line platinum- and fluoropyrimidine-based chemotherapy. This minimized treatment-related heterogeneity and allowed a more focused assessment of the baseline prognostic factors. Second, patients with impaired renal function (eGFR <60 mL/min/1.73 m²) were excluded to reduce potential confounding in the interpretation of creatinine-based indices. Multivariate analyses were performed to account for established clinical and laboratory variables, thereby supporting the robustness of the association between ACR and survival outcomes.

Study Limitations

Nevertheless, several limitations of this study should be acknowledged. The retrospective, single-center design may limit the generalizability of the findings and introduce inherent selection bias. Although adequate for exploratory purposes, the sample size was modest, particularly for subgroup analyses. ACR was dichotomized according to the cohort median because no validated cutoff value currently exists. Therefore, external validation in independent populations is required. Furthermore, the ACR was assessed only at baseline, prior to treatment initiation. Temporal changes in ACR during therapy and their potential association with treatment response or survival have not been evaluated, and the prognostic implications of longitudinal ACR dynamics remain to be clarified in prospective studies. In addition, the use of immune checkpoint inhibitors in this cohort was limited, which may restrict the applicability of these findings to contemporary immunotherapy-based treatment settings. Furthermore, programmed death-ligand 1 (PD-L1) combined positive score data were not systematically available in this cohort and were therefore not included in the analysis. Accordingly, we were unable to assess whether the prognostic impact of ACR was independent of PD-L1 status.

CONCLUSION

Baseline serum ACR was independently associated with survival outcomes in patients with *de novo* metastatic HER2-negative GC. As a simple and routinely available laboratory parameter, ACR may contribute to risk stratification in advanced disease. Further prospective studies are needed

to validate these findings and determine whether dynamic assessment of ACR during treatment provides additional prognostic value.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the Declaration of Helsinki and approved by the İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Ethics Committee (approval number: 1064826; date: 14.08.2024).

Informed Consent: Retrospective study.

Footnotes

Author Contributions

Surgical and Medical Practices: M.G., E.Ç., S.C., S.J., S.A., Ö.A., Concept: M.G., N.S.D., Ö.A., Design: M.G., Z.B., N.S.D., Ö.A., Data Collection or Processing: M.G., Z.B., V.A., H.A., S.C., S.J., E.Ç., Analysis or Interpretation: M.G., M.Gu., Literature Search: M.G., M.Gu., Writing: M.G., N.S.D., Ö.A.

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The Effect of Adjuvant Treatment Preferences on Survival in Elderly Patients (≥ 65 Years) with Early-stage Breast Cancer

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ABSTRACT

Objective: The optimal management of early-stage breast cancer in elderly patients remains challenging due to age-related comorbidities, limited life expectancy, and underrepresentation in clinical trials. This study aimed to evaluate the impact of adjuvant treatment preferences on survival outcomes in women aged 65 years and older with early-stage, node-negative, hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer.

Material and Methods: In this retrospective cohort study, 157 patients aged ≥ 65 years who underwent surgery between 2003 and 2022 were analyzed. Patients were categorized into two groups according to adjuvant treatment strategy: chemotherapy (CT) followed by hormonal therapy (HT), or HT alone. Clinicopathological characteristics, comorbidities, relapse-free survival (RFS), and overall survival (OS) were compared between groups. Survival outcomes were assessed using Kaplan-Meier analysis, and prognostic factors were evaluated using univariate and multivariate Cox proportional hazards regression models, including clinically relevant variables.

Results: Of the 157 patients, 49 (31.2%) received CT followed by HT, and 108 (68.8%) received HT alone. Patients treated with HT alone were significantly older and had smaller tumors and earlier-stage disease. Despite these favorable tumor characteristics, OS was significantly lower in the HT group compared with the CT group ($p=0.003$). No significant difference in RFS was observed between the groups ($p=0.782$). In multivariate analysis of OS, age [hazard ratio (HR): 1.17; 95% CI: 1.08-1.26; $p<0.001$] and multicentricity (HR: 6.51; 95% CI: 2.16-19.64; $p=0.001$) were independently associated with worse survival, while the treatment group was not independently associated with survival after adjustment. In multivariate analysis of RFS, age (HR: 1.24; 95% CI: 1.10-1.39; $p<0.001$) and the Charlson comorbidity score (HR: 2.07; 95% CI: 1.04-4.14; $p=0.038$) were independently associated with recurrence.

Conclusion: In elderly patients with early-stage hormone receptor-positive breast cancer, survival outcomes appear to be influenced more strongly by age and comorbidity burden than by treatment choice alone. While HT is frequently preferred, adjuvant CT may still be considered in carefully selected patients. A personalized, multidisciplinary approach is essential to optimize treatment decisions in this population.

Keywords: Breast cancer; aged; adjuvant chemotherapy; hormone therapy; comorbidity; survival

INTRODUCTION

Breast cancer (BC) is the most frequently diagnosed malignancy among women, and its incidence increases steadily with advancing age, particularly in countries with aging populations.¹ Despite this growing burden, elderly patients remain markedly underrepresented in clinical trials, resulting in limited high-level evidence to guide treatment decisions in this population.² Consequently, management

strategies for older patients often rely on retrospective data and clinical judgment rather than standardized, evidence-based recommendations.

BC in elderly women is commonly characterized by more favorable biological features, including higher hormone receptor positivity, lower histological grade, and reduced proliferative activity.^{3,4} While these characteristics are generally associated with better oncologic outcomes, their prognostic advantage may be offset by age-related factors such as comorbid conditions,

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reduced physiological reserve, and decreased tolerance to systemic therapies.^{5,6} As a result, overall survival (OS) in older patients is frequently influenced not only by cancer-related factors but also by competing causes of mortality.

Treatment decision-making for older patients with BC is complex and extends beyond tumor biology. Factors such as life expectancy, functional status, comorbidity burden, and patient preference play a central role in selecting optimal therapy.^{6,7} Although surgery remains the cornerstone of curative treatment in early-stage disease, adjuvant systemic therapy is often de-escalated in older patients. Endocrine therapy is frequently preferred due to its favorable toxicity profile, particularly in patients with hormone receptor-positive tumors and significant comorbidities.^{8,9} However, reliance on endocrine therapy alone—especially in patients who are otherwise fit—may raise concerns regarding potential undertreatment. However, these patients have been shown to experience higher rates of regional recurrence, often necessitating delayed surgical interventions later in life.^{10,11}

Radiotherapy and breast-conserving surgery (BCS) are also less frequently used among older adults, even when clinically indicated. Previous randomized trials have demonstrated that radiotherapy can be safely omitted in selected low-risk older patients following BCS without compromising OS.^{12,13} Nevertheless, treatment omission outside well-defined low-risk groups may contribute to suboptimal outcomes.

Adjuvant chemotherapy (CT) is particularly underutilized in elderly patients across all disease stages. Population-based studies have consistently shown a sharp decline in CT use with increasing age, independent of tumor stage or risk profile.^{14,15} This trend may reflect concerns regarding toxicity, comorbidities, and limited life expectancy, but also result in the exclusion of potentially beneficial therapy for carefully selected, fit older patients.

Given these challenges, there remains a need to better understand the impact of adjuvant treatment choices on survival outcomes in elderly patients with biologically favorable BC. This study aimed to evaluate the association between adjuvant treatment strategies—CT followed by hormonal therapy (HT) versus HT alone—and survival outcomes in women aged 65 years or older with early-stage, node-negative, hormone receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative BC.

MATERIAL AND METHODS

Study Design and Patient Selection

This retrospective cohort study included women aged 65 years or older who were diagnosed with early-stage, node-

negative, hormone receptor-positive, HER2-negative BC and who underwent primary surgical treatment between 2003 and 2022. Patients were identified through institutional medical records.

Patients with lymph node-positive disease were excluded, as adjuvant CT is routinely recommended in this group. In addition, patients who received neoadjuvant CT (NAC) were excluded to allow accurate evaluation of preferences for adjuvant treatment. After applying these criteria, a total of 157 patients were included in the final analysis.

Ethics committee approval was obtained from the University of Health Sciences Türkiye, Antalya Training and Research Hospital Scientific Research Ethics Committee (approval number: 8/3, date: 08.05.2025).

Treatment Groups

Patients were categorized into two groups according to the adjuvant systemic treatment received following surgery:

1. patients who received CT followed by HT, and
2. patients who received HT alone.

The choice of adjuvant treatment was based on tumor characteristics, patient age, comorbidities, and clinician judgment at the time of treatment.

Data Collection and Variables

Clinical and pathological data were collected retrospectively and included age, tumor size, pathological stage, histological subtype, tumor grade, Ki-67 proliferation index, E-cadherin status, tumor localization, multicentricity, type of surgery, receipt of radiotherapy, and adjuvant systemic therapy.

Comorbidities were assessed using the Charlson comorbidity index; individual comorbid conditions such as diabetes mellitus (DM), hypertension, coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), and cerebrovascular disease (CVD) were recorded.

Outcome Measures

The primary outcome of the study was OS, defined as the time from the date of surgery to death from any cause or to the last follow-up. The secondary outcome was relapse-free survival (RFS), defined as the time from surgery to the first documented local, regional, or distant recurrence or last follow-up.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as medians with interquartile ranges, and categorical variables were presented

as frequencies and percentages. The Mann-Whitney U test was used to compare continuous variables between treatment groups, while categorical variables were compared using the Pearson's chi-square test or Fisher's exact test, as appropriate.

Survival curves for OS and RFS were estimated using the Kaplan-Meier method and compared with the log-rank test.

Univariate Cox proportional hazards regression analyses were performed to identify factors associated with OS and RFS.

For OS, two multivariate Cox regression models were constructed. In Model 1, variables with a p-value <0.05 in the univariate analysis were included. In Model 2, clinically relevant variables were included irrespective of univariate significance to better account for potential confounding.

For RFS, a multivariate Cox regression model was constructed, including clinically relevant variables irrespective of univariate significance, in line with the approach used for Model 2 in the OS analysis.

Given the limited number of events, the multivariate models may be prone to overfitting, and the results should be interpreted with caution.

A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 157 patients aged 65 years and older who underwent surgery without prior NAC were included in the analysis. Among them, 49 patients (31.2%) received adjuvant CT followed by HT, while 108 patients (68.8%) received HT alone.

The median age was significantly higher in the HT group than in the CT group (82 vs. 73 years; $p<0.001$). Tumor size was significantly larger in patients who received CT (median 2.5 cm vs. 1.9 cm; $p=0.001$). Regarding stage distribution, stage IA disease was more common in the HT group (61.1%), whereas stage IIA disease was more common in the CT group (59.2%; $p=0.004$). Baseline clinicopathological characteristics are summarized in Table 1.

TABLE 1: Baseline clinicopathological characteristics of the study population.

	Total (n=157)	Chemotherapy (n=49)	Hormonal therapy (n=108)	p-value
Age, years, median	80.0 (10.0)	73.0 (12.0)	82.0 (11.8)	<0.001 ^a
65-70	13 (8.3)	13 (26.5)	0 (0.0)	<0.001 ^b
≥70	144 (91.7)	36 (73.5)	108 (100.0)	
Stage, n (%)				
1A	84 (53.5)	18 (36.7)	66 (61.1)	0.004 ^b
2A	62 (39.5)	29 (59.2)	33 (30.6)	
2B	6 (3.8)	2 (4.1)	4 (3.7)	
3B	5 (3.2)	0 (0)	5 (4.6)	
Tumor size (median)	2.0 (1.3)	2.5 (1.4)	1.9 (1.0)	0.001 ^a
Grade, n (%)				
1	25 (18.0)	8 (17)	17 (18.5)	0.150 ^c
2	100 (71.9)	31 (66)	69 (75)	
3	14 (10.1)	8 (17)	6 (6.5)	
E-cadherin, n (%)				
Negative	14 (21.5)	5 (21.7)	9 (21.4)	1.000 ^b
Positive	51 (78.5)	18 (78.3)	33 (78.6)	
Histology, n (%)				
Invasive ductal carcinoma	108 (68.7)	28 (57)	80 (74)	0.885 ^c
Invasive lobular carcinoma	19 (12.1)	5 (10.2)	14 (13)	
Others	30 (19.1)	16 (32.6)	14 (12.9)	
Ki-67, n (%)				
<20%	79 (61.2)	24 (54.5)	55 (64.7)	0.261 ^c
≥20%	50 (31.8)	20 (45.5)	30 (35.3)	

TABLE 1: Continued.

	Total (n=157)	Chemotherapy (n=49)	Hormonal therapy (n=108)	p-value
Tumor localization, n (%)				
Upper outer quadrant	66 (42.0)	19 (38.8)	47 (43.5)	0.378 ^b
Upper inner quadrant	44 (28.0)	11 (22.4)	33 (30.6)	
Lower outer quadrant	32 (20.4)	14 (28.6)	18 (16.7)	
Lower inner quadrant	13 (8.3)	5 (10.2)	8 (7.4)	
Retroareolar	2 (1.3)	0 (0)	2 (1.9)	
Multicentricity, n (%)				
No	141 (89.8)	43 (87.8)	98 (90.7)	0.577 ^b
Yes	16 (10.2)	6 (12.2)	10 (9.3)	
Surgery, n (%)				
BCS	55 (37.9)	18 (37.5)	37 (38.1)	0.940 ^c
MRM	90 (62.1)	30 (62.5)	60 (61.9)	
Radiotherapy, n (%)				
No	107 (68.2)	25 (51)	82 (75.9)	0.002 ^c
Yes	50 (31.8)	24 (49)	26 (24.1)	
Charlson comorbidity score		5.0 (2.0)	5.0 (2.0)	0.980 ^a
DM, n (%)				
No	98 (62.4)	31 (63.3)	67 (62)	0.883 ^c
Yes	59 (37.6)	18 (36.7)	41 (38)	
HT, n (%)				
No	75 (47.8)	23 (46.9)	52 (48.1)	0.888 ^c
Yes	82 (52.2)	26 (53.1)	56 (51.9)	
CVD, n (%)				
No	155 (98.7)	48 (98)	107 (99.1)	0.528 ^b
Yes	2 (1.3)	1 (2)	1 (0.9)	
CAD, n (%)				
No	136 (86.6)	47 (95.9)	89 (82.4)	0.021 ^c
Yes	21 (13.4)	2 (4.1)	19 (17.6)	
Hyperlipidemia, n (%)				
No	152 (96.8)	48 (98)	104 (96.3)	1.000 ^b
Yes	5 (3.2)	1 (2)	4 (3.7)	
COPD, n (%)				
No	141 (89.8)	41 (83.7)	100 (92.6)	0.096 ^b
Yes	16 (10.2)	8 (16.3)	8 (7.4)	
Hormonal therapy, n (%)				
Anastrozol	107 (70.4)	28 (63.6)	79 (73.1)	0.105 ^b
Letrozol	40 (26.3)	15 (34.1)	25 (23.1)	
Exemestan	1 (0.7)	1 (2.3)	0 (0)	
Tamoxifen	4 (2.6)	0 (0)	4 (3.7)	
Relapse, n (%)				
No	144 (91.7)	46 (93.9)	98 (90.7)	0.756 ^b
Yes	13 (8.3)	3 (6.1)	10 (9.3)	

TABLE 1: Continued.

TABLE 1. Continued.

	Total (n=157)	Chemotherapy (n=49)	Hormonal therapy (n=108)	p-value
Mortality, n (%)				
Alive	123 (79.4)	46 (95.8)	77 (72)	0.001 ^c
Dead	32 (20.6)	2 (4.2)	30 (28)	
Chemotherapy				
CA	14 (29.2)			
Weekly paclitaxel	3 (6.3)			
TC	15 (31.3)			
CA+T	8 (16.7)			
EC	4 (8.3)			
CEF+T	3 (6.3)			
Carboplatin+T	1 (2.1)			

^a: Mann-Whitney U test, ^b: Fisher's Exact test, ^c: Pearson chi-square test. BCS: Breast-conserving surgery; MRM: Modified radical mastectomy; DM: Diabetes mellitus; HT: Hypertension; COPD: Chronic obstructive pulmonary disease; CVD: Cerebrovascular disease; CAD: Coronary artery disease; CA: Doxorubicin plus cyclophosphamide; T: Paclitaxel; TC: Docetaxel plus cyclophosphamide; EC: Epirubicin plus cyclophosphamide; CEF: Cyclophosphamide, epirubicin, and 5-fluorouracil.

Tumor grade and Ki-67 proliferation index did not differ significantly between treatment groups, indicating comparable tumor biology across cohorts (Table 1). No significant differences were observed in histological subtype, E-cadherin status, tumor localization, multicentricity, or surgical approach.

Treatment Characteristics

Modified radical mastectomy (MRM) was performed in 62.1% of patients, while BCS was performed in 37.9%. Adjuvant radiotherapy was administered significantly more frequently in the CT group than in the HT group (49.0% vs. 24.1%; $p=0.002$).

Among patients receiving CT, the most commonly used regimens were docetaxel plus cyclophosphamide (TC) (31.3%) and doxorubicin plus cyclophosphamide (29.2%), followed by other anthracycline- or taxane-based combinations.

None of the patients in this cohort underwent genomic risk assessment using assays such as Oncotype DX or MammaPrint.

Comorbidities

Hypertension (52.2%) and DM (37.6%) were the most frequently observed comorbidities in the overall cohort. CAD was significantly more prevalent in the HT group than in the CT group (17.6% vs. 4.1%; $p=0.021$). Other comorbid conditions, including COPD, CVD, and hyperlipidemia, were similarly distributed between the groups. Despite differences in age and comorbidity profiles, the median Charlson comorbidity score was 5 in both treatment groups ($p=0.980$) (Table 1).

Survival Outcomes

During follow-up, disease relapse occurred in 13 patients (8.3%); there was no statistically significant difference in

relapse rates between the CT and HT groups (6.1% vs. 9.3%; $p=0.756$). A total of 32 patients (20.6%) died during the study period. Mortality was significantly higher in the HT group compared with the CT group (28.0% vs. 4.2%; $p=0.001$) (Table 1).

Kaplan-Meier analysis demonstrated a median OS of 172.3 months [95% confidence interval (CI): not reached (NR)] for the entire cohort. The 2 years and 5 years OS rates were 92.7% and 80.1%, respectively. OS was significantly longer in the CT group than in the HT group (log-rank $p=0.003$). Median OS was NR in the CT group, whereas in the HT group it was 172.3 months (95% CI: 81.87-262.72) (Table 2).

The median RFS was NR in both treatment groups. The 2 years and 5 years RFS rates for the entire cohort were 98% and 90%, respectively. No significant difference in RFS was observed between the treatment group arms (log-rank $p=0.782$) (Table 2).

Prognostic Factors

In univariate Cox regression analysis for OS, tumor stage, tumor size, and multicentricity were significantly associated with an increased risk of death. Stage IIIB disease was associated with the highest mortality risk [hazard ratio (HR): 9.52; 95% CI: 2.64-34.28; $p=0.001$], and multicentric disease was also associated with poorer OS (HR: 2.70; 95% CI: 1.01-7.20; $p=0.047$). Tumor grade, histology, Ki-67 proliferation index, type of surgery, receipt of radiotherapy, and Charlson comorbidity score were not significantly associated with OS (Table 3).

Two multivariate Cox regression models were constructed for OS. In Model 1, which included variables selected based on univariate analysis, multicentricity emerged as an

independent predictor of mortality (HR: 2.99; 95% CI: 1.10-8.09; $p=0.031$) (Table 4).

To better account for potential confounding, Model 2 included clinically relevant variables irrespective of univariate significance. These variables included treatment group, age, tumor size, stage, radiotherapy, Charlson comorbidity score, CAD, and multicentricity.

TABLE 2: Overall survival and relapse-free survival outcomes.		
OS (months)	Median (95% CI)	p-value
Overall	172.30 (NR)	
Treatment		
Chemotherapy	-	0.003
Hormonal therapy	172.30 (81.87-262.72)	
RFS (months)	Median(95% CI)	p-value
Overall	-	
Treatment		
Chemotherapy	-	0.782
Hormonal therapy	-	

Survival analyses were performed using the Kaplan-Meier method, and survival curves were compared using the log-rank test. A p-value <0.05 was considered statistically significant. CI: Confidence interval; OS: Overall survival; RFS: Relapse-free survival.

TABLE 3: Univariate Cox regression analysis for overall survival.		
OS	Univariate	
	HR (95% CI)	p-value
Stage (ref: 1A)		0.007
2A	1.31 (0.61-2.79)	0.482
2B	2.08 (0.27-15.97)	0.481
3B	9.52 (2.64-34.28)	0.001
Tumor size	1.29 (1.04-1.59)	0.018
Grade (ref: 1)		0.240
2	0.67 (0.27-1.67)	0.398
3	1.63 (0.46-5.71)	0.445
E-cadherin (ref: negative)	0.50 (0.12-2.02)	0.334
Histology (ref: others)		0.774
Invasive ductal carcinoma	0.77 (0.35-1.68)	0.515
Invasive lobular carcinoma	1.05 (0.34-3.17)	0.931
Ki-67 (ref: <20%)	1.57 (0.66-3.72)	0.297
Multicentricity (ref: no)	2.70 (1.01-7.20)	0.047
Surgery (ref: BCS)	0.87 (0.39-1.93)	0.744
Radiotherapy (ref: no)	0.50 (0.20-1.22)	0.130
Charlson comorbidity score	1.4 (0.90-1.70)	0.182
p<0.001, -loglikelihood=260.45		
HR: Hazard ratio; CI: Confidence interval; BCS: Breast-conserving surgery; OS: Overall survival.		

In Model 2, age (HR: 1.17; 95% CI: 1.08-1.26; $p<0.001$) and multicentricity (HR: 6.51; 95% CI: 2.16-19.64; $p=0.001$) were independently associated with worse OS. The treatment showed a trend toward improved survival with CT, but this did not reach statistical significance after adjustment (HR: 3.61; 95% CI: 0.79-16.45; $p=0.097$). Tumor size, stage, radiotherapy, Charlson comorbidity score, and CAD were not independently associated with OS (Table 5).

The results of Model 2 were considered the primary findings of the study. Given the limited number of events, these findings should be interpreted with caution due to the risk of overfitting.

For RFS, univariate analysis did not identify any clinical or pathological variables significantly associated with recurrence (Table 6).

A multivariate Cox regression model for RFS was subsequently fitted, including clinically relevant variables regardless of univariate significance. In this model, age (HR: 1.24; 95% CI: 1.10-1.39; $p<0.001$) and Charlson comorbidity score (HR: 2.07; 95% CI: 1.04-4.14; $p=0.038$) were independently associated with worse RFS. Treatment group and tumor-related variables were not significantly associated with recurrence (Table 7).

Given the limited number of relapse events ($n=13$), these findings should be interpreted with caution.

DISCUSSION

The incidence of BC continues to rise with advancing age, and management of older adults remains challenging due to age-related comorbidities, competing mortality risks, and limited representation in clinical trials. In this retrospective study, we evaluated the impact of adjuvant treatment strategies on survival outcomes in women aged 65 years and older with early-stage, node-negative, hormone receptor-positive, HER2-negative BC.

Our findings demonstrate a clear preference for endocrine therapy alone in the elderly population, consistent with previous reports showing declining use of adjuvant CT with increasing age.^{14,15} Patients treated with HT alone were older and had smaller tumors and earlier-stage disease, reflecting a tendency to avoid CT in those perceived to have lower oncologic risk. Tumor grade and Ki-67 proliferation index were comparable between treatment groups, suggesting that treatment selection was influenced predominantly by age and comorbidity considerations rather than intrinsic tumor biology.

Despite more favorable clinicopathological characteristics, the HT group had significantly lower OS. This observation should be interpreted with caution, as it likely reflects differences in

TABLE 4: Multivariate Cox regression analysis for overall survival (Model 1: univariate-based model).

OS	Multivariate	p-value
	HR (95% CI)	
Stage (ref: 1A)		0.035
2A	0.85 (0.32-2.12)	0.744
2B	0.36 (0.01-7.45)	0.509
3B	5.27 (0.92-30.07)	0.061
Tumor size	1.35 (0.91-2.01)	0.130
Multicentricity (ref: no)	2.99 (1.10-8.09)	0.031

HR: Hazard ratio; CI: Confidence interval; OS: Overall survival.

TABLE 5: Multivariate Cox regression analysis for overall survival (Model 2: fully adjusted model).

OS	Multivariate	p-value
	HR (95% CI)	
Hormonal therapy (ref: chemotherapy)	3.61 (0.79-16.45)	0.097
Age	1.17 (1.08-1.26)	<0.001
Tumor size	1.13 (0.81-1.57)	0.470
Radiotherapy (ref: no)	1.13 (0.42-3.03)	0.806
Charlson comorbidity score	1.13 (0.76-1.70)	0.548
CAD	1.91 (0.67-5.42)	0.226
Multicentricity (ref: no)	6.51 (2.16-19.64)	0.001
Stage	1.09 (0.58-2.03)	0.797

HR: Hazard ratio; CI: Confidence interval; CAD: Coronary artery disease; OS: Overall survival.

TABLE 6: Univariate Cox regression analysis for relapse-free survival.

RFS	Univariate	p-value
	HR (95% CI)	
Stage (ref: 1A)		0.306
2A	1.82 (0.55-5.97)	0.322
2B	4.67 (0.53-40.60)	0.162
3B	4.20 (0.48-36.35)	0.192
Tumor size	1.21 (0.91-1.62)	0.178
Grade (ref: 1)		0.055
2	0.29 (0.06-1.33)	0.114
3	2.04 (0.41-10.23)	0.382
E-cadherin (ref: negative)	29.18 (0.00-)	0.649
Histology (ref: others)		0.739
Invasive ductal carcinoma	1.54 (0.51-4.66)	0.437
Invasive lobular carcinoma	-	0.981
Ki-67 (ref: <20%)	2.81 (0.79-10.01)	0.109
Multicentricity (ref: no)	0.82 (0.11-6.36)	0.855
Surgery (ref: BCS)	0.80 (0.22-2.85)	0.735
RT (ref: no)	0.68 (0.18-2.47)	0.559
Charlson comorbidity score	1.63 (0.97-2.73)	0.064

HR: Hazard ratio; CI: Confidence interval; BCS: Breast-conserving surgery; RT: Radiotherapy; RFS: Relapse-free survival.

TABLE 7: Multivariate Cox regression analysis for relapse-free survival.

RFS	Multivariate	p-value
	HR (95% CI)	
Hormonal therapy (ref: chemotherapy)	0.79 (0.20-3.13)	0.733
Age	1.24 (1.10-1.39)	<0.001
Tumor size	1.16 (0.71-1.91)	0.550
Radiotherapy (ref: no)	0.84 (0.21-3.34)	0.800
Charlson comorbidity score	2.07 (1.04-4.14)	0.038
CAD	0.51 (0.09-2.85)	0.441
Multicentricity (ref: no)	3.10 (0.33-29.11)	0.322
Stage	1.04 (0.42-2.60)	0.927

HR: Hazard ratio; CI: Confidence interval; RFS: Relapse-free survival; CAD: Coronary artery disease.

baseline health status and competing risks of mortality rather than a direct causal effect of treatment choice.

Although CT appeared to be associated with improved OS in unadjusted analyses, this effect was no longer statistically significant after adjustment for clinically relevant variables. In the fully adjusted model, age and multicentricity emerged as independent predictors of mortality. These findings highlight the potential impact of confounding by indication and suggest that the observed differences in OS between treatment groups may largely reflect baseline patient characteristics rather than a direct treatment effect. They also underscore the importance of careful patient selection, indicating that treatment decisions in elderly patients should not rely solely on chronological age but should also incorporate the comorbidity burden and overall functional status.

In line with prior population-based studies, a substantial proportion of elderly BC patients die from non-cancer-related causes, most commonly cardiovascular disease.^{5,7,16} Supporting this finding, CAD was significantly more prevalent in the HT group in our cohort. However, CAD was not independently associated with OS in multivariate analyses. This finding suggests that, while comorbid conditions may influence treatment selection and baseline risk, they may not act as independent prognostic factors in this setting.

RFS did not differ significantly between treatment groups, indicating comparable disease control regardless of the choice of adjuvant systemic therapy. The low recurrence rate observed across the cohort likely reflects the favorable biological profile of hormone receptor-positive, node-negative disease. These findings suggest that, in carefully selected elderly patients, omission of CT may not adversely affect recurrence risk, although overall outcomes remain influenced by non-oncologic factors.

Consistent with these observations, multivariate analysis of RFS demonstrated that neither treatment group nor tumor-related variables were independently associated with recurrence. Instead, age and comorbidity burden, as reflected by the Charlson comorbidity score, emerged as significant predictors. These findings highlight the dominant role of patient-related factors in determining outcomes among elderly patients with BC. However, given the limited number of relapse events, these results should be interpreted with caution.

Surgical and radiotherapy patterns in our study also reflect real-world practice in elderly patients.^{17,18} MRM was performed more frequently than BCS, and radiotherapy was underutilized, particularly in patients receiving HT alone. Previous randomized trials have demonstrated that radiotherapy can be safely omitted in selected low-risk elderly patients without compromising OS.^{12,13} Given that a large proportion of our cohort met these low-risk criteria, the observed treatment patterns may be clinically justified.

In our study, radiotherapy was not independently associated with either OS or RFS in multivariate analyses. However, this finding should be interpreted cautiously, as differences in radiotherapy use between treatment groups and the retrospective nature of the study may still contribute to residual confounding.

Among patients who received CT, anthracycline- and taxane-based regimens were most commonly used, consistent with standard practice. The frequent use of TC aligns with evidence supporting its favorable efficacy-toxicity balance in older patients, particularly those with cardiovascular risk factors.^{19,20} These findings suggest that adjuvant CT can be safely administered to selected elderly patients when guided by careful clinical assessment.

These findings provide insight into real-world treatment patterns and outcomes in elderly patients with early-stage BC and may help inform clinical decision-making in this population. Given the retrospective design, non-randomized treatment allocation, and the absence of frailty and performance status measures, residual confounding by indication likely persists and should be considered when interpreting the results.

Study Limitations

Several limitations of this study should be acknowledged. First, the retrospective, and single-center design introduces potential selection bias, and treatment allocation was not randomized.

Second, the absence of performance status measures, such as Eastern Cooperative Oncology Group, represents an

important limitation, as frailty and functional capacity are key determinants of treatment selection among elderly patients and may have contributed to residual confounding by indication. In this context, prospective studies and real-world registries integrating Comprehensive Geriatric Assessment tools are warranted to better individualize and standardize adjuvant treatment decisions in older patients with BC.

Third, the absence of genomic risk assessment tools, such as multigene assays, limited the ability to further refine risk stratification for recurrence. During the study period, genomic risk assessment tools were not reimbursed by our national social security system, and their high out-of-pocket costs limited their routine use. Consequently, adjuvant treatment decisions were primarily based on clinicopathological factors and tumor biology, which remains common practice in our setting. This limitation reflects disparities in access to certain oncological approaches between developed and developing countries, and it may have influenced treatment selection and risk stratification within this cohort. In contemporary clinical practice, the routine integration of genomic assays may further refine risk stratification and help identify a more selective subgroup of older patients who are likely to benefit from adjuvant CT.

Fourth, the relatively small number of relapse events may have reduced the statistical power to identify independent predictors of recurrence. In addition, given the relatively low number of outcome events, particularly for RFS, the multivariate models may be prone to overfitting, which could limit the robustness of the identified associations. Although multivariate analyses were performed to adjust for clinically relevant variables, residual confounding cannot be fully excluded.

Fifth, the lack of quantitative estrogen and progesterone receptor expression data limited our ability to capture biological heterogeneity within hormone receptor-positive disease. Treating hormone receptor positivity as a binary variable may obscure clinically relevant differences, as tumors with low receptor expression may exhibit more aggressive behavior and differential responses to systemic therapies. Future studies that incorporate quantitative measurements of receptor expression are needed to refine treatment selection in this population.

Finally, cause-specific mortality data were not available, precluding a detailed analysis of cancer-related versus non-cancer-related deaths.

Despite these limitations, this study provides real-world data on adjuvant treatment patterns and outcomes in an understudied elderly BC population.

CONCLUSION

This study highlights the importance of individualized adjuvant treatment strategies in elderly patients with early-stage, node-negative, hormone receptor-positive, HER2-negative BC. While endocrine therapy alone is commonly preferred due to its favorable tolerability profile, our findings suggest that treatment selection should be guided primarily by patient-related factors rather than by treatment modality alone.

Importantly, survival outcomes in this population appear to be strongly influenced by age, comorbid conditions, and competing risks of mortality, rather than by cancer-related factors alone. Therefore, treatment decisions should not be based solely on chronological age but should integrate tumor characteristics, comorbidity burden, functional status, and life expectancy within a multidisciplinary framework. Such an approach may help optimize outcomes while minimizing both undertreatment and overtreatment in this heterogeneous patient population.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the University of Health Sciences Türkiye, Antalya Training and Research Hospital Scientific Research Ethics Committee (approval number: 8/3, date: 08.05.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: E.U., D.K.S., Design: E.U., D.K.S., Data Collection or Processing: E.U., Analysis or Interpretation: D.K.S., Literature Search: E.U., Writing: E.U.

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

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Prognostic Significance of Postoperative Edema in Surgically Treated Non-glioblastoma Diffuse Glioma

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ABSTRACT

Objective: Tumor-associated edema has been implicated as a marker of aggressive tumor biology in gliomas, yet most existing evidence is derived from preoperative imaging and glioblastoma-focused cohorts. The prognostic significance of postoperative edema in non-glioblastoma diffuse gliomas remains insufficiently characterized.

Material and Methods: This retrospective cohort study included patients with histologically confirmed non-glioblastoma diffuse gliomas who underwent surgical intervention at a single tertiary center between October 2010 and January 2024. Postoperative edema was assessed using routine magnetic resonance imaging and classified as present or absent. Overall survival (OS) was defined as the time from diagnosis to death from any cause or to last follow-up. Survival analyses were performed using Kaplan-Meier methods and Cox proportional hazards regression models. Multivariable models were constructed using clinically relevant covariates selected a priori, with sensitivity analyses incorporating performance status and comorbidity burden.

Results: A total of 79 patients were included. Postoperative edema was present in 34 patients (54.8%). Median OS was significantly shorter in patients with postoperative edema compared with those without edema (69 vs. 108 months; log-rank $p=0.007$). In multivariable Cox regression analysis, postoperative edema was independently associated with worse OS (hazard ratio: 2.86; 95% confidence interval: 1.41-5.88; $p=0.004$), after adjustment for tumor grade, tumor location, surgical procedure, and adjuvant treatment. Tumor grade and non-supratentorial location were also independently associated with poorer survival. The association between postoperative edema and OS remained significant in sensitivity analyses that included Eastern Cooperative Oncology Group performance status and the Charlson Comorbidity Index.

Conclusion: Postoperative edema is independently associated with OS in patients with non-glioblastoma diffuse glioma. These findings suggest that routine assessment of postoperative edema may provide clinically meaningful prognostic information beyond established clinicopathological factors and may support its potential role in postoperative risk stratification.

Keywords: Glioma; postoperative edema; overall survival; magnetic resonance imaging; prognosis; Cox regression

INTRODUCTION

Gliomas represent the most common primary malignant tumors of the central nervous system and encompass a biologically heterogeneous group of neoplasms with highly variable clinical outcomes. Despite advances in molecular classification and multimodal treatment strategies, high-grade gliomas remain associated with poor survival,

underscoring the need for improved prognostic stratification beyond traditional clinicopathological factors.^{1,2}

Current prognostic assessment in glioma relies on a combination of patient-related factors, tumor grade, molecular characteristics, and treatment-related variables.³ However, increasing evidence suggests that imaging-derived features may capture additional aspects of tumor biology

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that are not fully reflected by histopathology alone.⁴ Tumor-associated cerebral edema has gained attention as a potential marker of aggressive behavior and adverse outcome in malignant gliomas.

Peritumoral edema in glioma is predominantly vasogenic, arising from blood–brain barrier disruption driven by abnormal tumor angiogenesis and inflammatory signaling.⁵ Importantly, the edematous region may contain infiltrative tumor cells beyond contrast-enhancing margins, contributing to local recurrence and treatment resistance.⁶ Several studies have demonstrated that the extent of preoperative peritumoral edema is associated with reduced survival in glioblastoma, supporting its prognostic relevance.⁷

Although much of the existing evidence linking tumor-associated edema to prognosis originates from glioblastoma-focused studies, it remains unclear whether these observations extend to the broader spectrum of gliomas with varying histological grades and biological behavior. Glioblastoma represents the end of the glioma continuum and is characterized by pronounced vascular permeability and edema formation; findings derived from this subgroup may not be directly generalized to lower- and intermediate-grade gliomas. Moreover, the prognostic relevance of edema assessed in the postoperative setting—where imaging findings reflect a composite of residual infiltrative disease, surgical effects, and early postoperative tissue responses—has not been systematically evaluated across heterogeneous glioma populations.

Clarifying the prognostic significance of postoperative edema across the glioma spectrum may, therefore, provide clinically meaningful insights into disease biology and postoperative risk stratification, thereby addressing an important gap in the existing literature. This study aimed to examine the association between postoperative cerebral edema and overall survival (OS) in patients with surgically treated non-glioblastoma diffuse gliomas, and to determine whether postoperative edema provides independent prognostic value beyond established clinicopathological factors.

MATERIAL AND METHODS

Study Design, Setting, and Participants

This retrospective cohort study was conducted at Marmara University Hospital, a tertiary referral center. Patients treated between October 2010 and January 2024 were identified using the institutional oncology archive database and the electronic medical records. The initial screened population comprised 291 patients with a diagnosis of diffuse glioma.

To focus the analysis on non-glioblastoma diffuse gliomas and ensure consistency with the 2021 World Health Organization

(WHO) classification of central nervous system tumors, patients with glioblastoma (WHO grade 4) were excluded (n=180). Among the remaining 111 patients with non-glioblastoma diffuse glioma, 32 were additionally excluded because postoperative imaging was unavailable or not assessable for postoperative edema evaluation, or because survival follow-up data (vital status and/or date of last follow-up) could not be reliably retrieved. The final study cohort therefore consisted of 79 patients who were included in the survival analyses (Figure 1).

This study was approved by the Marmara University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 09.2024.1472, date: 21.12.2024).

Imaging Assessment and Definition of Postoperative Edema

Postoperative edema, the primary exposure of interest, was assessed using routine postoperative magnetic resonance imaging (MRI) performed as part of standard clinical care. Edema assessment was based primarily on T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences, in which vasogenic edema typically appears as a hyperintense signal in the surrounding brain parenchyma. Because non-

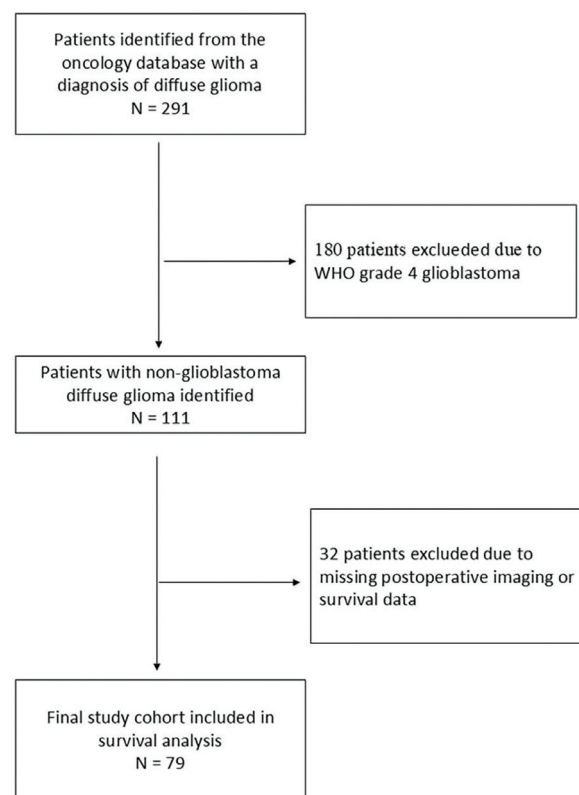


FIGURE 1: Flow diagram of patient selection for the study cohort.

enhancing tumors and edema can overlap on T2/FLAIR and postoperative reactive changes may confound interpretation, edema status was determined using a structured approach aligned with widely accepted neuro-oncology imaging principles. Postoperative edema was recorded as present when a perilesional T2/FLAIR hyperintense region, consistent with vasogenic edema, was visible adjacent to the operative bed and/or residual lesion, and as absent when no such edematous signal abnormality was identified. Postoperative edema was assessed using the first MRI, which was routinely performed within 72 hours after surgery as part of standard clinical care. It was coded as a binary variable (present vs. absent).

To reduce misclassification related to early postoperative effects, T2/FLAIR findings were interpreted in conjunction with other routinely available sequences, particularly diffusion-weighted imaging (DWI) when available, because evidence suggests that resection-related ischemia and postoperative tissue injury may increase non-enhancing FLAIR/T2 signal, which can be better contextualized by DWI. This is consistent with published observations that early postoperative MRI can overestimate non-enhancing abnormalities on FLAIR due to operative effects and ischemia, and that DWI can improve the distinction between postoperative change and residual disease. In addition, interpretation of non-enhancing T2/FLAIR signal changes is a recognized challenge in glioma imaging assessment frameworks, which emphasize qualitative evaluation of non-enhancing disease components.⁸ Edema status was assessed by two readers experienced in neuro-oncologic MRI interpretation who were blinded to survival outcomes. Discrepancies were resolved by consensus. Although no formal quantitative or volumetric framework was applied, the assessment approach was aligned with widely accepted neuro-oncology imaging principles used in routine clinical practice.

Outcome and Variables

The primary outcome was OS, defined as the time from initial diagnosis to death from any cause. Patients alive at the last clinical contact were censored at the date of the last follow-up. Demographic, clinical, tumor-related, imaging, and treatment-related variables were retrospectively abstracted from electronic medical records, operative reports, pathology reports, and oncology treatment records using prespecified definitions. Tumor grade was recorded based on histopathological assessment and categorized as grades 1-3, consistent with the non-glioblastoma composition of the study cohort. Tumor location was dichotomized into supratentorial and non-supratentorial (including deep, midline, and brainstem) to avoid sparse subgroups and to preserve model stability.

The type of surgical procedure was classified as biopsy or debulking, and adjuvant treatment was categorized as radiotherapy alone or radiotherapy combined with temozolomide. Eastern Cooperative Oncology Group (ECOG) performance status and the Charlson Comorbidity Index (CCI) were assessed at the postoperative referral time point, concurrently with the evaluation of postoperative edema. Given the potential temporal overlap between these variables and the primary exposure of interest, ECOG performance status and comorbidity burden were not included in the primary multivariable model, but were instead evaluated in prespecified sensitivity analyses to assess the robustness of the main findings.

Statistical Analysis

OS was summarized using the Kaplan-Meier method and compared between postoperative edema groups using the log-rank test. Univariable Cox proportional hazards regression analyses were performed to examine associations between individual covariates and OS, with results reported as hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). A multivariable Cox proportional hazards model was prespecified to include age, postoperative edema status, tumor grade, tumor location, surgical procedure, and adjuvant therapy. All variables were entered simultaneously using the enter method. The proportional hazards assumption was assessed using log-minus-log plots, and no major violations were observed. Primary multivariable analyses were conducted using complete-case data for the prespecified model. Exposure to bevacizumab was evaluated only in univariable analyses and was not included in multivariable survival models because of its post-baseline, time-dependent nature and the associated risk of immortal time bias. To assess the stability of the multivariable model and mitigate concerns regarding overfitting, internal validation was performed using bootstrap resampling with 1,000 iterations, focusing on the robustness of HR estimates. Prespecified sensitivity analyses were conducted by additionally including ECOG performance status and the CCI in the multivariable Cox model to assess the robustness of the association between postoperative edema and OS. In addition to the primary survival analyses, exploratory analyses were performed to examine the association between the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACE/ARB) and postoperative edema. Differences in the frequency of postoperative edema between ACE/ARB users and non-users were initially assessed using Fisher's exact test. A multivariable logistic regression model was subsequently constructed to evaluate the independent association between ACE/ARB use and the presence of postoperative edema, adjusting for age, tumor grade, tumor location, surgical procedure,

and adjuvant therapy. Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test. All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patient Characteristics

A total of 79 patients with non-glioblastoma diffuse glioma were included in the analysis. The median age at diagnosis was 35 years (interquartile range, 29-50), and 31 patients (39.2%) were male. With respect to tumor grade, 7 patients (10.1%) had grade 1 tumors, 36 (52.2%) had grade 2 tumors, and 26 (37.7%) had grade 3 tumors.

The majority (85.7%) of tumors were located in the supratentorial compartment, 9.1% in deep or midline structures, and 5.2% in the pons. Postoperative edema was present in 34 patients (54.8%). Most patients (91.0%) underwent debulking surgery rather than a biopsy.

Regarding adjuvant treatment, 46.8% of patients received radiotherapy alone and 53.2% received radiotherapy combined with temozolomide. ACE/ARB use was documented in 17.7% of patients, and 70.9% of patients had received bevacizumab at any point during their disease course. At the postoperative assessment, 67.1% of patients had a CCI of 0, and 50.8% had an ECOG performance status of 0 (Table 1).

Survival Outcomes and Prognostic Analyses

Median OS was 72.0 months (95% CI: 51.9-92.1) in the overall cohort. The median follow-up duration, estimated using the reverse Kaplan-Meier method, was 213.0 months (95% CI: 122.1-303.9). During follow-up, 59 deaths were observed, and 20 patients were censored at the last follow-up. Median OS differed significantly according to postoperative edema status. Kaplan-Meier analysis demonstrated that patients with postoperative edema had significantly shorter OS than those without edema: median OS was 69.0 months (95% CI: 51.4-86.6) for patients with postoperative edema and 108.0 months (95% CI: 56.8-159.2) for those without edema (log-rank $p=0.007$) (Figure 2).

In univariable Cox regression analyses, postoperative edema, tumor grade, tumor location, and ACE/ARB use were significantly associated with OS. Postoperative edema was associated with an increased risk of death (HR: 2.36; 95% CI: 1.24-4.49; $p=0.009$). In univariable analyses, tumor grade and tumor location demonstrated significant associations with OS, whereas age, sex, ECOG performance status, CCI, surgical procedure, adjuvant treatment modality, and

bevacizumab exposure were not significantly associated with OS (Table 2).

In multivariable Cox regression analysis, the presence of postoperative edema was independently associated with an increased risk of death (HR: 2.86; 95% CI: 1.41-5.88; $p=0.004$) (Table 3, Figure 3). Tumor grade remained a strong independent prognostic factor, with patients in the highest-grade group exhibiting a substantially higher risk of death compared with those with lower-grade tumors. Non-supratentorial tumor location was also independently associated with worse OS (HR: 5.26; 95% CI: 1.16-25.00; $p=0.031$). Age, type of surgical procedure, and adjuvant treatment modality were not independently associated OS in the multivariable model. ECOG performance status and CCI were not included in the primary multivariable model because their assessment was contemporaneous with postoperative edema, creating a potential risk of overadjustment; their impact was therefore evaluated in prespecified sensitivity analyses. The overall model was statistically significant

TABLE 1: Baseline demographic, clinical, and tumor characteristics of the study cohort (n=79).

Variable	Value
Age at diagnosis, years	35 (29-50)
Sex, male	31 (39.2%)
Tumor grade	
Grade 1	7 (10.1%)
Grade 2	36 (52.2%)
Grade 3	26 (37.7%)
Primary tumor location	
Supratentorial	66 (85.7%)
Deep/midline	7 (9.1%)
Pons	4 (5.2%)
Postoperative edema, present	34 (54.8%)
Surgical procedure, debulking	71 (91.0%)
Adjuvant treatment	
Radiotherapy alone	37 (46.8%)
Radiotherapy + temozolomide	42 (53.2%)
ACE/ARB use	14 (17.7%)
Bevacizumab exposure, ever	56 (70.9%)
Charlson Comorbidity Index, 0	
0	53 (67.1%)
≥1	26 (32.9%)
ECOG performance status	
0	31 (50.8%)
≥1	30 (49.2%)

Age is presented as median (interquartile range). RT: Radiotherapy; TMZ: Temozolomide; ACE/ARB: Angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; ECOG: Eastern Cooperative Oncology Group.

(Omnibus $\chi^2=31.82$; $p<0.001$), indicating good explanatory power. Bootstrap internal validation demonstrated consistent HR estimates across resampled datasets. In particular, postoperative edema remained independently associated with OS after bootstrap resampling (bootstrap $p=0.042$; BCa 95% CI: -2.01 to -0.69), supporting the robustness of the primary multivariable findings. In sensitivity analyses that additionally adjusted for ECOG performance status and CCI, postoperative edema remained independently associated with OS (HR: 3.34; 95% CI: 1.17-9.55; $p=0.024$), consistent with the primary multivariable analysis (Supplementary Table S1). Neither ECOG performance status nor comorbidity burden was independently associated with survival.

Exploratory Analysis: ACE/ARB Use and Postoperative Edema

In exploratory analyses, the association between use of ACE/ARB and postoperative edema was evaluated. In unadjusted analyses, postoperative edema was numerically less frequent among patients receiving ACE/ARB therapy compared with non-users; however, this difference did not reach statistical significance (Fisher's exact $p=0.220$).

In multivariable logistic regression analysis, adjusting for age, tumor grade, tumor location, surgical procedure, and adjuvant therapy, ACE/ARB use was not independently associated with

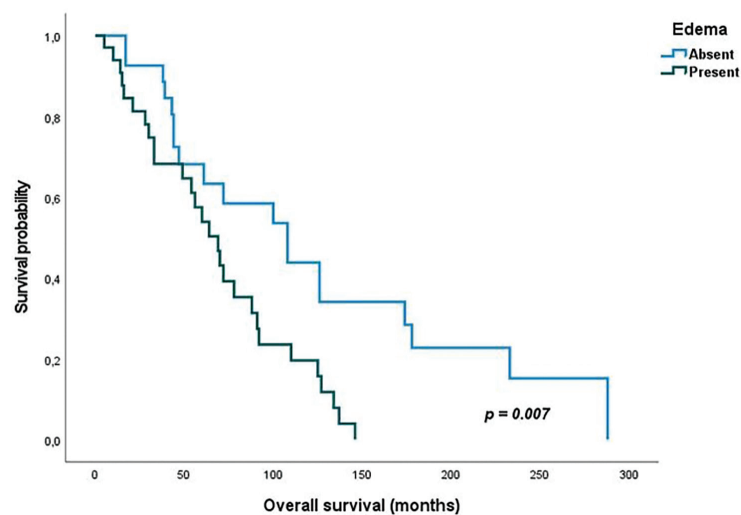


FIGURE 2: Kaplan-Meier curves for overall survival stratified by postoperative edema status. Patients with postoperative edema had significantly shorter overall survival compared with those without edema.

TABLE 2: Univariable Cox proportional hazards analysis for overall survival.

Variable	Hazard ratio (95% CI)	p-value
Age (per year increase)	1.01 (0.99-1.04)	0.375
Sex (male vs. female)	0.90 (0.53-1.53)	0.688
ECOG (≥ 1 vs. 0)	1.00 (0.55-1.84)	0.997
CCI (≥ 1 vs. 0)	2.90 (0.78-10.84)	0.113
Tumor grade (overall)	—	0.006
Grade 1 vs. grade 3	1.93 (0.77-4.87)	0.162
Grade 2 vs. grade 3	0.50 (0.27-0.91)	0.022
Tumor location (non-supratentorial vs. supratentorial)	2.22 (1.10-4.46)	0.026
Postoperative edema (present vs absent)	2.36 (1.24-4.49)	0.009
Surgical procedure (biopsy vs. debulking)	1.83 (0.77-4.30)	0.169
Adjuvant therapy (RT alone vs. RT+TMZ)	1.36 (0.81-2.29)	0.241
ACE/ARB use (yes vs. no)	2.66 (1.19-5.94)	0.017
Bevacizumab exposure (ever vs. never)	1.36 (0.73-2.54)	0.328

Reference categories for categorical variables were as follows: female for sex, ECOG 0 for performance status, Charlson Comorbidity Index 0, supratentorial location for tumor site, absence of postoperative edema, debulking for surgical procedure, radiotherapy plus temozolomide for adjuvant treatment, no use for ACE/ARB, and no exposure for bevacizumab. ACE/ARB: Angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; ECOG: Eastern Cooperative Oncology Group; RT: Radiotherapy; TMZ: Temozolomide; CI: Confidence interval.

postoperative edema (odds ratio: 2.21; 95% CI: 0.49-10.06; $p=0.304$) (Supplementary Table S1). Model calibration was acceptable based on the Hosmer-Lemeshow goodness-of-fit test ($p=0.437$).

DISCUSSION

In this retrospective cohort study of patients with histologically confirmed non-glioblastoma diffuse glioma, postoperative edema emerged as an independent factor associated with OS. Patients without postoperative edema experienced significantly longer survival compared with those with edema, and this association remained robust after adjustment for tumor grade, anatomical location, and treatment-related factors. Notably, the adverse prognostic impact of postoperative edema persisted after additional adjustment for functional status and comorbidity burden,

indicating that edema conveys prognostic information beyond general health status or baseline performance alone.

The prognostic relevance of tumor-associated edema has been investigated predominantly in the preoperative setting, with most available evidence derived from studies of glioblastoma and other high-grade gliomas. Several investigations have demonstrated that the presence and extent of peritumoral edema on baseline MRI are associated with shorter OS, independent of age, tumor grade, and extent of resection. Schoenegger et al.⁹ showed that preoperative peritumoral edema was an independent adverse prognostic factor in glioblastoma, while Wu et al.⁷ similarly reported poorer outcomes in glioma patients with more extensive edema on routine MRI. Collectively, these findings support the concept that edema reflects aggressive tumor biology rather than being a purely reactive imaging phenomenon.

TABLE 3: Multivariable Cox proportional hazards analysis of overall survival.

Variable	HR	95% CI	p-value
Age (per 1-year increase)	1.02	0.99-1.06	0.207
Postoperative edema (present vs. absent)	2.86	1.41-5.88	0.004
Grade (overall)	—	—	<0.001
Grade (highest vs. lower)	16.7	3.7-50.0	<0.001
Grade (highest vs. intermediate)	7.7	1.7-33.3	0.008
Adjuvant therapy (RT alone vs. RT + TMZ)	1.06	0.51-2.22	0.858
Surgical procedure (biopsy vs. debulking)	1.47	0.35-6.25	0.598
Tumor location (non-supratentorial vs. supratentorial)	5.26	1.16-25.00	0.031

RT: Radiotherapy; TMZ: Temozolomide; HR: Hazard ratio; CI: Confidence interval.

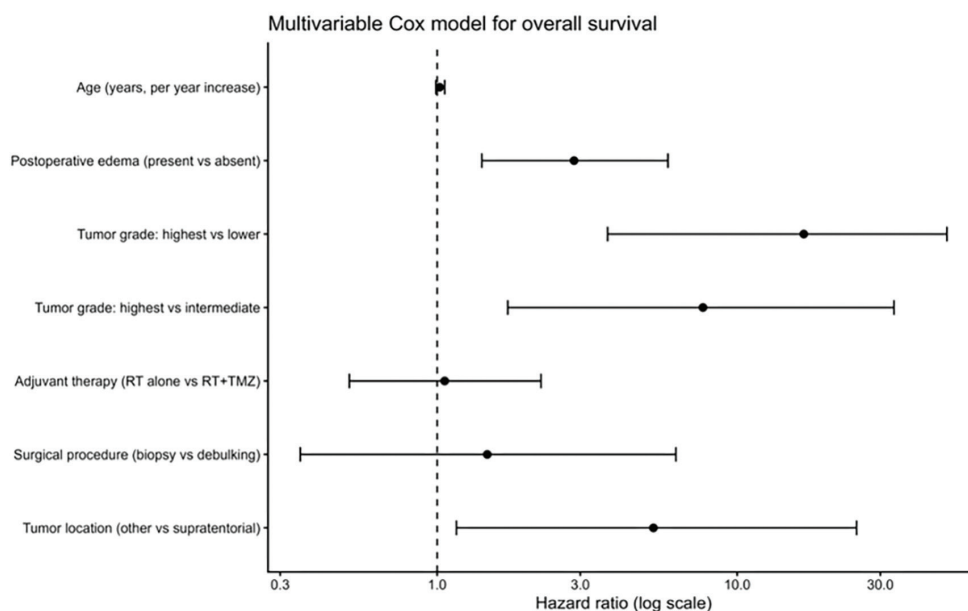


FIGURE 3: Forest plot showing hazard ratios from the multivariable Cox proportional hazards model for overall survival. Hazard ratios greater than 1 indicate increased risk of death relative to the reference category.

RT: Radiotherapy; TMZ: Temozolomide

Data addressing the prognostic role of edema in non-glioblastoma diffuse gliomas are limited, and most mechanistic and prognostic insights are extrapolated from high-grade disease. Nevertheless, the biological processes underlying edema formation—including blood-brain barrier disruption, abnormal tumor vasculature, and inflammatory signaling—are not unique to glioblastoma and are observed across the broader glioma spectrum.^{4,10,11} In this context, our findings extend observations in high-grade gliomas by demonstrating that postoperative edema retains independent prognostic significance in non-glioblastoma diffuse gliomas, supporting the generalizability of edema-associated adverse biology beyond WHO grade 4 disease.

The postoperative setting deserves consideration. Early postoperative MRI is routinely performed to assess residual tumor and surgical complications, and edema detected at this time may reflect persistent microvascular dysfunction, residual infiltrative tumor burden, or an early postoperative inflammatory milieu. Prior imaging studies have highlighted the complexity of interpreting non-enhancing T2/FLAIR abnormalities and edema in glioma patients, emphasizing their association with survival and treatment resistance.^{4,11} Our results suggest that postoperative edema, even when assessed qualitatively using routine clinical imaging, provides clinically meaningful prognostic information that may be readily incorporated into postoperative risk stratification frameworks.

Tumor grade remained one of the strongest independent predictors of survival in our cohort, consistent with extensive clinical evidence demonstrating progressively worse outcomes with increasing histological grade in diffuse gliomas.² Patients with lower- and intermediate-grade tumors exhibited substantially improved survival compared with those harboring higher-grade disease, in line with large cohort studies showing a strong and independent association between WHO grade and OS.¹² In addition, supratentorial tumor location was independently associated with better outcomes than non-supratentorial locations, a finding that has been previously attributed to differences in surgical accessibility, extent of resection, and the intrinsic biological behavior of deep and midline gliomas.¹³ The lack of a significant association between surgical procedure and survival in our cohort should be interpreted cautiously, because the simplified classification of surgical extent (biopsy vs. debulking) and the limited number of biopsy cases may have reduced the statistical power to detect a true effect.

Although use of ACE/ARB was associated with worse OS in univariable analyses, this relationship was attenuated after multivariable adjustment, suggesting confounding by baseline clinical and treatment-related factors.¹⁴⁻¹⁷ In our cohort, ACE/ARB exposure was not independently associated

with postoperative edema, and the observed survival signal did not persist after adjustment. Therefore, this exploratory finding should be considered hypothesis-generating rather than evidence of a direct causal effect.

The persistence of the postoperative edema-survival association after adjustment for the ECOG performance status and the CCI is noteworthy. Performance status and comorbidity burden are well-established prognostic factors in glioma and other solid tumors, with poorer ECOG scores and higher comorbidity indices consistently associated with inferior survival outcomes.^{18,19} The continued significance of postoperative edema after accounting for these variables suggests that edema captures disease-specific biological information rather than merely reflecting patient frailty or functional impairment. This observation is consistent with prior studies demonstrating that imaging-based biomarkers in glioma may provide incremental prognostic value beyond traditional clinical measures, reflecting underlying tumor biology and disease burden rather than patient frailty *per se*.^{4,20} To minimize the risk of model overfitting in this modest-sized cohort, a parsimonious multivariable strategy was deliberately employed, with clinically relevant covariates selected *a priori* and contemporaneous variables evaluated separately in sensitivity analyses, consistent with established methodological recommendations.^{21,22}

Study Limitations

Several limitations should be acknowledged. The retrospective, single-center design introduces potential selection bias and residual confounding despite multivariable adjustment. The relatively modest sample size limits statistical power for subgroup and exploratory analyses, and increases susceptibility to model instability. Postoperative edema was assessed on routine clinical MRI and evaluated qualitatively as a binary variable; this qualitative evaluation may be influenced by postoperative changes and does not capture volumetric or morphological heterogeneity. In addition, heterogeneity in adjuvant treatment strategies over the extended study period and the lack of uniformly available molecular tumor data may have limited comprehensive risk stratification and adjustment. Importantly, comprehensive molecular classification data, including IDH mutation status and 1p/19q co-deletion, were not uniformly available due to the retrospective design and long inclusion period. Given the central role of molecular markers in the current WHO classification of diffuse gliomas, this limitation may have introduced residual confounding. Prospective studies incorporating standardized volumetric edema assessment, serial postoperative imaging, and integrated molecular profiling may help address these limitations and further

clarify the biological underpinnings of edema-associated risk.

Despite these limitations, several strengths of this study deserve emphasis. First, the analysis focused on postoperative edema assessed using routinely acquired clinical MRI, which represents a clinically relevant and underexplored time point in glioma prognostication. Second, the study cohort predominantly comprised non-glioblastoma diffuse gliomas, thereby addressing an important gap in the literature, which has largely focused on glioblastoma and other high-grade tumors. Third, a rigorous and parsimonious multivariable modeling strategy was employed, with covariates selected a priori and sensitivity analyses performed to assess the robustness of the findings thereby minimizing the risk of overfitting. Edema assessment relied on standard imaging sequences without specialized software or advanced post-processing, enhancing applicability of the findings in routine clinical settings. Prospective studies incorporating standardized volumetric assessment of edema and integrated molecular profiling are warranted to validate these findings.

CONCLUSION

Postoperative edema is independently associated with OS in patients with non-glioblastoma diffuse glioma, retaining prognostic significance after comprehensive multivariable adjustment. Routine assessment of postoperative edema may therefore aid postoperative risk stratification, inform the intensity of follow-up and the planning of adjuvant treatment, and serve as a stratification factor in future clinical trials. Further studies are warranted to elucidate the biological mechanisms linking edema to adverse outcomes and to explore whether targeted interventions aimed at edema modulation can translate into improved survival.

Ethics

Ethics Committee Approval: This study was approved by the Marmara University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 09.2024.1472, date: 21.12.2024).

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Concept: E.K., Design: E.K., O.K., İ.V.B., Data Collection or Processing: E.K., G.Ö., M.H.F., A.P.A., Analysis or Interpretation: E.K., F.A., A.D., M.A.T., B.P.,

Y.A., P.E., A.K.G., O.K., İ.V.B., Literature Search: E.K., F.A., A.D., M.A.T., B.P., Y.A., P.E., A.K.G., O.K., İ.V.B., Writing: E.K., M.A.T., Y.A.

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Supplementary Table 1: <https://d2v96fxpocvxx.cloudfront.net/65bdf192-201e-4bb0-813a-d69339a28cf1/content-images/c93e7fb0-646b-4aa3-acad-dcb35bad0683.pdf>

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Evaluation of Age-related Clinical and Molecular Characteristics in a Single-center Real-world Breast Cancer Cohort

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ABSTRACT

Objective: Breast cancer exhibits biological heterogeneity that varies across age groups and influences treatment decisions. This study aimed to evaluate age-related differences in biomarker profiles, surrogate molecular subtypes, and treatment patterns in a single-center real-world breast cancer cohort.

Material and Methods: This retrospective cohort study included 1,124 patients with invasive breast cancer diagnosed or followed between January 2020 and June 2025. Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) status, and Ki-67 proliferation index were analyzed. Tumors were classified into surrogate molecular subtypes according to the St. Gallen and European Society for Medical Oncology recommendations. Biomarker distributions, molecular subtypes, and treatment modalities were compared across age groups. Multivariable logistic regression analyses were performed to identify independent predictors of HER2 positivity and triple-negative breast cancer (TNBC).

Results: Significant age-related differences were observed in ER status, HER2 status, Ki-67 index, and surrogate molecular subtype distribution. Younger patients more frequently exhibited ER negativity, HER2 positivity, high proliferative activity, and aggressive molecular subtypes, whereas older patients predominantly presented with hormone receptor-positive, lower-proliferation tumors. Neoadjuvant therapy was more frequently used in younger patients, while treatment de-escalation was observed in older age groups. The HER2-low phenotype was identified in 18.4% of the cohort. Age, PR status, Ki-67 level, and histological grade were independent predictors of HER2 positivity and TNBC.

Conclusion: Age-related biological differences in breast cancer are closely aligned with real-world treatment decisions. Treatment strategies are primarily guided by tumor biology rather than chronological age alone.

Keywords: Breast cancer; age groups; biomarkers; molecular subtypes; real-world data

INTRODUCTION

Breast cancer remains the most frequently diagnosed malignancy among women worldwide and is one of the leading causes of cancer-related mortality.¹ The disease exhibits marked biological heterogeneity, ranging from indolent hormone receptor-positive tumors to biologically aggressive subtypes. This diversity underscores the importance of biomarker-based risk assessment in diagnostic and therapeutic decision-making.

In routine clinical practice, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) status, and the Ki-67 proliferation index are widely used to assess prognosis and guide neoadjuvant and adjuvant treatment strategies.^{2,3} Because genomic profiling is not universally available, surrogate molecular subtyping based on the St. Gallen Consensus and European Society for Medical Oncology (ESMO) recommendations—namely luminal A, luminal B, HER2-positive non-luminal, and triple-negative breast cancer (TNBC)—is commonly applied in clinical settings.^{4,5}

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Within this framework, the Ki-67 proliferation index contributes to distinguishing biological behavior, particularly in luminal tumors. However, due to variability in assessment and interpretation, Ki-67 is generally considered a complementary marker rather than a standalone determinant of treatment decisions.⁶

In recent years, the HER2-low phenotype, defined by low levels of HER2 expression, has gained attention, particularly in the context of metastatic disease and targeted therapies. Current American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines emphasize that HER2-low should not be regarded as a distinct biological subtype but rather as a treatment-related classification.^{2,7}

Age is an important clinical factor associated with tumor biology and treatment patterns in breast cancer. Population-based studies have reported higher frequencies of HER2-positive and triple-negative tumors in younger patients, whereas hormone receptor-positive and lower-proliferation tumors are more common in older age groups.^{8,9} Although these biological features have been evaluated in various settings, data integrating age-related biomarker distribution, surrogate molecular subtypes, and real-world treatment approaches remain limited. Therefore, this study aimed to evaluate age-related biomarker profiles, surrogate molecular subtypes, and treatment patterns in a large, single-center real-world breast cancer cohort.

MATERIAL AND METHODS

Study Design and Patient Selection

This study was designed as a single-center retrospective cohort study. Data were obtained retrospectively from the electronic medical records of patients who were diagnosed with or were followed for breast cancer between January 2020 and June 2025. A total of 1,124 patients with invasive breast carcinoma were included. Eligible patients had complete immunohistochemical data on ER, PR, and HER2 status, and the Ki-67 proliferation index, as well as available systemic treatment information. Patients with ductal carcinoma in situ and those with missing clinical, pathological, or treatment-related data were excluded.

Clinical and Pathological Variables

Recorded clinical variables included age at diagnosis, age groups, menopausal status when available, presence of metastatic disease at diagnosis, and surgical treatment information. Pathological evaluation included histological subtype (invasive ductal, invasive lobular, or other), histological grade, lymph node status, and metastatic disease at diagnosis. Histological grade was classified according to the Nottingham grading system as Grade 1-3.¹⁰

Immunohistochemical Assessment

ER and PR expressions were reported as percentages of tumor cells with nuclear staining. Tumors with >10% staining were considered positive, while cases with 1-10% staining were classified as low-positive. HER2 status was evaluated according to ASCO/CAP guidelines.¹¹ Tumors with immunohistochemical scores of 0-1+ were considered negative, and those with 3+ were considered positive. For cases scored as 2+, fluorescence or silver *in situ* hybridization results were used for final classification.¹² HER2-low tumors were defined as cases showing HER2 1+ or 2+ expression without gene amplification. The HER2-low phenotype was assessed for descriptive purposes and was not considered a separate molecular subtype. The Ki-67 proliferation index was categorized as ≤20%, 20-50%, and >50% based on pathology reports.

Surrogate Molecular Subtypes

Based on immunohistochemical findings, tumors were classified into surrogate molecular subtypes.¹³ ER and/or PR-positive, HER2-negative tumors with low proliferation were classified as luminal A. Hormone receptor-positive tumors with high proliferation and/or HER2 positivity were classified as luminal B. Hormone receptor-negative and HER2-positive tumors were classified as HER2-positive (non-luminal), whereas tumors negative for ER, PR, and HER2 were classified as TNBC. Molecular subtype definitions followed the St. Gallen Consensus and ESMO recommendations.

Treatment Modalities

Treatment approaches were recorded as neoadjuvant, adjuvant, or palliative. Neoadjuvant therapy included systemic chemotherapy and/or targeted agents administered before surgery. Adjuvant therapy, administered after surgery, included chemotherapy, endocrine therapy, and targeted agents. Radiotherapy was evaluated separately.

Ethical Approval

This study was approved by the Çanakkale Onsekiz Mart University Rectorate Non-Interventional Clinical Research Ethics Committee (approval number: 2025-387, date: 10.12.2025). The study was conducted in accordance with the Declaration of Helsinki, and patient data were anonymized prior to analysis.

Statistical Analysis

Statistical analyses were performed using IBM SPSS, version 26 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean ± standard deviation or median (minimum-maximum), and categorical variables were presented as frequencies and percentages. Biomarker

distributions and molecular subtypes across age groups were summarized in tables. Group comparisons were performed using the chi-square test or Fisher's exact test when appropriate. For continuous variables that were not normally distributed, the Mann-Whitney U or Kruskal-Wallis tests were applied. Separate multivariable logistic regression models were constructed to identify independent predictors of HER2 positivity and TNBC. Variables with $p < 0.10$ in univariable analyses and variables considered clinically relevant were included in multivariable models. Results were reported as odds ratios with 95% confidence intervals. A p -value < 0.05 was considered statistically significant.

RESULTS

A total of 1,124 patients were included in the analysis. The mean age at diagnosis was 55.44 ± 12.20 years, with a median age of 55 years (range, 20-92) (Table 1). The largest proportion of patients was in the 50-59 age group ($n=315$), followed by the 60-69 ($n=293$) and 40-49 ($n=267$) age groups. There were 106 patients aged 18-39 years and 143 patients aged ≥ 70 years (Figure 1).

Distribution of Biomarkers by Age Group

ER status differed significantly across age groups ($p=0.009$) (Table 2). ER negativity was most frequent in the 18-39 years age group (33.0%) and decreased with advancing age. Overall, ER expression $>10\%$ was observed in 77.6% of the cohort (872/1,124). PR status did not differ significantly across age groups ($p=0.086$) (Table 2).

HER2 status was significantly associated with age ($p < 0.001$) (Table 2). HER2 positivity was highest in patients aged 18-39 years (43.4%), whereas rates ranged between 19.5% and

24.1% in patients aged 40-69 years and were 21.0% in those aged ≥ 70 years. The HER2-low phenotype was identified in 18.4% of the overall cohort, while 57.7% of tumors were HER2-negative.

The Ki-67 proliferation index differed significantly across age groups ($p=0.008$) (Table 2). High proliferation ($>50\%$) was more common in younger patients, observed in 23.6% of the 18-39 age group compared with 11.2% in patients aged ≥ 70 years. Histological grade distribution did not differ significantly among age groups ($p=0.702$) (Table 2).

Distribution of Surrogate Molecular Subtypes

The distribution of surrogate molecular subtypes varied significantly by age group ($p < 0.001$) (Table 3). In the overall cohort, luminal A accounted for 35.7%, luminal B (HER2-negative) for 29.4%, luminal B (HER2-positive) for 16.3%, HER2-positive non-luminal for 7.6%, and TNBC for 11.0%. The proportion of luminal A tumors was lowest in patients aged 18-39 years (19.8%) and highest in those aged 60-69 years (41.3%) and ≥ 70 years (40.6%).

TABLE 1: Descriptive statistics of age at diagnosis across age groups.

Age group (years)	Mean $\pm$ SD	Median (min-max)
18-39	34.74 $\pm$ 3.88	36 (20-39)
40-49	44.78 $\pm$ 2.77	45 (40-49)
50-59	54.49 $\pm$ 2.88	55 (50-59)
60-69	63.82 $\pm$ 2.84	63 (60-69)
≥ 70	75.64 $\pm$ 4.94	74 (70-92)
Total cohort	55.44$\pm$12.20	55 (20-92)

SD: Standard deviation; min-max: Minimum-maximum.

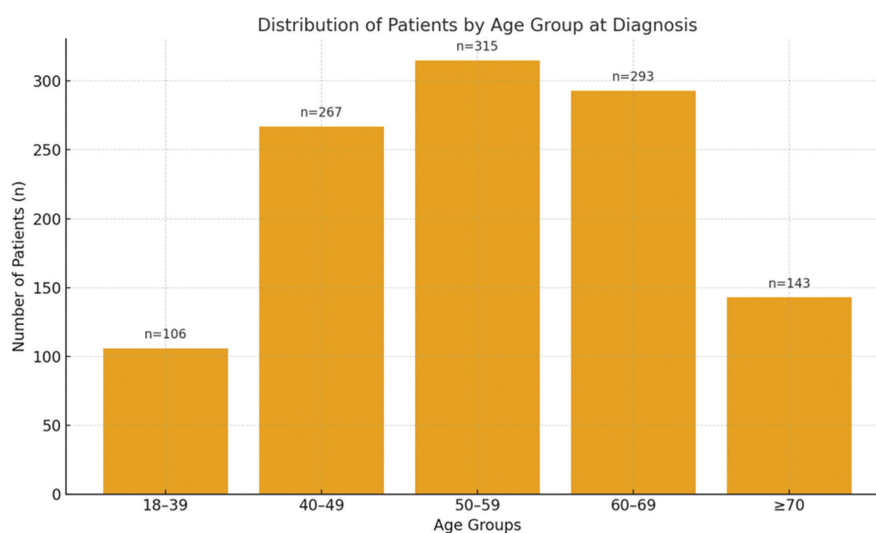


FIGURE 1: Distribution of patients by age group at diagnosis ($n=1,124$).

Tumor Characteristics and Treatment Patterns

Metastatic disease at diagnosis was present in 24.7% of the cohort (Table 4). The highest rate was observed in the 18-39 age group (33.0%), followed by patients aged ≥ 70 years (29.4%), whereas rates ranged from 21.0% to 23.5% in patients aged 40-69 years ($p=0.060$).

The use of neoadjuvant therapy differed significantly across age groups ($p<0.001$) (Table 4), with the highest rate in patients aged 18-39 years (43.4%) and the lowest in those aged ≥ 70 years (19.6%). No significant differences were observed among age groups regarding adjuvant therapy, surgical intervention, axillary lymph node dissection, or type of surgery (Table 4). The use of radiotherapy ($p<0.001$) and endocrine therapy ($p=0.018$) varied significantly by age group.

Multivariable Analyses

In the multivariable logistic regression model evaluating predictors of HER2 positivity (Table 5), PR negativity, younger age, higher Ki-67 levels, and higher histological grade were identified as independent predictors.

In the multivariable model for TNBC (Table 6), higher Ki-67 proliferation categories and grade 3 histology were independently associated with TNBC. Age group was not independently associated with TNBC. Comparative distributions of independent predictors across treatment models are presented in Figure 2.

TABLE 2: Distribution of ER, PR, HER2, Ki-67 and histological grade across age groups.

Biomarker/feature	18-39	40-49	50-59	60-69	≥70	Total cohort	p-value
ER status							
ER-	35 (33.0%)	50 (18.7%)	65 (20.6%)	44 (15.0%)	25 (17.5%)	219 (19.5%)	0.009
ER, 1-10%	2 (1.9%)	10 (3.7%)	9 (2.9%)	11 (3.8%)	1 (0.7%)	33 (2.9%)	
ER, >10%	69 (65.1%)	207 (77.5%)	241 (76.5%)	238 (81.2%)	117 (81.8%)	872 (77.6%)	
PR status							
PR-	40 (37.7%)	63 (23.6%)	90 (28.6%)	84 (28.7%)	43 (30.1%)	320 (28.5%)	0.086
PR, 1-10%	10 (9.4%)	17 (6.4%)	30 (9.5%)	19 (6.5%)	7 (4.9%)	83 (7.4%)	
PR, >10%	56 (52.8%)	187 (70.0%)	195 (61.9%)	190 (64.8%)	93 (65.0%)	721 (64.1%)	
HER2 status							
HER2-	46 (43.4%)	159 (59.6%)	178 (56.5%)	176 (60.1%)	90 (62.9%)	649 (57.7%)	<0.001
HER2-low	14 (13.2%)	49 (18.4%)	61 (19.4%)	60 (20.5%)	23 (16.1%)	207 (18.4%)	
HER2+	46 (43.4%)	59 (22.1%)	76 (24.1%)	57 (19.5%)	30 (21.0%)	268 (23.8%)	
Ki-67 index							
≤20%	28 (26.4%)	105 (39.3%)	124 (39.4%)	135 (46.1%)	67 (46.9%)	459 (40.8%)	0.008
20-50%	53 (50.0%)	125 (46.8%)	133 (42.2%)	113 (38.6%)	60 (42.0%)	484 (43.1%)	
>50%	25 (23.6%)	37 (13.9%)	58 (18.4%)	45 (15.4%)	16 (11.2%)	181 (16.1%)	
Histological grade							
Grade 1	7 (6.6%)	26 (9.7%)	27 (8.6%)	37 (12.6%)	11 (7.7%)	108 (9.6%)	0.702
Grade 2	71 (67.0%)	167 (62.5%)	203 (64.4%)	177 (60.4%)	93 (65.0%)	711 (63.3%)	
Grade 3	28 (26.4%)	74 (27.7%)	85 (27.0%)	79 (27.0%)	39 (27.3%)	305 (27.1%)	
ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; Ki-67: Proliferative index.							

ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; Ki-67: Proliferative index.

TABLE 3: Distribution of molecular subtypes across age groups.

Molecular subtype	18-39	40-49	50-59	60-69	≥ 70	Total cohort	p-value
Luminal A	21 (19.8%)	98 (36.7%)	103 (32.7%)	121 (41.3%)	58 (40.6%)	401 (35.7%)	<0.001
Luminal B (HER2-)	25 (23.6%)	82 (30.7%)	98 (31.1%)	86 (29.4%)	40 (28.0%)	331 (29.4%)	
Luminal B (HER2+)	26 (24.5%)	40 (15.0%)	52 (16.5%)	44 (15.0%)	21 (14.7%)	183 (16.3%)	
HER2-positive (non-luminal)	20 (18.9%)	19 (7.1%)	24 (7.6%)	13 (4.4%)	9 (6.3%)	85 (7.6%)	
Triple-negative	14 (13.2%)	28 (10.5%)	38 (12.1%)	29 (9.9%)	15 (10.5%)	124 (11.0%)	

HER2: Human epidermal growth factor receptor 2.

TABLE 4: Distribution of tumor morphology and treatment modalities across age groups.

Variable	18-39	40-49	50-59	60-69	≥70	Total cohort	p-value
Tumor morphology							
IDC	93 (87.7%)	216 (80.9%)	271 (86.0%)	239 (81.6%)	108 (75.5%)	927 (82.4%)	0.115
ILC	5 (4.7%)	21 (7.9%)	20 (6.3%)	29 (9.9%)	14 (9.8%)	89 (7.9%)	
Other	8 (7.5%)	30 (11.2%)	24 (7.6%)	25 (8.5%)	21 (14.7%)	108 (9.6%)	
Metastatic disease							
Absent	71 (67.0%)	210 (78.7%)	240 (76.2%)	226 (77.1%)	99 (69.2%)	846 (75.3%)	0.060
Present	35 (33.0%)	56 (21.0%)	74 (23.5%)	65 (22.2%)	42 (29.4%)	272 (24.7%)	
Neoadjuvant therapy							
No	60 (56.6%)	193 (72.3%)	225 (71.4%)	240 (81.9%)	115 (80.4%)	833 (74.1%)	<0.001
Yes	46 (43.4%)	74 (27.7%)	90 (28.6%)	53 (18.1%)	28 (19.6%)	291 (25.9%)	
Adjuvant therapy							
No	29 (27.4%)	52 (19.5%)	77 (24.4%)	68 (23.2%)	36 (25.2%)	262 (23.3%)	0.452
Yes	76 (71.7%)	213 (79.8%)	234 (74.3%)	223 (76.1%)	103 (72.0%)	849 (75.5%)	
Surgery performed							
No	10 (9.4%)	15 (5.6%)	24 (7.6%)	28 (9.6%)	17 (11.9%)	94 (8.4%)	0.209
Yes	96 (90.6%)	252 (94.4%)	291 (92.4%)	265 (90.4%)	126 (88.1%)	1030 (91.6%)	
ALND							
No	45 (42.5%)	126 (47.2%)	154 (48.9%)	153 (52.2%)	66 (46.2%)	544 (48.4%)	0.450
Yes	61 (57.5%)	141 (52.8%)	161 (51.1%)	140 (47.8%)	77 (53.8%)	580 (51.6%)	
Type of surgery							
None	9 (8.5%)	14 (5.2%)	24 (7.6%)	29 (9.9%)	14 (9.8%)	90 (8.0%)	0.508
Breast-conserving surgery	43 (40.6%)	124 (46.4%)	134 (42.5%)	119 (40.6%)	53 (37.1%)	473 (42.1%)	
Total mastectomy	54 (50.9%)	129 (48.3%)	157 (49.8%)	145 (49.5%)	76 (53.1%)	561 (49.9%)	
Radiotherapy							
No	18 (17.0%)	42 (15.7%)	64 (20.3%)	70 (23.9%)	48 (33.6%)	242 (21.5%)	<0.001
Yes	88 (83.0%)	225 (84.3%)	251 (79.7%)	223 (76.1%)	95 (66.4%)	882 (78.5%)	
Endocrine therapy							
No	34 (32.1%)	47 (17.6%)	63 (20.0%)	51 (17.4%)	29 (20.3%)	224 (19.9%)	0.018
Yes	72 (67.9%)	220 (82.4%)	252 (80.0%)	242 (82.6%)	114 (79.7%)	900 (80.1%)	
IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; ALND: Axillary lymph node dissection.							

IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; ALND: Axillary lymph node dissection.

DISCUSSION

Breast cancer is characterized by substantial biological heterogeneity, which directly influences clinical behavior and treatment strategies. Although biomarker-based risk stratification is well established in routine practice, data describing how age-related biological differences translate into real-world treatment patterns remain limited. In this large single-center cohort, we evaluated age-associated distributions of biomarkers and surrogate molecular subtypes to assess how guideline-defined biological risk profiles are reflected in daily clinical practice.¹⁴

The median age at diagnosis in our cohort was 55 years, with most patients clustered between 50 and 69 years, consistent with national data from Türkiye and global epidemiological reports.¹⁵ The distinctive strength of this study lies in the integrated evaluation of biological features and treatment patterns across age groups. The higher frequency of ER negativity, HER2 positivity, and elevated proliferative activity in younger patients, contrasted with the predominance of hormone receptor-positive and lower-proliferation tumors in older patients, aligns with previously reported population-based trends.^{16,17} Importantly, our findings demonstrate that these biological differences are accompanied by parallel differences in treatment intensity, particularly with respect to neoadjuvant systemic therapy.

TABLE 5: Independent predictors of HER2 positivity (multivariable logistic regression Model 1).			
Predictor	Category	OR (95% CI)	p-value
ER status	≥10% (ref)	—	0.249
	1-9%	1.74 (0.79-3.83)	0.171
	Negative	0.90 (0.55-1.48)	0.684
PR status	≥10% (ref)	—	<0.001
	1-9%	0.86 (0.46-1.60)	0.634
	Negative	0.43 (0.27-0.68)	<0.001
Age group	18-39 (ref)	—	0.002
	40-49	0.41 (0.24-0.68)	0.001
	50-59	0.44 (0.27-0.72)	0.001
	60-69	0.35 (0.21-0.59)	<0.001
	≥70	0.38 (0.21-0.70)	0.002
Ki-67 group	<20% (ref)	—	<0.001
	20-49%	2.69 (1.85-3.91)	<0.001
	≥50%	1.38 (0.84-2.26)	0.205
Histological grade	Grade 1 (ref)	—	0.001
	Grade 2	6.01 (1.84-19.69)	0.003
	Grade 3	8.95 (2.66-30.16)	<0.001
Tumor type	IDC (ref)	—	0.125
	ILC	0.74 (0.40-1.35)	0.324
	Other	0.58 (0.33-1.03)	0.064

ER: Estrogen receptor; PR: Progesterone receptor; IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; CI: Confidence interval; OR: Odds ratio; Ki-67: Proliferative index.

TABLE 6: Independent predictors of triple-negative phenotype (multivariable logistic regression Model 2).			
Predictor	Category	OR (95% CI)	p-value
Age group	18-39 (ref)	—	—
	40-49	0.86 (0.39-1.87)	0.699
	50-59	1.10 (0.52-2.29)	0.809
	60-69	0.94 (0.44-2.02)	0.876
	≥70	1.20 (0.51-2.85)	0.677
Ki-67 index	≤20% (ref)	—	—
	20-50%	2.87 (1.47-5.61)	0.002
	>50%	12.10 (6.06-24.17)	<0.001
Histological grade	Grade 2 (ref)	—	—
	Grade 3	2.79 (1.79-4.33)	<0.001
Tumor type	IDC (ref)	—	—
	ILC	0.67 (0.23-1.94)	0.457
	Other	1.81 (0.94-3.46)	0.074

Due to the absence of TNBC cases in the grade 1 category, grade 1 and grade 2 tumors were combined in the multivariable regression model. IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; OR: Odds ratio; CI: Confidence interval; TNBC: Triple-negative breast cancer; Ki-67: Proliferative index.

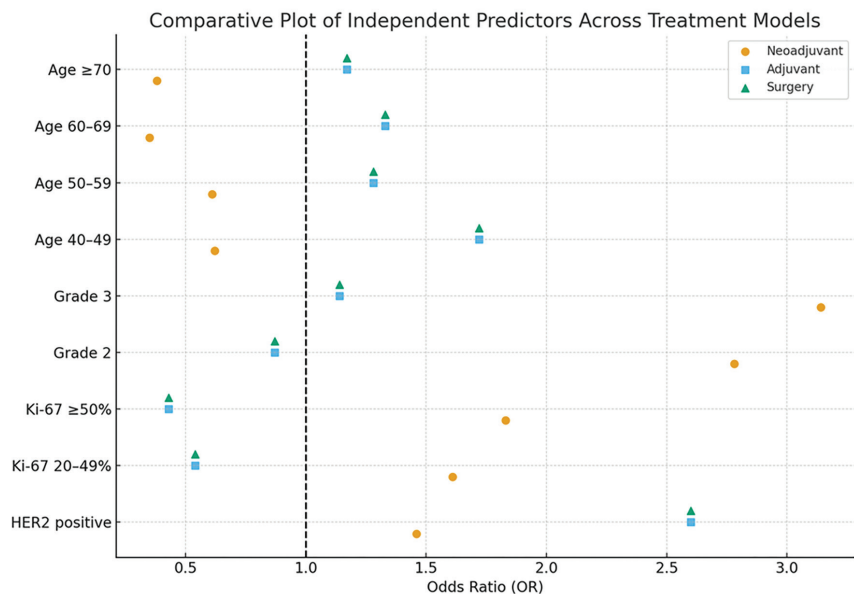


FIGURE 2: Comparative plot of independent predictors across neoadjuvant, adjuvant, and surgical treatment models. Comparative plot illustrating adjusted odds ratios (ORs) for key independent predictors across three multivariable logistic regression models: neoadjuvant therapy (orange circles), adjuvant therapy (blue squares), and surgical treatment (green triangles). The vertical dashed line at OR=1.0 represents the null effect. Each point reflects the estimated OR for the corresponding predictor relative to its reference category.

HER2: Human epidermal growth factor receptor 2; Ki-67: Proliferative index

In older age groups, the predominance of biologically indolent tumor phenotypes and the lower use of intensive systemic treatments are consistent with real-world series that include very elderly patients. Prior studies have shown that patients aged 80 years and older are more likely to harbor luminal tumors, to undergo de-escalated axillary surgery, and are less likely to receive chemotherapy. Moreover, non-breast cancer-related mortality represents a substantial proportion of deaths in this population, underscoring the importance of competing risks in prognostic assessment. Together, these observations support the notion that treatment decisions in older patients are guided not only by tumor biology but also by overall health status and comorbidity burden.^{18,19}

ER positivity increased with advancing age, consistent with national and international data. In contrast, PR expression did not differ significantly across age groups. This finding is notable, as PR is more sensitive than ER to pre-analytical variability and biological heterogeneity. The prognostic and predictive value of PR remains debated and is often context-dependent, emerging in conjunction with proliferation, HER2 status, and molecular subtype. Our results suggest that aggressive tumor biology in younger patients does not uniformly affect all biomarkers and that PR should be interpreted within a multidimensional biological framework rather than as an age-specific marker.^{20,21}

Marked age-related differences were observed in HER2 status and surrogate molecular subtype distribution. HER2-positive tumors were more frequent in younger patients, in line with population-based studies reporting higher rates of HER2-positive and triple-negative disease at younger ages. Similar to the findings of Turhan and Özyurt⁸, HER2-enriched and triple-negative subtypes were more common in patients under 40 years of age. In our cohort, the higher prevalence of HER2 positivity among patients receiving neoadjuvant therapy indicates that HER2 amplification is a key driver of treatment intensification in real-world practice, supporting biology-driven rather than age-driven treatment selection.^{17,22}

The Ki-67 proliferation index was a prominent indicator of age-related biological variation. Higher Ki-67 levels were more common among younger patients, reflecting a predominance of highly proliferative tumors and aligning with previous reports. Elevated Ki-67 was also associated with neoadjuvant treatment use, indicating concordance between proliferative activity and treatment intensity. However, Ki-67 was not used as a standalone determinant; instead, it contributed to integrated biological risk assessment. These findings support the use of Ki-67 as a complementary marker interpreted within the broader context of tumor biology rather than by fixed thresholds.²³⁻²⁵

In our cohort, the HER2-low phenotype (immunohistochemistry 1+ or 2+ with negative fluorescence *in situ* hybridization) was observed in 18.4% of patients, a rate consistent with those reported in real-world series. This finding indicates that HER2-low tumors constitute a clinically relevant proportion of breast cancer cases in routine practice. However, in line with current ASCO/CAP recommendations, HER2-low should be regarded as a treatment-oriented classification rather than an independent biological subtype, particularly in the metastatic setting.^{2,26} Accordingly, HER2-low status in this study was evaluated descriptively to reflect its real-world prevalence rather than for prognostic stratification. Given the known interobserver variability and technical limitations in assessing low-level HER2 expression, these findings should be interpreted with caution. Overall, our results align with contemporary literature emphasizing the need for diagnostic standardization rather than a biological redefinition of the HER2-low category.

Histological grade is a key pathological marker reflecting tumor aggressiveness in invasive breast cancer. Rakha et al.²⁷ demonstrated a strong association between Nottingham histological grade and both disease-free and breast cancer-specific survival. In our cohort, high-grade tumors clustered predominantly within biologically aggressive molecular subtypes, reinforcing the role of histological grade in integrated clinical and biological risk assessment. In contrast, the lack of age-related differences in PR expression suggests that risk stratification in breast cancer is driven primarily by ER status, HER2 expression, and proliferative activity rather than by PR alone.

Luminal B breast cancer warrants particular attention due to its heterogeneous biological behavior. Kang et al.²⁸ reported significantly different clinical outcomes when luminal B tumors were stratified by HER2 status. Similarly, Chen et al.²⁹ showed that HER2-negative luminal B1 tumors exhibit more aggressive pathological features and higher recurrence risk compared with luminal A tumors. The heterogeneity observed among luminal subtypes in our cohort is consistent with contemporary genomic and clinical data, indicating that high-proliferation luminal B tumors carry increased biological risk and may derive greater benefit from adjuvant chemotherapy.³⁰

Study Limitations

One of the major strengths of this study is the large, single-center real-world cohort. With 1,124 patients, it represents one of the most comprehensive datasets reported from Türkiye in this field. Nevertheless, the retrospective design and single-center nature of the study limit the ability to draw causal inferences. In addition, the lack of detailed data on

comorbidities and performance status restricts the scope of clinical interpretation.

Despite these limitations, the findings provide a solid basis for hypothesis generation and support the need for future prospective studies.

This large real-world cohort demonstrates the dynamic interplay between tumor biological risk profiles and treatment decisions in breast cancer. The alignment of aggressive tumor biology with more intensive treatment approaches in younger patients and the de-escalation of treatment parallel to indolent biology in older patients reflect the practical implementation of biology-driven individualized care. These findings underscore that treatment strategies in breast cancer are shaped primarily by tumor biology rather than chronological age alone.

CONCLUSION

This study demonstrates that age-related differences in breast cancer biology are not merely descriptive; they actively influence clinical decision-making in real-world practice. In this large cohort, biomarker profiles and surrogate molecular subtypes varied systematically across age groups and were closely aligned with the type and intensity of treatment strategies. These findings indicate that treatment selection in routine practice is guided primarily by tumor biological characteristics rather than chronological age alone.

The HER2-low phenotype was observed at a clinically meaningful frequency; however, our results support its interpretation as a treatment-oriented classification rather than an independent biological subtype. Overall, this study confirms that biology-driven individualized treatment approaches are effectively implemented in real-world breast cancer care and that age-related biological heterogeneity has tangible clinical implications. These findings provide a strong framework for future prospective and multicenter studies.

Ethics

Ethics Committee Approval: This study was approved by the Çanakkale Onsekiz Mart University Rectorate Non-Interventional Clinical Research Ethics Committee (approval number: 2025-387, date: 10.12.2025).

Informed Consent: Retrospective study.

Footnotes

Author Contributions

Surgical and Medical Practices: V.Ç., İ.G., S.C., E.Ö., Y.G.T., Ş.S.E., Y.Ç., Concept: V.Ç., İ.G., Y.Ç., Design: V.Ç., İ.G., Y.Ç., Data Collection or Processing: V.Ç., S.C., E.Ö., Y.G.T., Ş.S.E., Analysis or Interpretation: V.Ç., İ.G., S.C., Y.Ç., Literature Search: V.Ç., İ.G., S.C., Ş.S.E., Writing: V.Ç., İ.G., Y.Ç.

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Clinicopathologic Features and Survival Outcomes in Women Aged 80 Years or Older with Advanced Ovarian Cancer

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ABSTRACT

Objective: Women aged ≥ 80 years with ovarian cancer (OC) are significantly underrepresented in clinical trials, and their optimal management remains poorly defined. This study aimed to evaluate clinicopathologic characteristics and survival outcomes in this understudied population.

Material and Methods: This retrospective study included 43 women aged ≥ 80 years diagnosed with advanced OC between 2009 and 2024 at a tertiary care center. Clinical, pathological, and treatment data were collected. Kaplan-Meier and Cox regression analyses were used to evaluate time to progression (TTP) and overall survival (OS).

Results: The mean age was 83.4 ± 4.1 years (range: 80-101); 95.3% had at least one comorbidity, and 46.5% had polypharmacy. Despite advanced age, 86% of patients underwent cytoreductive surgery and 79.1% received chemotherapy. Optimal cytoreduction (≤ 1 cm) was achieved in 89.2% of surgically treated patients, with no gross residual disease (R0) obtained in 64.9%. During a median follow-up period of 21.4 months, 76.7% of patients died. The median TTP was 20.9 months [95% confidence interval (CI): 0.0-42.8], and the median OS was 27.1 months (95% CI: 14.9-39.2). The 2 years TTP and OS rates were $49.4\% \pm 9.3$ and $56.2\% \pm 7.8$, respectively. Notably, cytoreductive surgery was associated with improved OS (hazard ratio: 0.171; $p=0.001$).

Conclusion: Cytoreductive surgery can achieve high rates of optimal and complete resection in carefully selected women aged ≥ 80 years with advanced OC when performed at experienced, high-volume centers. These findings indicate that advanced chronological age alone should not be considered a contraindication to surgical evaluation in fit octogenarians.

Keywords: Ovarian cancer; elderly; cytoreductive surgery; polypharmacy

INTRODUCTION

Ovarian cancer (OC) is a major global health concern and remains one of the most lethal gynecologic malignancies.¹ The disease disproportionately affects older women, with the incidence increasing with age and peaking in the seventh decade of life.² Nearly half of all OC cases occur in women over 65, who also account for more than 70% of disease-related deaths.³ As global life expectancy rises, the number of elderly women diagnosed with OC is expected to increase, presenting a growing clinical challenge for gynecologic oncologists.⁴

Despite this high disease burden, older patients are often undertreated. They are less likely than younger patients to undergo optimal cytoreductive surgery or to receive standard chemotherapy.^{3,5} This trend is frequently attributed to concerns regarding comorbidities, functional limitations, and a higher perceived risk of treatment-related complications.^{6,7} Compounding this issue is the historical underrepresentation of elderly women in clinical trials that inform standard-of-care guidelines, leading to limited evidence-based strategies tailored to this population.^{4,8}

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Notably, women aged 80 years and older remain especially underrepresented in the literature, and their clinical characteristics and outcomes are poorly understood.⁹ The primary objective of this study was to evaluate the clinicopathologic features and survival outcomes in a cohort of Turkish women aged 80 years or older diagnosed with advanced OC. By providing real-world data on this distinct and understudied group, the study aims to inform more appropriate management strategies and contribute to the limited body of evidence in this field.

MATERIAL AND METHODS

Study Design and Patient Selection

This retrospective cohort study included women aged ≥ 80 years who were newly diagnosed, treatment-naïve, and had advanced [defined as International Federation of Gynecology and Obstetrics (FIGO) stage III or IV] OC, followed at our tertiary care center between July 2009 and December 2024. A total of 917 patients with OC were initially screened. Patients younger than 80 years, those with early-stage disease, or those with duplicate records, those with missing key clinical or pathological data, those lost to follow-up, those who received initial treatment at an external institution, or those with synchronous malignancies were excluded. Therefore, the final cohort consisted of patients with complete data for all analyzed variables. Treatment strategies, including the decision to proceed with cytoreductive surgery, were individualized. Surgical selection required that the patient have an acceptable clinical performance status (typically independent in activities of daily living), no severe uncompensated comorbidities (such as advanced organ failure), and a manageable estimated tumor burden. Final surgical decisions were based on multidisciplinary team tumor board consensus and pre-anesthesia evaluations. After these criteria were applied, 43 women constituted the final study cohort. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Başkent University Rectorate Medical and Health Sciences Research Board (approval number: KA25/254, date: 25.06.2025). Because the study was retrospective, the requirement for informed consent was waived.

Data Collection

All data were retrieved from the institutional electronic medical records. Variables collected included demographic and clinical information (age at diagnosis, presence and number of comorbidities, and number of regularly used medications). Additionally, baseline laboratory values, including serum albumin and total lymphocyte count, were collected to calculate the prognostic nutritional index (PNI),

a surrogate marker of nutritional and immunological status, with higher values indicating greater nutritional reserve and reduced frailty. PNI was calculated as $10 \times \text{serum albumin (g/dL)} + 0.005 \times \text{total lymphocyte count (per mm}^3\text{)}$ and dichotomized based on the median value. Pathologic features included histological subtype and tumor grade. In patients who underwent cytoreductive surgery [either primary debulking surgery (PDS), or interval debulking surgery (IDS)], additional data were collected on ovarian capsule rupture, lymphovascular invasion (LVI), lymph node involvement, and the surgical stage according to the 2014 FIGO classification. Data on systemic therapies across treatment lines were also collected.

Outcomes

The primary objective was to assess the clinicopathologic characteristics and survival outcomes among elderly women with OC. Polypharmacy was defined as the concurrent use of five or more medications. Time to progression (TTP) was defined as the interval between surgery and either radiological or clinical evidence of disease progression. TTP was chosen over standard progression-free survival to avoid counting non-cancer-related deaths in this elderly cohort as progression events. Because TTP was used to assess the durability of surgical cytoreduction, patients managed non-surgically were excluded from this analysis. Overall survival (OS), conversely, was calculated for the entire cohort and defined as the time from initial diagnosis to death from any cause or until last follow-up for censored cases.

Statistical Analysis

Statistical analyses were performed using IBM® SPSS Statistics, version 25. Baseline characteristics were summarized using descriptive statistics. Kaplan-Meier analysis was used to estimate survival curves, with group comparisons performed using the log-rank test. Univariable Cox proportional hazards regression was used to identify variables associated with TTP and OS. Hazard ratios (HRs) with 95% confidence intervals (CIs) were reported, and two-sided p-values <0.05 were considered statistically significant.

RESULTS

A total of 43 women with a mean age of 83.4 ± 4.1 years (range: 80-101) were included in the study. The majority of patients (95.3%) had at least one comorbid condition, and polypharmacy (≥ 5 medications) was observed in 46.5% of cases. The median baseline PNI of the cohort was 41.0 (range: 21.9-57.5). Despite advanced age and comorbidities, the rate of cytoreductive surgery was 86.0%. PDS was performed in 33 patients, while IDS was conducted in 4 patients. Thirteen patients (35.1%) underwent extended surgery, which

included standard cytoreduction plus major upper abdominal procedures such as splenectomy (n=7) and bowel resection (n=6). Optimal cytoreduction (≤ 1 cm residual disease) was achieved in 33 patients (89.2%), while no gross residual disease (R0) was obtained in 24 patients (64.9%). Stoma formation was required in 3 patients (8.1%). Additionally, 2 patients (4.7%) underwent secondary cytoreductive surgery after the first progression. The 30 days postoperative mortality rate among surgically treated patients was 8.1% (3/37). Baseline clinical and pathological characteristics are summarized in Table 1.

A total of 34 patients (79.1%) received at least one cycle of chemotherapy, with a median of 1 treatment line (range: 1-4). The most commonly administered first-line regimen was paclitaxel plus carboplatin (31/34, 91.2%); subsequent-line regimens varied. Based on their clinical response following completion of initial first-line platinum-based therapy, 31 (93.9%) patients were classified as platinum-sensitive, and 2 (6.1%) were classified as platinum-resistant after developing disease progression within 6 months. Second-line, third-line, and fourth-line treatment rates were 34.9% (n=15), 11.6% (n=5), and 4.7% (n=2), respectively.

During a median follow-up of 21.4 months (range: 0.4-132.7), 33 patients (76.7%) died. The median TTP was 20.9 months (95% CI: 0.0-42.8), while the median OS was 27.1 months (95% CI: 14.9-39.2). The 2 years TTP and OS rates were $49.4\% \pm 9.3$ and $56.2\% \pm 7.8$, respectively (Figure 1). In univariable analyses, age ≥ 85 years (vs. 80-84 years), ≥ 3 comorbid conditions, polypharmacy, PNI (<41 vs. ≥ 41), high-grade serous histology, LVI, para-aortic lymph node involvement, or FIGO stage IV disease (vs. III) were not significantly associated with TTP (Table 2). OS outcomes were generally consistent with TTP findings. Notably, the receipt of cytoreductive surgery was significantly associated with improved OS (HR: 0.171; 95% CI: 0.061-0.477; $p=0.001$) (Figure 2).

DISCUSSION

This study provides real-world data on the clinicopathologic features and survival outcomes of women aged 80 years or older with advanced OC, who remain significantly underrepresented in clinical research.^{10,11} Our findings demonstrate that in a carefully selected cohort, aggressive treatment is feasible and associated with meaningful outcomes. The median OS was 27.1 months, and univariable analysis showed that cytoreductive surgery was significantly associated with improved survival. These results challenge the common practice of undertreating the oldest old based solely on chronological age and highlight the importance of assessing physiological rather than chronological age.

TABLE 1: Baseline characteristics.

Characteristics	Total 43 patients
Age at diagnosis, mean $\pm$ SD, years	83.4 $\pm$ 4.1
Age categories, n (%)	
80-84 years	29 (67.4)
85-89 years	11 (25.6)
≥ 90 years	3 (7.0)
Presence of comorbid disease(s), n (%)	41 (95.3)
Comorbidities, n (%)	
Hypertension	37 (86.0)
Coronary artery disease	19 (44.2)
Diabetes mellitus	17 (39.5)
Malignancy	5 (11.6)
Chronic kidney disease	4 (9.3)
Heart failure	2 (4.7)
Number of comorbidities, median (range)	2 (0-5)
Number of drugs used, median (range)	4 (0-11)
Polypharmacy (≥ 5 drugs), n (%)	20 (46.5)
PNI, median (range)	41.0 (21.9-57.5)
Histological subtype, n (%)	
High-grade serous	39 (90.7)
Low-grade serous	2 (4.7)
Non-serous	2 (4.7)
Cytoreductive surgery, n (%)	
Primary debulking surgery	33/37 (89.2)
Interval debulking surgery	4/37 (10.8)
Extended surgery ^a , n (%)	13/37 (35.1)
Optimal cytoreduction (≤ 1 cm), n (%)	33/37 (89.2)
No gross residual disease (R0), n (%)	24/37 (64.9)
Secondary cytoreductive surgery, n (%)	2 (4.7)
Stoma formation, n (%)	3 (8.1)
Ruptured ovarian capsule*, n (%)	35/37 (94.6)
Lymphovascular invasion*, n (%)	29/37 (78.4)
Lymph node involvement*, n (%)	
Pelvic	11/37 (29.7)
Para-aortic	2/37 (5.4)
Both	8/37 (21.6)
FIGO surgical staging*, n (%)	
III	32/37 (86.5)
IV	5/37 (13.5)

^a Standard cytoreduction plus major upper abdominal organ resection.

* For patients who underwent debulking surgery.

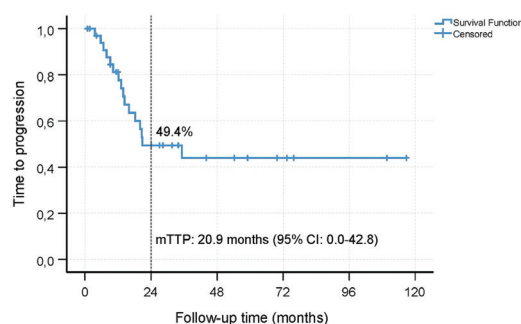
FIGO: International Federation of Gynecology and Obstetrics; PNI: Prognostic nutritional index; SD: Standard deviation.

TABLE 2: Univariate Cox regression analyses for time to progression and overall survival.

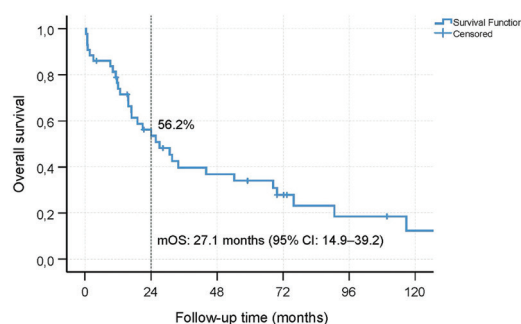
Parameters	Time to progression (events=16)			Overall survival (events=33)		
	HR	95% CI	p-value	HR	95% CI	p-value
Age ≥85 years (vs. 80-84 years)	1.894	0.685-5.236	0.219	1.443	0.694-2.999	0.326
≥3 comorbidities	1.365	0.495-3.765	0.548	1.766	0.874-3.566	0.113
Polypharmacy (≥5 drugs)	1.128	0.409-3.116	0.816	1.796	0.890-3.621	0.102
PNI ≥41 (vs. <41)	0.628	0.234-1.685	0.355	0.905	0.441-1.855	0.785
High-grade serous histology	1.127	0.254-5.005	0.876	1.164	0.397-3.411	0.782
Cytoreductive surgery	-	-	-	0.171	0.061-0.477	0.001
Lymphovascular invasion	2.907	0.657-12.86	0.159	1.122	0.443-2.843	0.808
Paraaortic LN involvement	0.307	0.069-1.372	0.122	0.826	0.342-1.996	0.672
FIGO stage IV (vs. III)	2.779	0.787-9.814	0.112	1.059	0.352-3.183	0.919

* Bold text indicates statistical significance at $p < 0.05$ level.

CI: Confidence interval; FIGO: International Federation of Gynecology and Obstetrics; HR: Hazard ratio; LN: Lymph node; PNI: Prognostic nutritional index.



A

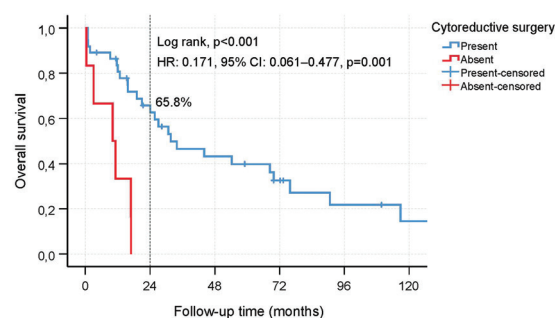


B

FIGURE 1: Kaplan-Meier curves for time to progression (A) and overall survival (B).

OS: Overall survival; CI: Confidence interval; TTP: Time to progression

A key finding is the significant survival benefit conferred by cytoreductive surgery (HR: 0.171; $p=0.001$). In our cohort, a notably high proportion (86%) underwent surgery, likely contributing to the favorable median OS. This contrasts sharply with population-based studies in which elderly patients are frequently denied surgery. For instance, a recent registry study reported that only 27% of women aged ≥ 80 received both surgery and chemotherapy,¹¹ while other reports consistently show lower rates of guideline-concordant care in older adults.^{12,13} Our results align with evidence from specialized centers demonstrating that selected elderly

**FIGURE 2: Kaplan-Meier curve for overall survival stratified by cytoreductive surgery.**

HR: Hazard ratio; CI: Confidence interval

(≥ 65) and very elderly (≥ 75) patients can tolerate radical procedures and achieve cytoreduction rates comparable to younger cohorts.¹⁴⁻¹⁶ These findings reinforce that maximal cytoreduction remains a cornerstone of OC management, even in the oldest old.^{17,18}

In our cohort, 89.2% of surgically treated patients achieved optimal cytoreduction (≤ 1 cm residual disease), with 64.9% having no gross residual disease (R0), rates that compare favorably with those reported in large multicenter analyses. For instance, a combined exploratory analysis of three randomized trials by du Bois et al.¹⁹ demonstrated that only 33.5% of patients achieved complete resection (R0), while 31.2% had small residual tumor burden (1-10 mm), and 35.3% had macroscopic residual disease exceeding 1 cm. Notably, their multivariate analysis showed that complete resection was associated with the most significant improvement in progression-free survival and OS compared with patients who had any residual disease, underscoring the critical prognostic impact of complete cytoreduction. Similarly, Bristow et al.²⁰, in a meta-analysis evaluating 81 cohorts encompassing 6,885 patients with stage III-IV ovarian carcinoma, reported

that each 10% increase in maximal cytoreduction rate was associated with a 5.5% increase in median survival time. Cohorts achieving $\leq 25\%$ maximal cytoreduction had a median survival of 22.7 months, whereas those with $>75\%$ maximal cytoreduction had a median survival of 33.9 months, representing a 50% improvement. Our cohort's optimal cytoreduction rate, exceeding 75%, likely contributed to the relatively favorable median OS of 27.1 months despite the advanced age and comorbidity burden of the population.

Additionally, Eisenhauer et al.²¹ reported that patients who required extensive upper abdominal procedures to achieve optimal cytoreduction had a median OS that was not reached at 68 months, compared to 84 months for those optimally cytoreduced with standard techniques and only 38 months for patients who were suboptimally cytoreduced. While our study did not include a formal comparison based on the extent of upper abdominal surgery, 35.1% of our patients underwent extensive surgical procedures, including splenectomy and bowel resection, supporting the feasibility of aggressive surgery in selected octogenarians. Importantly, their findings highlighted that patients requiring extensive procedures to achieve optimal cytoreduction had survival outcomes comparable to those optimally cytoreduced by standard surgery, reinforcing that tumor biology, rather than resection extent per se, drives prognosis.

Furthermore, Aletti et al.²² demonstrated that residual disease was the strongest independent predictor of survival in patients with stage IIIC OC, and minimizing residual disease through aggressive surgical resection was beneficial, especially in those with carcinomatosis. In their study, the use of radical procedures to achieve optimal cytoreduction was associated with a significant survival advantage, increasing five-year disease-specific survival from 17% to 44% among patients with peritoneal carcinomatosis. Taken together, these studies corroborate our findings that achieving optimal cytoreduction, particularly R0 resection, is critical for improving survival even in the oldest-old. Our cohort's high rates of optimal and complete cytoreduction are notable, likely reflecting surgical expertise at our tertiary center; optimal resection rates vary significantly between institutions and often exceed 75% only at specialized centers.²⁰

Interestingly, in univariable analyses, none of the traditional risk factors including age ≥ 85 years, number of comorbidities, high-grade serous histology, LVI, para-aortic lymph node involvement, or stage IV disease were significantly associated with TTP or OS. The prognostic impact of frailty, which encompasses factors beyond comorbidities, such as functional status, nutrition, and cognition, is increasingly recognized as more critical than age itself.^{23,24} Validated tools, such as the geriatric vulnerability score, have been shown to have

high prognostic performance for survival, superior to that of age.²⁵ Similarly, markers of nutritional status and sarcopenia are strongly associated with outcomes in OC patients.^{26,27} Although polypharmacy did not reach statistical significance as a predictor in our study, it remains an important clinical marker warranting further evaluation in larger cohorts.¹⁴

The survival outcomes in our cohort are noteworthy. The median OS of 27.1 months for women aged ≥ 80 is comparable to outcomes reported for younger "elderly" cohorts. For example, Liontos et al.²³ reported a median OS of 30.2 months for patients older than 70 years, while Ferrero et al.²⁴ observed a median survival of 30 months in patients older than 75. Importantly, all our patients had advanced-stage disease, yet their survival was comparable to that of younger elderly cohorts with a broader distribution of disease stages. The encouraging survival observed in our much older advanced-stage cohort likely reflects the high rates of standard treatment, including both surgery and platinum-based chemotherapy. This suggests that when treatment is not withheld because of age, women in their ninth decade of life and beyond can achieve outcomes similar to those of their younger counterparts. Furthermore, studies that have controlled for selection bias have shown that neoadjuvant chemotherapy and PDS yield similar survival in elderly women, particularly those over 80 or with stage IV disease, supporting the use of tailored surgical strategies in this population.^{28,29}

Study Limitations

This study has several limitations inherent in its design. Being a retrospective, single-center analysis, it is susceptible to selection bias. Patients treated at an experienced tertiary care center may be more motivated than the general population, which could explain the high rates of surgery and the favorable outcomes observed. The small sample size severely limited the statistical power of our analyses. Consequently, the lack of statistical significance for well-established prognostic factors, such as FIGO stage, LVI, and tumor histology, must be strictly interpreted as a type II error due to insufficient statistical power, rather than as a true absence of biological effect in this age group. Furthermore, since only cytoreductive surgery emerged as a significant predictor in the univariable analysis and the number of events was limited, a formal multivariable Cox regression was not performed to avoid statistical overfitting. Without multivariable adjustment, our finding that surgery improves survival is not definitive and must be interpreted strictly as hypothesis-generating. While representing a limitation to statistical power, the small sample size also reflects a broader clinical reality: even in high-volume centers like

ours, the number of patients over 80 receiving treatment is very low. This is often due to a prevailing tendency to avoid aggressive therapies in this age group. A major limitation of our study is that it does not define the specific criteria for selecting patients for surgery. Although our results show that cytoreductive surgery provides a significant survival benefit, they do not identify which patients are ideal candidates or what the optimal selection criteria should be. This limitation primarily stems from the retrospective nature of our analysis. While our surgical team undoubtedly performed thorough preoperative assessments, specific details such as functional status and comprehensive laboratory data were not systematically recorded, preventing a rigorous and standardized evaluation. The absence of formal geriatric assessment data is another major shortcoming; polypharmacy and PNI were used as proxies, but they cannot replace a comprehensive evaluation of frailty. Calculating TTP from the date of surgery introduces a non-uniform starting point between patients undergoing PDS and the small subset of patients (n=4) undergoing IDS because the duration of neoadjuvant chemotherapy is excluded from the time-to-event calculation. While the very low number of interval debulking cases likely minimizes the impact of this lead-time bias on the overall cohort estimates, it remains a methodological limitation. Despite these limitations, our study delivers a crucial message: successful cytoreductive surgery with high rates of optimal and R0 resection is achievable in patients aged 80 and over and is associated with improved OS in this selected cohort. The primary conclusion is that advanced age and the presence of large-volume disease at diagnosis should not be regarded as absolute contraindications to potentially curative-intent surgery. Instead, these patients warrant careful multidisciplinary evaluation. The critical next step for the field is to move beyond simply showing that surgery is possible and to focus on determining for whom it is appropriate. Larger-scale prospective studies are urgently needed to establish robust, evidence-based selection criteria that incorporate comprehensive geriatric assessments to ensure that appropriate patients receive appropriate treatment.

CONCLUSION

Our study demonstrates that selected women aged 80 years and older with advanced OC can tolerate standard treatment, which is associated with clinically meaningful survival outcomes. Chronological age alone should not be considered an absolute contraindication to curative-intent therapy. Instead, treatment decisions should be individualized on the basis of a comprehensive assessment of each patient's

physiological status, functional reserve, and overall fitness. Importantly, the high rates of surgery and favorable survival outcomes observed in this study reflect a carefully selected cohort of octogenarians treated at an experienced tertiary center. Accordingly, aggressive treatment strategies should be reserved for patients who are rigorously evaluated and managed in high-volume centers with multidisciplinary expertise. With meticulous preoperative assessment and optimal surgical management, selected women aged 80 years and older can achieve high rates of complete cytoreduction and survival outcomes that approach those reported in younger elderly cohorts.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Başkent University Rectorate Medical and Health Sciences Research Board (approval number: KA25/254, date: 25.06.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: R.I., H.A., A.F., Ö.A., N.Ö., Ü.E.K., Concept: R.I., H.A., Ö.A., N.Ö., Ü.E.K., Design: R.I., H.A., Ö.A., N.Ö., Ü.E.K., Data Collection or Processing: R.I., H.A., A.F., Analysis or Interpretation: R.I., H.A., Ö.A., N.Ö., Ü.E.K., Literature Search: R.I., H.A., Writing: R.I., H.A.

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

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Immunohistochemical Expression of EZH2 and INI-1 in Hepatocellular Carcinoma: Prognostic Significance in a Retrospective Cohort Study

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ABSTRACT

Objective: Enhancer of zeste homologue 2 (EZH2) serves as the enzymatic component of polycomb repressive complex 2, while SMARCB1/INI-1 is an essential subunit of the SWI/SNF chromatin remodeling complex; both function as epigenetic regulators with proposed roles in hepatocarcinogenesis. This study aimed to evaluate the expression of EZH2 and INI-1 by immunohistochemistry (IHC) in hepatocellular carcinoma (HCC) and to determine their association with clinicopathological features and survival outcomes.

Material and Methods: We retrospectively studied 103 patients with histologically proven HCC who were treated at a single center between 2011 and 2019. EZH2 and INI-1 were evaluated by IHC in archival, paraffin-embedded specimens, with a case considered positive when at least 1% of tumor nuclei showed distinct staining. Relationships with clinicopathological variables were tested using the chi-square test or Fisher's exact test, and overall survival (OS) and progression-free survival (PFS) were assessed using Kaplan-Meier estimation and Cox proportional hazards modeling.

Results: EZH2 positivity occurred in 45 patients (43.7%), whereas INI-1 loss occurred in 11 (10.7%). Over a median follow-up of 42.0 months, 71 deaths and 86 progression events were observed. EZH2-positive cases exhibited significantly shorter median OS (8.0 vs. 27.0 months; $p=0.007$) and PFS (5.0 vs. 9.0 months; $p=0.012$). In univariate Cox regression analysis, EZH2 positivity was a significant risk factor for both mortality [hazard ratio (HR): 1.88; 95% confidence interval (CI): 1.17-3.02; $p=0.009$] and disease progression (HR: 1.69; 95% CI: 1.10-2.60; $p=0.018$). However, EZH2 did not retain independent prognostic significance in multivariate analysis adjusted for Barcelona Clinic Liver Cancer (BCLC) stage for either OS (HR: 1.55; 95% CI: 0.95-2.53; $p=0.078$) or PFS (HR: 1.39; 95% CI: 0.89-2.17; $p=0.146$). INI-1 loss showed a trend toward longer PFS (22.0 vs. 6.0 months; $p=0.051$) and OS (30.0 vs. 15.0 months; $p=0.097$), but these differences did not reach statistical significance.

Conclusion: EZH2 positivity is associated with inferior survival in HCC; however, this association does not remain independent of BCLC stage, indicating a stage-dependent rather than independent prognostic effect. INI-1 loss showed only borderline exploratory associations with survival that require validation in larger series. Together, these observations support the biological relevance of epigenetic regulators in HCC.

Keywords: Hepatocellular carcinoma; EZH2; INI-1; SMARCB1; prognosis; immunohistochemistry; epigenetics; biomarker

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for a large share of malignancies and is among the leading contributors to cancer mortality worldwide. In 2020, approximately 906,000 individuals worldwide were newly diagnosed with liver cancer—of which HCC was the dominant histological type—and the disease ranked third in cancer-related deaths.

Given a five-year relative survival of approximately 18% and approximately 830,000 deaths recorded that same year, the small margin between new diagnoses and fatalities underscores the aggressive behavior of this tumor.¹⁻³

Efforts to deliver precision medicine in HCC are constrained by the disease's pronounced heterogeneity, both across different tumors and within a single lesion; the limited

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number of biomarkers validated for prognostic and treatment-selection purposes; and the drawbacks inherent to invasive tissue sampling. Consequently, identifying accessible and dependable molecular markers remains essential for improving risk stratification and tailoring therapy to the individual patient.⁴

HCC develops through a multistep sequence in which genetic and epigenetic alterations progressively accumulate. Among these, epigenetic dysregulation has been recognized as a central driver of tumor development, acting chiefly through disturbances in deoxyribonucleic acid (DNA) methylation, histone modifications, and non-coding ribonucleic acids (RNAs).^{5,6} Two histone-modifying systems exert opposing effects on chromatin: polycomb repressive complex 2 (PRC2) silences transcription, while the SWI/SNF remodeling complex renders chromatin accessible for transcriptional activation.^{7,8}

As the catalytic subunit of PRC2, enhancer of zeste homologue 2 (EZH2) represses transcription by trimethylating histone H3 at lysine 27 (H3K27me3)—a modification that condenses chromatin and inactivates genes.⁹ Because many of the genes silenced are tumor suppressors, this process constitutes an important oncogenic driver in hepatocarcinogenesis.^{10,11} Accumulating evidence implicates EZH2 as a regulator of cell proliferation and disease progression in HCC, primarily through the silencing of tumor suppressor genes such as chromodomain helicase DNA binding protein 5 (CHD5), a confirmed suppressor that favors senescence and restrains proliferation. By trimethylating H3K27 at the CHD5 promoter, *EZH2* silences this protective gene and thereby supports tumor growth.^{12,13}

Opposing PRC2, the SWI/SNF complex restores an open, transcription-permissive chromatin configuration. A central component of this complex, SMARCB1/INI-1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1) behaves as a tumor suppressor across a range of cancers.⁸ Although the loss of INI-1 has been thoroughly described in rhabdoid and other tumors, little is known about INI-1 expression in HCC or whether it has prognostic relevance.¹⁴

Therefore, this study aimed to evaluate the expression of EZH2 and INI-1 by immunohistochemistry (IHC) in a single-institution cohort of HCC patients and to determine their association with clinicopathological features and survival outcomes, and to assess their value as prognostic biomarkers.

MATERIAL AND METHODS

Study Design and Population

This single-institution, retrospective cohort study was conducted following approval from the Çukurova University

Faculty of Medicine Clinical Research Ethics Committee (approval number: 38; date: 13.04.2018) and in accordance with the Declaration of Helsinki. Reporting adheres to the STROBE statement. This study was funded by the Çukurova University Scientific Research Projects Unit (project number: TTU-2018-10738).

The study cohort comprised 103 patients with pathologically confirmed HCC who were managed at our institution between 2011 and 2019. Because the analysis was retrospective and relied on fully de-identified records, the ethics committee waived the need for individual informed consent. Clinicopathological information—age at diagnosis, sex, etiology, Barcelona Clinic Liver Cancer (BCLC) stage, and Child-Pugh class—was retrieved from patient charts. Treatment modalities administered during the disease course, including surgical resection, liver transplantation, locoregional therapies (transarterial chemoembolization, transarterial radioembolization, and percutaneous thermal ablation), systemic therapy (sorafenib), cytotoxic chemotherapy, and best supportive care were recorded where available.

Immunohistochemical Analysis

IHC analysis was performed on archival formalin-fixed, paraffin-embedded tissue blocks from all 103 cases. Five-micron-thick sections were processed on a Ventana BenchMark XT automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) using the ultraView Universal DAB Detection Kit.

To assess EZH2 protein expression, sections were incubated with a primary mouse monoclonal antibody against EZH2 (Clone 11; Cat. No. 415M-15; Cell Marque, Rocklin, CA, USA). For INI-1 assessment, a primary mouse monoclonal antibody targeting SMARCB1/INI-1 was used (Clone: MRQ-27, Cat. No. 272M-15; Cell Marque, Rocklin, CA, USA). All stained slides were evaluated by an experienced pathologist who was blinded to the clinical data. For both markers, a case was considered positive if distinct nuclear staining was observed in $\geq 1\%$ of the tumor cells. Loss of INI-1 expression was defined as the absence of nuclear staining in tumor cells, with retained expression in surrounding non-neoplastic cells serving as an internal positive control. A $\geq 1\%$ nuclear threshold was applied because EZH2 nuclear staining in HCC is frequently heterogeneous and focal, and a low cut-off maximizes sensitivity for detecting any tumoral EZH2 expression in a routine diagnostic setting. Because staining intensity and H-score were not systematically recorded, we use the term “EZH2 positivity” rather than “overexpression” throughout, to reflect a binary, presence-based scoring approach.

Statistical Analysis

All statistical analyses were conducted using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Associations between biomarker expression status and clinicopathological parameters were assessed using the chi-square test or Fisher's exact test. Overall survival (OS) and progression-free survival (PFS) were estimated by the Kaplan-Meier method and compared using the log-rank test. The prognostic value of EZH2 was further evaluated by univariate and multivariate Cox proportional hazards regression. OS was measured from the date of diagnosis until death from any cause, whereas PFS was measured from the start of treatment until radiological progression, recurrence, or death, whichever occurred first. A two-sided p-value of <0.05 was considered statistically significant for all analyses.

The multivariate Cox model included EZH2 status and BCLC stage, with 71 OS events and 86 PFS events for four estimated parameters (approximately 17.8 and 21.5 events per variable, respectively), exceeding the recommended minimum of ten. Complete-record analysis was used without imputation. The proportional hazards assumption was assessed using scaled Schoenfeld residuals and was satisfied for all covariates in both the OS and PFS models (all $p > 0.05$). Child-Pugh class was not entered into the multivariate model because liver function and performance status are already incorporated within BCLC staging, thereby avoiding collinearity. The median follow-up duration was estimated using the reverse Kaplan-Meier method.

RESULTS

Patient Characteristics

A total of 103 patients with pathologically confirmed HCC were included in this retrospective study. The baseline clinicopathological characteristics of the cohort are summarized in Table 1. The cohort was predominantly male (86.4%; $n=89$), with a median age at diagnosis of 68 years (range, 17-89). The most common underlying etiology was hepatitis B (57.3%), followed by non-viral causes (29.1%) and hepatitis C (13.6%). At diagnosis, the majority of patients were classified as Child-Pugh class A (58.3%), and early-stage disease (BCLC stages 0 and A) comprised 43.7% of the cohort (Table 1). With respect to treatment, 43 patients (41.7%) received locoregional therapy with or without sorafenib; the remaining patients received surgical, systemic, or supportive approaches, alone or in combination, according to BCLC stage and liver function. Detailed patient-level treatment allocation could not be uniformly retrieved for all cases owing to the retrospective design.

EZH2 and INI-1 Expression

IHC analysis was performed on all 103 tumor specimens. Representative IHC images are shown in Figure 1A-D. Using the predefined cut-off ($\geq 1\%$ distinct nuclear staining), EZH2 positivity was detected in 45 patients (43.7%), while 58 patients (56.3%) were classified as EZH2-negative. INI-1 nuclear expression was retained in 92 patients (89.3%), while loss of expression was observed in 11 patients (10.7%).

Association of EZH2 Expression with Clinicopathological Features

The association between EZH2 positivity and clinicopathological parameters is presented in Table 2. No statistically significant association was observed between EZH2 status and underlying etiology ($p=0.789$). Although a higher proportion of EZH2 positivity was observed in advanced BCLC stages (65.2% in stage C vs. 30.0% in stage

TABLE 1: Baseline clinicopathological characteristics of the study cohort ($n=103$).

Characteristic	Values ^a
Age (years), median (range)	68 (17-89)
Sex, n (%)	
Male	89 (86.4)
Female	14 (13.6)
Etiology, n (%)	
Hepatitis B	59 (57.3)
Hepatitis C	14 (13.6)
Non-viral	30 (29.1)
Child-Pugh score, n (%)	
Class A	60 (58.3)
Class B	30 (29.1)
Class C	13 (12.6)
BCLC stage, n (%)	
Stage 0	5 (4.9)
Stage A	40 (38.8)
Stage B	14 (13.6)
Stage C	23 (22.3)
Stage D	21 (20.4)
EZH2 expression, n (%)	
Positive	45 (43.7)
Negative	58 (56.3)
INI-1 (SMARCB1) expression, n (%)	
Retained	92 (89.3)
Loss	11 (10.7)

^a Data are presented as number (percentage) unless otherwise indicated. INI-1/SMARCB1, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1.

BCLC: Barcelona Clinic Liver Cancer; EZH2: Enhancer of zeste homologue 2.

A), this trend did not reach statistical significance ($p=0.064$) (Table 2).

Survival Analysis

At the time of analysis, 71 of 103 patients (68.9%) had died, and 86 (83.5%) had experienced disease progression, resulting in 71 OS events and 86 PFS events. The median follow-up duration, estimated by the reverse Kaplan-Meier method, was 42.0 months. Median OS and PFS for the entire cohort were 16.0 months [95% confidence interval (CI): 9.8-22.2] and 6.0 months (95% CI: 3.7-8.3), respectively. Kaplan-Meier survival analysis demonstrated that EZH2 positivity was significantly associated with inferior survival outcomes. EZH2-positive patients had a significantly shorter median OS than the EZH2-negative group (8.0 vs. 27.0 months; log-rank $p=0.007$) (Figure 2). A similar association was observed for PFS, with a median of 5.0 months in the EZH2-positive group compared with 9.0 months in the EZH2-negative group (log-rank $p=0.012$) (Figure 3). As expected, worsening Child-Pugh class and advanced BCLC stage were also significantly associated with reduced median OS and PFS (Table 3).

In an exploratory analysis of INI-1 expression, the INI-1-loss group demonstrated numerically longer median PFS compared with the INI-1-retained group (22.0 vs. 6.0 months); however, this difference did not reach statistical significance (log-rank $p=0.051$). Similarly, median OS was numerically longer in the INI-1-loss group (30.0 vs. 15.0 months; log-rank $p=0.097$). Among the 11 patients with INI-1 loss, 6 died and 8 progressed; the median OS and PFS were 30.0 and 22.0 months, respectively. Notably, this subgroup was enriched for early-stage disease: 7 of 11 patients were classified as BCLC stage 0 or A (stage 0, $n=2$; stage A, $n=5$), with only 2 patients in stage B and 1 patient each in stages

C and D. This stage imbalance may largely account for the numerically longer survival observed in the INI-1-loss group. Given the small subgroup size and this distributional skew, these findings are strictly exploratory and hypothesis-generating. In a further exploratory analysis restricted to the 43 patients who received locoregional therapy with or without sorafenib, EZH2 positivity remained associated with significantly shorter median PFS (4.0 vs. 14.0 months; log-rank $p=0.014$) and numerically shorter OS (16.0 vs. 41.0 months; $p=0.077$), supporting the relevance of EZH2 within a treated subgroup.

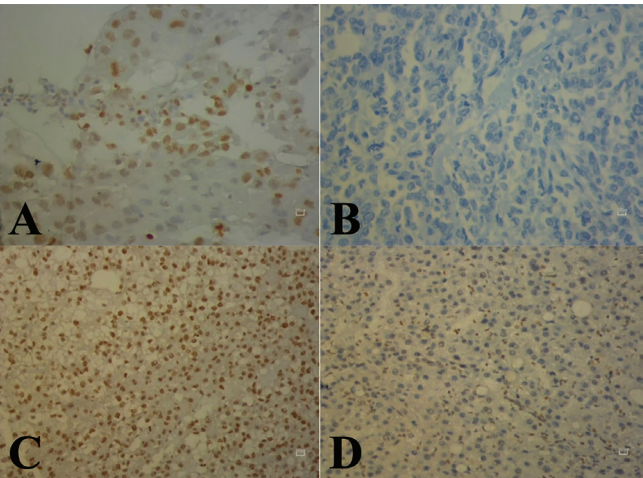


FIGURE 1: Representative immunohistochemical staining patterns for EZH2 and INI-1 in hepatocellular carcinoma. (A) EZH2-positive tumor demonstrating distinct nuclear staining in tumor cells (scale bar = 100 μ m). (B) EZH2-negative tumor with absent nuclear immunoreactivity (scale bar = 100 μ m). (C) Retained INI-1 expression showing diffuse nuclear staining in tumor cells (scale bar = 100 μ m). (D) Loss of INI-1 expression in tumor cells with retained nuclear staining in adjacent non-neoplastic cells serving as an internal positive control (scale bar = 100 μ m).

EZH2: Enhancer of zeste homologue 2

TABLE 2: Association between EZH2 positivity and clinicopathological parameters.

Characteristic	Total (n=103)	EZH2 positive (n=45)	EZH2 negative (n=58)	p-value ^a
Etiology, n (%)				0.789
Hepatitis B	59	26 (44.1)	33 (55.9)	
Hepatitis C	14	5 (35.7)	9 (64.3)	
Non-viral	30	14 (46.7)	16 (53.3)	
BCLC stage, n (%)				0.064
Stage 0	5	1 (20.0)	4 (80.0)	
Stage A	40	12 (30.0)	28 (70.0)	
Stage B	14	7 (50.0)	7 (50.0)	
Stage C	23	15 (65.2)	8 (34.8)	
Stage D	21	10 (47.6)	11 (52.4)	

^a Chi-square test or Fisher's exact test. EZH2: Enhancer of zeste homologue 2.

Cox Regression Analysis

Univariate and multivariate Cox proportional hazards regression analyses were performed to identify prognostic factors for both OS and PFS (Table 3). In univariate analysis, EZH2 positivity was a significant risk factor for both shorter OS [hazard ratio (HR): 1.88; 95% CI: 1.17-3.02; $p=0.009$] and shorter PFS (HR: 1.69; 95% CI: 1.10-2.60; $p=0.018$). Advanced BCLC stage was also significantly associated with poorer outcomes in a stage-dependent manner: compared with stage A, stage C was associated with substantially increased risks of death (HR: 3.79; 95% CI: 1.96-7.32; $p<0.001$) and of progression (HR: 2.59; 95% CI: 1.44-4.67; $p=0.002$), while stage D conferred the highest risk for both OS (HR: 23.44; 95% CI: 10.41-52.76; $p<0.001$) and PFS (HR: 8.50; 95% CI: 4.44-16.28; $p<0.001$). Worsening Child-Pugh class was significantly associated with inferior OS (class B vs. A: HR: 2.11; 95% CI: 1.23-3.61; $p=0.007$; class C vs. A: HR: 11.94; 95% CI: 5.61-25.42; $p<0.001$) and PFS (class C vs. A: HR: 6.50; 95% CI: 3.30-12.81; $p<0.001$).

In multivariate analysis adjusted for BCLC stage, EZH2 positivity did not retain independent prognostic significance for either OS (HR: 1.55; 95% CI: 0.95-2.53; $p=0.078$) or PFS (HR:

1.39; 95% CI: 0.89-2.17; $p=0.146$); BCLC stage remained the dominant independent predictor of survival (Table 3).

In a sensitivity analysis that combined BCLC stages 0 and A into a single early-stage reference category (thereby retaining all 103 patients), the direction and magnitude of the EZH2 effect were similar. EZH2 reached nominal significance for OS (HR: 1.71; 95% CI: 1.05-2.79; $p=0.030$) but not for PFS (HR: 1.51; 95% CI: 0.97-2.35; $p=0.067$); BCLC stage remained the dominant predictor. Given that the point estimate hovered near the significance threshold across model specifications, we interpret EZH2 as indicating a borderline stage-dependent prognostic signal rather than a robust independent effect. To examine whether EZH2 retained discriminatory value within a single stage, we additionally analyzed the BCLC stage C subgroup ($n=23$), the largest advanced-stage group. EZH2 positivity was associated with significantly shorter median OS (7.0 vs. 23.0 months; log-rank $p=0.002$) and a non-significant trend for PFS (4.0 vs. 6.0 months; $p=0.058$), indicating that EZH2 may carry prognostic information even within advanced-stage disease.

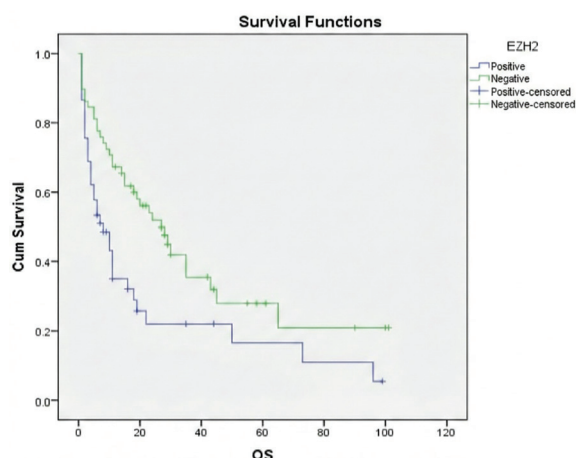


FIGURE 2: Kaplan-Meier curves for OS according to EZH2 expression status. EZH2-positive patients ($n=45$) demonstrated significantly shorter median OS compared with EZH2-negative patients ($n=58$) (8.0 vs. 27.0 months; log-rank $p=0.007$).

EZH2: Enhancer of zeste homologue 2; OS: Overall survival

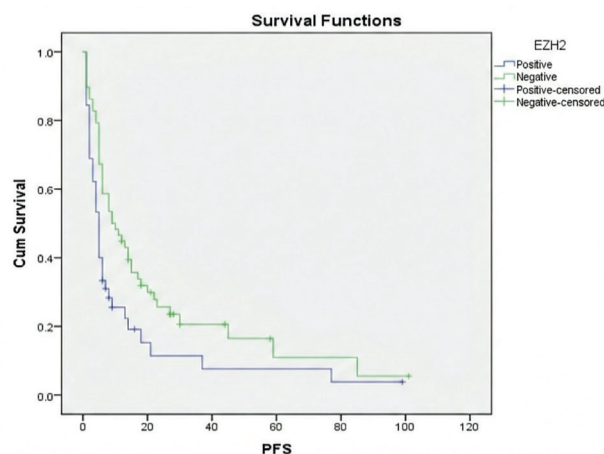


FIGURE 3: Kaplan-Meier curves for progression-free survival according to EZH2 expression status. Median PFS was 5.0 months in the EZH2-positive group vs. 9.0 months in the EZH2-negative group (log-rank $p=0.012$).

EZH2: Enhancer of zeste homologue 2; PFS: Progression-free survival

TABLE 3: Survival analysis and Cox proportional hazards regression for overall survival and progression-free survival.

Panel A. Kaplan-Meier survival estimates.

Variable	n	Median PFS (months)	p-value	Median OS (months)	p-value
EZH2 expression					0.007
Positive	45	5.0	0.012	8.0	
Negative	58	9.0		27.0	
INI-1 expression					0.097
Retained	92	6.0	0.051	15.0	
Loss	11	22.0		30.0	

TABLE 3: Continued.

Panel A. Kaplan-Meier survival estimates.					
Variable	n	Median PFS (months)	p-value	Median OS (months)	p-value
Child-Pugh class					<0.001
A	60	9.0	<0.001	29.0	
B	30	6.0		9.0	
C	13	1.0		1.0	
BCLC stage					<0.001
A	40	15.0	<0.001	45.0	
B	14	5.0		15.0	
C	23	5.0		11.0	
D	21	1.0		1.0	
Panel B. Cox proportional hazards regression analysis.					
Variable	PFS HR (95% CI)		p-value	OS HR (95% CI)	p-value
Univariate analysis					
EZH2 (positive vs. negative)	1.69 (1.10-2.60)		0.018	1.88 (1.17-3.02)	0.009
Age (per year)	1.01 (0.99-1.02)		0.532	1.01 (0.99-1.03)	0.284
Sex (female vs. male)	1.05 (0.57-1.94)		0.880	0.91 (0.45-1.83)	0.785
Etiology (overall)	—		>0.16	—	>0.16
INI-1 loss vs. retained	0.49 (0.23-1.02)		0.056	0.50 (0.21-1.16)	0.104
BCLC B vs. A	1.88 (0.97-3.62)		0.060	2.20 (1.03-4.72)	0.043
BCLC C vs. A	2.59 (1.44-4.67)		0.002	3.79 (1.96-7.32)	<0.001
BCLC D vs. A	8.50 (4.44-16.28)		<0.001	23.44 (10.41-52.76)	<0.001
Child-Pugh B vs. A	1.37 (0.85-2.23)		0.199	2.11 (1.23-3.61)	0.007
Child-Pugh C vs. A	6.50 (3.30-12.81)		<0.001	11.94 (5.61-25.42)	<0.001
Multivariate analysis (EZH2 + BCLC)					
EZH2 (positive vs. negative)	1.39 (0.89-2.17)		0.146	1.55 (0.95-2.53)	0.078
BCLC B vs. A	1.75 (0.90-3.39)		0.100	2.03 (0.94-4.38)	0.071
BCLC C vs. A	2.49 (1.37-4.51)		0.003	3.69 (1.90-7.18)	<0.001
BCLC D vs. A	8.28 (4.31-15.92)		<0.001	23.55 (10.34-53.61)	<0.001
Sensitivity analysis (BCLC 0 + A combined reference)					
EZH2 (positive vs. negative)	1.51 (0.97-2.35)		0.067	1.71 (1.05-2.79)	0.030
P-values were determined by the log-rank test (Panel A) and Cox proportional hazards regression (Panel B). Bold p-values indicate statistical significance (p<0.05). BCLC stage (n=5; 1 death, 1 progression event) was excluded from the primary Cox regression owing to limited events; a sensitivity analysis combining BCLC C and A is shown. The proportional hazards assumption was verified (scaled Schoenfeld residuals, all p>0.05). Child-Pugh class was not included in the multivariate model as it is incorporated within the BCLC staging system. BCLC: Barcelona Clinic Liver Cancer; CI: Confidence interval; HR: Hazard ratio; OS: Overall survival; PFS: Progression-free survival; EZH2: Enhancer of zeste homologue 2.					

DISCUSSION

In this single-institution retrospective study of 103 HCC patients, we found that EZH2 positivity, detected by IHC in 43.7% of cases, was significantly associated with poorer survival outcomes. Specifically, EZH2 positivity was associated with markedly reduced median OS (8.0 vs. 27.0 months; $p=0.007$) and median PFS (5.0 vs. 9.0 months; $p=0.012$). Although in univariate analysis EZH2 positivity emerged as a significant prognostic factor for both OS (HR: 1.88; 95%

CI: 1.17-3.02; $p=0.009$) and PFS (HR: 1.69; 95% CI: 1.10-2.60; $p=0.018$), it did not retain independent prognostic value in multivariate analysis adjusted for BCLC stage (OS: HR: 1.55; 95% CI: 0.95-2.53; $p=0.078$; PFS: HR: 1.39; 95% CI: 0.89-2.17; $p=0.146$), suggesting that its prognostic impact may be mediated, at least in part, through its association with tumor burden and disease stage. Accordingly, EZH2 positivity should be regarded as a marker associated with more aggressive, advanced-stage disease rather than as a stage-independent prognostic factor.

The observed EZH2 positivity rate of 43.7% aligns with previously reported frequencies in HCC. A recent study analyzing mitotic cell cycle genes in HCC identified EZH2 as one of the key diagnostic biomarkers with individual diagnostic performance (area under the curve >0.81) across multiple datasets.¹⁵ Similarly, Wang et al.¹⁶ reported that EZH2 was overexpressed in HCC and significantly associated with advanced tumor stage and poor prognosis. Our findings are consistent with and extend this body of evidence. Bae et al.¹⁷ demonstrated that EZH2 overexpression was an independent biomarker for poor outcomes in HCC, with high expression being negatively correlated with patient age and positively associated with alpha-fetoprotein (AFP) levels >20 ng/mL; in keeping with this, EZH2-positive tumors in our cohort also showed higher AFP levels (median 43 vs. 13 ng/mL). Chien et al.¹⁸ revealed that EZH2-mediated stage-dependent methylation patterns correlate with immune polarization, metabolic suppression, and unfavorable outcomes, providing biological insights into EZH2-driven disease progression.

The biological basis for these findings is well supported. EZH2 mediates transcriptional repression through H3K27me3, leading to silencing of critical genes involved in cell cycle control, apoptosis, and differentiation. Multiple pathways have been implicated, including EZH2-mediated SOCS3 silencing through the CTCF-vigilin complex, activating STAT3 signaling,¹⁹ and epigenetic modulation of *miR-1224* forming a positive feedback circuit with CREB to activate YAP signaling.²⁰ Wu et al.²¹ further demonstrated that *SIRT7* super-enhancer-driven reprogramming involves EZH2-dependent H3K27me3 at tumor suppressor promoters. Beyond direct tumor cell regulation, emerging single-cell RNA-seq data indicate that EZH2 modulates the MIF-CD74 axis, affecting T-cell activation and exhaustion,²² while glutamate-NMDAR signaling influences tumor-associated macrophage infiltration through EZH2 regulation.²³ These findings suggest that EZH2 may simultaneously drive tumor cell proliferation and modulate anti-tumor immune responses.

Importantly, our multivariate analysis revealed that EZH2 did not function as an independent prognostic factor for either OS (HR: 1.55; 95% CI: 0.95-2.53; $p=0.078$) or PFS (HR: 1.39; 95% CI: 0.89-2.17; $p=0.146$) when BCLC stage was included in the model. This finding suggests that the prognostic value of EZH2 may be confounded by or closely associated with tumor stage and overall disease burden. One plausible interpretation is that EZH2 positivity is a molecular feature enriched in more advanced tumors, and thus its prognostic signal is captured by established staging systems. This interpretation is supported by the observed trend toward higher EZH2 positivity in advanced BCLC stages (65.2% in stage C vs. 30.0% in stage A; $p=0.064$), which was not statistically significant,

likely due to limited sample size. Li et al.²⁴ similarly identified EZH2 as a prognostically relevant gene in HBV-related HCC with enhanced stratification when combined with clinical parameters, and Liu et al.²⁵ demonstrated improved prognostic accuracy when integrating EZH2 with clinicopathological features in an oxidative stress risk model.

As an exploratory analysis, we also assessed the expression of SMARCB1/INI-1, a core subunit of the SWI/SNF chromatin remodeling complex, which functionally antagonizes PRC2-mediated gene silencing. Loss of INI-1 expression was observed in only 10.7% of cases. Although a numerical trend toward longer survival was noted in the INI-1-loss group for both PFS ($p=0.051$) and OS ($p=0.097$), these differences did not reach statistical significance. With only 11 INI-1-loss cases, the analysis is underpowered and the CIs are wide; moreover, 7 of these 11 patients had early-stage disease, suggesting that the paradoxical direction of the association most likely reflects stage imbalance rather than a protective biological effect, and these observations should be regarded strictly as hypothesis-generating. The high rate of retained INI-1 expression (89.3%) in our cohort contrasts with the findings of Mochizuki et al.²⁶, who reported that only 1 of 30 HCC cases demonstrated nuclear SMARCB1 staining in their diagnostic study; this discrepancy likely reflects several factors. First, the two cohorts were assembled in fundamentally different clinical contexts: Mochizuki et al.²⁶ examined SMARCB1 specifically as a diagnostic discriminator between alpha-fetoprotein-producing gastric carcinoma and HCC, whereas our cohort comprised unselected, pathologically confirmed cases of HCC. Second, differences in antibody clones, staining platforms, and—most importantly—the threshold used to define retained versus lost expression may substantially alter the reported frequency of INI-1 loss. Such methodological heterogeneity in SMARCB1/INI-1 interpretation is well recognized and underscores the need for standardized scoring before cross-study comparisons can be made reliably. To our knowledge, this is the first report to examine the prognostic implications of INI-1 expression in HCC, and future studies with larger sample sizes are warranted to clarify its potential role.

Emerging evidence supports the potential therapeutic relevance of targeting EZH2 in HCC. Salani et al.²⁷ demonstrated that circulating H3K27me3/H3K36me3 ratios, reflecting EZH2 activity, predicted response to sorafenib in advanced HCC. Additionally, disruption of EZH2-dependent regulatory networks, such as the lncRNA-AC079061.1/VIPR1 axis, has been shown to suppress HCC development.²⁸ These findings suggest that pharmacological inhibition of EZH2, potentially in combination with immunotherapy or targeted agents, merits further clinical investigation.

Study Limitations

Our study has several strengths, including complete clinicopathological annotation, consistent survival follow-up, and standardized IHC methodology with blinded pathological evaluation. Furthermore, the simultaneous assessment of two functionally antagonistic epigenetic regulators (EZH2 and INI-1) provides a broader perspective on the role of chromatin remodeling in HCC. However, several limitations warrant acknowledgment. The retrospective design and modest sample size (n=103) may limit statistical power, particularly for the INI-1-loss subgroup (n=11), as evidenced by the observed borderline associations. The binary $\geq 1\%$ cut-off, while clinically pragmatic, does not capture intensity or H-score information; although the proportion of positive nuclei was recorded (median 30%, range 1-80% among positive cases), intensity-based scoring was not available. The limited number of events also constrained the number of covariates that could be modeled simultaneously. Although AFP values were available for most patients and were discussed descriptively, AFP was not incorporated into the formal survival analyses because it lies outside the primary EZH2-INI-1 focus of this study and because the limited number of events constrained the multivariate model. In addition, several other standard prognostic variables—including ECOG performance status, tumor size and number, vascular invasion, extrahepatic spread, and histological grade—were not consistently documented and could not be reliably analyzed. As BCLC staging already incorporates performance status, tumor burden, and vascular/extrahepatic involvement, it captures much of the information carried by these individual variables. Detailed treatment-stratified modeling was not feasible, limiting our ability to assess the predictive value for therapeutic response. Finally, our cohort was treated between 2011 and 2019, predating the routine use of immune checkpoint inhibitor-based regimens such as atezolizumab plus bevacizumab, now a first-line standard for advanced HCC. Whether EZH2 expression influences outcomes or responses in the contemporary immunotherapy era—particularly given evidence that EZH2 modulates anti-tumor immunity—remains an open and clinically relevant question. Prospective multicenter studies with larger cohorts, refined cut-off values, integration of complementary biomarkers, and evaluation of EZH2-targeted therapies are needed to validate and extend these findings.

CONCLUSION

EZH2 positivity, detected in 43.7% of HCC patients, was significantly associated with inferior OS and PFS. Although EZH2 demonstrated prognostic value in univariate analysis, it did not retain independent significance after adjustment

for BCLC stage, indicating that its prognostic value is stage-dependent and does not constitute an independent prognostic effect. INI-1 loss, observed in 10.7% of cases, showed only borderline, exploratory associations with survival that did not reach statistical significance. These findings, together with consistent evidence from the broader literature, support the biological relevance of epigenetic regulators in HCC and warrant further investigation in larger prospective studies to clarify the roles of EZH2 and INI-1 as prognostic biomarkers and potential therapeutic targets.

Ethics

Ethics Committee Approval: This single-institution, retrospective cohort study was conducted following approval from the Çukurova University Faculty of Medicine Clinical Research Ethics Committee (approval number: 38; date: 13.04.2018).

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Concept: M.M.K., B.Ş., Design: M.M.K., F.D., Data Collection or Processing: M.M.K., F.D., Analysis or Interpretation: M.M.K., B.Ş., Literature Search: M.M.K., Writing: M.M.K., B.Ş.

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The Impact of KRAS Mutation on Treatment Response and Survival in Patients with Metastatic Lung Adenocarcinoma Receiving First-line Systemic Therapy

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ABSTRACT

Objective: The prognostic and predictive significance of kirsten rat sarcoma viral oncogene homologue (KRAS) mutations in metastatic non-small cell lung cancer remains controversial, particularly in the immunotherapy era. Most previous studies compared KRAS-mutant tumors with heterogeneous KRAS wild-type populations that included other oncogenic driver alterations. This study aimed to evaluate the clinical impact of isolated KRAS mutations in patients with metastatic lung adenocarcinoma receiving first-line systemic therapy.

Material and Methods: This retrospective single-center study included patients with metastatic lung adenocarcinoma who underwent next-generation sequencing (NGS) between January 2023 and December 2025. Patients were categorized into two groups: those with isolated KRAS-mutant tumors and those with pan-wild tumors without any detectable oncogenic alterations on NGS. Patients with co-occurring oncogenic alterations were excluded. Progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method and Cox regression analysis.

Results: A total of 75 patients were included, comprising 28 with isolated KRAS mutations and 47 without detectable oncogenic alterations. Programmed death-ligand 1 expression $\geq 50\%$ was significantly more frequent in the KRAS-mutant group ($p=0.001$). However, KRAS mutation status was not significantly associated with PFS or OS in either univariate or multivariate analyses. Median PFS was 10.77 months in the KRAS-mutant group and 7.84 months in the pan-wild group ($p=0.597$), while median OS was 18.97 months in the KRAS-mutant group and 18.27 months in the pan-wild group ($p=0.926$). Similarly, no significant differences in survival were observed between the immunotherapy-containing and chemotherapy-alone subgroups.

Conclusion: Isolated KRAS mutations were not associated with significantly different survival outcomes compared with tumors lacking detectable oncogenic alterations. These findings suggest that KRAS mutation status alone may have limited prognostic value in metastatic lung adenocarcinoma.

Keywords: Immunotherapy; KRAS mutation; metastatic lung adenocarcinoma; survival outcomes

INTRODUCTION

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, accounting for a substantial proportion of cancer deaths despite advances in diagnosis and treatment.¹ Non-small cell lung cancer (NSCLC) constitutes approximately 80-85% of all lung cancer cases, with adenocarcinoma being the most common histological subtype.² A significant proportion of patients are diagnosed

at an advanced or metastatic stage, where curative treatment options are limited and the prognosis remains poor. In patients with stage IV disease, the 5 years overall survival (OS) rate is generally below 10%, underscoring its aggressive nature.^{3,4}

In recent years, the management of metastatic NSCLC has been transformed by the integration of molecular profiling and the development of targeted therapies and immune

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checkpoint inhibitors. While patients with actionable driver alterations, such as epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) and ROS1 rearrangements, benefit significantly from targeted therapies, a substantial proportion lack these alterations. For these patients, platinum-based chemotherapy, alone or combined with immunotherapy, remains the cornerstone of first-line treatment. However, treatment responses remain heterogeneous, highlighting the need for reliable biomarkers to better predict therapeutic efficacy and survival outcomes.⁵

Kirsten rat sarcoma viral oncogene homologue (KRAS) mutations represent the most common oncogenic driver alterations in NSCLC, particularly in lung adenocarcinoma, where they are detected in approximately 20-40% of cases.^{6,7} These mutations are strongly associated with tobacco exposure and predominantly occur at codons 12 and 13, with G12C, G12V, and G12D being the most frequent subtypes.⁸ KRAS encodes a small GTPase that plays a central role in regulating key intracellular signaling pathways involved in cell proliferation, differentiation, and survival. Activating mutations in KRAS lead to constitutive pathway activation, resulting in uncontrolled tumor growth and disease progression.⁹

Despite its high prevalence, the prognostic and predictive role of KRAS mutations in metastatic NSCLC remains controversial. In the era of cytotoxic chemotherapy, several studies suggested that KRAS mutations were associated with poorer survival outcomes and reduced response to platinum-based regimens.^{10,11} However, since the introduction of immune checkpoint inhibitors, emerging evidence indicates that KRAS-mutant tumors may exhibit enhanced sensitivity to immunotherapy, potentially due to a higher tumor mutational burden and increased immunogenicity.^{12,13} Nevertheless, real-world studies and retrospective analyses have reported inconsistent findings, with some demonstrating improved outcomes in KRAS-mutant patients receiving immunotherapy, while others have shown no significant difference or even worse survival compared to KRAS wild-type patients.¹⁴⁻¹⁶

Moreover, most of the existing studies have compared KRAS-mutant tumors with heterogeneous KRAS wild-type groups that may include other oncogenic driver alterations, potentially confounding the interpretation of results.¹⁴ Therefore, the independent clinical impact of KRAS mutations, particularly in the absence of other detectable driver alterations, remains unclear. In this context, the present study aimed to evaluate whether the presence of an isolated KRAS mutation influences treatment response and survival outcomes in patients with metastatic lung adenocarcinoma receiving first-line systemic therapy.

MATERIAL AND METHODS

This study was a retrospective, single-center, observational analysis conducted at a tertiary referral center. Patients diagnosed with metastatic lung adenocarcinoma between January 2023 and December 2025 were screened for eligibility. Only patients who underwent next-generation sequencing (NGS) as part of routine clinical practice were included.

Targeted DNA- and RNA-based NGS was performed using the Archer VariantPlex Expanded panel (Invitae, Boulder, CO, USA). The panel routinely assessed clinically relevant genomic alterations in NSCLC, including *EGFR*, *BRAF*, *KRAS*, *ERBB2*, *PIK3CA*, *STK11*, *KEAP1*, *TP53*, *ALK*, *NTRK1*, *NTRK2*, *NTRK3*, *RET*, *ROS1*, *NRG1*, *MET*, *FGFR1*, *FGFR2*, *FGFR3*, and *NF1*. The molecular findings were used to classify patients as having isolated KRAS-mutant tumors or pan-wild tumors without detectable oncogenic driver alterations.

Eligible patients were required to have histologically confirmed stage IV lung adenocarcinoma and to have received first-line systemic therapy consisting of platinum-based chemotherapy alone or combined with immune checkpoint inhibitors. To ensure adequate evaluation of treatment response, only patients who received at least four cycles of first-line therapy and had a radiological response assessment were included in the analysis.

Patients were categorized into two groups according to their molecular status: those harboring isolated KRAS mutations (KRAS-mutant group) and those without any detectable oncogenic alterations by NGS (pan-wild group). To minimize potential confounding effects, patients with co-occurring oncogenic driver alterations, including EGFR mutations, ALK and ROS1 rearrangements, BRAF mutations, and other actionable genomic alterations, were excluded. A total of 75 patients were included in the final analysis, of whom 28 were in the KRAS-mutant group and 47 were in the pan-wild group.

Clinical and pathological data, including age, sex, smoking status, Eastern Cooperative Oncology Group (ECOG) performance status, tumor histology, sites of metastasis, and treatment regimens, were collected retrospectively from electronic medical records. Treatment response was evaluated radiologically according to the Response Evaluation Criteria in Solid Tumors version 1.1.

Univariate and multivariate analyses were performed using the Cox proportional hazards regression model to identify factors associated with OS and PFS. Variables with a p value <0.20 in univariate analysis, along with clinically relevant factors, were included in the multivariate model. Hazard ratios with 95% confidence intervals (CIs) were calculated.

A two-sided p -value <0.05 was considered statistically significant.

This retrospective study was approved by the Ethics Committee of the University of Health Sciences Türkiye, Prof. Dr. Cemil Taşcıoğlu City Hospital (approval number: 174, date: 16.04.2026). The study was conducted in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived by the same Ethics Committee because the study was retrospective.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation or median (range), while categorical variables were summarized as frequencies and percentages. Comparisons between groups were performed using the chi-square test or Fisher's exact test for categorical variables, and the Student's t -test or Mann-Whitney U test for continuous variables, as appropriate.

OS was defined as the time from initiation of first-line therapy to death from any cause or to last follow-up. Progression-free survival (PFS) was defined as the time from initiation of first-line therapy to disease progression or death, whichever occurred first. Follow-up duration was calculated from initiation of first-line systemic therapy to the date of death or last clinical follow-up. The median follow-up duration was calculated from the individual follow-up times. Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test.

RESULTS

A total of 75 patients with metastatic lung adenocarcinoma who met the inclusion criteria were included in the study. Among these, 28 patients (37.3%) had tumors harboring isolated KRAS mutations, while 47 patients (62.7%) had no detectable oncogenic alterations by NGS (pan-wild group). The median follow-up time was 17.03 months, and the mean age of the study population was 63.33 ± 8.69 years. The baseline demographic and clinical characteristics of the study population are summarized in Table 1.

Among the 28 patients with KRAS-mutant tumors, KRAS G12C was the most common subtype, accounting for 13 cases (46.4%). The remaining KRAS mutation subtypes included G12V ($n=4$; 14.3%), G12D ($n=4$; 14.3%), G12A ($n=2$; 7.1%), G12S ($n=1$; 3.6%), G12R ($n=1$; 3.6%), G13D ($n=1$; 3.6%), Q61H ($n=1$; 3.6%), and Q61L ($n=1$; 3.6%). Overall, 15 patients (53.6%) harbored non-G12C KRAS variants.

The majority of patients in both groups were male, and the sex distribution was comparable between groups. Similarly, smoking status, ECOG performance status, comorbidity, and stage at diagnosis were balanced, with no statistically significant differences observed.

However, a significant difference was noted in programmed death-ligand 1 (PD-L1) expression levels between the groups ($p=0.001$), with a higher proportion of patients with PD-L1 $\geq 50\%$ in the KRAS-mutant group. In addition, distant lymph node metastases were significantly more frequent in the pan-wild group ($p=0.018$). No significant differences were observed between groups regarding other metastatic sites, including brain, liver, bone, adrenal, and contralateral lung. Likewise, the distribution of first-line treatment regimens and the best overall response rates were similar between the two groups.

Univariate and multivariate Cox regression analyses were performed to identify factors associated with PFS (Table 2). In univariate analysis, treatment with chemotherapy plus immunotherapy was significantly associated with improved PFS compared to chemotherapy alone ($p<0.001$). In contrast, KRAS mutation status was not significantly associated with PFS ($p=0.597$). Similarly, none of the other evaluated variables, including ECOG performance status, PD-L1 expression, and metastatic sites, showed a significant association with PFS.

In multivariate analysis, treatment with chemotherapy plus immunotherapy remained an independent predictor of improved PFS compared to chemotherapy alone ($p<0.001$). In contrast, KRAS mutation status was not independently associated with PFS ($p=0.264$). Likewise, ECOG performance status and PD-L1 expression did not demonstrate a statistically significant association with PFS in the multivariate model.

Univariate and multivariate Cox regression analyses were performed to identify factors associated with OS (Table 3). In univariate analysis, treatment with chemotherapy plus immunotherapy was significantly associated with improved OS compared with chemotherapy alone ($p=0.017$). In contrast, KRAS mutation status was not significantly associated with OS ($p=0.926$). None of the other evaluated variables demonstrated a statistically significant association with OS in the univariate analysis.

In multivariate analysis, treatment with chemotherapy plus immunotherapy remained an independent predictor of improved OS ($p=0.003$). ECOG performance status was also identified as an independent prognostic factor, with worse performance status associated with shorter OS ($p=0.020$). In addition, the absence of bone metastasis was independently associated with improved OS ($p=0.030$). KRAS mutation status

TABLE 1: Baseline demographic and clinical characteristics of patients with metastatic lung adenocarcinoma according to KRAS mutation status.

		KRAS-mutant (n=28)	Pan-wild (n=47)	Total (n=75)	p-value
Sex	Female	5 (17.9%)	8 (17.0%)	13 (17.3%)	0.926
	Male	23 (82.1%)	39 (83.0%)	62 (82.7%)	
Smoking status	Yes	25 (89.3%)	45 (95.7%)	70 (93.3%)	0.278
	No	3 (10.7%)	2 (4.3%)	5 (6.7%)	
ECOG-PS	0	17 (60.7%)	28 (59.6%)	45 (60.0%)	0.922
	1	11 (39.3%)	19 (40.4%)	30 (40.0%)	
Comorbidity	Yes	23 (82.1%)	31 (66.0%)	54 (72.0%)	0.131
	No	5 (17.9%)	16 (34.0%)	21 (28.0%)	
Stage at diagnosis	I-III	5 (17.9%)	7 (14.9%)	12 (16.0%)	0.400
	IV	23 (82.1%)	40 (85.1%)	63 (84.0%)	
PD-L1 expression	0%	5 (17.9%)	24 (51.1%)	29 (38.7%)	0.001
	1-49%	12 (42.9%)	20 (42.6%)	32 (42.7%)	
	≥50%	11 (39.3%)	3 (6.4%)	14 (18.7%)	
Contralateral lung metastasis	Yes	15 (53.6%)	20 (42.6%)	35 (46.7%)	0.355
	No	13 (46.4%)	27 (57.4%)	40 (53.3%)	
Brain metastasis	Yes	6 (21.4%)	15 (31.9%)	21 (28.0%)	0.328
	No	22 (78.6%)	32 (68.1%)	54 (72.0%)	
Liver metastasis	Yes	1 (3.6%)	6 (12.8%)	7 (9.3%)	0.186
	No	27 (96.4%)	41 (87.2%)	68 (90.7%)	
Bone metastasis	Yes	17 (60.7%)	32 (68.1%)	49 (65.3%)	0.516
	No	11 (39.3%)	15 (31.9%)	26 (34.7%)	
Adrenal metastasis	Yes	3 (10.7%)	14 (29.8%)	17 (22.7%)	0.056
	No	25 (89.3%)	33 (70.2%)	58 (77.3%)	
Distant lymph node metastasis	Yes	14 (50.0%)	36 (76.6%)	50 (66.7%)	0.018
	No	14 (50.0%)	11 (23.4%)	25 (33.3%)	
First-line treatment	Chemotherapy	14 (50.0%)	32 (68.1%)	46 (61.3%)	0.298
	Chemo + IO	13 (46.4%)	14 (29.8%)	27 (36.0%)	
	IO	1 (3.6%)	1 (2.1%)	2 (2.7%)	
Best response	PR	19 (67.9%)	31 (66.0%)	50 (66.7%)	0.700
	SD	1 (3.6%)	4 (8.5%)	5 (6.7%)	
	PD	8 (28.6%)	12 (25.5%)	20 (26.7%)	
Progression	Yes	18 (64.3%)	28 (59.6%)	46 (61.3%)	0.685
	No	10 (35.7%)	19 (40.4%)	29 (38.7%)	

Bold p-values indicate statistically significant results ($p < 0.05$). ECOG-PS: Eastern Cooperative Oncology Group performance status; KRAS: Kirsten rat sarcoma viral oncogene homologue; PD-L1: Programmed death-ligand 1; IO: Immunotherapy; PR: Partial response; SD: Stable disease; PD: Progressive disease.

was not significantly associated with OS in the multivariate model ($p=0.085$).

Kaplan-Meier survival analyses were performed to compare PFS and OS between patients with isolated KRAS mutations and those without detectable oncogenic alterations (Figure 1).

The median PFS was 10.77 months (95% CI: 7.18-14.35) in the KRAS-mutant group and 7.84 months (95% CI: 6.12-

9.55) in the pan-wild group, with no statistically significant difference observed between the groups ($p=0.597$) (Figure 1A).

Similarly, the median OS was 18.97 months (95% CI: 11.56-26.37) in the KRAS-mutant group and 18.27 months (95% CI: 15.02-21.52) in the pan-wild group, with no significant difference between the groups ($p=0.926$) (Figure 1B).

TABLE 2: Univariate and multivariate Cox regression analysis of factors associated with PFS.

Variable	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value
Sex				
Female	Reference	0.401		
Male	0.74 (0.37-1.47)			
Age				
≥65 years	Reference	0.683		
<65 years	1.14 (0.60-2.16)			
ECOG-PS				
0	Reference	0.446	Reference	0.081
1	1.29 (0.67-2.45)		1.83 (0.93-3.61)	
Comorbidity				
No	Reference	0.400		
Yes	0.75 (0.38-1.47)			
KRAS status				
Pan-wild	Reference	0.597	Reference	0.264
Mutant	1.18 (0.64-2.17)		0.66 (0.32-1.36)	
PD-L1				
0	Reference	0.117	Reference	0.124
1-49	0.59 (0.31-1.14)		0.58 (0.29-1.16)	
≥50	0.68 (0.29-1.57)		0.66 (0.24-1.80)	
Contralateral lung metastasis				
No	Reference	0.898		
Yes	1.04 (0.57-1.88)			
Brain metastasis				
No	Reference	0.825		
Yes	0.93 (0.48-1.78)			
Liver metastasis				
No	Reference	0.728		
Yes	1.17 (0.49-2.80)			
Bone metastasis				
No	Reference	0.501		
Yes	0.81 (0.45-1.48)			
Adrenal metastasis				
No	Reference	0.927		
Yes	1.03 (0.49-2.16)			
Distant lymph node metastasis				
No	Reference	0.207		
Yes	1.54 (0.79-3.03)			
Treatment group				
Chemotherapy	Reference	<0.001	Reference	<0.001
Chemo + IO	0.23 (0.10-0.53)		0.20 (0.08-0.48)	
IO	1.37 (0.18-10.24)		1.35 (0.15-11.83)	

Bold p-values indicate statistically significant results (p<0.05). CI: Confidence interval; HR: Hazard ratio; KRAS: Kirsten rat sarcoma viral oncogene homologue; ECOG-PS: Eastern Cooperative Oncology Group performance status; IO: Immunotherapy; PD-L1: Programmed death-ligand 1; PFS: Progression-free survival; OS: Overall survival.

TABLE 3: Univariate and multivariate Cox regression analysis of factors associated with OS.

Variable	Univariate HR (95% CI)	p-value	Multivariate HR (95% CI)	p-value
Sex				
Female	Reference	0.357		
Male	0.69 (0.31-1.52)			
Age				
≥65 years	Reference	0.250		
<65 years	1.51 (0.75-3.04)			
ECOG-PS				
0	Reference	0.148	Reference	0.020
1	1.67 (0.83-3.34)		2.40 (1.15-5.01)	
Comorbidity				
No	Reference	0.964		
Yes	1.02 (0.47-2.20)			
KRAS status				
Pan-wild	Reference	0.926	Reference	0.085
Mutant	0.97 (0.48-1.95)		0.47 (0.20-1.11)	
PD-L1				
0	Reference	0.608	Reference	0.453
1-49	0.82 (0.39-1.74)		0.73 (0.33-1.64)	
≥50	0.63 (0.22-1.84)		0.61 (0.17-2.19)	
Contralateral lung metastasis				
No	Reference	0.621		
Yes	1.19 (0.60-2.33)			
Brain metastasis				
No	Reference	0.429		
Yes	0.72 (0.32-1.62)			
Liver metastasis				
No	Reference	0.687		
Yes	0.81 (0.29-2.23)			
Bone metastasis				
No	Reference	0.110	2.40 (1.08-5.31)	0.030
Yes	1.84 (0.87-3.90)			
Adrenal metastasis				
No	Reference	0.623		
Yes	1.22 (0.55-2.72)			
Distant lymph node metastasis				
No	Reference	0.247		
Yes	1.58 (0.73-3.42)			
Treatment group				
Chemotherapy	Reference	0.017	Reference	0.003
Chemo + IO	0.23 (0.07-0.77)		0.14 (0.04-0.52)	
IO	0.40 (0.05-3.17)		0.21 (0.02-2.55)	

Bold p-values indicate statistically significant results (p<0.05). CI: Confidence interval; HR: Hazard ratio; KRAS: Kirsten rat sarcoma viral oncogene homologue; ECOG-PS: Eastern Cooperative Oncology Group performance status; IO: Immunotherapy; PD-L1: Programmed death-ligand 1; PFS: Progression-free survival; OS: Overall survival.

A subgroup analysis was performed in patients who received immunotherapy-containing regimens ($n=14$ in the KRAS-mutant group and $n=15$ in the pan-wild group). In this subgroup, the median PFS was 14.07 months (95% CI: 13.17-14.96) in the KRAS-mutant group and 13.53 months (95% CI: 2.52-24.55) in the pan-wild group, with no statistically significant difference between the groups ($p=0.633$) (Figure 2). OS data in this subgroup were immature at the time of analysis and were therefore not reported.

A separate subgroup analysis was performed among patients who received chemotherapy alone ($n=14$ in the KRAS-mutant group and $n=32$ in the pan-wild group). In this subgroup, the median PFS was 4.97 months (95% CI: 0-11.32) in the KRAS-mutant group and 7.23 months (95% CI: 6.14-8.32) in the pan-wild group, with no statistically significant difference between the groups ($p=0.709$). These findings were consistent with those observed in the overall cohort.

At the time of analysis, 13 patients (46.4%) in the KRAS-mutant group had died and 15 patients (53.6%) were alive, whereas 21 patients (44.7%) in the pan-wild group had died and 26 patients (55.3%) were alive.

DISCUSSION

In the present study, we evaluated the impact of isolated KRAS mutations on treatment response and survival outcomes in patients with metastatic lung adenocarcinoma receiving first-line systemic therapy. Our findings demonstrated that KRAS mutation status was not significantly associated with PFS or OS in either univariate or multivariate analyses. Similarly, Kaplan-Meier analyses showed no statistically significant differences in survival outcomes between the KRAS-mutant and pan-wild groups. In contrast, the use of chemoimmunotherapy was independently associated with improved PFS and OS

compared with chemotherapy alone. Notably, although patients with KRAS-mutant tumors had significantly higher PD-L1 expression levels, this did not translate into superior survival outcomes.

Previous studies investigating the prognostic role of KRAS mutations in metastatic NSCLC have reported conflicting results. In the pre-immunotherapy era, several studies suggested that KRAS mutations were associated with poorer clinical outcomes and reduced sensitivity to platinum-based chemotherapy. Marabese et al.¹⁰ demonstrated that KRAS-mutant NSCLC patients receiving first-line platinum-containing chemotherapy had significantly shorter survival outcomes compared with KRAS wild-type patients. Similarly, Eklund et al.¹¹ reported that KRAS mutations were associated with inferior survival in metastatic NSCLC, supporting the hypothesis that KRAS-driven tumors may exhibit more aggressive biological behavior. However, more recent studies conducted in the immunotherapy era have produced inconsistent findings regarding the prognostic and predictive value of KRAS mutations.¹⁴

In our study, KRAS mutation status was not independently associated with either PFS or OS. These findings are in line with several contemporary real-world analyses demonstrating that KRAS mutations alone may not confer a distinct survival disadvantage in patients receiving modern systemic therapies.^{17,18} Importantly, unlike many previous reports, our study excluded patients harboring other actionable oncogenic alterations and compared KRAS-mutant tumors with a pan-wild population lacking detectable driver mutations on NGS. This more homogeneous comparator group may have reduced potential molecular confounding and allowed a more accurate assessment of the independent clinical impact of KRAS mutations.

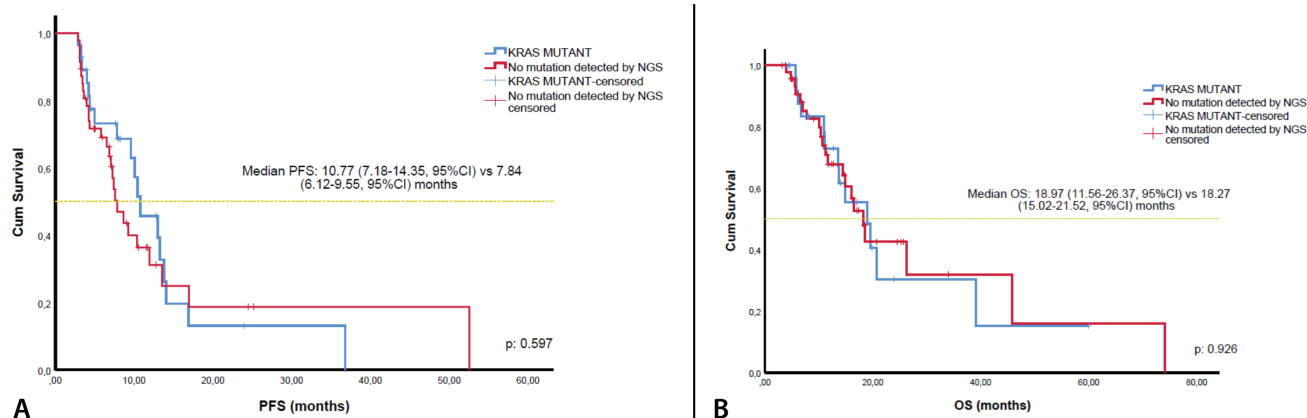


FIGURE 1: Kaplan-Meier curves for progression-free survival (PFS) and overall survival (OS) according to KRAS mutation status in the overall study population ($n=75$; KRAS-mutant, $n=28$; pan-wild, $n=47$). (A) PFS; (B) OS.

KRAS: Kirsten rat sarcoma viral oncogene homologue; CI: Confidence interval

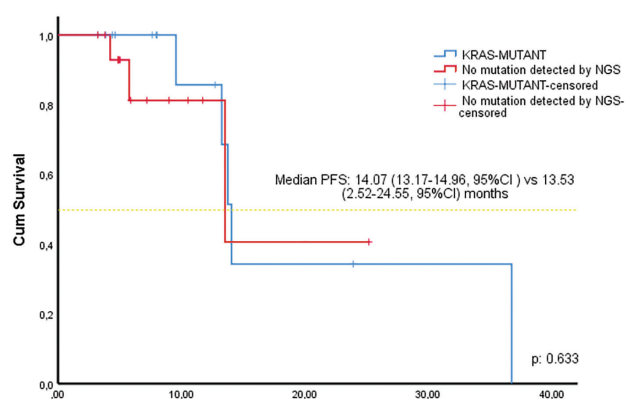


FIGURE 2: Kaplan-Meier curve for progression-free survival (PFS) according to KRAS mutation status in patients receiving immunotherapy-containing regimens (n=29; KRAS-mutant, n=14; pan-wild, n=15).

NGS: Next-generation sequencing; CI: Confidence interval; KRAS: Kirsten rat sarcoma viral oncogene homologue

Notably, patients with KRAS-mutant tumors in our cohort exhibited significantly higher PD-L1 expression levels compared with the pan-wild group. This finding is consistent with previous studies reporting an association between KRAS mutations and increased tumor immunogenicity.¹⁹ KRAS-mutant NSCLC has been associated with smoking-related mutational signatures, higher tumor mutational burden, and a more inflamed tumor microenvironment, all of which may contribute to increased PD-L1 expression and enhanced immune recognition.²⁰ In particular, several studies have demonstrated that PD-L1 $\geq 50\%$ is more frequently observed in KRAS-mutant tumors than in KRAS wild-type populations.²¹ Despite this biological association, the higher PD-L1 expression observed in our KRAS-mutant cohort did not translate into superior survival outcomes, suggesting that PD-L1 expression alone may be insufficient to predict prognosis in this molecular subgroup.

In addition to KRAS mutation status, accumulating evidence suggests that co-occurring genomic alterations may substantially influence the biological behavior of KRAS-mutant NSCLC. In particular, concurrent STK11 and KEAP1 mutations have consistently been associated with an immunosuppressive tumor microenvironment, reduced responsiveness to immune checkpoint inhibitors, and poorer survival outcomes, whereas TP53 co-mutations have been linked to increased tumor immunogenicity and may be associated with improved responses to immunotherapy.²²⁻²⁴ Unfortunately, information regarding co-mutation status was not routinely available in our cohort; therefore, the potential impact of these alterations could not be evaluated. Future studies incorporating comprehensive genomic profiling are warranted to better define the prognostic and predictive

significance of these co-mutations in patients with KRAS-mutant NSCLC.

In the subgroup analysis of patients receiving immunotherapy-containing regimens, no statistically significant difference in PFS was observed between the KRAS-mutant and pan-wild groups. Several previous studies have suggested that KRAS-mutant NSCLC may derive greater benefit from immune checkpoint inhibitors compared with KRAS wild-type tumors, potentially due to higher PD-L1 expression, smoking-related mutational signatures, and increased tumor immunogenicity.^{19,25} However, despite these findings, other studies have reported neutral survival outcomes between KRAS-mutant and KRAS wild-type populations in the immunotherapy era.^{18,26} Differences in study populations, co-occurring genomic alterations, KRAS mutation subtypes, and treatment strategies may partly explain these heterogeneous findings across studies. In our cohort, the exclusion of patients with other concomitant oncogenic alterations may have contributed to the relatively comparable outcomes observed between the two groups.

A separate subgroup analysis among patients treated with chemotherapy alone also demonstrated no significant difference in PFS between the KRAS-mutant and pan-wild groups. In the pre-immunotherapy era, KRAS mutations were frequently considered a negative prognostic factor and were associated with reduced sensitivity to platinum-based chemotherapy in several studies.^{10,27} However, our findings suggest that isolated KRAS mutations alone may not substantially influence treatment outcomes in patients receiving conventional chemotherapy. These results further support the notion that the clinical impact of KRAS mutations may be strongly influenced by accompanying molecular alterations and tumor biological heterogeneity rather than by KRAS status alone.

Study Limitations

This study has several strengths. First, unlike many previous studies, patients with other concomitant oncogenic alterations were excluded, allowing a more homogeneous comparison between KRAS-mutant and pan-wild populations. Second, all patients underwent NGS, enabling more accurate molecular characterization of the study cohort. In addition, both OS and PFS were evaluated in the overall cohort as well as in treatment-specific subgroups, providing a more comprehensive assessment of the clinical impact of KRAS mutations in metastatic lung adenocarcinoma.

Several limitations of this study should be acknowledged. First, the retrospective and single-center design may have introduced potential selection bias. Second, the relatively small sample size, particularly in subgroup analyses, may have

limited the statistical power to detect modest differences in survival outcomes between groups. Therefore, the absence of statistically significant differences should be interpreted with appropriate caution. In addition, KRAS mutation subtypes and co-mutation profiles were not analyzed separately, which may have influenced treatment response and survival outcomes. KRAS G12C, which was the most common KRAS subtype in our cohort, was also not evaluated separately despite the ongoing development of first-line treatment strategies combining KRAS G12C inhibitors with immunotherapy or chemotherapy. Finally, the relatively short follow-up duration and immature OS data in some subgroups may limit the interpretation of long-term outcomes.

Overall, isolated KRAS mutations were not significantly associated with PFS or OS when compared with patients without detectable oncogenic alterations in metastatic lung adenocarcinoma receiving first-line systemic therapy. Although KRAS-mutant tumors exhibited higher PD-L1 expression, this did not translate into superior survival outcomes. These findings suggest that KRAS mutation status alone may have limited prognostic value in the absence of other concomitant oncogenic alterations.

CONCLUSION

In this retrospective single-center study, isolated KRAS mutations did not appear to significantly influence survival outcomes in patients with metastatic lung adenocarcinoma receiving first-line systemic therapy. No significant differences in survival outcomes were observed between the KRAS-mutant and pan-wild groups in either the immunotherapy-containing regimen subgroup or the chemotherapy-alone subgroup. Further prospective studies with larger patient populations and comprehensive molecular analyses are needed to better clarify the clinical significance of KRAS mutations in metastatic NSCLC.

Ethics

Ethics Committee Approval: This retrospective study was approved by the Ethics Committee of the University of Health Sciences Türkiye, Prof. Dr. Cemil Taşcıoğlu City Hospital (approval number: 174, date: 16.04.2026).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: O.A., Ş.D.S., A.E.Ö., Concept: O.A., M.E., K.E., Design: O.A., K.E., Data Collection or Processing: O.A., Ş.D.S., A.E.Ö., Analysis or Interpretation: O.A., F.A., Z.S.İ., Literature Search: O.A., M.E., F.A., Writing: O.A., Z.S.İ.

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

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Pathological Response and Survival Outcomes Following Neoadjuvant Chemotherapy in HR+/HER2- Breast Cancer: Real-world Experience

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ABSTRACT

Objective: Hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer (BC) is the most common subtype but shows limited sensitivity to neoadjuvant chemotherapy (NAC), and its clinical benefit remains controversial. This study evaluated clinicopathological characteristics, pathological response patterns, and survival outcomes in HR+/HER2- BC patients treated with NAC.

Material and Methods: This retrospective cohort study included 186 women with stage II-III HR+/HER2- BC who received NAC followed by surgery between 2015 and 2024 at two tertiary centers. Pathological responses, event-free survival (EFS), and overall survival (OS) were analyzed. Logistic regression identified predictors of pathological complete response (pCR), and Cox models evaluated prognostic factors for survival.

Results: After a median follow-up of 30.6 months, pCR was achieved in 13.4% of patients and 42.5% showed a partial pathological response, resulting in a total pathological response rate of 55.9%. Grade 3 tumors and estrogen receptor (ER) expression <90% independently predicted pCR. Five-year EFS and OS rates were 77% and 88%, respectively. High ER expression (≥90%) and any pathological response were strong independent predictors of improved EFS and OS, whereas pCR alone was not associated with survival.

Conclusion: In HR+/HER2- BC treated with NAC, overall pathological response and ER expression provide greater prognostic value than pCR alone.

Keywords: Hormone receptor-positive breast cancer; neoadjuvant chemotherapy; pathological response; prognostic factors

INTRODUCTION

Hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer (BC) represents the most common molecular subtype of invasive

BC, accounting for approximately 70% of all newly diagnosed cases.^{1,2} Despite generally favorable long-term outcomes, HR+/HER2- BC is biologically heterogeneous and encompasses tumors with varying proliferation rates, endocrine sensitivity, recurrence risks, and treatment responses.^{3,4} In particular,

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luminal B tumors are associated with higher proliferative activity, more aggressive clinical behavior, and poorer prognosis compared with luminal A tumors.⁵

Neoadjuvant therapy has become increasingly utilized in early-stage disease.⁶ In HR+/HER2- patients, neoadjuvant chemotherapy (NAC) may facilitate surgical resection by downstaging the tumor, increase the feasibility of breast-conserving surgery, and provide early assessment of treatment efficacy to guide subsequent adjuvant therapy.^{7,8} However, compared to other subtypes, HR+/HER2- tumors generally exhibit lower chemosensitivity, with pathological complete response (pCR) rates significantly lower than those seen in triple-negative or HER2-positive disease.^{9,10} Although pCR has traditionally been considered a surrogate marker for improved survival in aggressive BC subtypes, its prognostic significance in HR+/HER2- disease remains controversial.¹¹⁻¹³ Several studies have suggested that long-term outcomes in HR+/HER2- tumors may depend more strongly on endocrine responsiveness and tumor biology than on pCR achievement alone.^{14,15} Consequently, concerns regarding overtreatment and the optimal selection of patients for NAC continue to be debated in clinical practice.¹⁶

In recent years, the use of NAC in HR+/HER2- BC has increased, particularly among patients with high-risk clinicopathological features.^{17,18} Nevertheless, most available evidence originates from clinical trials with highly selected patient populations, whereas real-world data reflecting routine oncology practice remain relatively limited.^{19,20} Therefore, further studies are needed to better define pathological response (pR) patterns, prognostic factors, and survival outcomes in this heterogeneous subgroup.

Accordingly, the present study aimed to evaluate the clinicopathological characteristics, pR patterns, and survival outcomes of HR+/HER2- BC patients treated with NAC in a real-world, multicenter cohort.

MATERIAL AND METHODS

Study Population

This retrospective study included 186 consecutive female patients diagnosed with stage II-III HR+/HER2- BC cancer who received NAC between January 2015 and September 2024 at University of Health Sciences Türkiye, Bağcılar Training and Research Hospital and University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital.

The inclusion criteria were as follows:

1. Age ≥ 18 years.
2. Histologically confirmed invasive breast carcinoma.

3. HR-positive status was determined via immunohistochemistry (IHC).

4. HER2- status confirmed via IHC and/or fluorescence *in situ* hybridization (FISH).

5. Completion of surgery following NAC.

The exclusion criteria were as follows:

1. Stage IV disease at diagnosis.
2. No surgery after NAC.
3. Incomplete follow-up data (Figure 1).

The indication for NAC was determined by multidisciplinary BC teams at each participating institution. NAC was generally preferred for patients with locally advanced disease, large primary tumors, clinically positive axillary lymph nodes, high-risk biological features such as high histologic grade or elevated Ki-67 index, and tumor downstaging when considered necessary to improve operability or to increase the feasibility of breast-conserving surgery.

Clinical, pathological, and treatment-related data were collected from the electronic medical records, including age, menopausal status, body mass index (BMI), histologic subtype, clinical T and N stages, estrogen receptor (ER) and

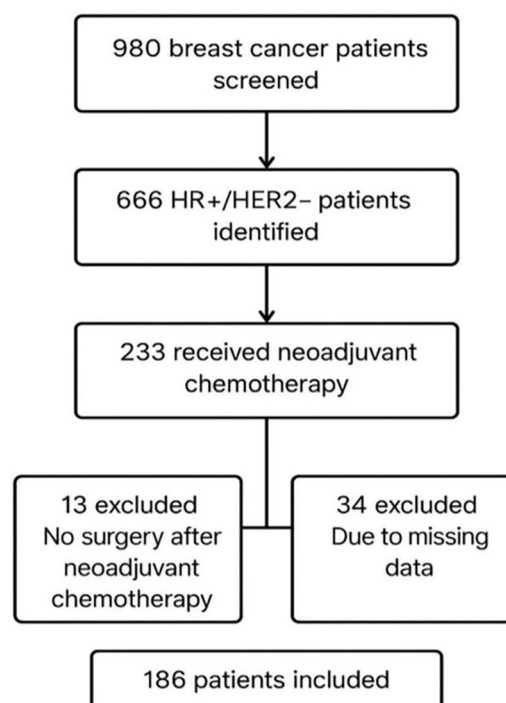


FIGURE 1: Patient selection flowchart for this study.

HER2: Human epidermal growth factor receptor 2; HR+: Hormone receptor positive

progesterone receptor (PR) status, HER2 status, Ki-67 index, chemotherapy regimen, pCR status, event-free survival (EFS), and overall survival (OS).

Adjuvant endocrine therapy was administered according to menopausal status and institutional treatment protocols. Postmenopausal patients received aromatase inhibitor therapy, whereas premenopausal patients received ovarian suppression combined with aromatase inhibitors. Perimenopausal patients received tamoxifen-based endocrine therapy.

Definitions

Staging was based on the eighth edition of the American Joint Committee on Cancer tumor, lymph node, and metastasis classification. HR positivity was defined as $\geq 1\%$ tumor cells showing nuclear staining via IHC, according to American Society of Clinical Oncology/College of American Pathologists guidelines.^{21,22} An ER expression level of 90% was used as the cut-off value based on previous studies demonstrating prognostic differences according to very high ER expression levels.¹⁵ A PR cut-off value of 20% and a Ki-67 cut-off value of 20% were selected according to commonly used thresholds in luminal BC classification and St. Gallen consensus recommendations.^{5,23} HER2 was assessed via IHC, with scores of 3+ considered positive and 0-1+ considered negative; 2+ cases were further evaluated using FISH. HER2 negativity was defined as 2+ without amplification on FISH.^{24,25}

Assessment of pR was based on postoperative pathology reports obtained after completion of NAC. pCR was defined as the absence of residual invasive carcinoma in both the breast and axillary lymph nodes (ypT0/TisN0).

Because the study was retrospective and multicenter, pR evaluation was not fully standardized across institutions. In some centers, pathological regression was assessed using the Miller-Payne grading system, whereas in others, residual disease was evaluated according to the residual cancer burden (RCB) classification. Therefore, partial pR (pPR) was defined as residual invasive disease characterized by documented pathological evidence of treatment-related tumor regression, including decreased tumor cellularity and/or a reduction in residual invasive tumor burden in the surgical specimen, without fulfilling the pCR criteria. Overall pR was defined as either pCR or pPR.

EFS was defined as the time from diagnosis to recurrence or death from any cause. OS was defined as the time from diagnosis to death from any cause.

Ethical Considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics

Committee of the University of Health Sciences Türkiye, Bağcılar Training and Research Hospital (approval number: 2025/02/09/026, date: 28.02.2025). Written informed consent was obtained from all participants.

Statistical Analysis

All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Univariate and multivariate logistic regression analyses were used to identify predictors of pCR, with odds ratios (ORs) and 95% confidence intervals (CIs) reported. Survival curves for EFS and OS were estimated using the Kaplan-Meier method and compared using the log-rank test. Independent prognostic factors for EFS and OS were determined using Cox proportional hazards regression models, and hazard ratios and 95% CIs were calculated. A two-sided p-value < 0.05 was considered statistically significant. Variables demonstrating statistical significance in univariate analysis ($p < 0.05$) were included in the multivariate Cox regression models.

RESULTS

Patient Characteristics

A total of 186 female patients with HR+/HER2- BC who received NAC were included, with a median follow-up duration of 30.6 months. The mean age was 48.5 years (range: 28-74), and 82.3% had a BMI ≥ 25 kg/m². Menopausal status was pre-/perimenopausal in 51.1% of participants and postmenopausal in 48.9%. Histologically, 61.8% of tumors were grade 2, and 57.0% were invasive ductal carcinomas. A Ki-67 index $\geq 20\%$ was observed in 72.0% of patients (median: 25%). Stage III disease was present in 59.7% of cases. ER expression $\geq 90\%$ was detected in 60.2%, and PR expression $\geq 20\%$ in 71.0%. Based on the molecular classification, 78.5% of tumors were classified as luminal B. Anthracycline- and taxane-based regimens were administered to 98.4% of patients. Total mastectomy was performed in 64.5% of patients, and axillary dissection in 81.7% of patients (Table 1).

pR

Overall, 25 patients (13.4%) achieved pCR. In the univariate analysis, ductal histology, grade 3 tumors, high Ki-67, ER $< 90\%$, and luminal B subtype were significantly associated with pCR. In multivariate logistic regression analysis, grade 3 tumors (OR: 4.49; 95% CI: 1.74-11.63; $p = 0.002$) and ER $< 90\%$ (OR: 2.64; 95% CI: 1.01-6.89; $p = 0.047$) remained independent predictors of pCR.

TABLE 1: Demographic and clinical characteristics of patients.

Variable	Category	n (%)
Age	<50 years	102 (54.8)
	≥50 years	84 (45.2)
BMI	<25	33 (17.7)
	≥25	153 (82.3)
Menopausal status	Pre-/perimenopausal	95 (51.1)
	Postmenopausal	91 (48.9)
Histological subtype	Ductal carcinoma	106 (57.0)
	Lobular carcinoma	26 (14.0)
	Others	54 (29.0)
Histologic grade	Grade I	29 (15.6)
	Grade II	115 (61.8)
	Grade III	42 (22.6)
Ki-67 proliferation index	<20%	52 (28.0)
	≥20%	134 (72.0)
ER	<90%	74 (39.8)
	≥90%	112 (60.2)
PR	<20%	54 (29.0)
	≥20%	132 (71.0)
Molecular subtype	Luminal A	40 (21.5)
	Luminal B	146 (78.5)
Clinical stage (AJCC 8 th)	Stage II	75 (40.3)
	Stage III	111 (59.7)

AJCC: American Joint Committee on Cancer; BMI: Body mass index; ER: Estrogen receptor; PR: Progesterone receptor; HR: Hazard ratio; CI: Confidence interval.

Survival Outcomes

During follow-up, 28 patients experienced recurrence or metastasis, and 18 died from BC. The median EFS was 92.02 months, while the median OS was not reached. The estimated 5 years EFS and OS rates were 77% and 88%, respectively.

Univariate Cox regression analysis identified ER ≥90%, PR ≥20%, and pR as favorable prognostic factors for EFS. In the multivariate analysis, ER ≥90% (hazard ratio: 2.70; 95% CI: 1.21-6.03; p=0.015) and pR (hazard ratio: 4.22; 95% CI: 1.40-12.74; p=0.011) were independently associated with improved EFS. The Kaplan-Meier curves demonstrated significant associations between the EFS and both ER ≥90% and pR. No significant association was found between pCR and EFS.

For OS, univariate Cox regression analysis showed that ER ≥90% and pR were favorable prognostic factors (Table 2).

In the multivariate model, ER ≥90% (hazard ratio: 3.159; 95% CI: 1.152-8.660; p=0.025) and pR (hazard ratio: 12.516; 95% CI: 2.921-53.627; p<0.001) remained independent predictors

TABLE 2: Univariate Cox regression analysis for overall survival.

Variable	Univariate HR (95% CI)	p-value*
Age		
<50 years	1.175 (0.465-2.972)	0.733
≥50 years		
Menopausal status		
Pre-/perimenopausal	1.626 (0.627-4.216)	0.317
Postmenopausal		
BMI		
<25	2.125 (0.485-9.306)	0.317
≥25		
Histology		
Ductal	1.033 (0.385-2.771)	0.949
Others		
Histologic grade		
Grade III	1.212 (0.393-3.738)	0.738
Others		
ER		
<90%	2.680 (0.999-7.186)	0.042*
≥90%		
PR		
<20%	1.761 (0.690-4.492)	0.236
≥20%		
Ki-67		
<20	1.614 (0.565-4.612)	0.371
≥20		
Luminal groups		
Luminal A	1.265 (0.360-4.446)	0.714
Luminal B		
Clinical stage		
Stage II	1.438 (0.564-3.667)	0.447
Stage III		
pCR		
Yes	2.624 (0.348-19.805)	0.350
No		
pR		
Yes	9.860 (2.408-40.376)	<0.001*
No		
* Indicates statistically significant results. BMI: Body mass index; ER: Estrogen receptor; PR: Progesterone receptor; pR: Pathologic response; pCR: Pathologic complete response; HR: Hazard ratio; CI: Confidence interval.		

* Indicates statistically significant results. BMI: Body mass index; ER: Estrogen receptor; PR: Progesterone receptor; pR: Pathologic response; pCR: Pathologic complete response; HR: Hazard ratio; CI: Confidence interval.

(Table 3). Kaplan-Meier analysis confirmed significant associations between OS and both ER ≥90% and pR (Figures 2 and 3), whereas no statistically significant correlation was observed between pCR and OS.

TABLE 3: Multivariate Cox regression analysis for overall survival.		
Variable	Multivariate HR (95% CI)	p-value*
ER		
<90%	3.159 (1.152-8.660)	0.025*
≥90%		
pR		
Yes	12.516 (2.921-53.627)	<0.001*
No		
* Indicates statistically significant results. ER: Estrogen receptor; pR: Pathologic response.		

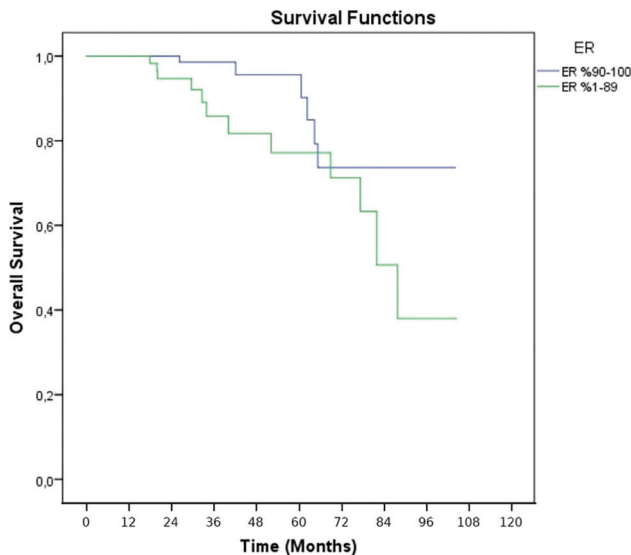


FIGURE 2: Kaplan-Meier curves for OS according to ER expression levels. Patients with high ER expression (90-100%, blue line) demonstrated significantly improved OS compared with those with lower ER expression (1-89%, green line) (log-rank test, $p=0.042$).
OS: Overall survival; ER: Estrogen receptor

DISCUSSION

This retrospective study evaluated pCR rates, survival outcomes, and prognostic factors among patients with HR+/HER2- BC treated with NAC. The pCR rate of 13.4% in our cohort is consistent with previously reported ranges (9.1-17%).¹⁸⁻²⁰ Differences in the pCR rates across studies may be attributed to variations in patient characteristics, treatment regimens, and pathological assessment criteria.

Our findings indicate that an ER expression <90% was independently associated with higher pCR rates, aligning with prior studies.^{14,20} Lower ER expression may reflect a more proliferative tumor phenotype and potentially increase chemosensitivity. Similarly, grade 3 tumors were significantly associated with pCR, consistent with the previous literature.¹⁸ These tumors generally have higher proliferation rates, rendering them more susceptible to cytotoxic agents.

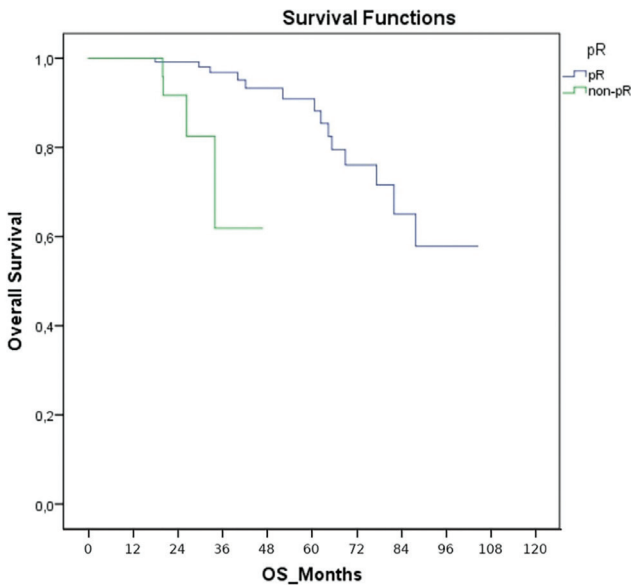


FIGURE 3: Kaplan-Meier curves for OS according to pR status. Patients achieving pR (blue line) demonstrated significantly improved OS compared with those without pR (green line) (log-rank test, $p<0.001$).
OS: Overall survival; pR: Pathologic response

Although pCR has traditionally been regarded as a surrogate marker for improved survival, our results show no statistically significant association between pCR and either EFS or OS in HR+/HER2- disease. However, when complete and pPR were combined (pR), both EFS and OS improved significantly. This finding suggests that in this subtype, achieving any degree of pathological tumor reduction may have prognostic relevance, as supported by von Minckwitz et al.¹¹

The absence of a significant association between pCR and survival outcomes in our cohort may reflect the unique biological characteristics of HR+/HER2- BC. Unlike triple-negative and HER2-positive subtypes, HR+/HER2- tumors generally exhibit lower chemosensitivity and lower pCR rates following NAC.^{12,13} Furthermore, several studies have suggested that pCR may not serve as a sufficiently robust surrogate marker for long-term survival in luminal BC, where endocrine responsiveness and intrinsic tumor biology play a more substantial prognostic role.^{11,15}

In contrast, our findings demonstrated that overall pR, including both complete and partial pathological regression, was associated with improved EFS and OS. This observation suggests that even partial tumor reduction following NAC may indicate treatment sensitivity and less aggressive tumor biology in HR+/HER2- disease. Given the relatively indolent clinical course of many luminal tumors, modest pathological regression may still translate into favorable long-term outcomes despite residual disease.

Additionally, the limited number of patients achieving pCR in our cohort may have reduced the statistical power to detect a survival difference. Therefore, the prognostic significance of pCR alone in HR+/HER2- BC should be interpreted cautiously, particularly in retrospective real-world studies with heterogeneous pR patterns.

Our findings suggest that broader pR assessment beyond strict pCR definitions may provide additional prognostic information in HR+/HER2- BC. This may be particularly relevant in luminal tumors, where substantial but incomplete tumor regression could still reflect biologically meaningful treatment sensitivity.

Importantly, an ER $\geq 90\%$ emerged as an independent predictor of longer EFS and OS, consistent with prior studies.^{14,15} HR+ tumors typically exhibit less aggressive biology, and the prolonged benefit from adjuvant endocrine therapy likely contributes to improved outcomes in this subgroup.

Study Limitations

The limitations include its retrospective design, the absence of multigene assay data, and a relatively short median follow-up, which may limit the assessment of late recurrences, events that are typical of HR+/HER2- tumors. In addition, pR assessment was not completely standardized across participating centers, as Miller-Payne grading and RCB classifications were used according to institutional routine practice. Therefore, pPR evaluations may have been subject to interobserver variability.

CONCLUSION

HR+/HER2- BCs generally achieve lower pCR rates following NAC. Nevertheless, the presence of a pR, high ER expression, and adjuvant endocrine therapy were associated with improved survival outcomes. Prospective studies are warranted to validate these findings and refine NAC selection criteria in this patient population.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Health Sciences Türkiye, Bağcılar Training and Research Hospital (approval number: 2025/02/09/026, date: 28.02.2025).

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ş.B., U.A.Y., B.Ç.D., M.T., N.B., G.U.E., Concept: Ş.B., U.A.Y., B.Ç.D., M.T., N.S.A., A.M., N.E., E.Ç., E.D., N.Ş., G.U.E., Design: Ş.B., B.Ç.D., N.S.A., A.M., A.Ö., N.E., E.Ç., E.D., N.Ş., Data Collection or Processing: Ş.B., U.A.Y., B.Ç.D., N.S.A., A.Ö., E.Ç., N.B., G.U.E., Analysis or

Interpretation: Ş.B., U.A.Y., M.T., A.M., N.E., E.D., N.Ş., N.B., G.U.E., Literature Search: Ş.B., A.Ö., E.D., G.U.E., Writing: Ş.B.

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

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Neoadjuvant TCHP for Stage II-III HER2-positive Breast Cancer: A Real-world Cohort

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ABSTRACT

Objective: The neoadjuvant combination regimen of taxane, carboplatin, trastuzumab, and pertuzumab (TCHP) is widely used for stage II-III human epidermal growth factor receptor 2 (HER2)-positive breast cancer; however, real-world pathologic complete response (pCR) rates and predictors vary. We evaluated pCR, response correlates, and treatment outcomes in a two-center cohort.

Material and Methods: Consecutive non-metastatic HER2-positive patients with clinical T2-4 and/or N1-3 who were treated with neoadjuvant TCHP at two centers from 2022 to 2025 were retrospectively analyzed. pCR was defined as ypT0/is ypN0. Groups were compared, and univariable logistic regression was used to assess factors associated with pCR.

Results: Among 43 patients (median age, 48 years), pCR occurred in 24 patients (55.8%). Hormone receptor (HR)-negative tumors were more frequent in the pCR group (66.7% vs. 31.6%; $p=0.033$). HR positivity predicted lower odds of pCR (odds ratio: 0.231; 95% confidence interval: 0.064-0.836; $p=0.026$), whereas age, baseline T/N stage, grade, and Ki-67 were not significant predictors. T- and N- downstaging occurred in 81.4% and 74.4%, respectively. Radiologic-pathologic agreement was limited (breast $\kappa=0.272$; axilla $\kappa=0.081$). Grade 3-4 toxicity was 11.6% (cardiotoxicity 4.7%). Breast-conserving surgery was performed in 72.1%; sentinel lymph node biopsy was performed in 79.1%. Median follow-up was 18 months, with one local recurrence and no deaths.

Conclusion: Real-world neoadjuvant TCHP achieved a 55.8% pCR rate with substantial downstaging. HR-negative disease was the key correlate, and imaging CR was an unreliable surrogate for pCR.

Keywords: Breast neoplasms; receptor, ErbB-2; neoadjuvant therapy; treatment outcome

INTRODUCTION

Breast cancer remains the leading malignancy among women globally, with more than 25,000 new diagnoses reported annually in Türkiye.¹ Human epidermal growth factor receptor 2 (HER2)-positive disease represents approximately 15-20% of breast cancers.² Despite major improvements in outcomes with HER2-directed therapies, stage II-III HER2-positive breast cancer continues to encompass a biologically and clinically heterogeneous population. In this setting,

residual invasive disease after neoadjuvant therapy identifies patients who may benefit from post-neoadjuvant treatment escalation.³ Neoadjuvant systemic therapy offers several clinical advantages, including downstaging of the breast tumor and axilla, facilitation of breast-conserving surgery (BCS), and direct assessment of treatment responsiveness *in vivo*.^{3,4} Pathologic complete response (pCR) is associated with improved long-term outcomes, particularly in patients with HER2-positive, hormone receptor (HR)-negative tumors.⁵ For patients with residual invasive disease, post-

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neoadjuvant escalation has become an established strategy; in the KATHERINE trial, adjuvant trastuzumab emtansine significantly improved invasive disease-free survival compared with continued trastuzumab.⁶ Accordingly, pCR has become a clinically meaningful endpoint that also guides post-neoadjuvant management.

For stage II-III HER2-positive breast cancer, standard treatment combines chemotherapy with HER2-directed therapy, with dual HER2 blockade using trastuzumab and pertuzumab recommended for tumors >2 cm and/or node-positive disease.³ The addition of pertuzumab improved pCR in the NeoSphere trial; the trastuzumab, docetaxel, and pertuzumab arm achieved a pCR rate of approximately 46%.⁷ In clinical practice, the anthracycline-free taxane, carboplatin, trastuzumab, and pertuzumab (TCHP) regimen has since become widely used in routine practice, with pCR rates of approximately 66% in TRYPHAENA⁸ and 55.7% in KRISTINE.⁹ However, pCR rates are strongly influenced by tumor biology, with HR-negative tumors consistently showing a higher likelihood of pCR.¹⁰

The shift toward anthracycline-free strategies is driven by efforts to maintain efficacy while reducing toxicity. Anthracyclines are associated with cardiotoxicity when used alongside HER2-targeted therapy and, less commonly, with secondary hematologic malignancies.^{11,12} In TRAIN-2, the addition of anthracyclines to dual HER2 blockade did not provide a clinically meaningful efficacy advantage, further supporting anthracycline-free strategies in appropriately selected patients.¹³

Against this background, we conducted a two-center retrospective study of patients with stage II-III HER2-positive breast cancer who were treated with neoadjuvant TCHP in routine clinical practice. In addition to evaluating real-world pCR rates and clinicopathologic correlates of response, we assessed the concordance of radiologic complete response with surgical pathology findings in the breast and axilla to inform interpretation of post-treatment imaging for surgical decision-making.

MATERIAL AND METHODS

Study Design and Population

This two-center retrospective cohort study evaluated patients with non-metastatic, HER2-positive breast cancer who were treated with neoadjuvant TCHP between 2022 and 2025. Patients were identified from institutional oncology records at the two participating centers. Eligible patients had clinical T2-4 and/or N1-3 disease and received neoadjuvant TCHP as part of routine care. Clinical, imaging, pathology, treatment, toxicity, and follow-up data were collected from electronic medical records.

Eligibility Criteria

Inclusion criteria were age ≥ 18 years, histologically confirmed HER2-positive breast carcinoma, absence of distant metastasis at baseline staging, and available post-neoadjuvant surgical pathology for response assessment. HER2 positivity was defined according to immunohistochemistry (IHC) and/or fluorescence *in situ* hybridization (FISH) results available in pathology reports. Patients were excluded if they had a synchronous malignancy, lacked key clinical or pathologic data required for response assessment, had unavailable radiologic or surgical pathology reports, or had incomplete follow-up information. During the study period, 242 patients with early-stage or locally advanced HER2-positive breast cancer who were receiving neoadjuvant treatment were screened. After excluding patients with missing key variables ($n=18$), those who received non-TCHP neoadjuvant regimens ($n=172$), and those with ongoing treatment, no surgery, or non-assessable pCR status ($n=9$), the final analytic cohort comprised 43 patients. Patient selection is summarized in Figure 1.

Definitions and Study Variables

The primary outcome was pCR. pCR was defined as the absence, on examination of the surgical specimen, of residual invasive carcinoma in the breast and axillary lymph nodes (ypT0/is ypN0). Patients were categorized into pCR and non-pCR groups based on final surgical pathology.

Prespecified covariates included patient-related characteristics (age, sex, performance status, comorbidities, menopausal status); tumor-related factors (HR status, histologic grade, Ki-67); HER2 category (IHC 3+; IHC 2+ with FISH amplification); and post-neoadjuvant/adjuvant treatment modalities (radiotherapy; anti-HER2 therapy; and endocrine therapy). HER2 expression categories were not included in comparative analyses because non-IHC 3+ cases were rare, and their inclusion would have produced unstable estimates. Treatment-related adverse events were captured from routine clinical documentation and analyzed only for grade 2-4 toxicities; grade 1 events were not systematically collected.

Radiologic Response Assessment

Systemic staging at diagnosis was performed according to routine institutional practice. Baseline local breast and axillary staging modalities were recorded; these included breast magnetic resonance imaging (MRI), breast and axillary ultrasonography (US), or both US and MRI. Radiologic response following neoadjuvant therapy was assessed using imaging modalities commonly used in routine clinical practice, including MRI, breast and axillary US, positron

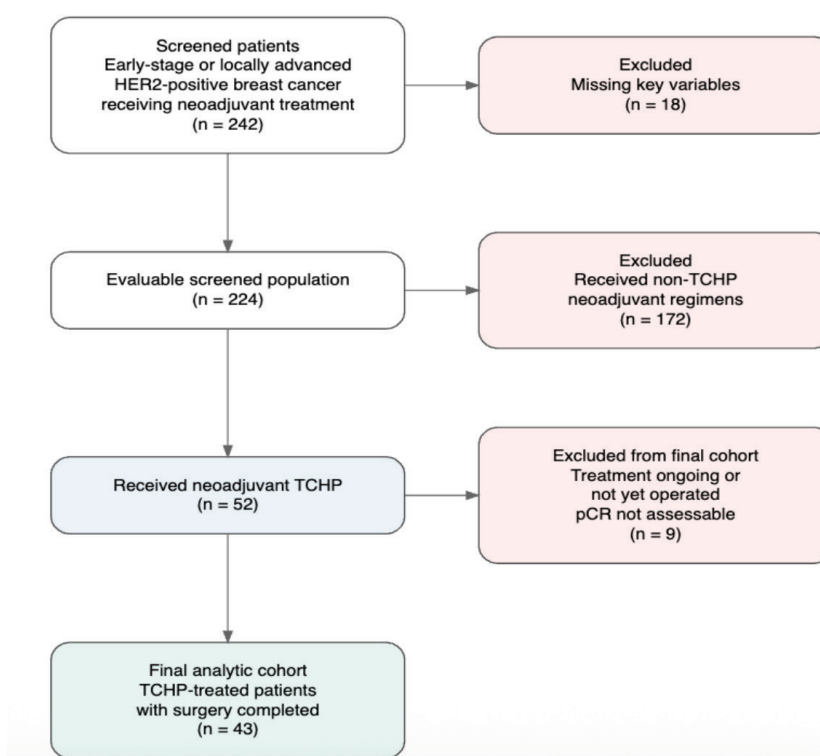


FIGURE 1: Flow diagram of patient selection.

HER2: Human epidermal growth factor receptor 2; TCHP: Docetaxel, carboplatin, trastuzumab, and pertuzumab; pCR: Pathologic complete response

emission tomography/computed tomography (PET/CT), or combined US and PET/CT. Imaging modality selection was based on institutional practice rather than a predefined study protocol.

Radiologic response was retrospectively abstracted from formal radiology reports. Central radiologic re-review was not performed. Standardized RECIST or PERCIST measurements were not uniformly available across all patients and imaging modalities; therefore, radiologic CR was defined pragmatically as the absence of a visible residual primary breast lesion on post-treatment imaging. Axillary radiologic CR was defined as the absence of suspicious residual axillary lymph nodes on post-treatment imaging, based on the reporting radiologist's assessment.

Statistical Analysis

All analyses were performed using IBM SPSS Statistics version 30.0 and R. Distributional assumptions for continuous variables were assessed using the Kolmogorov-Smirnov test. Continuous variables are presented as mean $\pm$ standard deviation when normally distributed and as median (range or interquartile range) when non-normally distributed; categorical variables are reported as frequencies and

percentages. Baseline and treatment-related characteristics were compared between the pCR and non-pCR groups using the Student's t-test or the Mann-Whitney U test for continuous variables and the chi-square test or the Fisher's exact test for categorical variables, as appropriate. Univariable logistic regression was used to explore factors associated with pCR, and results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). Because of the small sample size, multivariable modeling was not performed. A two-sided p-value of <0.05 was considered statistically significant. Survival outcomes were not formally analyzed because follow-up was not sufficiently mature, and the number of events was very low.

Ethical Approval

The study protocol was reviewed and approved by the Dokuz Eylül University Non-Interventional Clinical Research Ethics Committee (approval number: 2026/09-10, date: 02.03.2026). Given the retrospective design and the use of routinely collected clinical data, the requirement for written informed consent was waived. The study was conducted in accordance with the Declaration of Helsinki, and all data were anonymized and de-identified prior to analysis to ensure patient confidentiality.

RESULTS

Among the 43 patients included in the study, the median age was 48 years (range, 28-78), and menopausal status was evenly distributed (51.2% premenopausal and 48.8% postmenopausal). Most patients (95.3%) underwent PET-based initial staging. Baseline local staging was performed using MRI alone in 21 patients (48.8%), US alone in 18 patients (41.9%), and combined US and MRI in 4 patients (9.3%). At presentation, the clinical T stage was most commonly T2 (53.5%), followed by T3 (20.9%), T1 (18.6%), and T4 (7.0%).

Multicentric or multifocal disease was present in 27.9% of cases. Clinical nodal stages were predominantly N1 (51.2%) and N2 (27.9%). HR status was balanced (51.2% HR-negative; 48.8% HR-positive); the median Ki-67 was 35% (range, 10-90); the grade distribution was similar (G2: 51.2%; G3: 48.8%) (Table 1).

pCR was achieved in 24 patients, corresponding to a pCR rate of 55.8% (24/43), while 19 patients (44.2%) had residual disease. Baseline characteristics were largely similar between the pCR and non-pCR groups; however, HR status differed

TABLE 1: Baseline clinicopathologic characteristics of the cohort according to pCR status.

Variable	All patients (n=43)	pCR (n=24)	Non-pCR (n=19)	p-value
Age, years	48 (28-78)	53 (29-78)	45 (28-69)	0.359
Menopausal status				
Premenopause	22 (51.2)	12 (50.0)	10 (52.6)	1.000
Postmenopause	21 (48.8)	12 (50.0)	9 (47.4)	
BMI (n=36)	27.14 (19.95-37.11)	26.48 (19.95-35.55)	27.49 (21.08-37.11)	0.824
Baseline systemic staging modality				
PET/CT	41 (95.3)	23 (95.8)	18 (94.7)	1.000
Conventional CT	2 (4.7)	1 (4.2)	1 (5.3)	
Baseline local staging modality				
MRI	21 (48.8)	14 (58.3)	7 (36.8)	0.158
US	18 (41.9)	7 (29.2)	11 (57.9)	
US + MRI	4 (9.3)	3 (12.5)	1 (5.3)	
Multicentric/multifocal tumor	12 (27.9)	5 (20.8)	7 (36.8)	0.314
Primary tumor size, mm	28 (12-70)	28 (12-70)	28 (12-52)	0.569
Clinical T stage				
T1	8 (18.6)	3 (12.5)	5 (26.3)	0.430
T2	23 (53.5)	12 (50.0)	11 (57.9)	
T3	9 (20.9)	7 (29.2)	2 (10.5)	
T4	3 (7.0)	2 (8.3)	1 (5.3)	
Clinical N stage				
N0	5 (11.6)	4 (16.7)	1 (5.3)	0.472
N1	22 (51.2)	12 (50.0)	10 (52.6)	
N2	12 (27.9)	7 (29.2)	5 (26.3)	
N3	4 (9.3)	1 (4.2)	3 (15.8)	
HR status				
HR negative	22 (51.2)	16 (66.7)	6 (31.6)	0.033
HR positive	21 (48.8)	8 (33.3)	13 (68.4)	
Ki-67, %	35 (10-90)	32.5 (15-90)	35 (10-80)	0.815
Grade				
G2	22 (51.2)	13 (54.2)	9 (47.4)	0.763
G3	21 (48.8)	11 (45.8)	10 (52.6)	
Baseline CA15-3 (n=35)	18.6 (6.0-64.6)	15.7 (6.9-24.4)	21.5 (6.0-64.6)	0.092
BMI: Body mass index; HR: Hormone receptor; MRI: Magnetic resonance imaging; pCR: Pathologic complete response; PET/CT: Positron emission tomography computed tomography; US: Ultrasonography.				

BMI: Body mass index; HR: Hormone receptor; MRI: Magnetic resonance imaging; pCR: Pathologic complete response; PET/CT: Positron emission tomography/computed tomography; US: Ultrasonography.

significantly: HR-negative tumors were more frequent among patients achieving pCR (66.7% vs. 31.6%; $p=0.033$) (Table 1 and Figure 2). Because of the small number of patients in some stage subgroups, this comparison was interpreted as exploratory.

Univariable logistic regression analyses for pCR are presented in Table 2. HR positivity was associated with significantly lower odds of achieving pCR compared with HR-negative disease (OR: 0.231; 95% CI: 0.064-0.836; $p=0.026$). In contrast, age, menopausal status, multicentric/multifocal disease, clinical T stage, clinical N stage, tumor grade, Ki-67, and body mass index were not significantly associated with pCR (all $p>0.05$).

Beyond pCR, neoadjuvant treatment led to clinically meaningful stage migration of both the primary tumor and the axilla, with T downstaging in 35/43 (81.4%) and N downstaging in 32/43 (74.4%), including transitions to ypT0 in 25/43 (58.1%), of whom 21 originated from $cT\geq 2$, and to ypN0 in 31/43 (72.1%), including 27 conversions from $cN\geq 1$ (Figure 3).

Post-treatment radiologic response was assessed in 35 patients (81.4%), most commonly by MRI (40.0%) or ultrasound (34.3%), with no difference in imaging modality distribution between groups ($p=0.404$). Primary breast radiologic CR was observed in 15/35 patients (42.9%) and was numerically more frequent among patients achieving pCR (57.9% vs. 25.0%),

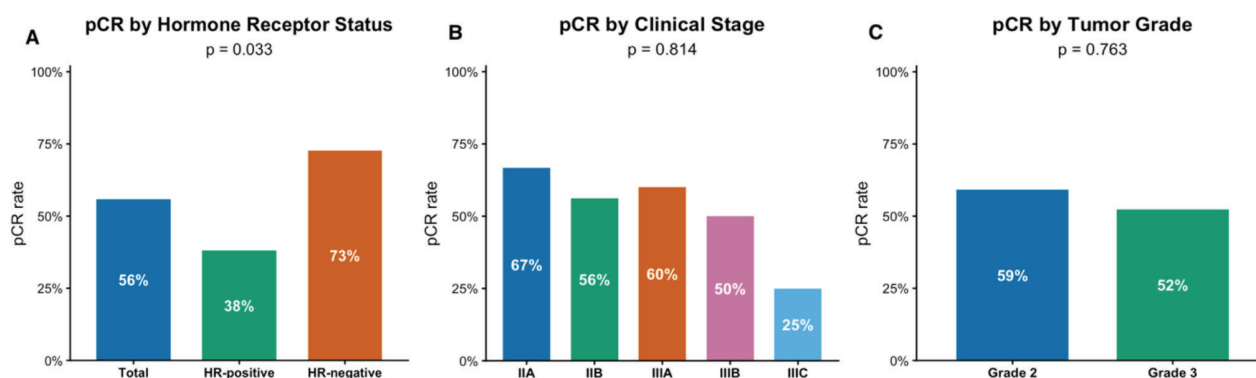


FIGURE 2: pCR rates stratified by (A) HR status, (B) clinical stage, and (C) tumor grade. The comparison across clinical-stage subgroups was considered exploratory because of the small numbers of patients in some stage categories.

HR: Hormone receptor; pCR: Pathologic complete response

TABLE 2: Univariate regression analyses for pCR.

Variable	Comparison/unit	OR (95% CI)	p-value
Age	Per 1 year increase	1.023 (0.967-1.081)	0.433
Menopausal status	Post- vs. pre- (ref)	1.111 (0.333-3.706)	0.864
Multicentric/multifocal tumor	Yes vs. no (ref)	0.451 (0.116-1.751)	0.250
Clinical T stage	Overall (ref=T1)	—	0.406
T2 vs. T1		1.818 (0.350-9.455)	0.477
T3 vs. T1		5.833 (0.696-48.873)	0.104
T4 vs. T1		3.333 (0.204-54.532)	0.398
Clinical N stage	Overall (ref=N0)	—	0.486
N1 vs. N0		0.300 (0.029-3.135)	0.315
N2 vs. N0		0.350 (0.029-4.153)	0.406
N3 vs. N0		0.083 (0.004-1.945)	0.122
HR status	HR+ vs HR- (ref)	0.231 (0.064-0.836)	0.026
Grade	G3 vs. G2 (ref)	0.762 (0.228-2.545)	0.658
Ki-67 (%)	per 1% increase	0.997 (0.971-1.025)	0.855
BMI	per 1 kg/m ² increase	1.010 (0.864-1.180)	0.904

BMI: Body mass index; HR: Hormone receptor; OR: Odds ratio; CI: Confidence interval.

but the difference did not reach statistical significance ($p=0.088$; dichotomized $p=0.087$). Axillary radiologic CR was noted in 16/34 patients (47.1%) and was more common in the pCR group (57.9% vs. 33.3%); however, this difference was not statistically significant ($p=0.372$; dichotomized $p=0.185$) (Table 3).

Among patients with available post-treatment radiologic response assessment ($n=35$), baseline local staging was

performed with MRI alone in 14 (40.0%), with US alone in 17 (48.6%), and with combined US and MRI in 4 (11.4%). The same imaging modality was used for both baseline local staging and post-treatment response assessment in 23 patients (65.7%): MRI-to-MRI in 14 (40.0%) and US-to-US in 9 (25.7%). When combined modalities that shared the same local imaging component were considered, an overlapping local modality was observed in 30 patients (85.7%). In the

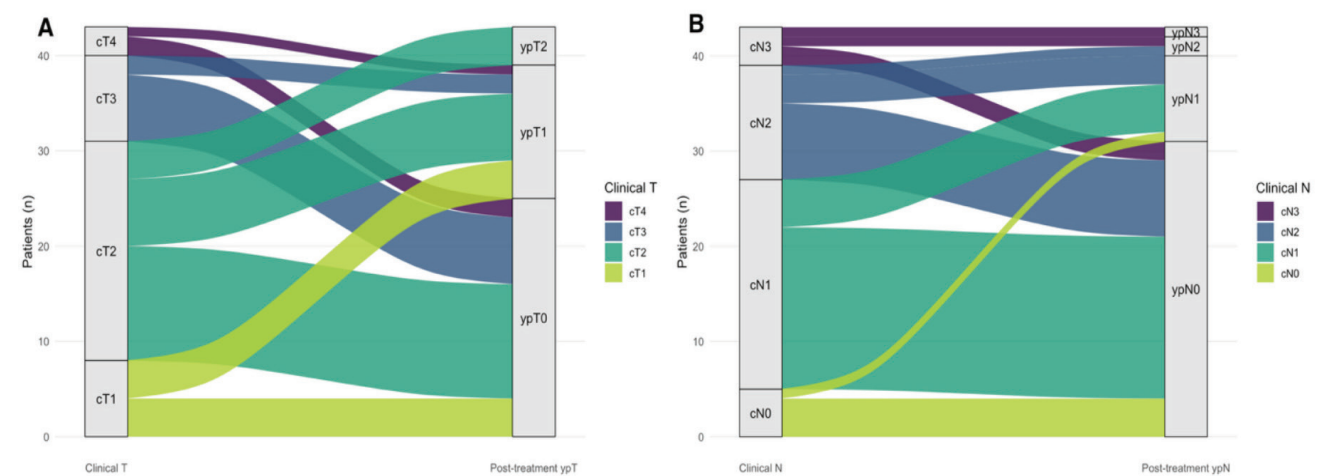


FIGURE 3: Sankey diagrams showing transitions from (A) clinical T to post-treatment pathologic ypT and (B) clinical N stage to post-treatment pathologic ypN stage after neoadjuvant TCHP.

TCHP: Docetaxel, carboplatin, trastuzumab, and pertuzumab

TABLE 3: Neoadjuvant treatment exposure, toxicity, and radiologic response assessment according to pCR status.

Variable	All patients	pCR	Non-pCR	p-value
Number of TCHP doses (n=42)	6 (3-6)	6 (3-6)	6 (6-6)	0.072
Grade 2 toxicity	12 (27.9)	5 (20.8)	7 (36.8)	0.626
Grade 3-4 toxicity	5 (11.6)	3 (12.5)	2 (10.5)	0.918
Radiologic response assessment	35 (81.4)	19 (79.2)	16 (84.2)	1.000
MRI	14 (40.0)	9 (47.4)	5 (31.3)	0.404
US	12 (34.3)	7 (36.8)	5 (31.3)	
PET/CT	5 (14.3)	1 (5.3)	4 (25.0)	
US + PET/CT	4 (11.4)	2 (10.5)	2 (12.5)	
Primary breast radiologic response (n=35)				
CR	15 (42.9)	11 (57.9)	4 (25.0)	0.088
PR	19 (54.3)	8 (42.1)	11 (68.8)	
PD	1 (2.9)	0 (0.0)	1 (6.3)	
Axillary radiologic response (n=34)				
CR	16 (47.1)	11 (57.9)	5 (33.3)	0.372
PR	12 (35.3)	6 (31.6)	6 (40.0)	
SD	5 (14.7)	2 (10.5)	3 (20.0)	
PD	1 (2.9)	0 (0.0)	1 (6.7)	
CR: Complete response; MRI: Magnetic resonance imaging; TCHP: Docetaxel, carboplatin, trastuzumab, and pertuzumab; pCR: Pathologic complete response; PET/CT: Positron emission tomography/computed tomography; PD: Progressive disease; PR: Partial response; SD: Stable disease; US: Ultrasonography.				

remaining 5 patients (14.3%), post-treatment response assessment was based on PET/CT because the same baseline local imaging modality was not available.

Agreement between radiologic and pCRs was modest. For the primary breast tumors (n=35), radiologic CR showed an observed agreement of 62.9% with pathologic ypT0 status, corresponding to fair agreement (Cohen's $\kappa=0.272$). This concordance did not reach statistical significance (κ , $p=0.094$ by asymptotic testing; $p=0.162$ by Monte Carlo). For the axilla (n=34), agreement between radiologic axillary CR and pathologic ypN0 status was low (observed agreement, 52.9%) and negligible beyond chance ($\kappa=0.081$), with no evidence of statistically significant concordance ($p=0.595$).

Primary breast surgery favored breast-conserving approaches in the overall cohort: 31 of 43 patients (72.1%) underwent BCS, while 12 patients (27.9%) underwent mastectomy. Axillary management was predominantly de-escalated: 34 of 43 patients (79.1%) underwent sentinel lymph node biopsy, whereas only 9 (20.9%) required axillary lymph node dissection (ALND).

Adjuvant anti-HER2 treatment patterns diverged sharply by response status ($p<0.001$). Patients who achieved pCR almost uniformly received adjuvant trastuzumab (23/24; 95.8%), with one patient receiving trastuzumab plus pertuzumab (1/24; 4.2%); none received T-DM1. In contrast, among patients without pCR, adjuvant T-DM1 was the most common regimen (11/19; 57.9%), while trastuzumab alone

was used less frequently (5/19; 26.3%), and trastuzumab plus pertuzumab was uncommon (1/19; 5.3%). Two non-pCR patients (2/19; 10.5%) did not receive adjuvant anti-HER2 therapy: one because of cardiotoxicity and the other because reassessment of the primary pathology and review of the surgical specimen demonstrated HER2 negativity. Surgical and adjuvant treatments are summarized in Table 4.

Neoadjuvant treatment exposure and toxicity were comparable between groups. Among patients with available cycle data (n=42), the median number of TCHP cycles was 6 (range, 3-6) both overall and in the pCR group; all non-pCR patients received 6 cycles, which represented a non-significant trend ($p=0.072$). Overall, grade 2 toxicity occurred in 12/43 patients (27.9%) and grade 3-4 toxicity occurred in 5/43 patients (11.6%). The most frequent grade 2 events were neutropenia (4/43; 9.3%) and diarrhea (3/43; 7.0%), followed by nausea (2/43; 4.7%) and isolated cases of hepatotoxicity, fatigue, and anemia-thrombocytopenia (each 1/43; 2.3%). Grade 3-4 toxicities were uncommon and included cardiotoxicity (2/43; 4.7%), neutropenia (1/43; 2.3%), fatigue (1/43; 2.3%), and neurologic toxicity (1/43; 2.3%). Only one patient discontinued treatment because of neurologic adverse events and proceeded directly to surgery after three cycles of treatment.

Median follow-up was 18 months, during which no deaths were observed. Only one patient experienced local disease recurrence; therefore, survival outcomes were considered immature and not formally analyzed.

TABLE 4: Surgical management and adjuvant treatments according to pCR status.

Variable	All patients	pCR	Non-pCR	p-value
Primary surgery				
BCS	31 (72.1)	17 (70.8)	14 (73.7)	1.000
Mastectomy	12 (27.9)	7 (29.2)	5 (26.3)	
Axillary surgery				
SLNB	34 (79.1)	21 (87.5)	13 (68.4)	0.127
ALND	9 (20.9)	3 (12.5)	6 (31.6)	
Adjuvant anti-HER2				
None	2 (4.7)	0 (0.0)	2 (10.5)	<0.001
Trastuzumab	28 (65.1)	23 (95.8)	5 (26.3)	
Trastuzumab + pertuzumab	2 (4.7)	1 (4.2)	1 (5.3)	
Trastuzumab emtansine	11 (25.6)	0 (0.0)	11 (57.9)	
Adjuvant radiotherapy	40 (93.0)	22 (91.7)	18 (94.7)	1.000
Adjuvant endocrine therapy (n=21)				
Aromatase inhibitor	10 (47.6)	3 (37.5)	7 (53.8)	0.010
Tamoxifen	4 (19.0)	0 (0.0)	4 (30.8)	
LHRH agonist + aromatase inhibitor/tamoxifen	7 (33.3)	5 (62.5)	2 (15.4)	
ALND: Axillary lymph node dissection; BCS: Breast-conserving surgery; HER2: Human epidermal growth factor receptor 2; LHRH: Luteinizing hormone-releasing hormone; SLNB: Sentinel lymph node biopsy.				

ALND: Axillary lymph node dissection; BCS: Breast-conserving surgery; HER2: Human epidermal growth factor receptor 2; LHRH: Luteinizing hormone-releasing hormone; SLNB: Sentinel lymph node biopsy.

DISCUSSION

In this two-center, retrospective, real-world cohort of patients with stage II-III HER2-positive breast cancer treated with neoadjuvant TCHP, the pCR rate was 55.8%. More than two-thirds of patients underwent BCS, and axillary management was frequently de-escalated, reflecting a substantial treatment effect in routine practice. Toxicity was generally manageable, with grade 3-4 events uncommon and cardiotoxicity observed in a small minority.

Although the overall pCR rate in our cohort (55.8%) supports the activity of neoadjuvant TCHP in routine practice, it was numerically lower than the 66.2% reported in the phase II TRYPHAENA study.⁸ However, our finding is closely aligned with the 55.7% pCR rate observed in the standard TCHP arm of the Phase III KRISTINE trial.⁹ This difference from TRYPHAENA should not be interpreted solely as a shortfall in real-world treatment delivery; it may also be partly explained by baseline disease burden and by the less-selected nature of our cohort. Specifically, 88.4% of our patients had clinically N+ disease at presentation. The nodal burden in our cohort was higher than that reported in KRISTINE and more similar to that observed in the high-risk population evaluated in TRAIN-2.^{9,13} Furthermore, nearly half of our cohort (48.8%) had HR+ disease. Across real-world cohorts treated with TCHP, pCR rates have ranged between 48% and 64%, highlighting that pCR rates vary substantially across populations even under the same regimen.¹⁴⁻¹⁷

Tumor biology appeared to be the clearest factor associated with response in our cohort. HR status was the only factor that significantly differed between the pCR and non-pCR groups, with the pCR rate dropping from 72.7% in HR-negative patients to 38.1% in HR-positive patients. This pattern is consistent with large TCHP cohorts that demonstrate markedly higher pCR rates in ER-negative compared with ER-positive disease (77.9% vs. 47.5%), which emphasizes that endocrine signaling modulates responsiveness to dual HER2 blockade.^{10,18} Prior biomarker studies suggest that response heterogeneity within ER-positive/HER2-positive disease may relate to HER2 expression intensity and immune contexture, such as tumor-infiltrating lymphocytes.¹⁸ These factors were not available in our dataset, and therefore should be evaluated in larger cohorts.

From a surgical standpoint, our high breast-conservation rate and low ALND utilization are clinically meaningful because they reflect patient-centered benefits, including effective neoadjuvant therapy, less extensive breast and axillary surgery, and potential reductions in morbidity. This aligns with real-world TCHP cohorts, in which breast-conservation rates

are approximately 70%, and outcomes differ by pCR status, favoring patients with pCR.¹⁴ Moreover, our cohort reflects contemporary post-neoadjuvant management: adjuvant anti-HER2 strategies diverged substantially according to response status, with T-DM1 used exclusively in non-pCR patients, which is consistent with the availability of escalation approaches for residual disease in modern practice.³

Beyond the pCR rate itself, the most distinctive contribution of this cohort is the evaluation, in routine practice, of radiologic-pathologic concordance in both the breast and axilla after neoadjuvant TCHP. A key pragmatic finding was the limited agreement between radiologic and pathologic responses. In our cohort, post-treatment radiologic response assessment was available in 35 patients and was most commonly based on breast MRI or breast/axillary US, with PET/CT used in a smaller proportion of patients. Importantly, baseline and post-treatment imaging showed modality continuity in most patients: the same imaging modality was used in 23 of 35 patients, and an overlapping local imaging component was present in 30 of 35 patients when combined modalities were considered. Radiologic CR showed only fair concordance with ypT0 in the breast ($\kappa=0.272$) and negligible concordance with ypN0 in the axilla ($\kappa=0.081$), indicating that an imaging complete response should not be used as a surrogate for pCR and cannot reliably exclude microscopic residual disease.

In our routine institutional practice, post-neoadjuvant imaging is used as a complementary tool to guide breast surgical planning and axillary evaluation, rather than as a stand-alone determinant of response. For axillary management, decisions are generally based on baseline nodal status, clinical examination, and surgical/pathologic assessment; radiologic CR alone is not considered sufficient to omit pathologic evaluation. Although baseline and post-treatment imaging showed modality overlap in most patients, imaging was selected according to routine clinical practice rather than a predefined protocol. Therefore, the observed discordance should be interpreted as a pragmatic estimate of radiologic-pathologic agreement in routine practice rather than as a modality-specific assessment of imaging accuracy.

With respect to safety, neoadjuvant TCHP was generally well tolerated in our cohort, with grade 3-4 toxicity and cardiotoxicity observed in 11.6% and 4.7% of patients, respectively. The relatively low rate of grade 3-4 toxicity may partly reflect patient selection, as the cohort had a young median age and included patients who were considered suitable for neoadjuvant TCHP. However, detailed data on dose reductions, treatment delays, G-CSF use, and other supportive care interventions were not uniformly available, limiting our ability to determine their contribution to toxicity

outcomes. Compared with a large real-world Korean TCHP experience (n=447), in which adverse events, particularly anemia and non-hematologic toxicities such as diarrhea, were commonly documented,¹⁴ our observed high-grade event rates appeared numerically lower; however, direct comparisons are limited by differences in toxicity capture, grading practices, and treatment modification strategies. Importantly, grade 1 adverse events were not systematically captured in the present study, and toxicity data were extracted from routine clinical documentation, which may have led to an underestimation of the toxicity burden. Nevertheless, the overall pattern supports the feasibility of anthracycline-free TCHP delivery in real-world settings with routine cardiac monitoring and proactive supportive care.

Study Limitations

This study has limitations inherent to its retrospective design. The modest sample size and two-center setting introduce potential selection bias, limit generalizability, reduce statistical power, and preclude robust multivariable modeling. Detailed data for patients receiving non-TCHP neoadjuvant regimens were not systematically available, precluding comparative assessment of selection bias. In addition, radiologic assessment was not standardized across centers, imaging modalities varied, and central radiologic review was not performed. Toxicity may have been underestimated because adverse events were collected from routine clinical documentation, and grade 1 events were not systematically captured. Finally, the follow-up was short, with only one recurrence and no deaths; therefore, survival outcomes remain immature. Despite these constraints, the real-world setting, the contemporary systemic and surgical management patterns, and the detailed characterization of pCR, surgical outcomes, and radiologic-pathologic concordance strengthen the clinical relevance of our findings.

CONCLUSION

Neoadjuvant TCHP achieved a pCR rate of 55.8% in a real-world cohort of stage II-III HER2-positive breast cancer. HR-negative disease was associated with higher pCR rates, underscoring biological heterogeneity in response to dual HER2 blockade. The regimen was generally well tolerated and associated with high rates of breast conservation and low ALND rates, supporting meaningful downstaging in routine practice. Given the limited radiologic-pathologic concordance, surgical pathology remains essential for definitive response assessment; larger cohorts with standardized biomarker assessment and longer follow-up are needed to refine predictors of long-term outcomes.

Ethics

Ethics Committee Approval: The study protocol was reviewed and approved by the Dokuz Eylül University Non-Interventional Clinical Research Ethics Committee (approval number: 2026/09-10, date: 02.03.2026).

Informed Consent: Retrospective study.

Footnotes

The single center results of this study have been submitted as an abstract to Turkish Medical Oncology Congress 2026.

Authorship Contributions

Surgical and Medical Practices: K.C., A.T., M.K., T.Y., Concept: K.C., M.K., T.Y., Design: K.C., T.Y., Data Collection or Processing: K.C., E.D., A.M.A., A.T., Analysis or Interpretation: K.C., E.D., M.K., Literature Search: K.C., E.D., A.M.A., A.T., M.K., T.Y., Writing: K.C., E.D., A.M.A., A.T., M.K., T.Y.

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Clinicopathological Correlates of High Stromal Tumor-infiltrating Lymphocytes in Luminal Breast Cancer: A Real-world Study

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ABSTRACT

Objective: The prognostic role of stromal tumor-infiltrating lymphocytes (sTIL) is well recognized in triple-negative and human epidermal growth factor receptor 2 (HER2)-positive breast cancer (BC), but their meaning in hormone receptor-positive, HER2-negative tumors is still debated. In luminal BC, lymphocytic infiltration may reflect biologically aggressive disease rather than an effective antitumor response. We investigated clinicopathological factors associated with high sTIL and examined their relationships with disease-free survival (DFS) in an early-stage luminal BC cohort treated in routine clinical practice.

Material and Methods: This retrospective single-center study included 120 patients with early-stage estrogen receptor-positive, HER2-negative BC who were treated surgically between 2017 and 2021. Hematoxylin and eosin-stained slides were used to evaluate sTIL in accordance with International TILs Working Group recommendations. A 10% cut-off was used to define low-sTIL and high-sTIL categories. The relationship between sTIL category and clinicopathological variables was tested in univariable analyses and then explored using multivariable logistic regression. DFS was analyzed using the Kaplan-Meier method and groups were compared with the log-rank test.

Results: Twenty-three patients (19.2%) were classified as having high sTIL. In univariable analysis, high sTIL status was associated with grade 3 tumors, lymphovascular invasion, and Ki-67 $\geq 20\%$. In the adjusted model, Ki-67 $> 20\%$ remained the only independent factor associated with high sTIL (odds ratio: 8.68; 95% confidence interval: 1.07-70.45; $p=0.043$). During a median follow-up of 56.5 months, DFS did not differ significantly between patients with low and high sTIL ($p=0.37$); however, the high-sTIL group showed a numerically longer DFS.

Conclusion: In this real-world cohort of early-stage luminal BC, high sTIL density was most strongly associated with proliferative activity, but not with a statistically significant DFS benefit. These results suggest that immune infiltration in luminal tumors, when assessed by conventional pathology, may be interpreted primarily as a feature of aggressive tumor biology rather than a consistent marker of effective antitumor immunity.

Keywords: Breast cancer; disease-free survival; Ki-67; stromal tumor-infiltrating lymphocytes; tumor microenvironment

INTRODUCTION

Breast cancer (BC) represents a biologically diverse group of tumors characterized by distinct molecular profiles, clinical behaviors and patient outcomes.¹ Tumor-immune interactions are increasingly recognized as key determinants of disease progression and treatment response. Among immune-related markers, stromal tumor-infiltrating lymphocytes (sTIL) have demonstrated reproducible prognostic and predictive value

in early triple-negative BC (TNBC) and human epidermal growth factor receptor 2 (HER2)-positive disease, where greater lymphocytic infiltration is generally linked to more favorable treatment outcomes.²⁻⁵ Retrospective datasets have also suggested that incorporating sTIL may improve risk stratification in TNBC.⁶

The clinical significance of sTIL in hormone receptor (HR)-positive/HER2-negative BC remains controversial. In this

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subtype, several reports have described a paradoxical association between high sTIL levels and poorer outcomes, challenging the conventional view that immune infiltration consistently indicates a favorable prognosis.⁷ These findings indicate that in luminal BC, sTIL may represent subtype-specific biological activity rather than a direct marker of effective antitumor immune response.

In luminal BC, increased sTIL density has been associated with unfavorable tumor characteristics, including higher grade, greater proliferative activity and activation of oncogenic signaling pathways such as PI3K/AKT.^{8,9} The immune contexture of luminal tumors may also be shaped by immunosuppressive lymphocyte subsets, including regulatory T-cells (Tregs) and Th17 subsets.^{10,11} These findings suggest that immune infiltration in luminal BC may represent a surrogate marker of tumor aggressiveness rather than a protective immune phenotype. In addition, higher sTIL levels and immune-related gene expression have been linked to weaker endocrine therapy response, especially to aromatase inhibitors, suggesting a possible connection between immune activation and endocrine resistance.¹²

Despite growing interest in this topic, much of the existing evidence comes from mixed cohorts, post-hoc analyses, or selected trial populations. Data derived from unselected clinical practice and specifically focused on sTIL correlates in luminal BC remain scarce. For this reason, we aimed to determine which clinicopathological variables were associated with high sTIL levels in a real-world early-stage luminal BC cohort. We also assessed Disease-free survival (DFS) by sTIL status to determine whether routine visual sTIL scoring provides clinically relevant prognostic information in this setting.

MATERIAL AND METHODS

Study Design and Patient Population

This retrospective study was performed at a single center and included 120 patients with early-stage HR-positive/HER2-negative BC who underwent surgery at Ankara University Faculty of Medicine between 2017 and 2021. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ankara University Faculty of Medicine Human Research Ethics Committee (approval number: İ07-464-22, date: 02.09.2022). The requirement for informed consent was waived because of the retrospective design. Patients were eligible if they were at least 18 years old, had histologically confirmed primary invasive breast carcinoma, HR-positive status [estrogen receptor (ER) and/or progesterone receptor (PR) $\geq 1\%$ by immunohistochemistry (IHC)], HER2-negative status (IHC 0/1+ or IHC 2+ with

negative *in situ* hybridization), and available surgical tumor tissue adequate for sTIL evaluation on hematoxylin and eosin (H&E)-stained slides. Exclusion criteria were: sTIL level $<1\%$; receipt of neoadjuvant systemic therapy or radiotherapy; *de novo* metastatic disease; or missing clinicopathological or follow-up information required for classification or outcome analyses.

Evaluation of sTIL levels was performed on H&E-stained slides in accordance with the guidelines of the International TILs Working Group.¹³ TIL evaluation was independently performed by experienced breast pathologists who were not involved in patient management. In accordance with the International TILs Working Group's recommendations, sTIL were analyzed primarily as a continuous variable. For categorical analyses, patients were grouped using a 10% threshold, defining low sTIL as 1-10% and high sTIL as $>10\%$, a commonly used cut-off in luminal BC cohorts to ensure adequate group sizes. Menopausal status was defined clinically; patients were considered postmenopausal if they had ≥ 12 months of amenorrhea not attributable to pregnancy, lactation, or hormonal treatment or if they had undergone bilateral oophorectomy; all others were classified as premenopausal. Luminal subtypes were assigned using clinicopathologic surrogate definitions in HR+/HER2- tumors: luminal A-like tumors were defined as Ki-67 $<14\%$ or Ki-67 14-19% with PR $\geq 20\%$, whereas luminal B-like tumors were defined as Ki-67 $\geq 20\%$ or Ki-67 14-19% with PR $<20\%$.¹⁴ Variables associated with sTIL status in unadjusted analyses were first explored using chi-square or Mann-Whitney U tests, as appropriate. Factors showing significant or borderline associations were subsequently entered into multivariable logistic regression models. Given the limited number of cases with high sTIL, multivariable models were restricted to a small number of covariates to avoid overfitting. Variables reflecting overlapping biological information (e.g., grade and Ki-67) were not included simultaneously. DFS was defined as the time from surgery to disease recurrence, death from any cause, or last follow-up. DFS outcomes were compared between patients with low and high sTIL using the log-rank test.

Statistical Analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Inc., Armonk, NY, USA), and a two-sided p-value of <0.05 was considered statistically significant.

RESULTS

A total of 120 patients diagnosed with early-stage luminal-type BC were included in the study. The median age was 53 years (interquartile range, 45-64), and 52.9% of the patients were postmenopausal. The predominant histological subtype was invasive (ductal) carcinoma of no special type

(76.7%), followed by invasive lobular carcinoma (10%) and other special types (13.3%). At diagnosis, 57.1% of patients presented with stage II disease, while stages I and III accounted for 21.8% and 21.0%, respectively. The majority of tumors were T2 (56.3%), and 47.5% of patients were node-negative. Regarding histologic grade, 5.8% of tumors were grade 1, 52.5% were grade 2, and 41.7% were grade 3. Lymphovascular invasion (LVI) and perineural invasion (PNI) were observed in 38.1% and 13.3% of cases, respectively. All tumors were ER-positive with 94.2% exhibiting ER expression >50% and 66.4% exhibiting PR expression >50%. The entire cohort consisted of HER2-negative tumors (IHC 0-2+). The Ki-67 proliferation index exceeded 20% in 66% of patients (Table 1).

TABLE 1: Baseline clinicopathological characteristics of the study population (n=120).

Variables	n (%)	Variables	n (%)
Age, years (median, IQR)	53 (45-64)	Grade	
Menopausal status		G1	7 (5.8)
Pre-menopausal	56 (47.1)	G2	63 (52.5)
Post-menopausal	63 (52.9)	G3	50 (41.7)
Histological type		LVI	
IC-NST	92 (76.7)	Yes	43 (35.8)
ILC	12 (10.0)	No	70 (58.3)
Others	16 (13.3)	Unknown	7 (5.9)
Stage at diagnosis		PNI	
Stage 1	26 (21.8)	Yes	16 (13.3)
Stage 2	68 (57.1)	No	83 (69.2)
Stage 3	25 (21.0)	Unknown	21 (17.5)
T stage		ER status, %	
T1	41 (34.5)	ER ≤50	7 (5.8)
T2	67 (56.3)	ER >50	113 (94.2)
T3	11 (9.2)	PR status, %	
N stage		PR ≤50	40 (33.6)
N0	56 (46.6)	PR >50	79 (66.4)
N1	37 (30.8)	HER2 status, IHC	
N2	15 (12.5)	HER2 0	56 (46.7)
N3	10 (8.3)	HER2 1-2	64 (53.3)
Unknown	2 (1.6)	Ki-67, %	
		<20	32 (26.7)
		≥20	62 (51.6)
		Unknown	26 (21.7)

Values are median (IQR) or n (%). "Unknown" indicates missing data. Analyses were performed using available cases; evaluable n varied by variable (Ki-67 n=94; PNI n=99; LVI n=113). IQR: Interquartile range; IC-NST: Invasive carcinoma of no special type; ILC: Invasive lobular carcinoma; LVI: Lymphovascular invasion; PNI: Perineural invasion; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry.

When stratified by sTIL density, 97 patients (80.8%) were categorized as having low sTIL and 23 (19.2%) as having high sTIL. High sTIL were significantly associated with higher histologic grade (G3) ($p=0.011$), LVI ($p=0.046$), and elevated Ki-67 index ($\geq 20\%$) ($p=0.010$). Although the difference did not reach statistical significance, sTIL density tended to be higher in younger patients and in tumors with a luminal B-like subtype. No significant associations were found between sTIL levels and menopausal status, tumor stage, nodal status, PNI, HR expression, and HER2 status (all $p>0.05$) (Table 2).

In multivariable logistic regression analysis, high proliferative activity (Ki-67 $\geq 20\%$) was independently associated with high sTIL, with an approximately 8.7-fold increased odds [odds ratio: 8.68; 95% confidence interval (CI): 1.07-70.45; $p=0.043$]. LVI was not independently associated with high sTIL (Table 3).

At a median follow-up of 56.5 months, disease recurrence occurred in 12 of 120 patients (10%). Although the median DFS was not reached, stratification by sTIL status revealed no statistically significant difference between the groups ($p=0.37$). However, a numerically longer DFS was observed in patients with high sTIL levels (Figure 1).

DISCUSSION

In this real-world cohort of early-stage luminal BC, we demonstrate that elevated sTIL are predominantly associated with markers of aggressive tumor biology—most notably high proliferative activity—rather than with a clear survival

TABLE 2: Comparison of clinicopathological characteristics between low and high sTIL groups.

Characteristics	sTIL low, n=97	sTIL high, n=23	p-value
Age, years (median, IQR)	55 (46-64)	47 (41-62)	0.1
Menopausal status (pre; post)	43/53	13/10	0.31
Stage at diagnosis (3; 1-2)	20/76	5/18	0.92
T stage (T1; T2-3)	35/61	6/17	0.34
N stage (positive, negative)	50/45	12/11	0.96
Grade (3; ≤1-2)	35/62	15/8	0.01
LVI (positive, negative)	31/61	12/9	0.04
PNI (positive, negative)	13/67	3/16	0.96
ER status (≤50; >50)	4/93	3/20	0.10
PR status (≤50; >50)	31/66	9/13	0.42
HER2 status (IHC 0; IHC+1-2)	46/51	10/13	0.73
Ki-67 (<20; ≥20)	31/47	1/15	0.01
Luminal A/B like subtype	20/57	1/15	0.08

Analyses were performed using available cases; evaluable n varied by variable. sTIL: Stromal tumor-infiltrating lymphocytes; IQR: Interquartile range; LVI: Lymphovascular invasion; PNI: Perineural invasion; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry.

TABLE 3: Multivariable analysis of clinicopathological determinants of high sTIL.			
Variable	OR	95% CI	p-value
Ki-67 ≥20%	8.68	1.07-70.45	0.043
LVI (yes)	1.96	0.61-6.31	0.260

OR: Odds ratio; CI: Confidence interval; sTIL: Stromal tumor-infiltrating lymphocytes; LVI: Lymphovascular invasion.

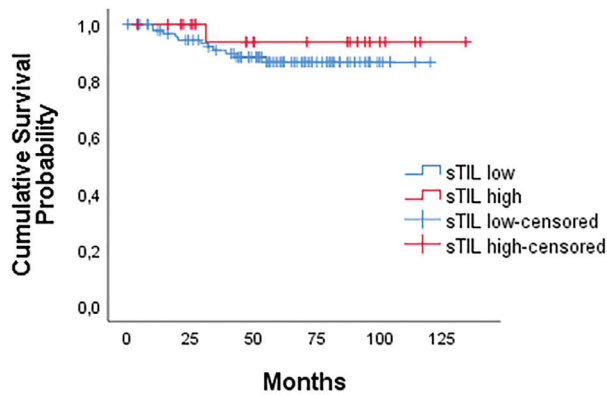


FIGURE 1: Kaplan-Meier curves for DFS according to sTIL status (low vs. high). Kaplan-Meier curves showing DFS according to sTIL status (low: 0-10% vs. high: >10%). Tick marks indicate censored observations.

DFS: Disease-free survival; sTIL: Stromal tumor-infiltrating lymphocytes

advantage. Among all clinicopathological variables examined, Ki-67 ≥20% emerged as the sole independent determinant of high sTIL density, whereas classical adverse features such as LVI lost significance in multivariable analysis.

Luminal BC is generally characterized by low baseline immune infiltration, a finding consistently reported across large pooled analyses and systematic reviews. Stanton et al.¹⁵ demonstrated that HR+/HER2-tumors exhibit the lowest incidence of lymphocyte-predominant BC among all molecular subtypes, underscoring the intrinsically “immune-desert” nature of luminal disease. Our observation that only 19.2% of patients displayed high sTIL levels is, therefore, consistent with the existing literature and supports the biological plausibility of our cohort. Although this proportion may appear relatively high compared with previous reports, patients without sTIL were not included in our cohort. The overall low prevalence of elevated sTIL in luminal disease is likely related to ER-mediated immune modulation, as ERα signaling has been shown to impair CD8⁺ T-cell-mediated cytotoxicity and reduce interferon-γ-dependent antigen presentation, thereby facilitating immune escape.^{16,17} Higher sTIL levels observed in highly proliferative tumors may reflect an increased antigenic burden and a relative reduction in

ER-mediated immunosuppression, potentially resulting in a more immune-infiltrated tumor microenvironment.¹⁸

While high sTIL levels correlated with adverse pathological features, no statistically significant difference in DFS was observed between groups. In line with this finding, Criscitiello et al.⁸ reported no overall association between TIL levels and distant DFS in luminal BC; however, subgroup analyses suggested a favorable impact of high TIL levels among patients with Ki-67 ≥20%. In contrast, Fujimoto et al.¹⁸ identified higher TIL levels as a marker of worse DFS in luminal BC with low Ki-67 expression. Similarly, in our cohort, elevated sTIL levels were observed almost exclusively among patients with high Ki-67 expression and were associated with a numerical improvement in DFS. These data support the notion that the prognostic relevance of TILs is influenced by tumor biological features—especially proliferative activity—and appears to be most evident in ER+/HER2-negative BC.¹⁹

Recent artificial intelligence-based studies have suggested that more sophisticated computational approaches, particularly those incorporating the spatial distribution of TILs, may enhance the prognostic value of immune infiltration in luminal BC. By moving beyond simple quantification and capturing aggregated versus distributed immune patterns, these artificial intelligence-driven analyses have reported favorable survival associations in selected luminal subgroups, implying that spatial immune architecture may reflect biologically meaningful tumor-immune interactions that are not fully appreciated by conventional visual assessment.²⁰ These findings have generated significant interest, especially given the longstanding uncertainty surrounding the prognostic role of TILs in HR+ disease.

Study Limitations

Several limitations merit consideration. The retrospective, single-center design and the limited number of survival events restricted the statistical power of survival analyses and precluded more complex multivariable modeling for DFS. Moreover, the high-sTIL group comprised only 23 patients, which likely contributed to imprecision in the effect estimates; accordingly, the association observed for Ki-67 was accompanied by a wide CI, reflecting the limited sample size and number of events. In addition, the lack of assessment by a single dedicated pathologist and the lack of analyses distinguishing between effector and suppressive immune cell subsets represent major limitations of the present study. Finally, the median follow-up of 56 months may be relatively short for HR-positive BC, in which late recurrences are common, and may therefore underestimate long-term DFS differences. Nevertheless, the consistency of our findings

with those from large real-world cohorts and pooled analyses strengthens the validity of our conclusions.

CONCLUSION

The present study was designed to reflect real-world clinical practice. Strictly according to the International TILs Working Group recommendations, sTIL were assessed using standard visual evaluation without computational or spatial augmentation. Our results demonstrate that high sTIL density in early-stage luminal BC predominantly mirrors increased tumor proliferative activity—most notably high Ki-67 expression—rather than conferring an independent survival advantage. This observation suggests that, when evaluated using conventional pathology-based methods, immune infiltration in luminal tumors may function more as a surrogate marker of aggressive tumor biology than as an indicator of effective antitumor immunity.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ankara University Faculty of Medicine Human Research Ethics Committee (approval number: İ07-464-22, date: 02.09.2022).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

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

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Perioperative Nivolumab Combined with Neoadjuvant Chemotherapy in Locally Advanced Gastric/ Gastroesophageal Junction Adenocarcinoma

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ABSTRACT

Objective: The exact role of nivolumab in the perioperative systemic treatment of locally advanced-gastric/gastroesophageal junction (LA-G/GEJ) adenocarcinoma remains unclear. This study aimed to evaluate the real-world effectiveness and safety of perioperative nivolumab in patients with LA-G/GEJ adenocarcinoma.

Material and Methods: Clinical and pathological data were retrospectively collected for previously untreated patients with human epidermal growth factor receptor 2-negative, clinical stage T2-4bN0-3M0 (stage II-III) G/GEJ adenocarcinoma, either microsatellite instability-high (MSI-H)/deficient mismatch repair (dMMR) or microsatellite stable (MSS)/proficient MMR (pMMR) with a programmed death-ligand 1 (PD-L1) combined positive score (CPS) of ≥ 5 , who received perioperative nivolumab in combination with chemotherapy or ipilimumab between January 2021 and April 2025. Kaplan-Meier survival analysis was used to estimate disease-free survival (DFS) and overall survival (OS).

Results: Fifteen eligible patients were identified among 134 with stage II-III G/GEJ adenocarcinoma. Seven patients (46.7%) were classified as MSI-H/dMMR, and 11 patients (73.3%) had a PD-L1 CPS of ≥ 5 . The median numbers of immunotherapy and chemotherapy cycles prior to surgery were 6 (range, 3-8) and 8 (range, 1-8), respectively. Objective response and disease control rates were 60% and 100%, respectively. All patients underwent surgical resection, and the R0 resection rate was 93.3%. Pathological complete response and major pathological response rates were both 71.4% in the MSI-H/dMMR subgroup, compared to 25% and 37.5%, respectively, in MSS/pMMR patients with a PD-L1 CPS ≥ 5 . During follow-up [median 17.0 months (95% confidence interval: 13.0-20.9)], four patients experienced tumor recurrence, and three died. All cases of disease recurrence and death occurred in the MSS/pMMR subgroup, with 18 months DFS and OS rates of 35.7% and 47.6%, respectively.

Conclusion: For the treatment of LA-G/GEJ adenocarcinoma, perioperative nivolumab combined with total neoadjuvant chemotherapy might be effective for both MSI-H/dMMR patients and MSS/pMMR patients with a PD-L1 CPS of ≥ 5 , and have an acceptable safety profile.

Keywords: Neoadjuvant therapy; immunotherapy; perioperative treatment; gastric cancer

INTRODUCTION

Gastric/gastroesophageal junction (G/GEJ) cancers account for approximately 5% of newly diagnosed malignancies and remain a major global cause of cancer-related mortality,

ranking fifth worldwide.¹ Adenocarcinoma is the predominant subtype, comprising nearly 95% of all cases.² While surgical resection remains a key component of curative-intent treatment, high recurrence rates exceeding 60% and poor survival outcomes following upfront surgery have prompted

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a shift towards perioperative therapy, which has become the standard of care in locally advanced-G/GEJ (LA-G/GEJ) adenocarcinoma.^{3,4} The incorporation of both neoadjuvant and adjuvant chemotherapy has been shown to improve tumor regression, increase the likelihood of complete (R0) resection, and raise pathological complete response (pCR) rates, overall survival (OS), and disease-free survival (DFS).⁵⁻⁷

Recent advances have highlighted the potential of immune checkpoint inhibitors (ICIs) in the neoadjuvant treatment of various solid tumors, prompting interest in their application to G/GEJ adenocarcinomas.⁸ In a meta-analysis of 33 studies including 1,074 patients whose tumors were either microsatellite instability-high (MSI-H)/deficient mismatch repair (dMMR) or microsatellite stable (MSS)/proficient MMR (pMMR) tumors, neoadjuvant immunotherapy was associated with promising outcomes, including a pCR rate of 24%, a major pathological response (MPR) rate of 49%, and an R0 resection rate of 89%.⁹ A pooled analysis of phase I and II trials evaluating neoadjuvant ICIs in MSS/pMMR LA-G/GEJ adenocarcinoma also reported encouraging rates of pCR (21%), MPR (47%), and R0 resection (92%).¹⁰ Consistent with these findings, a meta-analysis of randomized controlled trials including 2,718 patients showed that adding ICIs to perioperative chemotherapy significantly increased pCR rates compared to chemotherapy alone.¹¹ The addition of durvalumab to the perioperative fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT) regimen has led to improved survival outcomes and pathological response rates, establishing this combination as the current standard of care.^{12,13} Despite these promising results, the optimal incorporation of ICIs into the systemic treatment of LA-G/GEJ adenocarcinoma has yet to be clearly defined. Furthermore, nivolumab was infrequently used in these trials, underscoring a notable gap in evidence specific to this agent.

In this study, we aimed to assess the real-world effectiveness and safety of perioperative nivolumab administered in combination with either chemotherapy or ipilimumab in patients with LA-G/GEJ adenocarcinoma.

MATERIAL AND METHODS

Patient Selection

This study included previously untreated patients with clinical stage T2-4bN0-3M0 (stage II-III) G/GEJ adenocarcinoma who received neoadjuvant ICIs at two tertiary centers between January 2021 and April 2025. Eligibility criteria included histologically confirmed human epidermal growth factor receptor 2 (HER2)-negative G/GEJ adenocarcinoma, MSI-H/dMMR status or a programmed death-ligand 1 (PD-L1) combined positive score (CPS) of ≥ 5 in patients with

MSS/pMMR tumors, clinical stage T2-4bN0-3M0 per the 8th edition of the American Joint Committee on Cancer (AJCC) gastric cancer staging system, age ≥ 18 years, Eastern Cooperative Oncology Group performance status of 0-1, and administration of neoadjuvant ICI therapy, either alone or in combination with chemotherapy. Patients with coexisting malignancies or compromised organ function were excluded.

Ethical Approval

The study was approved by the Koç University Ethics Committee (approval number: 2025.036.IRB2.024, date: 22.01.2025); the requirement for informed consent was waived because of its retrospective design. The study was performed in compliance with the principles of the Declaration of Helsinki.

Treatment Protocols

Neoadjuvant chemotherapy regimens included FLOT (oxaliplatin 85 mg/m² on day 1, docetaxel 50 mg/m² on day 1, leucovorin 200 mg/m² on day 1, and fluorouracil 2,600 mg/m² as a 24 hours continuous infusion, administered biweekly) and FOLFOX (fluorouracil 400 mg/m² as a bolus on day 1 followed by 2,400 mg/m² as a 46 hours continuous infusion, leucovorin 400 mg/m² on day 1, oxaliplatin 85 mg/m², administered biweekly). One patient with GEJ adenocarcinoma received concurrent chemoradiotherapy with weekly carboplatin (area under the curve 2 mg/mL/min) and paclitaxel (50 mg/m²). Immunotherapy regimens included nivolumab (240 mg biweekly), either combined with chemotherapy or administered as dual immune checkpoint blockade with ipilimumab (1 mg/kg every six weeks). Although total neoadjuvant therapy (TNT) is not currently standard for LA-G/GEJ adenocarcinoma, participating institutions adopted a center-specific approach in which all planned perioperative systemic treatment was administered before surgery. The rationale for this approach was to maximize treatment completion and minimize the risk of postoperative treatment omission. Tumor response was assessed two weeks after the final neoadjuvant systemic treatment using computed tomography (CT) scans of the abdomen and chest, magnetic resonance imaging of the abdomen, and/or floride 18-fluorodeoxyglucose positron emission tomography-CT scan.

All patients underwent gastrectomy with lymphadenectomy following neoadjuvant systemic treatment. The surgical extent was individualized based on tumor characteristics to ensure negative margins. Of the 15 patients, 12 completed chemotherapy in the neoadjuvant setting (the total neoadjuvant chemotherapy approach), and none received adjuvant chemotherapy. The only adjuvant immunotherapy

regimen was nivolumab, administered as either 240 mg every two weeks or 480 mg every four weeks. Regardless of pathological response status, continuation of nivolumab for a treatment duration of one year was recommended in routine clinical practice. Routine follow-up included CT scans at three-month intervals.

Outcome Assessment

Radiologic and pathologic responses were defined as the primary endpoints of the study. Radiologic response to neoadjuvant therapy was assessed according to the Response Evaluation Criteria in Solid Tumors version 1.1 and classified as CR, partial response (PR), stable disease (SD), or progressive disease.¹⁴ The objective response rate (ORR) and the disease control rate (DCR) were defined as the proportions of patients achieving CR or PR and CR, PR, or SD, respectively. Surgical specimens were evaluated for pathological response using the Becker tumor regression grade (TRG) criteria, defined as follows:¹⁵ TRG1a (no residual tumor cells), TRG1b (<10% residual tumor cells), TRG2 (10-50% residual tumor cells), and TRG3 (>50% residual tumor cells). TRG1a was classified as pCR, and TRG1a/1b was classified as MPR. cTNM staging was determined based on the 8th edition of the AJCC staging system.

Secondary outcomes included OS, defined as the time from initiation of neoadjuvant therapy to death from any cause, and DFS, defined as the time from initiation of neoadjuvant therapy to disease recurrence or death from any cause. Treatment-related adverse events (TRAEs) were graded using the Common Terminology Criteria for Adverse Events, version 5.0.¹⁶

MSI/MMR status was determined by immunohistochemistry for MSH2, MSH6, MLH1, and PMS2. PD-L1 expression was assessed through immunohistochemical staining with the SP263 anti-PD-L1 antibody (VENTANA PD-L1 Assay, Roche), and expression levels were quantified by the CPS. CPS was calculated as the ratio of PD-L1-expressing cells (including tumor cells, lymphocytes, and macrophages) to the total number of viable tumor cells, multiplied by 100. HER2 expression was assessed by immunohistochemistry.

Statistical Analysis

SPSS software version 24.0 (IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Continuous variables were presented as medians with ranges, while categorical variables were reported as frequencies with percentages. The reverse Kaplan-Meier approach was applied to estimate follow-up duration. DFS and OS were calculated using Kaplan-Meier methods.

RESULTS

Patient Characteristics

Of the 134 patients with previously untreated clinical stage T2-4bN0-3M0 (stage II-III) G/GEJ adenocarcinoma, 24 underwent upfront surgery, whereas 110 received neoadjuvant systemic treatment. Among them, 15 eligible patients who received perioperative nivolumab, either in combination with chemotherapy or with ipilimumab, were included in the analysis. The median age was 60 years (range, 55-70), and 12 patients (80.0%) were male (Table 1). Gastric adenocarcinoma was diagnosed in 13 patients (86.7%); only two patients (13.3%) had tumors located at the GEJ. All patients were HER2 negative; seven (46.7%) were classified as MSI-H/dMMR; and 11 (73.3%) had a PD-L1 CPS of ≥ 5 . Six patients (40.0%) had signet ring cell carcinoma.

The median time interval between the initiation of neoadjuvant treatment and surgery was 4.3 months (range, 2.1-4.9 months). Median numbers of immunotherapy and chemotherapy cycles prior to surgery were 6 (range, 3-8) and 8 (range, 1-8), respectively. One patient with MSI-H/dMMR gastric adenocarcinoma received only dual immunotherapy with nivolumab and ipilimumab after a prolonged hospitalization for febrile neutropenia following the first cycle of the FLOT regimen. All other patients were treated with both chemotherapy and immunotherapy before surgery. Additionally, one patient with GEJ adenocarcinoma received radiotherapy in the neoadjuvant setting, along with immunotherapy and concomitant chemotherapy.

Tumor Response and Survival Outcomes

Radiological evaluation after the last cycle of neoadjuvant therapy revealed that two patients (13.3%) achieved CR and seven patients (46.7%) achieved PR. SD was observed in six patients (40%), with no cases of progressive disease. ORR and DCR were 60% and 100%, respectively. All patients underwent surgical resection, with an R0 resection rate of 93.3% (14 out of 15 patients). The median interval between completion of neoadjuvant treatment and surgery was 25 days (range, 16-43 days). Pathological examination showed rates of TRG1a, TRG1b, TRG2, and TRG3 of 46.7%, 6.7%, 13.3%, and 33.3%, respectively; the corresponding pCR and MPR rates were 46.7% and 53.3%.

Within our cohort, we observed a notably high pCR rate of 71.4% (5 out of 7) in the MSI-H/dMMR subgroup (Table 1). One patient with MSI-H/dMMR gastric adenocarcinoma and a PD-L1 CPS of 60 experienced grade 4 febrile neutropenia following the first cycle of the FLOT regimen. Subsequently, treatment was switched to dual immunotherapy with nivolumab and ipilimumab, without chemotherapy (Patient 7 in Table 1).

TABLE 1: Data on clinical and pathological characteristics for the entire cohort.

	Age/ sex	Clinical stage	Histological diagnosis	Tumor location	MSI status	PD-L1 CPS	Neoadjuvant treatment	Pathological response	Adjuvant treatment	FU (months)	DFS (months)	OS (months)	Last status
1	58/M	cT3N0	Adenocarcinoma, moderately differentiated	Antrum	MSI-H/ dMMR	0	8x FLOT + 7x nivolumab	TRG1a	Nivolumab	25.4	N/A	N/A	Remission
2	57/M	cT3N0	Adenocarcinoma, moderately differentiated	Antrum	MSI-H/ dMMR	Unknown	8x FLOT + 6x nivolumab	TRG1a	Nivolumab	47.6	N/A	N/A	Remission
3	59/M	cT3N+	Adenocarcinoma, poorly differentiated	GEJ	MSS/pMMR	10	2x FOLFOX + 1x nivolumab, then 5x weekly carboplatin plus paclitaxel concurrent with radiotherapy, then 2x FOLFOX + 2x nivolumab	TRG1a	Nivolumab	8.9	N/A	N/A	Remission
4	70/M	cT3N+	Adenocarcinoma, moderately differentiated	Corpus	MSI-H/ dMMR	5	8x FLOT + 7x nivolumab	TRG1a	Not received	10.3	N/A	N/A	Remission
5	54/F	cT3N+	Signet ring cell carcinoma	Antrum	MSS/pMMR	21	8x FLOT + 5x nivolumab	TRG1b	Nivolumab	16.1	10.2	16.1	Exitus
6	55/M	cT3N+	Tubulopapillary adenocarcinoma	Antrum	MSI-H/ dMMR	0	3x FOLFOX + 3x nivolumab	TRG2	Nivolumab	14.3	N/A	N/A	Remission
7	65/F	cT4aN+	Adenocarcinoma, poorly differentiated	Antrum	MSI-H/ dMMR	60	1x FLOT, then 6x nivolumab + 2x ipilimumab	TRG1a	Nivolumab	24.8	N/A	N/A	Remission
8	55/M	cT4aN+	Signet ring cell carcinoma	Corpus	MSS/pMMR	5	8x FLOT + 3x nivolumab	TRG3	Not received	9.5	8.3	9.5	Exitus
9	85/M	cT4bN+	Signet ring cell carcinoma	Antrum	MSS/pMMR	5	8x FOLFOX + 7x nivolumab	TRG3	Nivolumab	10.0	8.9	10.0	Exitus
10	60/M	cT4aN+	Adenocarcinoma, poorly differentiated	Antrum	MSS/pMMR	30	8x FLOT + 7x nivolumab	TRG3	Nivolumab (postoperative radiotherapy due to R2 resection)	15.3	14.4	N/A	Receiving subsequent treatment
11	81/M	cT4bN+	Signet ring cell carcinoma	Corpus	MSS/pMMR	7	7x FOLFOX + 6x nivolumab	TRG1a	Not received	34.1	N/A	N/A	Remission
12	40/M	cT3N+	Signet ring cell carcinoma	Corpus	MSS/pMMR	15	8x FLOT + 8x nivolumab	TRG2	Nivolumab	18.0	N/A	N/A	Remission
13	67/M	cT3N+	Adenocarcinoma, poorly differentiated	Corpus	MSI-H/ dMMR	70	8x FLOT + 7x nivolumab	TRG1a	Nivolumab	17.0	N/A	N/A	Remission
14	66/F	cT3N+	Signet ring cell carcinoma	Antrum	MSI-H/ dMMR	0	8x FLOT + 5x nivolumab	TRG3	Not received	11.7	N/A	N/A	Remission
15	70/M	cT2N+	Adenocarcinoma, moderately differentiated	GEJ	MSS/pMMR	13	6x FOLFOX + 4x nivolumab	TRG3	Not received	10.1	N/A	N/A	Remission

DFS: Disease-free survival; F: Female; FU: Follow-up; GEJ: Gastroesophageal junction; M: Male; MPR: Major pathological response; N/A: Not applicable; OS: Overall survival; pCR: Pathological complete response; TRG: Tumor response grade; MSI: Microsatellite instability; MSS: Microsatellite stable; MMR: Mismatch repair.

pCR was achieved in this patient, and adjuvant nivolumab monotherapy was continued for up to 1 year following surgery. One patient with MSI-H/dMMR gastric adenocarcinoma underwent surgery after three cycles of FOLFOX and nivolumab because of chemotherapy-induced myelosuppression, following the multidisciplinary tumor board's recommendation (Patient 6). Post-surgical pathology revealed a TRG2 response, and adjuvant nivolumab monotherapy was continued for up to 1 year. In the MSI-H/dMMR subgroup, a TRG3 response was observed in a single patient, who was also the only individual with signet ring cell carcinoma (Patient 14).

In MSS/pMMR patients with a PD-L1 CPS ≥ 5 , the pCR and MPR rates remained modest, observed in only 25% (2 out of 8) and 37.5% (3 out of 8) of cases, respectively. One patient with GEJ adenocarcinoma (Patient 3) received neoadjuvant radiotherapy in addition to chemotherapy and nivolumab and achieved pCR. Only one patient with an R2 resection received adjuvant radiotherapy (Patient 10). Of the four patients with a poor pathological response to neoadjuvant therapy (TRG3 response), three experienced recurrence (Patients 8, 9, and 10) and two (Patients 8 and 9) died from cancer progression, with OSs of 9.5 and 10 months, respectively. A patient with a TRG1b response to neoadjuvant therapy (Patient 5) also experienced disease recurrence and died from cancer progression, with an OS of 16.1 months. Signet-ring cell carcinoma was present in three of the four patients with disease recurrence (Patients 5, 8, and 9). Across the cohort, three of the six patients with signet ring cell carcinoma (Patients 11, 12, and 14) were in remission at last follow-up.

By the data cut-off date of April 30, 2025, the median follow-up duration was 18.0 months [95% confidence interval (CI): 10.5-25.5] in the MSS/pMMR subgroup and 17.0 months

(95% CI: 13.0-20.9) in the MSI-H/dMMR subgroup. During this period, four patients experienced tumor recurrence, three died, and all other patients remained in remission. All cases of disease recurrence and death occurred in the MSS/pMMR subgroup, with 18 months DFS and OS rates of 35.7% and 47.6%, respectively (Figure 1). In contrast, no recurrences or deaths were observed in the MSI-H/dMMR subgroup, resulting in 100% DFS and OS at 18 months. Although the number of patients was limited in each subgroup, the log-rank test demonstrated a statistically significant difference in DFS ($p=0.034$) between the MSI-H/dMMR and MSS/pMMR subgroups and a trend toward better OS ($p=0.062$) in the MSI-H/dMMR subgroup.

Safety

TRAEs were observed in all patients during neoadjuvant treatment (Table 2). The most common TRAEs ($>10\%$) were anemia (93.3%), thrombocytopenia (80%), fatigue (80%), neutropenia (60%), increased aspartate aminotransferase (60%), increased alanine aminotransferase (53.3%), peripheral neuropathy (46.7%), nausea or vomiting (20%), and fever (13.3%). Neutropenia accounted for the largest proportion of grade 3 or higher TRAEs (46.7%), while other grade 3 or higher TRAEs were anemia (20%), thrombocytopenia (20%), febrile neutropenia (6.7%), diarrhea (6.7%), peripheral neuropathy (6.7%), fever (6.7%), and myasthenia gravis (6.7%).

DISCUSSION

This retrospective analysis demonstrated that perioperative nivolumab combined with neoadjuvant chemotherapy for patients with LA-G/GEJ adenocarcinoma may improve pathological responses and is associated with an acceptable safety profile. This benefit might be particularly notable in

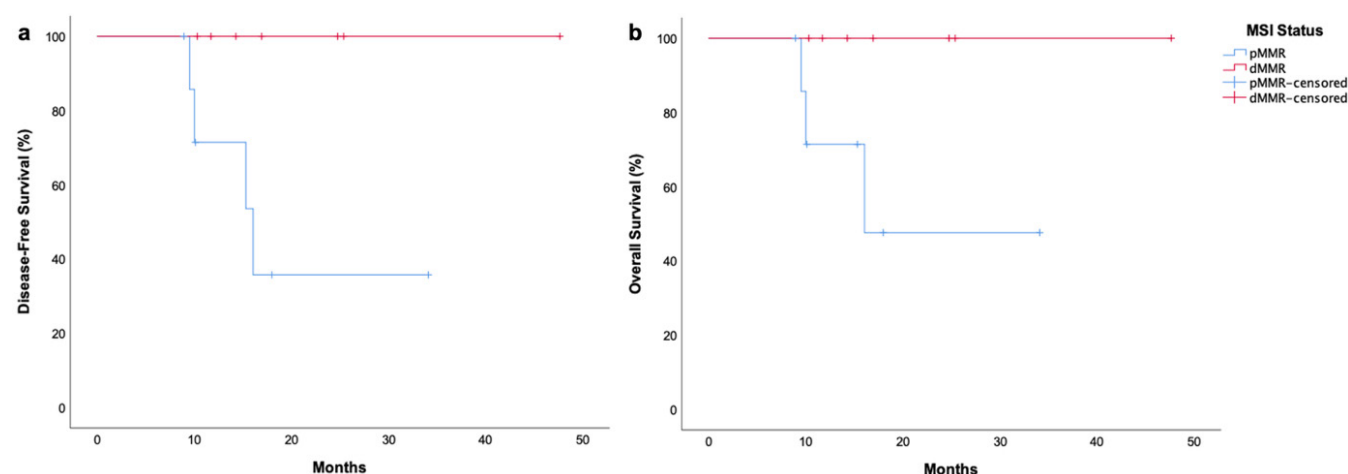


FIGURE 1: Kaplan-Meier curves for (a) disease-free survival and (b) overall survival according to microsatellite instability status

MSI: Microsatellite instability; MMR: Mismatch repair

TABLE 2: Treatment-related adverse events during neoadjuvant treatment.

	Grade 1-2	Grade 3	Grade 4
All events	15 (100%)	11 (73.3%)	2 (13.3%)
Anemia	11 (73.3%)	3 (20.0%)	0
Thrombocytopenia	9 (60.0%)	3 (20.0%)	0
Neutropenia	2 (13.3%)	5 (33.3%)	2 (13.3%)
Febrile neutropenia	0	0	1 (6.7%)
Alanine aminotransferase increase	8 (53.3%)	0	0
Aspartate aminotransferase increase	9 (60.0%)	0	0
Nausea or vomiting	3 (20.0%)	0	0
Diarrhea	0	1 (6.7%)	0
Peripheral neuropathy	6 (40.0%)	1 (6.7%)	0
Fever	1 (6.7%)	1 (6.7%)	0
Fatigue	12 (80.0%)	0	0
Rash	2 (13.3%)	0	0
Pneumonitis	1 (6.7%)	0	0
Hypophysitis	1 (6.7%)	0	0
Myasthenia gravis	0	1 (6.7%)	0

MSI-H/dMMR patients and in MSS/pMMR patients with a PD-L1 CPS of ≥ 5 . A total neoadjuvant chemotherapy approach, rather than perioperative chemotherapy, might further enhance both pathological and radiological outcomes.

The current standard of care for LA-G/GEJ adenocarcinoma is perioperative therapy. Following the results of the phase II/III FLOT4 trial (NCT01216644), which demonstrated a significant improvement in OS with perioperative FLOT compared to epirubicin, cisplatin, fluorouracil/epirubicin, cisplatin, capecitabine, FLOT has become the preferred perioperative chemotherapy regimen.⁷ Nevertheless, fewer than half of patients diagnosed with LA-G/GEJ adenocarcinoma survive beyond five years.^{7,17} Given the efficacy of perioperative immunotherapy across different tumor types, clinical trials were initiated to evaluate the safety and efficacy of adding ICIs to perioperative systemic treatment in patients with LA-G/GEJ adenocarcinoma.^{8,18,19} In phase II/III DANTE trial (NCT03421288), Lorenzen et al.²⁰ reported that adding atezolizumab to perioperative FLOT improved tumor downstaging and increased the pCR rate (24% vs. 15%), particularly in patients with PD-L1 CPS ≥ 10 (33% vs. 12%) and MSI-H/dMMR tumors (63% vs. 27%). The Phase III KEYNOTE-585 study (NCT03221426) evaluated the efficacy of combining pembrolizumab with either perioperative cisplatin-based doublet chemotherapy (main cohort) or FLOT (FLOT cohort).²¹ Although the addition of pembrolizumab increased pCR rates both in the main cohort (13.4% vs. 2.0%) and the main plus FLOT cohort (14.2% vs. 2.8%), there was no benefit in event-free survival (EFS) or OS.²² However, it should be noted that pembrolizumab demonstrated a trend

toward improved EFS in patients with PD-L1 CPS ≥ 10 (hazard ratio: 0.69; 95% CI: 0.48-1.01), despite the majority of patients receiving cisplatin-based doublet chemotherapy rather than the FLOT regimen.²¹ Recently, the MATTERHORN study (NCT04592913) demonstrated that adding durvalumab to perioperative FLOT significantly improved EFS, OS, and the pCR rate (19.2% vs. 7.2%).^{12,13} The survival benefit of adding durvalumab was observed regardless of the PD-L1 tumor area positivity score.¹³ Based on these data, durvalumab in combination with the FLOT regimen received U.S. Food and Drug Administration approval for perioperative treatment of resectable G/GEJ adenocarcinoma.²³

Neoadjuvant ICIs are significantly more efficacious in patients with MSI-H/dMMR disease than in patients with MSS/pMMR LA-G/GEJ adenocarcinoma, in whom only limited but encouraging pathological responses have been observed. The phase II GERCOR NEONIPIGA study (NCT04006262), assessing the efficacy of dual immune checkpoint blockade with nivolumab and ipilimumab in patients with resectable MSI-H/dMMR G/GEJ adenocarcinoma, successfully met its primary endpoint, achieving a pCR rate of 58.6%.²⁴ The Phase II INFINITY trial (NCT04817826), evaluating the combination of durvalumab plus tremelimumab in patients with resectable MSI-H/dMMR G/GEJ adenocarcinoma, recently reported a pCR rate of 60% and an MPR rate of 80%.²⁵ These findings suggest that patients with resectable MSI-H/dMMR G/GEJ adenocarcinoma may be suitable candidates for non-operative management using dual immune checkpoint blockade in future treatment strategies. Within our cohort, only one patient with MSI-H/dMMR gastric adenocarcinoma

received dual immune checkpoint blockade with nivolumab and ipilimumab, and this patient achieved pCR.

In the Phase III CheckMate 649 study (NCT02872116), the addition of nivolumab to chemotherapy significantly improved both OS and progression-free survival in patients with previously untreated advanced G/GEJ adenocarcinoma, with a more substantial benefit observed in those with PD-L1 CPS ≥ 5 .²⁶ Although nivolumab has been widely used in patients with advanced G/GEJ adenocarcinoma, particularly in those with PD-L1 CPS ≥ 5 based on these data, its efficacy in the preoperative or perioperative setting in patients with LA-G/GEJ adenocarcinoma remains unclear. Currently, only a few studies are investigating the addition of neoadjuvant nivolumab to perioperative chemotherapy in patients with LA-G/GEJ adenocarcinoma. In the phase II single-arm STIMULATE-01 trial (n=46), perioperative nivolumab combined with the SOX regimen (oxaliplatin and S-1) for LA-G/GEJ adenocarcinoma yielded a pCR rate of 28.3%, an MPR rate of 41.3%, and a 100% R0 resection rate, following three cycles of preoperative nivolumab plus SOX.²⁷ PD-L1 CPS status (≥ 5 vs. < 5) was not associated with significant differences in MPR (42.9% vs. 38.9%, $p=0.79$). A large-scale observational study (NCT06235164) is currently underway to assess the efficacy and safety of neoadjuvant chemotherapy combined with immunotherapy, including nivolumab, in patients with cT2-4a and/or node-positive gastric cancer.²⁸

Although adjuvant nivolumab significantly improved DFS in patients with resected esophageal or GEJ cancer who had residual disease following neoadjuvant chemoradiotherapy, it did not demonstrate a similar benefit as adjuvant treatment for gastric cancer.^{29,30} The efficacy of adding nivolumab to adjuvant chemotherapy in patients with pathological stage III disease who underwent upfront gastrectomy with D2 or a more extensive lymph node dissection was evaluated in the ATTRACTION-5 (NCT03006705) study; no improvement in 3 years relapse-free survival was observed.³⁰ However, a majority of patients in this trial had low PD-L1 expression, and subgroup analysis suggested that patients with PD-L1 expression of $\geq 1\%$ might benefit from the addition of nivolumab. Lower PD-L1 expression has been consistently associated with reduced therapeutic benefit from the addition of ICIs across various trials.^{26,31} This observation could partly explain the negative outcomes in the ATTRACTION-5 study, as a significant reduction in PD-L1-positive tumor cells after surgery could limit nivolumab's ability to effectively activate the immune system in the postoperative adjuvant setting. Moreover, neoadjuvant chemotherapy, rather than adjuvant therapy, could enhance immunogenic cell death and promote antigen release, potentially improving treatment outcomes when combined with nivolumab.³²

Therefore, using nivolumab in the perioperative setting, combined with chemotherapy, particularly in patients with PD-L1 CPS ≥ 5 , might yield better results than its use as postoperative adjuvant treatment.

Taken together, the previously discussed data indicate that the primary rationale for choosing nivolumab as an ICI in combination with perioperative chemotherapy is the inability to reliably exclude peritoneal metastases in every patient with LA-G/GEJ adenocarcinoma because diagnostic laparoscopy was used selectively. Hence, extrapolating the results of the CheckMate 649 study to treat this subgroup characterized by PD-L1 CPS ≥ 5 or MSI-H/dMMR is a reasonable approach. Additionally, ICIs are not currently reimbursed for LA-G/GEJ adenocarcinoma by Türkiye's healthcare system, and nivolumab is more financially accessible to patients than other ICIs. However, some otherwise eligible patients with MSI-H/dMMR tumors or MSS/pMMR tumors with a PD-L1 CPS ≥ 5 may still not have received this treatment because of financial constraints. Consequently, selection bias related to treatment accessibility cannot be excluded.

In our cohort, another important approach that could potentially increase pCR rates is completing all planned chemotherapy cycles before surgery as TNT rather than perioperative (neoadjuvant and adjuvant) chemotherapy. TNT has been adopted in locally advanced rectal cancer due to its potential advantages including a higher likelihood of completing all planned chemotherapy cycles, more effective management of micro-metastatic disease, enhanced tumor downstaging, and a greater chance of achieving R0 resection.^{33,34} This approach also seems to be applicable to LA-G cancer.³⁵⁻³⁷ Despite the historically low pCR rates, several studies have reported that achieving pCR could be a useful surrogate endpoint for predicting long-term survival in patients with LA-G/GEJ adenocarcinoma.^{38,39} While this retrospective study involved a limited number of patients, our findings suggest that perioperative nivolumab combined with total neoadjuvant chemotherapy may increase pCR rates in MSI-H/dMMR patients and in MSS/pMMR patients with a PD-L1 CPS of ≥ 5 .

Study Limitations

Despite these encouraging findings, several important limitations should be acknowledged. The retrospective nature of the study and its restriction to two centers may introduce selection bias, while unmeasured confounders may have impacted the observed outcomes. In addition to the retrospective design, the small sample size, variability in chemotherapy regimens, and heterogeneity in histopathological characteristics may further limit the robustness of the conclusions. Furthermore, the survival

findings should be interpreted cautiously because the survival data remain immature, with limited follow-up duration and a small number of events. Although continuation of adjuvant nivolumab to complete a total treatment duration of one year was recommended, access to treatment was influenced by financial constraints. As a result, not all patients were able to receive the planned duration of adjuvant immunotherapy, which may also have affected long-term outcomes. PD-L1 assessment was performed using the SP263 assay, primarily because of local availability, rather than the more commonly used 22C3 assay employed in G/GEJ cancer clinical trials. Although previous studies have demonstrated a high degree of concordance between these assays for PD-L1 assessment in G/GEJ adenocarcinomas, minor differences in CPS classification may occur in individual cases.⁴⁰ Large, well-designed prospective trials are needed to further clarify the potential impact of perioperative nivolumab in combination with total neoadjuvant chemotherapy on pathological responses and survival outcomes.

CONCLUSION

In the treatment of LA-G/GEJ adenocarcinoma, perioperative nivolumab combined with total neoadjuvant chemotherapy might be an effective approach for both MSI-H/dMMR patients and MSS/pMMR patients with a PD-L1 CPS of ≥ 5 , with an acceptable safety profile.

Ethics

Ethics Committee Approval: The study was approved by the Koç University Ethics Committee (approval number: 2025.036.IRB2.024, date: 22.01.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.K., C.I.K., F.K., N.D., P.F.Y., Ş.L., M.K., N.M.M., F.S., Concept: B.K., F.S., Design: B.K., F.S., Data Collection or Processing: B.K., C.I.K., F.K., R.E., N.D., Analysis or Interpretation: B.K., C.I.K., F.K., R.E., N.D., Literature Search: B.K., Writing: B.K., C.I.K., F.K., R.E., Ö.A., N.D., P.F.Y., Ş.L., M.K., N.M.M., F.S.

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Real-world Outcomes of Second-line Irinotecan Plus Bevacizumab in Recurrent High-grade Gliomas: A Single-center Retrospective Study

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ABSTRACT

Objective: High-grade gliomas (HGGs), particularly glioblastoma, remain among the most aggressive primary central nervous system malignancies. Despite standard treatment consisting of maximal safe resection followed by radiotherapy with concurrent and adjuvant temozolomide, disease recurrence is nearly inevitable. Therapeutic options after progression remain limited, and the optimal management of recurrent HGGs continues to be debated. This study aimed to evaluate the real-world efficacy of second-line irinotecan plus bevacizumab in patients with recurrent HGGs.

Material and Methods: This retrospective single-center study included patients with recurrent HGGs treated at University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital between January 2020 and December 2024. Demographic, clinical, pathological, treatment, and survival data were collected from institutional medical records. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method. Objective response rates (ORRs) were assessed according to radiological evaluations documented in routine clinical practice.

Results: A total of 55 patients were included. The median age was 50 years, and 76% of patients had glioblastoma. Following first-line chemoradiotherapy, 48 patients received adjuvant temozolomide, with a median PFS of 5.0 months. Fifty patients subsequently received second-line irinotecan plus bevacizumab, achieving an ORR of 46%. The median PFS associated with irinotecan plus bevacizumab was 8.8 months. At the final analysis, the median OS for the entire cohort was 23.4 months.

Conclusion: Second-line irinotecan plus bevacizumab demonstrated meaningful clinical activity in recurrent HGGs, yielding encouraging response rates and disease control in a real-world setting. These findings support the continued use of bevacizumab-based strategies for selected patients with recurrent disease.

Keywords: High-grade glioma; glioblastoma; bevacizumab; irinotecan; recurrent glioma; temozolomide; progression-free survival

INTRODUCTION

High-grade gliomas (HGGs) are among the most aggressive primary malignancies of the central nervous system and remain associated with poor clinical outcomes despite advances in surgical techniques, radiotherapy, molecular diagnostics, and systemic therapies. According to the 2021 World Health Organization (WHO) Classification of Tumors of the Central Nervous System, adult-type diffuse gliomas are classified as astrocytoma, isocitrate dehydrogenase (IDH)-mutant; oligodendroglioma, IDH-mutant and

1p/19q-codeleted; and glioblastoma, IDH-wildtype, with molecular features playing a central role in diagnosis and prognostication.¹ Among these entities, glioblastoma represents the most common and biologically aggressive subtype, accounting for approximately 45-50% of all malignant primary brain tumors in adults.²

The current standard-of-care for newly diagnosed glioblastoma consists of maximal safe surgical resection followed by radiotherapy with concurrent and adjuvant temozolomide, commonly referred to as the Stupp regimen.³

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This multimodal approach has significantly improved survival outcomes compared with radiotherapy alone and has remained the cornerstone of treatment for nearly two decades. Nevertheless, recurrence remains almost inevitable, with the majority of patients experiencing disease progression within the first year after completing initial therapy.^{3,4} Consequently, recurrent HGGs continue to represent a major therapeutic challenge in neuro-oncology.

Management strategies for recurrent HGGs are considerably less well-defined than those used in the frontline setting. Treatment decisions are influenced by multiple factors, including patient age, performance status (PS), extent of prior treatment, tumor location, resectability, molecular characteristics, and patterns of recurrence.^{5,6} Potential therapeutic options include repeat surgical resection, re-irradiation, alkylating agents such as lomustine, bevacizumab-containing regimens, regorafenib, enrollment in clinical trials, or best supportive care.⁵⁻⁸ However, no universally accepted standard treatment exists following failure of temozolomide-based chemoradiotherapy.

Tumor angiogenesis plays a pivotal role in glioma growth and progression. Glioblastomas are characterized by extensive neovascularization and overexpression of vascular endothelial growth factor (VEGF), which contributes to tumor proliferation, invasion, and treatment resistance.⁹ Bevacizumab, a monoclonal antibody targeting VEGF-A, was therefore investigated as a potential therapeutic strategy in recurrent gliomas. Early-phase studies demonstrated promising radiographic response rates, improvements in peritumoral edema, and prolonged progression-free survival (PFS), leading to regulatory approval of bevacizumab for recurrent glioblastoma in several countries.^{10,11}

Irinotecan, a topoisomerase I inhibitor, has also demonstrated activity against HGGs and has frequently been combined with bevacizumab based on potential synergistic effects.^{12,13} Several prospective and retrospective studies have reported encouraging response rates and disease control with irinotecan plus bevacizumab for recurrent glioblastoma, although survival benefits have varied across studies.¹⁰⁻¹³ Despite widespread clinical use, real-world evidence regarding the effectiveness of this combination remains limited, particularly outside clinical trial settings.

Furthermore, the evolving molecular classification of gliomas and the increasing heterogeneity of treatment approaches have highlighted the importance of institutional experience in understanding treatment outcomes in routine clinical practice. Real-world studies may provide clinically meaningful information regarding treatment effectiveness, tolerability, and survival outcomes among patient populations that are often underrepresented in prospective trials.

Therefore, the present study aimed to evaluate the real-world efficacy of second-line irinotecan plus bevacizumab in patients with recurrent HGGs treated at a tertiary referral center. We analyzed treatment response, PFS, and overall survival (OS) in a contemporary cohort managed in routine clinical practice.

MATERIAL AND METHODS

Study Design and Patient Population

This retrospective single-center study was conducted at University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital. Patients diagnosed with recurrent high-grade glioma who were treated between January 2020 and December 2024 were screened for eligibility. Histopathological diagnoses were established according to the WHO classification of central nervous system tumors.

Eligible patients were required to have:

1. Histologically confirmed high-grade glioma,
2. Receipt of first-line radiotherapy with or without concurrent and adjuvant temozolomide,
3. Radiologically confirmed disease progression
4. Availability of complete clinical and follow-up data.

Patients with insufficient medical records or incomplete follow-up information were excluded.

The study was conducted in accordance with the Declaration of Helsinki and approved by the University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital Clinical Research Ethics Committee (approval number: 2024-25, date: 07.11.2024).

Data Collection

Clinical data were retrospectively collected from electronic medical records. Variables included: age, sex, Eastern Cooperative Oncology Group-PS (ECOG-PS), comorbidities, family history of cancer, tumor histology, WHO grade, IDH mutation status, tumor size at diagnosis, extent of lobar involvement, number of lesions, surgical intervention, radiotherapy dose, treatment characteristics, radiological response, and survival outcomes.

Treatment Procedures

Patients underwent treatment according to institutional standards and at the physician's discretion. Initial management consisted of a maximal safe surgical resection or biopsy, followed by radiotherapy, with or without concurrent and adjuvant temozolomide. Following disease progression, second-line systemic treatment was administered. The majority of patients received irinotecan plus bevacizumab,

while alternative regimens were used in a limited number of cases. Subsequent therapies were administered according to clinical judgment and patient PS.

Outcome Measures

The primary objective of the study was to evaluate the efficacy of second-line irinotecan plus bevacizumab in recurrent HGGs.

PFS was defined as the interval between second-line treatment initiation and radiologically or clinically documented disease progression or death from any cause. OS was defined as the interval between initial diagnosis and death from any cause or last follow-up. Patients without documented events were censored on the date of last contact.

Radiological response assessments were performed according to routine institutional practice, using magnetic resonance imaging. Objective response rate (ORR) was defined as the proportion of patients achieving a complete or partial radiological response.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as median values with corresponding ranges or 95% confidence intervals (CIs), while categorical variables were presented as frequencies and percentages. Survival analyses were conducted using the Kaplan-Meier method. Median survival estimates were reported with 95% CIs. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 55 patients with recurrent HGGs were included in the study. The median follow-up duration was 36.1 months (95% CI: 29.5-42.7 months). The median age at diagnosis was 50 years (range, 41-56 years). Most patients were male (64%), had an ECOG-PS of 0-1 (89%), and had glioblastoma histology (76%). WHO grade 4 tumors were present in 91% of the study population, while IDH mutations were identified in 14% of patients. Additional baseline demographic and disease characteristics are summarized in Table 1.

Regarding tumor-related features, 56% of patients had tumors measuring ≥ 4 cm at diagnosis, whereas 34% demonstrated multilobar involvement. Most patients presented with a single lesion (89%) and underwent complete surgical resection (64%) as the initial intervention (Table 1).

Treatment Characteristics

Treatment characteristics are presented in Table 2. Following surgical management, 48 patients (87%) received standard

chemoradiotherapy followed by adjuvant temozolomide, whereas 7 patients received chemoradiotherapy alone. Radiotherapy was administered at a dose of 6000 cGy to 69% of patients.

TABLE 1: Baseline patient and disease characteristics.

Characteristic	Patient number (n=55)	Percentage (%)
Age, years		
<50	25	45
≥ 50	30	55
Gender		
Male	35	64
Female	20	36
Familial history of cancer		
Yes	22	40
No	33	60
Comorbidity		
Yes	17	31
No	38	69
ECOG-PS		
0	15	27
1	34	62
2	6	11
Histology		
Glioblastoma	42	76
Astrocytoma	9	16
ODG	2	4
DMG	2	4
WHO grade		
3	5	9
4	50	91
IDH mutation		
Present	8	14
Absent	47	86
Initial tumor size, cm		
<4	24	44
≥ 4	31	56
Lobar involvement		
Unilobar	36	66
Multilobar	19	34
Lesion number		
1	49	89
2-3	7	11
DMG: Diffuse midline glioma; ECOG-PS: Eastern Cooperative Oncology Group performance status; IDH: Isocitrate dehydrogenase; ODG: Oligodendroglioma; WHO: World Health Organization.		

TABLE 2: Treatment characteristics.

Characteristic	Patient number	Percentage
Initial surgical intervention		
Biopsy only	12	22
Incomplete resection	8	14
Complete resection	35	64
First-line treatment		
CRT + TMZ	48	87
CRT only	7	13
Radiotherapy dosing, cGy		
6000	38	69
<6000	10	18
>6000	7	13
Objective response to TMZ		
Yes	16	29
No	32	58
NE (only CRT)	7	13
Re-resection		
Yes	17	31
No	38	69
Second-line treatment		
Irinotecan+beva	50	91
BCNU+beva	3	5
BCNU/CCNU+proc	1	2
CCNU	1	2
Objective response to second-line Irinotecan+beva treatment		
Yes	23	46
No	27	54
Third-line treatment		
None	42	76
BCNU	7	13
Regorafenib	3	5
Irinotecan+beva	1	2
TMZ	1	2
Carboplatin	1	2

Beva: Bevacizumab; BCNU: Carmustine; CCNU: Lomustine; CRT: Chemoradiotherapy; NE: Not evaluated, proc, procarbazine; TMZ: Temozolomide.

Among patients receiving adjuvant temozolomide, the median number of treatment cycles was 5 (range, 3-6 cycles). Objective radiological responses were observed in 16 patients (29%), whereas 32 patients (58%) experienced disease progression despite treatment. All patients ultimately developed recurrent disease requiring second-line therapy.

Second-line Treatment Outcomes

All patients received second-line systemic treatment after disease progression. Irinotecan plus bevacizumab was the predominant treatment regimen, administered to 50 patients (91%); alternative regimens were used in a limited number of cases (Table 2).

Among patients treated with second-line irinotecan plus bevacizumab, 23 achieved an objective response, corresponding to an ORR of 46%. The median number of administered cycles was 6 (range, 4-12 cycles). Fifteen patients, all of whom belonged to the irinotecan plus bevacizumab group, remained progression-free during or after second-line treatment.

The median PFS associated with second-line irinotecan plus bevacizumab was 8.8 months (95% CI: 5.1-12.4 months) (Figure 1).

Survival Outcomes

Among the 48 patients who received adjuvant temozolomide following chemoradiotherapy, the median PFS was 5.0 months (95% CI: 3.2-6.8 months) (Figure 2).

At the time of the final analysis, 35 deaths had occurred. The median OS for the entire cohort was 23.4 months (95% CI: 15.5-31.3 months) (Figure 3).

Third-line therapy was administered to 13 patients. One patient achieved an objective response, whereas disease progression occurred in 11 patients despite treatment.

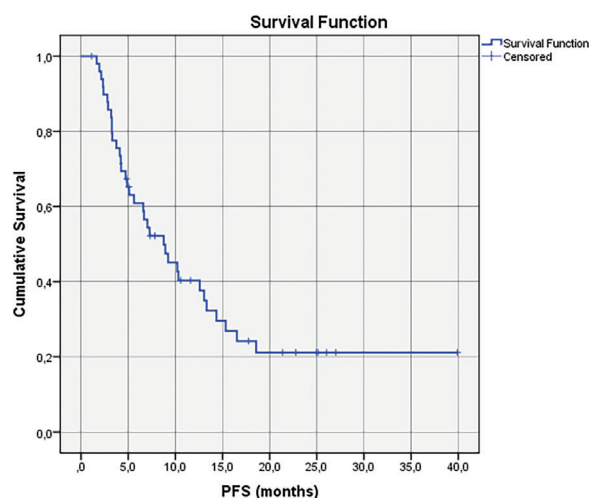


FIGURE 1: PFS plot of patients who had received second-line irinotecan plus bevacizumab.

PFS: Progression-free survival

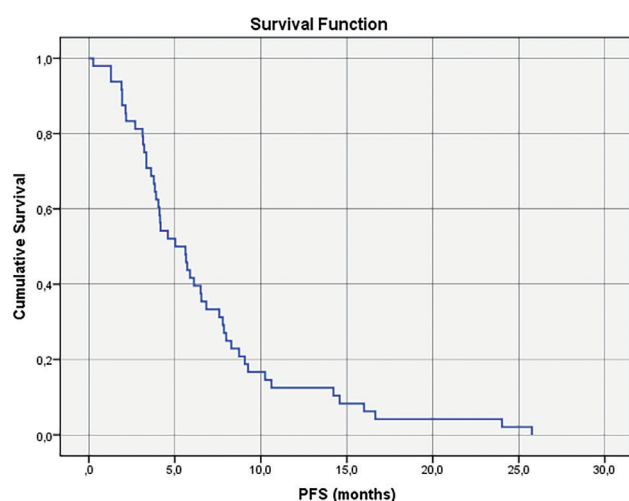


FIGURE 2: PFS plot of patients who had received temozolomide following chemoradiotherapy.

PFS: Progression-free survival

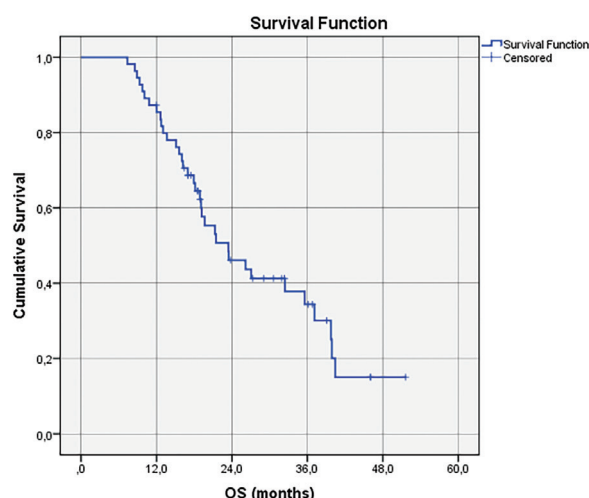


FIGURE 3: OS plot of all patients.

OS: Overall survival

DISCUSSION

In this retrospective real-world study, we evaluated the outcomes of second-line irinotecan plus bevacizumab in patients with recurrent HGGs who were treated at a tertiary referral center. The principal findings of our analysis were a 46% ORR, a median PFS of 8.8 months with irinotecan plus bevacizumab, and a median OS of 23.4 months for the entire cohort. These findings suggest that bevacizumab-based therapy continues to provide meaningful clinical benefit in selected patients with recurrent HGGs.

Despite substantial advances in neuro-oncology, recurrence remains nearly universal among patients with HGGs.

Following the establishment of the Stupp regimen as the standard frontline treatment, recurrent disease has emerged as the major cause of treatment failure and mortality.^{3,4,7} Therapeutic options after progression remain limited, and no universally accepted standard of care has been established.^{5,6}

The antiangiogenic rationale for bevacizumab is particularly compelling in glioblastoma because VEGF overexpression is a hallmark of tumor biology and contributes to angiogenesis, edema formation, and tumor progression.⁹ Several clinical studies have investigated bevacizumab alone or in combination with irinotecan in patients with recurrent glioblastoma.

One of the landmark studies evaluating bevacizumab-based treatment was conducted by Friedman et al.¹⁰, who reported ORRs of 28.2% with bevacizumab monotherapy and 37.8% with bevacizumab plus irinotecan, with median PFSs of approximately 4-5 months. Similarly, Vredenburgh et al.¹³ reported response rates approaching 57% and median PFS of approximately 6 months in patients with recurrent glioblastoma treated with bevacizumab-containing regimens.

Our observed ORR of 50% compares favorably with these historical studies and may reflect careful patient selection, advances in supportive care, and improvements in radiological monitoring. Furthermore, the median PFS of 8.8 months observed in our cohort appears numerically longer than that reported in several pivotal bevacizumab studies.^{10,12,13} Although cross-study comparisons should be interpreted cautiously, our findings support the continued effectiveness of bevacizumab-based strategies in routine clinical practice.

More recently, alternative therapeutic approaches have been explored in recurrent glioblastoma. The REGOMA trial demonstrated improved OS with regorafenib compared with lomustine, establishing regorafenib as an important treatment option in recurrent disease.¹⁴ Nevertheless, bevacizumab-based therapy remains widely used due to its ability to achieve rapid symptom control, reduce corticosteroid requirements, improve radiographic responses, and maintain quality of life in appropriately selected patients.^{10,15,16}

The median OS of 23.4 months, observed in our study, is generally consistent with outcomes reported in contemporary high-grade glioma cohorts receiving multimodal treatment.¹⁷⁻¹⁹ Importantly, most patients in our cohort had glioblastoma histology and WHO grade 4 disease, representing a population with an inherently poor prognosis. Therefore, the survival outcomes observed in our study further support the potential clinical value of bevacizumab-containing salvage therapy.

Study Limitations

Several limitations should be acknowledged. First, the retrospective nature of the study introduces the possibility of selection bias and unmeasured confounding factors. Second, the sample size was relatively small and was drawn from a single institution, potentially limiting generalizability. Third, molecular information beyond IDH status was unavailable for a substantial proportion of patients, precluding detailed molecular subgroup analyses. The absence of a control group receiving alternative second-line therapies limits direct comparisons of treatment efficacy.

Despite these limitations, our study reflects real-world clinical practice and provides contemporary evidence regarding the effectiveness of irinotecan plus bevacizumab in recurrent HGGs. Given the scarcity of prospective randomized data in this setting, such institutional experiences remain clinically relevant.

CONCLUSION

Second-line irinotecan plus bevacizumab demonstrated clinically meaningful antitumor activity in patients with recurrent HGGs, achieving an ORR of 46% and a median PFS of 8.8 months. The observed survival outcomes are favorable compared with those reported in previously published studies and support the continued use of bevacizumab-based treatment strategies in appropriately selected patients. Prospective multicenter studies incorporating contemporary molecular classification systems are warranted to further define the optimal role of irinotecan plus bevacizumab in recurrent HGGs.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and approved by the University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital Clinical Research Ethics Committee (approval number: 2024-25, date: 07.11.2024).

Informed Consent: Due to the retrospective nature of the study, the requirement for informed consent was waived.

Footnotes

Authorship Contributions

Concept: A.Ö., G.U.E., Design: A.Ö., G.U.E., Data Collection or Processing: A.Ö., G.U.E., Analysis or Interpretation: A.Ö., G.U.E., Literature Search: A.Ö., Writing: A.Ö.

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

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Comparative Efficacy of Second-line Anti-VEGF Continuation and Anti-EGFR Switch Strategies in RAS Wild-type Metastatic Colorectal Cancer Patients Progressing on First-line Anti-VEGF Therapy: A Retrospective Single-center Study

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ABSTRACT

Objective: The optimal selection of second-line biological therapy remains unclear in patients with RAS wild-type metastatic colorectal cancer (mCRC) who develop disease progression following first-line anti-vascular endothelial growth factor (VEGF)-based treatment. This study aimed to compare the efficacy and safety outcomes of continuation of second-line anti-VEGF therapy versus switching to anti-epidermal growth factor receptor (EGFR) therapy.

Material and Methods: This retrospective single-center study included 38 patients with RAS wild-type mCRC who were treated between January 2015 and December 2025. All patients received bevacizumab-based chemotherapy in the first-line setting and subsequently developed disease progression. Patients were divided into two groups according to the second-line biological therapy administered concomitantly with chemotherapy: anti-VEGF continuation (n=20) and anti-EGFR switch (cetuximab/panitumumab; n=18). The primary endpoints were second-line progression-free survival (PFS2) and overall survival (OS).

Results: Median PFS2 was 7.1 months in the anti-VEGF group and 10.0 months in the anti-EGFR group (p=0.404). Median OS were 28.2 months and 25.5 months, respectively (p=0.752). No significant differences were observed between the groups in survival outcomes. Left-sided colon tumors were more frequent in the anti-EGFR group, whereas right-sided colon tumors were more frequent in the anti-VEGF group. Skin rash was significantly more common with anti-EGFR therapy (22.2%; p=0.041), while other toxicities were manageable.

Conclusion: In patients with RAS wild-type mCRC progressing after first-line anti-VEGF therapy, second-line anti-VEGF continuation and anti-EGFR switch strategies demonstrated comparable survival outcomes. Treatment selection should be individualized based on tumor sidedness, prior treatment response, toxicity profile, and patient characteristics.

Keywords: Metastatic colorectal cancer; RAS wild-type; second-line treatment; progression-free survival; real-world data

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy worldwide and the second leading cause of cancer-related mortality.¹ Approximately 25% of patients present with metastatic disease at initial diagnosis, and nearly half develop metastases during the course of the disease.²

Metastatic CRC (mCRC) continues to represent a substantial global clinical burden due to its aggressive disease biology and limited therapeutic options.³ In the management of mCRC, systemic therapies supported by randomized clinical trials and meta-analyses constitute the cornerstone of treatment.⁴ With the widespread implementation of extended

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molecular testing and genomic profiling, contemporary mCRC treatment has become increasingly personalized. Despite these scientific advances, determining the most effective treatment sequence and defining the optimal therapeutic strategy remain major unresolved clinical challenges.⁵

Precision oncology approaches in patients with RAS wild-type mCRC require the integration of molecular biomarkers, primary tumor location, and patient-related characteristics into the therapeutic decision-making process.⁶ Monoclonal antibodies targeting the epidermal growth factor receptor (EGFR), including panitumumab and cetuximab, currently represent important first-line treatment options with demonstrated survival benefit.⁷ In parallel, the addition of antibodies targeting the vascular endothelial growth factor (VEGF) signaling pathway to chemotherapy has been shown to improve response rates across all molecular subgroups, regardless of RAS mutation status.⁸ Current guidelines identify primary tumor sidedness as a key determinant of treatment selection, recommending anti-EGFR agents preferentially for left-sided tumors and anti-VEGF agents for right-sided tumors.⁹ Nevertheless, national registry data suggest that anti-EGFR-based therapies may provide advantages in progression-free survival (PFS) and overall survival (OS) compared with bevacizumab-based regimens in real-world practice.¹⁰ Therefore, the selection of the optimal biological agent and treatment combination in the RAS wild-type population represents a multidimensional decision-making process requiring comprehensive evaluation of efficacy, clinical pharmacology, and toxicity profiles.^{2,5}

In routine clinical practice, anti-VEGF agents, such as bevacizumab, when combined with cytotoxic chemotherapy, constitute one of the most frequently used biological strategies in the first-line treatment of mCRC because of their efficacy irrespective of RAS mutation status.^{1,2,11} However, the optimal therapeutic approach for patients with RAS wild-type disease who experience progression during first-line anti-VEGF therapy remains unclear.^{5,11,12} This uncertainty creates a critical clinical gap in optimizing treatment sequencing to maximize survival outcomes in patients with RAS wild-type mCRC.⁵

Among second-line treatment strategies, the potential of anti-EGFR agents to improve response rates and the rationale for continued anti-VEGF inhibition have yielded conflicting results in the literature.^{5,11} The PRODIGE-18 study suggested a numerical survival advantage with bevacizumab continuation compared with switching to anti-EGFR therapy, whereas the SPIRITT trial demonstrated no significant differences in PFS or OS between the two approaches.^{13,14} Such heterogeneity in available data complicates the translation of guideline recommendations into real-world practice and highlights the

need to evaluate local treatment patterns in light of national registry data. Consequently, there is an increasing need for additional real-world evidence directly comparing these two biological strategies in patients with RAS wild-type mCRC who progress following first-line anti-VEGF therapy.

In the present study, using real-world clinical data, we aimed to compare the efficacy of continuing anti-VEGF as second-line therapy versus switching to anti-EGFR therapy in patients with RAS wild-type mCRC who developed disease progression after receiving first-line anti-VEGF-based chemotherapy. The primary endpoints were second-line PFS (PFS2) and OS, while secondary endpoints included treatment response rates and toxicity profiles. We anticipate that the findings of this study will address the current clinical uncertainty and strengthen the evidence base regarding optimal second-line biological agent selection in patients with RAS wild-type mCRC.

MATERIAL AND METHODS

Study Design and Patient Selection

This study was designed as a single-center, retrospective, observational analysis. The study protocol was approved by the İstanbul University, İstanbul Faculty of Medicine Clinical Research Ethics Committee (approval number: 2026/136, date: 06.02.2026), and all procedures were conducted in accordance with the Declaration of Helsinki. Due to the retrospective nature of the study, the requirement for informed consent was waived.

Patients diagnosed with mCRC and with histopathologically confirmed colorectal adenocarcinoma between January 2015 and December 2025 were included in the study. The inclusion criteria were defined as follows: (1) confirmed RAS wild-type and BRAF wild-type molecular status, (2) receipt of a standard first-line chemotherapy regimen containing bevacizumab, (3) radiological disease progression following first-line therapy with subsequent initiation of second-line systemic treatment, and (4) an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2. Patients with unknown RAS/BRAF mutation status, those with BRAF-mutant tumors, those who died before initiation of second-line therapy, or those with insufficient follow-up data were excluded from the study.

Eligible patients were categorized into two groups according to the biological agent administered during second-line treatment: the anti-VEGF group (bevacizumab-based regimen, n=20) and the anti-EGFR group (cetuximab- or panitumumab-based regimen, n=18). The selection of biological therapy was determined by the treating oncologist based on the patient's clinical condition, first-line chemotherapy regimen, and individual patient characteristics.

Assessment of Clinical and Treatment Characteristics

Clinical, demographic, and treatment-related data were retrospectively collected from the institutional electronic medical record system. The evaluated parameters included age at diagnosis, sex, body mass index (BMI), ECOG performance status, presence of comorbidities, primary tumor localization and clinical stage, histological subtype, status and intent of primary tumor surgery, type of metastasis (synchronous/metachronous), interval from diagnosis to metastatic disease, number of metastatic organs and their sites, number of liver metastases, RAS/BRAF mutation status and microsatellite instability (MSI) status, serum carcinoembryonic antigen and carbohydrate antigen 19-9 levels, chemotherapy protocol administered, type of biological agent, number of treatment cycles, treatment response according to RECIST version 1.1, and reasons for treatment discontinuation. Toxicity data were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0.

Follow-up and Study Endpoints

The primary endpoints of the study were PFS2 and OS. PFS2 was defined as the interval between the date of radiological progression following first-line treatment and the date of progression during second-line therapy or death from any cause. Radiologically confirmed first-line progression was selected as the starting point because it represented the predefined clinical event prompting the initiation of second-line treatment in all eligible patients. OS was defined as the interval from the initiation of first-line treatment for metastatic disease to death from any cause. This definition was chosen to evaluate the OS associated with the complete treatment strategy, rather than the isolated effect of second-line

biological therapy. Patients without documented progression or death during follow-up were censored on the date of the last clinical evaluation.

The secondary endpoints included first-line PFS (PFS1) and toxicity profiles associated with each treatment group.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software, version 28.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation or median (minimum-maximum), whereas categorical variables were presented as frequencies and percentages (%). Comparisons between groups were performed using the Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables, as appropriate.

Survival analyses were conducted using the Kaplan-Meier method, and survival curves were compared using the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using univariable Cox proportional hazards models. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

The patient selection process is illustrated in Figure 1. A total of 38 patients with confirmed RAS- wild-type and BRAF- wild-type mCRC who met the study eligibility criteria and were followed at our center between January 2015 and December 2025 were retrospectively analyzed. Of these patients, 20 continued anti-VEGF therapy in the second-line setting, whereas 18 switched to anti-EGFR therapy. Baseline patient characteristics are summarized in Table 1.

TABLE 1: Baseline demographic and clinical characteristics of patients according to second-line biological treatment strategy.

Variable	Anti-VEGF (n=20)	Anti-EGFR (n=18)	p-value
Demographic characteristics			
Age (years)			
Mean ± SD	60.4±10.7	59.4±11.7	0.682 ¹
Median (min-max)	61 (37-77)	58 (36-78)	
Age group			
<65 years	12 (60.0%)	13 (72.2%)	0.652 ²
≥65 years	8 (40.0%)	5 (27.8%)	
Sex			
Female	3 (15.0%)	8 (44.4%)	0.074 ²
Male	17 (85.0%)	10 (55.6%)	
BMI (kg/m²)			
Mean ± SD	25.0±5.1	29.1±6.7	0.034 ¹
Median (min-max)	24.6 (15.6-36.6)	29.7 (15.1-41.1)	

TABLE 1: Continued.			
Variable	Anti-VEGF (n=20)	Anti-EGFR (n=18)	p-value
ECOG performance status			
ECOG 0	18 (90.0%)	14 (77.8%)	0.450 ²
ECOG 1	2 (10.0%)	3 (16.7%)	
ECOG 2	0 (0.0%)	1 (5.6%)	
Smoking status			
Never	7 (35.0%)	5 (27.8%)	1.000 ²
Ever	10 (50.0%)	8 (44.4%)	
Pack-years (mean ± SD)	29.4±12.0	31.2±8.3	0.622 ¹
Comorbidity	15 (75.0%)	6 (33.3%)	0.024 ²
Family history			
None	8 (40.0%)	8 (44.4%)	0.598 ²
Family history of CRC	3 (15.0%)	2 (11.1%)	
Family history of non-CRC cancer	5 (25.0%)	2 (11.1%)	
Unknown	3 (15.0%)	5 (27.8%)	
Second primary malignancy	2 (10.0%)	0 (0.0%)	0.488 ²

¹: Independent samples Mann-Whitney U test; ²: Pearson chi-square test/Fisher's exact test, p<0.05 was considered statistically significant.
VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor; SD: Standard deviation; BMI: Body mass index; ECOG: Eastern Cooperative Oncology Group; CRC: Colorectal cancer.

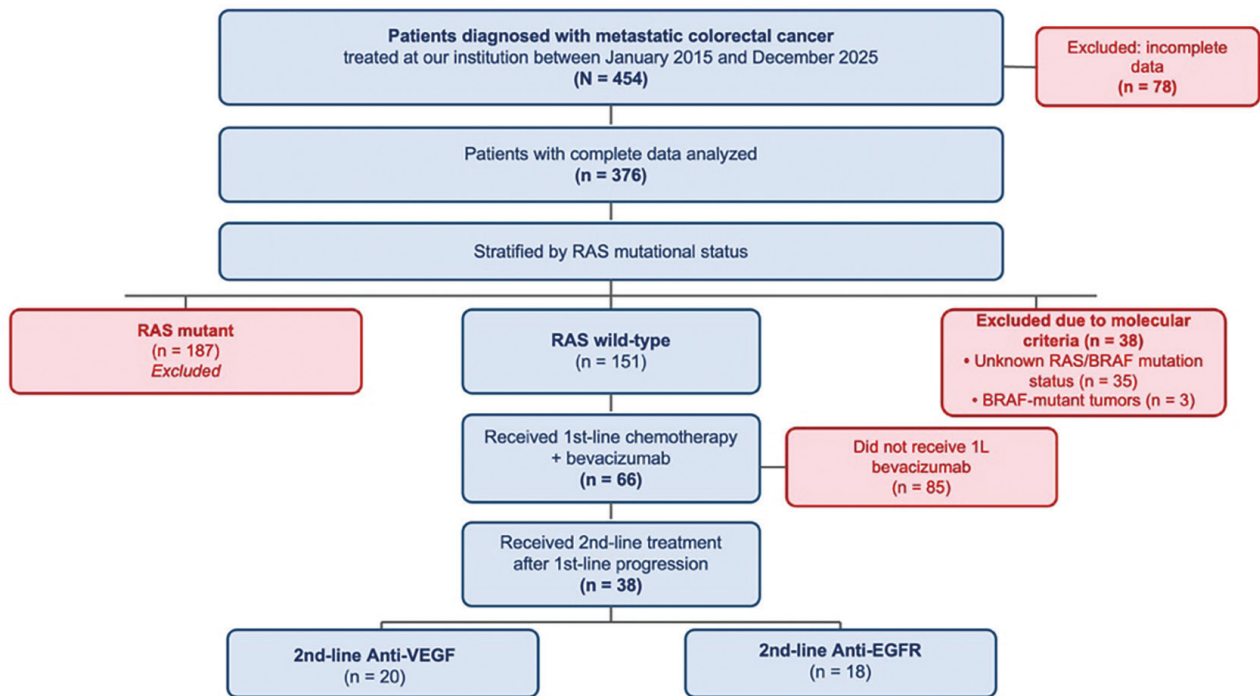


FIGURE 1: Flow diagram of patient selection.
VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor

No statistically significant differences were observed between the groups in demographic characteristics, including age, sex, ECOG performance status, smoking history, height and weight (Table 1). However, BMI was significantly higher in the anti-EGFR group compared with the anti-VEGF group

(29.1±6.7 vs. 25.0±5.1 kg/m²; p=0.034). The presence of comorbidities was more frequent in the anti-VEGF group than in the anti-EGFR group (75.0% vs. 33.3%; p=0.024), whereas no other significant differences in baseline clinical characteristics were identified.

Regarding primary tumor localization, right-sided colon tumors were more common in the anti-VEGF group (70.0%), whereas left-sided colon and rectal tumors predominated in the anti-EGFR group (77.8%). Detailed localization analysis demonstrated a statistically significant difference in tumor distribution between the groups ($p=0.024$). In terms of

metastatic disease characteristics, liver metastasis was the most common metastatic site in both groups. Although lung and peritoneal metastases were observed more frequently in the anti-VEGF group, the differences did not reach statistical significance (both $p=0.067$) (Table 2).

TABLE 2: Tumor and metastatic disease characteristics according to second-line biological treatment strategy.

Variable	Anti-VEGF (n=20)	Anti-EGFR (n=18)	p-value
Clinical and pathological characteristics			
Clinical stage at initial diagnosis			
Stage 4A	7 (35.0%)	8 (44.4%)	0.559 ²
Stage 4B	4 (20.0%)	4 (22.2%)	
Stage 4C	6 (30.0%)	1 (5.6%)	
Stage 3C	1 (5.0%)	2 (11.1%)	
Stage 3B	1 (5.0%)	1 (5.6%)	
Stage 3A	0 (0.0%)	1 (5.6%)	
Stage 2A	1 (5.0%)	1 (5.6%)	
Tumor localization (general)			
Right colon	14 (70.0%)	4 (22.2%)	
Left colon	6 (30.0%)	14 (77.8%)	
Both sides	0 (0.0%)	0 (0.0%)	
Tumor localization (detail)			
Cecum	6 (30.0%)	2 (11.1%)	0.024 ²
Ascending colon	8 (40.0%)	1 (5.6%)	
Transverse colon	3 (15.0%)	2 (11.1%)	
Descending colon	1 (5.0%)	1 (5.6%)	
Sigmoid colon	3 (15.0%)	1 (5.6%)	
Rectum	1 (5.0%)	7 (38.9%)	
Rectosigmoid	1 (5.0%)	4 (22.2%)	
Primary tumor surgery			
Performed	10 (50.0%)	12 (66.7%)	0.478 ²
Not performed	10 (50.0%)	6 (33.3%)	
Surgical intent			
Curative	4 (20.0%)	5 (27.8%)	1.000 ²
Palliative	6 (30.0%)	7 (38.9%)	
Surgical procedure			
Right hemicolectomy	6 (30.0%)	2 (11.1%)	0.219 ²
Anterior resection	1 (5.0%)	1 (5.6%)	
Laparoscopic anterior resection	0 (0.0%)	2 (11.1%)	
Abdominoperineal resection	0 (0.0%)	1 (5.6%)	
Total colectomy	0 (0.0%)	2 (11.1%)	
Segmental resection	3 (15.0%)	4 (22.2%)	
Time from diagnosis to metastasis (months)			
Mean ± SD	2.2±5.7	7.1±12.4	0.176 ¹
Median (min-max)	0 (0-20)	0 (0-40)	

TABLE 2: Continued.			
Variable	Anti-VEGF (n=20)	Anti-EGFR (n=18)	p-value
Metastasis type			
Synchronous	17 (85.0%)	12 (66.7%)	0.260 ²
Metachronous	3 (15.0%)	6 (33.3%)	
Number of metastatic sites			
Mean ± SD	1.6±0.9	1.3±0.5	0.318 ¹
Median (min-max)	1 (1-4)	1 (1-2)	
Metastatic sites			
Liver	14 (70.0%)	14 (77.8%)	0.719 ²
Lung	8 (40.0%)	2 (11.1%)	0.067 ²
Peritoneum	8 (40.0%)	2 (11.1%)	0.067 ²
Bone	1 (5.0%)	1 (5.6%)	1.000 ²
Distant lymph node	2 (10.0%)	4 (22.2%)	0.395 ²
Liver metastasis count (among patients with liver metastases)			
Mean ± SD	1.9±1.1	2.2±1.8	0.940 ¹
Median (min-max)	1 (1-4)	2 (1-7)	
Multiple (≥4)	4 (20.0%)	3 (16.7%)	1.000 ²
Baseline CEA (ng/mL)			
Mean ± SD	211.1±641.3	122.6±231.8	0.855 ¹
Median (min-max)	14.1 (0.8-2834.0)	6.0 (1.2-658.0)	
Baseline CA 19-9 (U/mL)			
Mean ± SD	122.6±229.9	107.4±340.1	0.293 ¹
Median (min-max)	30.8 (0.6-820.0)	7.8 (0.6-1418.0)	
1: Independent samples Mann-Whitney U test, 2: Pearson chi-square test/Fisher's exact test, log-rank test, p<0.05 was considered statistically significant. Clinical stage refers to the stage at the time of the initial colorectal cancer diagnosis, before the development of metastatic disease in patients with metachronous metastases. VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor; SD: Standard deviation; CEA: Carcinoembryonic antigen; CA 19-9: Carbohydrate antigen 19-9.			

All patients in both groups received bevacizumab-based treatment in the first-line setting. FOLFOX was the most commonly administered chemotherapy regimen. The number of first-line treatment cycles was comparable between the groups (8.3 $\pm$ 4.1 vs. 9.7 $\pm$ 3.5; p=0.500). A statistically significant difference was observed in first-line treatment response rates between the groups (p=0.047). The anti-VEGF group demonstrated a higher rate of partial response (40.0% vs. 5.6%), whereas progressive disease was more frequent in the anti-EGFR group (66.7% vs. 30.0%) (Table 3).

In the second-line setting, all patients in the anti-VEGF group continued bevacizumab therapy, while 66.7% of patients in the anti-EGFR group received cetuximab and 33.3% received panitumumab. Significant differences were observed between the groups in the second-line chemotherapy regimens administered (p=0.031), with FOLFIRI more frequently used in the anti-EGFR group (77.8%). No statistically significant difference was found between the groups in second-line treatment response (p=0.483) (Table 3).

Evaluation of treatment-line transitions revealed a numerically higher rate of progression to third-line therapy in the anti-VEGF group compared with the anti-EGFR group (65.0% vs. 50.0%); however, this difference was not statistically significant (p=0.544).

Survival analyses are presented in Table 4 and Figures 2-4. The median PFS1 was 12.3 months in the anti-VEGF group versus 9.1 months in the anti-EGFR group; the difference was not statistically significant (HR: 0.68; 95% CI: 0.32-1.44; p=0.311) (Figure 2). The median PFS2 durations were 7.1 months and 10.0 months in the anti-VEGF and anti-EGFR groups, respectively (HR: 1.42; 95% CI: 0.62-3.23; p=0.404) (Figure 3). Median OS durations were 28.2 months in the anti-VEGF group and 25.5 months in the anti-EGFR group (HR: 1.12; 95% CI: 0.54-2.32; p=0.752) (Figure 4). At the end of follow-up, mortality rates were 80.0% and 88.9% in the anti-VEGF and anti-EGFR groups, respectively.

TABLE 3: Treatment characteristics and response outcomes according to second-line biological treatment strategy.

Variable	Anti-VEGF (n=20)	Anti-EGFR (n=18)	p-value
1 st line treatment characteristics			
1 st line chemotherapy regimen			
XELOX	7 (35.0%)	8 (44.4%)	0.363 ²
FOLFOX	11 (55.0%)	7 (38.9%)	
FOLFIRI	2 (10.0%)	1 (5.6%)	
FOLFIRINOX	0 (0.0%)	2 (11.1%)	
1 st line anti-VEGF agent			
Bevacizumab	20 (100.0%)	18 (100.0%)	
Number of 1 st line cycles			
Mean ± SD	8.3±4.1	9.7±3.5	0.500 ¹
Median (min-max)	8 (1-14)	10 (3-18)	
1 st line treatment response			
CR	2 (10.0%)	3 (16.7%)	0.047 ²
PR	8 (40.0%)	1 (5.6%)	
Stable disease	1 (5.0%)	2 (11.1%)	
PD	6 (30.0%)	12 (66.7%)	
2 nd line treatment characteristics			
2 nd line chemotherapy regimen			
XELOX	2 (10.0%)	0 (0.0%)	0.031 ²
XELIRI	6 (30.0%)	1 (5.6%)	
FOLFOX	5 (25.0%)	3 (16.7%)	
FOLFIRI	5 (25.0%)	14 (77.8%)	
FOLFIRINOX	1 (5.0%)	0 (0.0%)	
XELOXIRI	1 (5.0%)	0 (0.0%)	
2 nd line biological agent			
Bevacizumab	20 (100.0%)	0 (0.0%)	<0.001 ²
Panitumumab	0 (0.0%)	6 (33.3%)	
Cetuximab	0 (0.0%)	12 (66.7%)	
Number of 2 nd line cycles			
Mean ± SD	6.0±3.6	7.8±4.4	0.162 ¹
Median (min-max)	5 (1-12)	6 (2-18)	
2 nd line treatment response			
CR	1 (5.0%)	0 (0.0%)	0.483 ²
PR	1 (5.0%)	2 (11.1%)	
Stable disease	0 (0.0%)	1 (5.6%)	
PD	14 (70.0%)	12 (66.7%)	
Treatment line transition summary			
Received 1 st line treatment	20 (100.0%)	18 (100.0%)	
Proceeded to 2 nd line	20 (100.0%)	18 (100.0%)	1.000 ²
Proceeded to 3 rd line	13 (65.0%)	9 (50.0%)	0.544 ²

¹: Independent samples Mann-Whitney U test, ²: Pearson chi-square test/Fisher's exact test, log-rank test, p<0.05 was considered statistically significant.
 VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor; SD: Standard deviation; CR: Complete response; PR: Partial response; PD: Progressive disease.

TABLE 4: Survival outcomes according to second-line biological treatment strategy.			
Variable	Anti-VEGF (n=20)	Anti-EGFR (n=18)	p-value
PFS1			
Definition: from 1 st line start to 1 st line progression			
Evaluable patients	20	18	0.311 ³
Events (progression/death)	12	15	
Censored patients	8	3	
Median PFS1 (months)	12.3	9.1	
95% CI	8.7-13.6	4.5-11.5	
HR (95% CI)	0.68 (0.32-1.44)	—	
Mean PFS1 (months)	10.8±7.3	10.2±7.1	
6-month PFS1 rate	94.4%	61.1%	
12-month PFS1 rate	52.5%	37.0%	
24-month PFS1 rate	16.4%	14.8%	
PFS2			
Definition: from 1 st line progression to 2 nd line progression			
Evaluable patients	20	18	0.404 ³
Events (progression/death)	13	10	
Censored patients	7	8	
Median PFS2 (months)	7.1	10.0	
95% CI	4.5-8.3	6.8-11.0	
HR (95% CI)	1.42 (0.62-3.23)	—	
Mean PFS2 (months)	6.1±3.8	6.8±3.7	
6-month PFS2 rate	67.0%	70.6%	
12-month PFS2 rate	8.4%	30.9%	
24-month PFS2 rate	8.4%	15.4%	
OS			
Definition: from 1 st line start to death from any cause			
Evaluable patients	20	18	0.752 ³
Events (deaths)	16	16	
Censored patients	4	2	
Median OS (months)	28.2	25.5	
95% CI	21.3-31.4	19.5-32.6	
HR (95% CI)	1.12 (0.54-2.32)	—	
Mean OS (months)	27.1±11.8	28.0±17.2	
12-month OS rate	90.0%	88.9%	
24-month OS rate	54.2%	51.4%	
36-month OS rate	34.4%	25.7%	
60-month OS rate	0.0%	6.4%	
Survival status			
Alive	4 (20.0%)	2 (11.1%)	0.663 ²
Deceased	16 (80.0%)	16 (88.9%)	
² : Pearson chi-square test/Fisher's exact test, ³ : Kaplan-Meier survival analysis, log-rank test, p<0.05 was considered statistically significant. VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor; SD: Standard deviation; OS: Overall survival; CI: Confidence interval; PFS: Progression-free survival; HR: Hazard ratio.			

²: Pearson chi-square test/Fisher's exact test; ³: Kaplan-Meier survival analysis, log-rank test, p<0.05 was considered statistically significant.
 VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor; SD: Standard deviation; OS: Overall survival; CI: Confidence interval; PFS: Progression-free survival; HR: Hazard ratio.

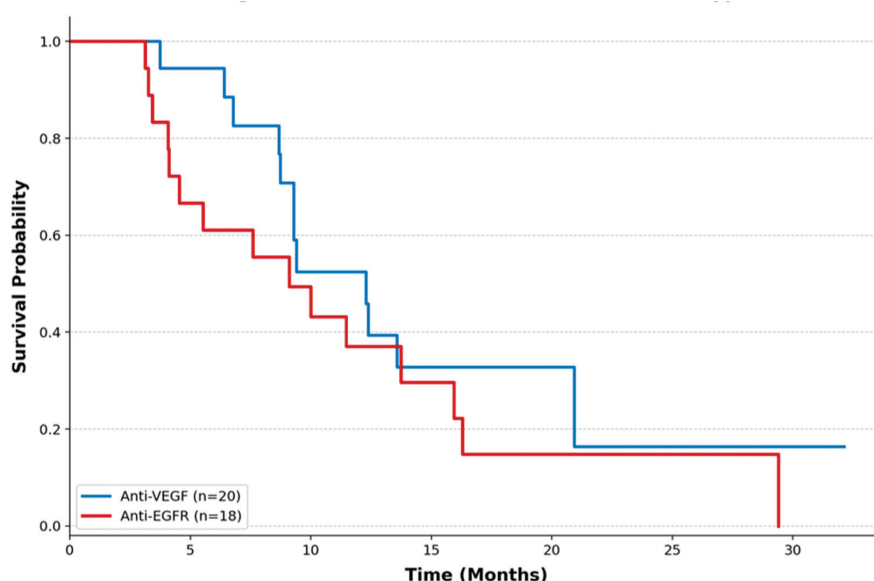


FIGURE 2: Kaplan-Meier analysis of first-line progression-free survival according to second-line biological treatment strategy.

VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor

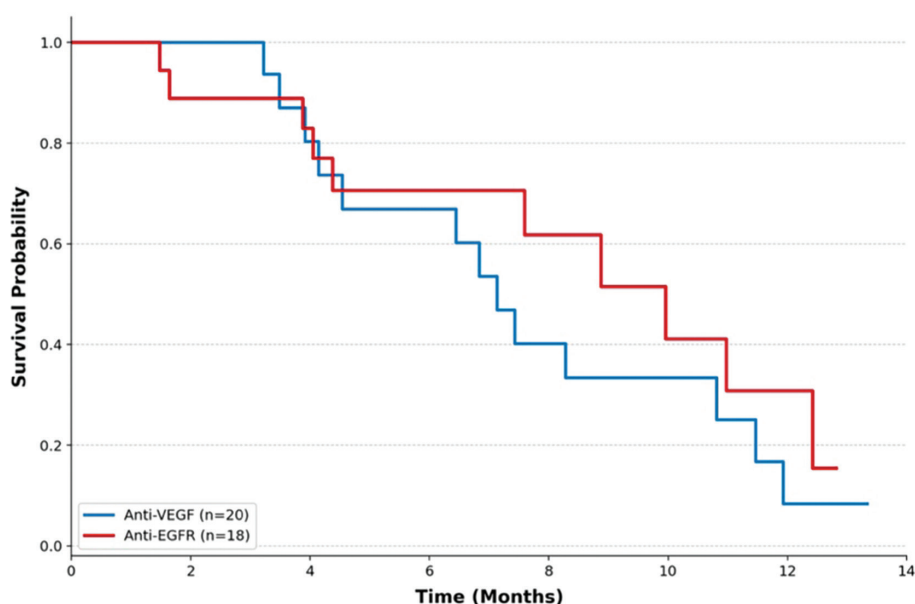


FIGURE 3: Kaplan-Meier analysis of second-line progression-free survival according to second-line biological treatment strategy.

VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor

Toxicities related to first-line treatment were summarized to provide clinical context for treatment tolerability prior to the initiation of second-line therapy. In the first-line toxicity analysis, the incidence of any adverse event was 50.0% in the anti-VEGF group and 33.3% in the anti-EGFR group, with no statistically significant difference ($p=0.478$) (Table 5). Peripheral neuropathy was the most common chemotherapy-related toxicity and tended to occur more frequently in the anti-VEGF group (30.0% vs. 5.6%; $p=0.093$).

In the second-line toxicity analysis, the overall incidence of adverse events was comparable between the groups (40.0% vs. 61.1%; $p=0.330$). Biologic agent-related skin rash was significantly more common in the anti-EGFR group than in the anti-VEGF group (22.2% vs. 0.0%; $p=0.041$). Apart from this finding, no significant differences were observed between the groups in chemotherapy-related or biologic agent-related toxicities (Table 5).

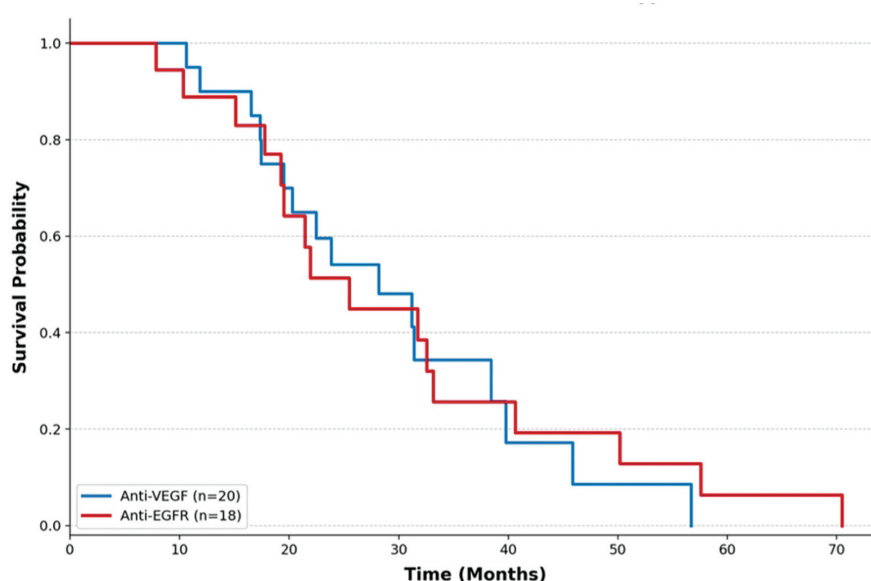


FIGURE 4: Kaplan-Meier analysis of overall survival according to second-line biological treatment strategy.

VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor

TABLE 5: First- and second-line treatment-related toxicities according to second-line biological treatment strategy.

Variable	Anti-VEGF (n=20)	Anti-EGFR (n=18)	p-value
1st line treatment toxicities			
Patients with adverse events	10 (50.0%)	6 (33.3%)	0.478 ²
Chemotherapy-related toxicities (1st line)			
Peripheral neuropathy	6 (30.0%)	1 (5.6%)	0.093 ²
Diarrhea	0 (0.0%)	2 (11.1%)	0.218 ²
Neutropenia	2 (10.0%)	1 (5.6%)	1.000 ²
Thrombocytopenia	4 (20.0%)	2 (11.1%)	0.663 ²
No chemotherapy toxicity	11 (55.0%)	12 (66.7%)	
Anti-VEGF agent-related toxicities (1st line)			
Hypertension	0 (0.0%)	1 (5.6%)	0.474 ²
Thromboembolic event	1 (5.0%)	0 (0.0%)	1.000 ²
Perforation	1 (5.0%)	0 (0.0%)	1.000 ²
Diarrhea	0 (0.0%)	2 (11.1%)	0.218 ²
No biological agent toxicity	19 (95.0%)	16 (88.9%)	
Maximum grade of biological agent toxicity			
Grade 1	0 (0.0%)	0 (0.0%)	
Grade 2	0 (0.0%)	1 (5.6%)	
Grade 3	1 (5.0%)	1 (5.6%)	
Grade 4	0 (0.0%)	0 (0.0%)	
2nd line treatment toxicities			
Patients with adverse events	8 (40.0%)	11 (61.1%)	0.330 ²
Chemotherapy-related toxicities (2nd line)			
Peripheral neuropathy	3 (15.0%)	2 (11.1%)	1.000 ²
Diarrhea	1 (5.0%)	4 (22.2%)	0.170 ²
Neutropenia	2 (10.0%)	0 (0.0%)	0.488 ²

TABLE 5: Continued.

Variable	Anti-VEGF (n=20)	Anti-EGFR (n=18)	p-value
Thrombocytopenia	3 (15.0%)	0 (0.0%)	0.232 ²
Infusion reaction	1 (5.0%)	0 (0.0%)	1.000 ²
Nausea/vomiting	1 (5.0%)	1 (5.6%)	1.000 ²
Constipation	0 (0.0%)	1 (5.6%)	0.474 ²
No chemotherapy toxicity	11 (55.0%)	11 (61.1%)	
Anti-EGFR/anti-VEGF agent-related toxicities (2nd line)			
Proteinuria	0 (0.0%)	1 (5.6%)	0.474 ²
Bleeding	1 (5.0%)	1 (5.6%)	1.000 ²
Skin rash	0 (0.0%)	4 (22.2%)	0.041 ²
Diarrhea	0 (0.0%)	2 (11.1%)	0.218 ²
No biological agent toxicity	19 (95.0%)	10 (55.6%)	
Maximum grade of biological agent toxicity			
Grade 1	0 (0.0%)	1 (5.6%)	
Grade 2	1 (5.0%)	5 (27.8%)	
Grade 3	0 (0.0%)	2 (11.1%)	
Grade 4	0 (0.0%)	0 (0.0%)	

²: Pearson chi-square test/Fisher's exact test, log-rank test, p<0.05 was considered statistically significant.

VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor.

DISCUSSION

In this retrospective, real-world study, we compared continuation of anti-VEGF therapy as second-line treatment with switching to anti-EGFR therapy in patients with RAS wild-type mCRC who developed disease progression during first-line anti-VEGF-based treatment. The principal finding of our study was that there was no statistically significant difference between the two treatment strategies for second-line PFS2 and OS. Similarly, first-line PFS outcomes were comparable between the groups. These findings suggest that the optimal sequencing of biological agents in patients with RAS wild-type mCRC remains undefined, and they further support the ongoing debate in the current literature regarding the most effective treatment sequence. Because the objective of the present study was to compare alternative second-line biological treatment strategies rather than the isolated efficacy of individual second-line biological agents, survival endpoints were intentionally defined to reflect the clinical outcome of the entire treatment sequence. Accordingly, OS was calculated from initiation of first-line metastatic treatment. We acknowledge that this definition may be influenced by the duration of first-line treatment and by treatment response; however, we consider this approach more appropriate for evaluating the long-term outcomes associated with alternative second-line treatment strategies following first-line anti-VEGF-based therapy. Particularly in light of the heterogeneous results observed across randomized clinical

trials and real-world studies, our findings underscore the importance of individualized treatment selection based on patient- and tumor-specific characteristics.

In our cohort, median PFS2 was 7.1 months in the anti-VEGF group and 10.0 months in the anti-EGFR group, while median OS was 28.2 months in the anti-VEGF group and 25.5 months in the anti-EGFR group. These results indicate no significant survival advantage for either continuing second-line anti-VEGF therapy or switching to anti-EGFR therapy. Our findings are consistent with those of the SPIRITT trial, which compared panitumumab-based and bevacizumab-based second-line strategies and reported no significant differences in PFS or OS between the two strategies.¹⁴ In contrast, the PRODIGE-18 study demonstrated a numerically longer OS with continuation of bevacizumab than with switching to cetuximab following disease progression (15.8 vs. 10.4 months).¹³ Although a numerically longer PFS2 was observed in the anti-EGFR arm in our cohort, this difference did not reach statistical significance. This discrepancy may be attributable to the limited sample size, selection bias inherent to the retrospective design, clinical and molecular heterogeneity of the patient population, and differences in treatment sequencing strategies.

One of the most notable clinical differences in our study was the distribution of primary tumor sidedness between the treatment groups. While the majority of patients in the anti-VEGF group had right-sided primary tumors (70.0%),

left-sided colon and rectal tumors predominated in the anti-EGFR group (77.8%). Primary tumor location is recognized as one of the most important prognostic and predictive factors guiding the selection of biological agents in RAS wild-type mCRC. Large meta-analyses and randomized studies have consistently demonstrated that anti-EGFR-based strategies confer a substantial survival advantage in left-sided RAS wild-type tumors, whereas anti-VEGF-based strategies may represent a more appropriate option for right-sided tumors.¹⁵ In this context, the predominance of left-sided tumors in the anti-EGFR group may partially explain the numerically longer PFS2 observed in this cohort.

The higher rate of partial response achieved during first-line therapy in the anti-VEGF group may also support the clinical rationale for the “bevacizumab beyond progression” strategy. Previous studies have shown that continued VEGF inhibition after disease progression may provide an additional survival benefit for patients who derived clinical benefit from first-line bevacizumab therapy.⁵

Despite a higher rate of progressive disease during first-line treatment in the anti-EGFR group, the achievement of a median PFS2 of 10.0 months in the second-line setting is noteworthy. This finding suggests that targeting an alternative biological pathway following first-line angiogenesis inhibition may restore tumor sensitivity, particularly in molecularly selected subgroups. The potential benefit associated with switching from anti-VEGF to anti-EGFR therapy may be related to dynamic alterations in the tumor microenvironment and signaling networks induced by treatment pressure. Therefore, biologic-agent crossover may represent a rational therapeutic strategy to improve second-line treatment efficacy in selected patients with RAS wild-type disease.^{5,15,16}

In the second-line treatment setting for mCRC, careful evaluation of toxicity profiles is as important as efficacy outcomes. Particularly in patients receiving palliative treatment, maintaining quality of life and treatment adherence are critical considerations influencing biological agent selection. In our study, skin rash was significantly more common in the anti-EGFR group (22.2%), consistent with the literature identifying cutaneous toxicity as the most characteristic class-specific adverse event associated with EGFR inhibitors.^{2,10} Although anti-EGFR-related dermatologic toxicities are generally manageable, they may adversely affect daily activities and treatment compliance. Accordingly, patient education, early dermatologic assessment, and proactive supportive care strategies should be implemented at treatment initiation.¹⁷

In contrast, the higher incidence of peripheral neuropathy observed in the anti-VEGF group was likely attributable to the accompanying cytotoxic chemotherapy, particularly exposure to oxaliplatin. The absence of a substantial increase in severe

toxicities related to biologic agents during continued anti-VEGF therapy suggests that this approach maintains a manageable safety profile. In patients with limited performance status or frailty, continuation of anti-VEGF therapy may represent a clinically preferable option because of its lower burden of cutaneous toxicity.^{9,11}

Study Limitations

Several limitations of our study should be acknowledged. First, the retrospective single-center design inherently introduces the possibility of selection bias and incomplete registry-based data collection. The relatively limited sample size reduced the statistical power of the study and may have prevented clinically meaningful differences between the treatment strategies from reaching statistical significance. In addition, because biological agent selection was determined by physician preference rather than by randomization, heterogeneity in patient characteristics and treatment sequencing was unavoidable. The marked imbalance in primary tumor sidedness distribution and the heterogeneity of second-line chemotherapy regimens may have been confounding factors influencing survival outcomes. Because different chemotherapy backbones were used across the treatment groups, the observed clinical outcomes cannot be attributed solely to the biological agents; the potential contribution of the accompanying chemotherapy regimens should also be considered when interpreting the results. Because of the limited sample size, additional multivariable adjustments or subgroup analyses stratified by primary tumor sidedness were not considered statistically reliable, as these analyses would have led to substantial model instability and a high risk of overfitting. Therefore, the observed survival outcomes should be interpreted with caution, and the potential influence of tumor sidedness cannot be excluded. Comprehensive molecular profiling data, including MSI status, human epidermal growth factor receptor 2 amplification, neurotrophic tyrosine receptor kinase fusions, and detailed RAS submutation analyses, were not systematically available because molecular testing reflected routine clinical practice during the study period, thereby limiting a more refined evaluation of biologically distinct subgroups. Importantly, the absence of statistically significant differences between treatment groups should not be interpreted as evidence of therapeutic equivalence, as the study was not powered to detect or exclude clinically meaningful differences between the treatment strategies. Accordingly, our findings should be considered exploratory and hypothesis-generating, and require validation in larger prospective studies.

Nevertheless, the real-world nature of our study and the fact that it represents one of the limited analyses directly comparing second-line biological strategies following first-line anti-VEGF therapy enhance the clinical relevance of our findings.

CONCLUSION

In patients with RAS wild-type mCRC who developed disease progression during first-line anti-VEGF-based therapy, no significant differences in PFS or OS were observed between continuing anti-VEGF as second-line therapy and switching to anti-EGFR therapy. However, these findings should not be interpreted as evidence of therapeutic equivalence, given the study's retrospective design and limited sample size. Treatment decisions should therefore be individualized according to primary tumor sidedness, molecular characteristics, prior treatment response, toxicity profile, and patient-related factors. Although a numerically longer PFS2 was observed in the anti-EGFR group, this finding should be considered exploratory and hypothesis-generating. Larger prospective randomized studies are warranted to determine the optimal second-line biological treatment strategy in patients with RAS wild-type mCRC.

Ethics

Ethics Committee Approval: The study protocol was approved by the Istanbul University, Istanbul Faculty of Medicine Clinical Research Ethics Committee (approval number: 2026/136, date: 06.02.2026).

Informed Consent: Due to the retrospective nature of the study, the requirement for informed consent was waived.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.Ç., Concept: S.Ç., H.U.B., D.T., S.K., Design: S.Ç., H.U.B., D.T., S.K., Data Collection or Processing: S.Ç., E.N.S., İ.S.B., M.Ş., H.U.B., Analysis or Interpretation: S.Ç., E.N.S., İ.S.B., M.Ş., D.T., S.K., Literature Search: S.Ç., E.N.S., İ.S.B., M.Ş., D.T., S.K., Writing: S.Ç.

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Effect of Prognostic Nutritional Index and Systemic Immune-inflammation Index Changes on Progression-free Survival in Patients Treated with CDK4/6 Inhibitors and Letrozole

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ABSTRACT

Objective: CDK4/6 inhibitors are the standard of care for metastatic hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative breast cancer. Markers for predicting prognosis and survival during follow-up of these patients are not yet available. Therefore, this study aimed to examine the effect of changes in prognostic nutritional index (PNI) and systemic immune-inflammation index (SII) on survival in patients treated with CDK4/6 inhibitors plus letrozole.

Material and Methods: This was a retrospective study of 88 patients. Both PNI and SII values were calculated just before, at 3 months, and at 6 months. Changes at 0-3 months and 0-6 months were divided into two groups: increasing and decreasing. Changes in progression-free survival (PFS) between these two groups were analyzed.

Results: PFS was 28.2 months [95% confidence interval (CI): 23.6-32.89] in the PNI-increasing group and 23.3 months (95% CI: 15.55-31.21) in the PNI-decreasing group at the 3rd month of treatment ($p=0.048$). At 6 months, PFS was 29.1 months (95% CI: 24.2-33.9) in the PNI increasing group and 23.2 months (95% CI: 15.9-30.6) in the PNI decreasing group. In the group with decreased SII at 3 months, PFS was almost 7 months longer than in the group with increased SII. At month 6, PFS was 27.7 months (95% CI: 23.4-32.1) in the SII-decreasing group and 14.8 months (95% CI: 10.5-19.1) in the SII-increasing group ($p=0.017$). In multivariate Cox regression analysis, only a reduction in SII between 0-6 months was a negative independent risk factor for PFS, hazard ratio 0.24 (95% CI: 0.06-0.87), $p=0.031$.

Conclusion: A decrease in PNI during follow-up is associated with poor survival in metastatic HR-positive and HER2 negative patients receiving letrozole in combination with a CDK4/6 inhibitor, whereas a decrease in SII may be a prognostic indicator in these patients.

Keywords: Breast cancer; CDK4/6 inhibitors; PNI; SII

INTRODUCTION

Breast cancer remains the most common cancer in the world, accounting for approximately 30% of all cancers in women, with a mortality rate of 15%.¹ Breast cancer is very heterogeneous and is clinically divided into three main subtypes based on the presence or absence of molecular markers for estrogen or progesterone receptors and human epidermal growth factor receptor 2 [Erb-B2 receptor tyrosine

kinase 2 (ERBB2); formerly HER2]: hormone receptor (HR)-positive /ERBB2 negative (70% of patients), ERBB2 positive (15-20%), and triple negative (15%).² Therapeutic options have increased substantially in recent years for both locoregional and systemic therapy, and avoiding both overtreatment and undertreatment has become a major focus. Therapeutic approaches need to be decided in a multidisciplinary setting, taking into account molecular subtype and locoregional tumour burden. Endocrine therapy is the standard treatment

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for hormone-positive breast cancer unless the tumour burden is high. In patients with estrogen receptor-positive and HER2-negative metastatic breast cancer, a CDK4/6 inhibitor (ribociclib, palbociclib or abemaciclib) in combination with endocrine therapy should be considered standard therapy.¹ In comparison with endocrine therapy alone, this combination results in a higher response rate, a progression-free survival (PFS) benefit, and a substantial increase in overall survival (OS) while maintaining or improving quality of life. In *de novo* or recurrent metastatic breast cancer, CDK4/6 inhibitors may be combined with an aromatase inhibitor (anastrozole and letrozole; preferably in the setting of endocrine-sensitive disease) or fulvestrant (in endocrine-resistant disease), in first-line or subsequent lines.³

Nutrition influences the incidence, natural history, and therapeutic response of malignant diseases in both humans and preclinical animal models. Progressive weight loss is common in patients with advanced cancer, leading to a decline in performance status and quality of life. However, the management of these problems has not been generalized due to a lack of a clear understanding of the underlying mechanisms.^{4,5} Nevertheless, several preclinical studies have shown that dietary interventions potentiate anticancer therapy and improve cancer outcomes. These findings are in the process of being translated into practice, with early-stage clinical data supporting their feasibility and safety.⁶ Not only nutrition but also inflammatory processes play a role in cancer development.⁷ The tumor microenvironment is strongly inflammatory, and small changes in inflammatory cell profiles can influence tumor development and progression, including tumor cell proliferation, invasion, migration, and metastasis. Recent clinical and epidemiological studies have also revealed that the inflammatory response is associated with breast cancer and could potentially be targeted for tumor treatment or measured as a prognostic indicator.^{8,9}

The prognostic nutritional index (PNI), which was used many years ago to determine the nutritional status of patients undergoing surgery in the postoperative period, has been simplified as a calculation of serum albumin concordance and peripheral blood lymphocyte count, it is calculated as $[10 \times \text{serum albumin (g/dL)}] + [\text{total lymphocyte count} (\times 10^9/\text{L}) \times 5]$, and in the last few years it has been examined in studies to determine whether it can also indicate prognosis in malignancies. Although there are differences between studies, low PNI has been associated with poor prognosis.¹⁰⁻¹² The systemic immune-inflammation index (SII) is defined as the ratio of neutrophils $\times$ platelets/lymphocytes in peripheral blood. In several studies, increasing SII values have been associated with reduced survival and poorer prognosis.^{13,14}

In metastatic HR-positive and HER2-negative disease, patients without hormone resistance were selected; therefore, the study was designed to include patients receiving a combination of letrozole and CDK4/6 inhibitors. In our study, we aimed to investigate whether changes in PNI and SII at the beginning of treatment and during follow-up affect PFS in patients receiving letrozole with CDK4/6 inhibitors.

MATERIAL AND METHODS

Study Population

In this retrospective study, we evaluated patients with HR-positive HER2-negative metastatic breast cancer who were followed up at the Department of Medical Oncology of Necmettin Erbakan University Faculty of Medicine between June 2020 and June 2023 and received CDK4/6 inhibitors (ribociclib or palbociclib) and letrozole as first-line treatment. Age at diagnosis, presence of comorbidities, Eastern Cooperative Oncology Group (ECOG) performance status, pathological subtype, tumor grade, and sites of metastasis were recorded. Patients under 18 years of age, HR-positive, HER2-positive patients, and patients receiving chemotherapy before CDK4/6 inhibitor therapy were excluded. However, patients were excluded if they met any of the following criteria: recent blood transfusions (within the last month); hematologic disorders; inflammatory or autoimmune diseases; bone marrow metastases; or use of medications that could affect the blood values related to these indices.

Variable Measurement and Definition

PNI was calculated as $[10 \times \text{serum albumin (g/dL)}] + [\text{total lymphocyte count} (\times 10^9/\text{L}) \times 5]$ and SII as $\text{neutrophils} \times \text{platelets/lymphocytes}$, both from peripheral blood results. PNI and SII were calculated 1 week before starting letrozole with a CDK4/6 inhibitor and at 3 and 6 months after treatment. At 3 and 6 months, two groups were defined based on changes in PNI and SII relative to pretreatment values: a group with increasing PNI and SII and a group with decreasing PNI and SII.

PFS was defined as the time to progression while receiving a CDK4/6 inhibitor or to death occurring during treatment. In patients without progression, PFS was calculated as the time to data entry.

This study approved by the Necmettin Erbakan University, Non-Drug and Non-Medical Device Research Ethics Committee (approval number: 2024/4787, date: 02.02.2024) and due to the retrospective nature of the study, the requirement for informed consent was waived.

Statistical Analysis

Clinical, pathologic, and demographic characteristics and blood sample results were obtained from the patient files and the hospital database. Since our study was characterized as retrospective and cross-sectional, no sample size calculation was required. Chi-square and Fisher’s exact tests were used for descriptive statistics. For measurable evaluations, Student’s t-test was performed for variables with a normal distribution, with results reported as mean and standard deviation (SD), and the Mann-Whitney U test was performed for variables with a non-normal distribution, with results reported as the median. Non-parametric tests: two or more related samples were used for dependent statistics with more than two variables. A Kaplan-Meier survival curve was used to estimate survival, and the log-rank test was used to compare survival times. All variables affecting prognosis and PFS were evaluated first by univariate and then by multivariate Cox regression analyses. The chi-squared test or Fisher’s exact test was used to assess the association between categorical variables. All statistical analyses were performed using IBM SPSS Statistics 22.0 (Armonk, New York,United States, USA). Two-sided p-values <0.05 were considered statistically significant.

It was seen that the accepted limit value for PNI was taken different cut-off values by receiver operating curve analysis in the various studies. Although the generally accepted limit for SII was not clearly defined in the studies, different cut-off values were determined using receiver operating characteristic (ROC) curve analysis according to each study’s design. Considering that this approach is not sufficient as a prognostic criterion for treatment follow-up, instead of determining a cut-off point, we compared changes in the patients’ indices at the 3rd and 6th months with those at the beginning of treatment and categorized the changes as increasing or decreasing. Survival analyses were performed for these two groups.

RESULTS

A total of 88 patients who received letrozole with a CDK4/6 inhibitor in the first-line setting were women. Sixty-six (75%) of the patients were receiving ribociclib and twenty-two (25%) were receiving palbociclib. The mean age of all patients was 57.57 years(SD=14.1). The most common pathology was invasive ductal carcinoma (n=77; 87.5%).

Grade 2 tumors were the most common (39 patients; 44.3%), and the median value of Ki-67 was 20 (10-40). The mean body mass index (BMI) was 28.2 (SD=6.85), and 39 (44.3%) of the patients had at least one comorbidity. HER2 low status was observed in 51.1% of cases, and the two most common synchronous metastatic sites were bone (67; 76.1%) and lung

(31; 35.2%). Nineteen (21.4%) patients died during follow-up (Table 1).

Tables 2 and 3 show the changes in PNI and SII, respectively. As shown in Figure 1, the median PFS was 28.2 months [95% confidence interval (CI): 23.6-32.89] in patients with a positive change in PNI at 3 months, compared with 23.3 months (95% CI: 15.55-31.21) in those with a negative change in PNI. This difference was statistically significant (p=0.048). In patients receiving ribociclib, PFS was 33.4 months (95% CI: 27.9-39) in the group with a positive PNI change and 24.7 months (95% CI: 14.9-34.5) in the group with a negative PNI change between baseline and month 3 (p=0.035). In patients receiving palbociclib, PFS was 21.1 months (95% CI: 15-27.1) in the positively changed group and 14 months (95% CI: 8.7-19.2) in the negatively changed group; however, the difference was not statistically significant because the curves crossed early.

As shown in Figure 2, PFS was 29.1 months (95% CI: 24.2-33.9) in patients with a positive change in PNI at 6 months compared with baseline, and 23.2 months (95% CI: 15.9-30.6)

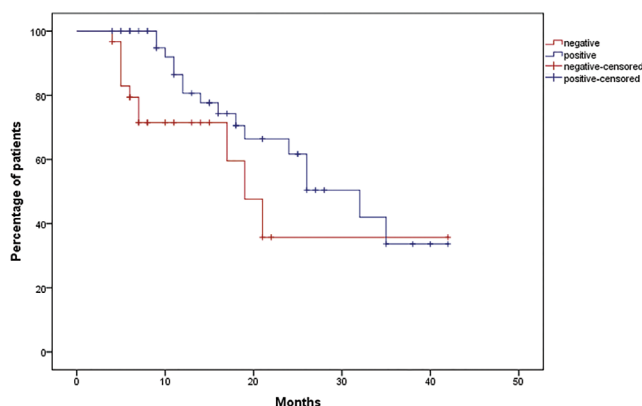
TABLE 1: Demographic, clinical, and pathologic characteristics.	
All patients	n=88
Age	57.57 (SD=14.1)
Age at diagnosis	54.88 (SD=15.6)
ECOG-PS- no. (%)	
0	53 (60)
1	24 (27)
2	11 (13)
At least one comorbidity- no. (%)	39 (44.3)
Pathology- no. (%)	
Invasive ductal carcinoma	77 (87.5)
Invasive lobular carcinoma	9 (10.2)
Mixed type (IDC + ILC)	2 (2.3)
Tumor grade- no. (%)	
1	13 (14.8)
2	51 (57.9)
3	24 (27.3)
Metastasis site- no. (%)	
Bone	67 (76.1)
Lung	31 (35.2)
Brain	10 (11.3)
Liver	20 (22.7)
Adrenal gland	4 (4.6)
Death- no. (%)	19 (21.4)
SD: Standard deviation; IDC: Invasive ductal carcinoma; ILC: Invasive lobular carcinoma; ECOG-PS: Eastern Cooperative Oncology Group performance score.	

TABLE 2: Prognostic nutritional index (PNI).

	All patients, n=88
Pre-treatment PNI, median (IQR)	52.2 (46.6-56.3)
3 rd month PNI, median (IQR)	52 (48-55.7)
6 th month PNI, median (IQR)	51.9 (49.1-55.4)
p-value, median (IQR)	0.031
When all patients were considered, the PNI value decreased at 3 and 6 months after the start of treatment; this decrease was statistically significant (p=0.031). IQR: Interquartile range.	

TABLE 3: Systemic immune-inflammatory index (SII).

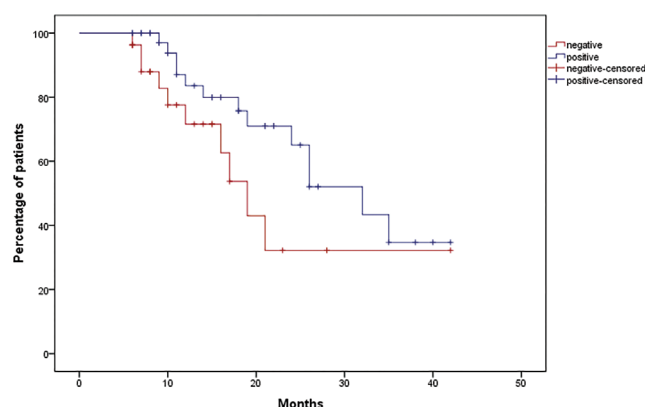
	All patients, n=88
Pre-treatment SII, median (IQR)	595.3 (352.1-1171.1)
3 rd month SII, median (IQR)	261 (166.5-496.9)
6 th month SII, median (IQR)	272.6 (161.5-468.3)
p-value, median (IQR)	<0.001
When all patients were evaluated, a significant decrease in SII was observed after the start of treatment and this was statistically significant (p=0<0.001). IQR: Interquartile range.	

**FIGURE 1: PFS in patients with positive or negative change of PNI at 3 months compared to baseline.**

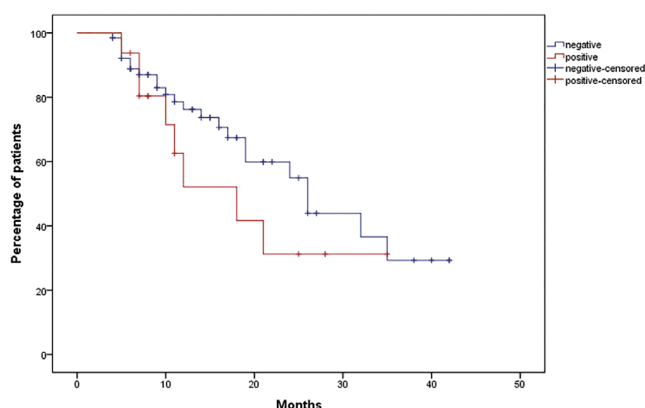
PNI: Prognostic nutritional index; PFS: Progression-free survival

in patients with a negative change; $p=0.091$, which was not statistically significant. In patients receiving ribociclib, PFS was 33.5 (95% CI: 27.5-39.5) months in the group with a positive change in PNI at 6 months and 28.7 (95% CI: 19.7-37.7) months in the group with a negative change; the p value was not statistically significant. In patients receiving palbociclib, PFS was 22.8 (95% CI: 16.1-29.5) months in the group with a positive change in PNI at 6 months, whereas it was 12.4 (95% CI: 7.7-17.2) months in those with a negative change; the difference was statistically significant ($p=0.031$).

As shown in Figure 3, for all patients, PFS was 25.8 (95% CI: 21.4-30.3) months in those with decreasing SII at 3 months compared to baseline, while PFS was 19.3 (95% CI: 12.7-26) months in those with increasing SII. Although PFS

**FIGURE 2: PFS in patients with positive or negative change of PNI at 6 months compared to baseline.**

PNI: Prognostic nutritional index; PFS: Progression-free survival

**FIGURE 3: PFS in patients with positive or negative change of SII at 3 months compared to baseline.**

SII: Systemic immune-inflammation index; PFS: Progression-free survival

was numerically better in patients with decreasing SII, the difference was not statistically significant ($p=0.306$). Figure 3 shows that the curves crossed each other early. Among patients receiving palbociclib, PFS was 21.7 (95% CI: 15.6-27.9) months for those with decreasing SII at 3 months and 10.7 (95% CI: 5.6-15.8) months for those with increasing SII ($p=0.023$). In patients receiving ribociclib, PFS was 23.4 (95% CI: 15-31.7) in patients with increasing SII and 28.6 (95% CI: 22.9-34.3) in patients with decreasing SII at 3 months; the difference was not statistically significant.

As shown in Figure 4, among all patients, PFS was 14.8 months (95% CI: 10.5-19.1) in patients with increased SII at 6 months, compared with 27.7 months (95% CI: 23.4-32.1) in patients with decreased SII; the difference was statistically significant ($p=0.017$). In patients receiving palbociclib, at month 6, PFS was 9 months (95% CI: 5-12.9) in those with increasing SII and 20.8 months (95% CI: 15-26.5) in those with decreasing SII ($p=0.028$). This was not statistically significant for ribociclib,

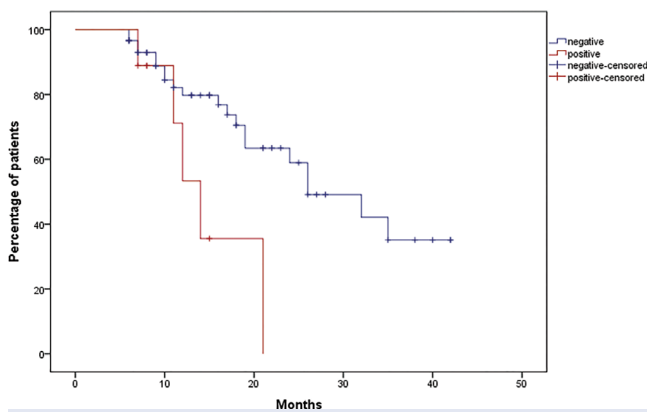


FIGURE 4: PFS in patients with positive or negative change of SII at 6 months compared to baseline.

SII: Systemic immune-inflammation index; PFS: Progression-free survival

although numerically PFS was higher in patients with decreasing SII [32.3 months (95% CI: 27-37.5) vs. 17 months (95% CI: 12.1-21.8); $p=0.065$].

Factors affecting prognosis were evaluated first by univariate and then by multivariate Cox regression analyses. A model was created including the following variables: age at diagnosis; ECOG 0 performance status; presence of comorbidity; presence of invasive ductal carcinoma; presence of bone, lung, and liver metastases; PNI 0-3 months difference; PNI 0-6 months difference; SII 0-3 months difference; and SII 0-6 months difference. Only a decrease in SII between 0-6 months was an independent risk factor for PFS, with a hazard ratio of 0.24 (95% CI: 0.06-0.87; $p=0.031$). Correlation was analyzed for the change in SII from 0 to 6 months, which was the only independent variable in terms of PFS. The correlations were 0.385 ($p<0.001$) for SII 0-3 months change, -0.082 ($p=0.404$) for PNI 0-3 months change, and -0.095 ($p=0.323$) for PNI 0-6 months change.

DISCUSSION

In this study, patients with HR-positive and HER2-negative metastatic breast cancer who received letrozole in combination with a CDK4/6 inhibitor had better PFS and showed increased PNI and decreased SII during follow-up. This is the first study in the literature to examine both SII and PNI at specific time points and to evaluate their effects on PFS in this patient group.

According to the World Cancer Research Fund/American Institute for Cancer Research recommendations, maintaining a healthy body weight, being physically active, eating a diet high in fiber and limiting fat intake (saturated fatty acids if possible) can improve OS after a breast cancer diagnosis.¹⁵ In studies, both low BMI and the amount of weight loss independently predicted poorer OS. There is a large body

of evidence supporting the clinical relevance of nutritional intervention in patients with cancer, which aims to ensure adequate energy and nutrient intake during anticancer treatment, which can lead to improved response to treatment and reduce the side effects of these therapies.¹⁶ Malnutrition decreases survival and worsens tumor prognosis; albumin levels are known to decrease in malnutrition. Low albumin levels in cancer patients are not only associated with malnutrition but also with inhibition of albumin synthesis due to cancer-related systemic inflammation and increased albumin turnover by tumors.¹⁷ Lymphopenia also affects prognosis in patients with metastatic cancer. The reasons for this include destruction of lymphocytes stimulated by tumor cells with proapoptotic ligands, alteration in T-cell receptor (TCR) expression and consequent decrease in the capacity of lymphocytes to respond to TCR stimulation, increase in Tregs (regulatory T-cells) that play a suppressive role in the immune system and activation-induced cell death.¹⁸ Neutrophils in the tumor microenvironment, which are involved in systemic inflammation, have also been shown to increase tumor cell proliferation, stimulate angiogenesis, contribute to deoxyribonucleic acid damage, and be associated with metastasis.¹⁹ Several mechanisms have been proposed to explain the relationship between high platelet counts and cancer, including the recruitment of cancer cells by platelets, increased extravasation or permeability of the basement membrane, and protection of cancer cells from the immune response in the bloodstream.²⁰ Considering all these biological processes, it is clear why changes in both PNI and SII can predict the behavior of tumor cells and response to therapy.

The effects of inflammation on carcinogenesis may be due to continuous and uncontrolled stimulation of the cell cycle, resulting in increased stem cell divisions and local mutagenic effects. Mutagenesis may occur as a by-product of the inflammatory agent itself, as a by-product of increased metabolism, or as a result of increased production of reactive oxygen radicals produced by cells involved in the inflammatory response.⁵ In particular, monitoring the primary immune response and alterations in systemic immune status can influence both response to treatment and prognosis in patients with breast cancer.²¹ One of the best and simplest indicators of this is the SII, which is derived solely from blood test results. It has been studied many times before to see if it can predict prognosis in chronic non-malignant diseases.^{22,23} Recently, it has also been examined in solid tumors for which surgery is a treatment option. In breast cancer, limited studies have been conducted in the literature and high SII has been an indicator of poor prognosis in these studies.^{24,25} In these studies, different SII cut-off points were used. Accordingly, patients were divided into two groups and were evaluated.

We found it appropriate to evaluate SII by grouping subjects according to whether SII increased or decreased from the start of treatment to follow-up, as was done for PNI.

PNI has long been used in patients undergoing gastrointestinal surgery because of its predictive and prognostic significance in cancer patients. It has been reported that surgery can be performed in patients with normal or low normal PNI values, and surgery should be avoided in very low patients because it will not contribute to life.¹¹ In the early 2000s, it was again used as a prognostic marker during the perioperative period for colon and esophageal cancers.^{26,27} In the last few years, several studies have shown that PNI in breast cancer predicts prognosis. With the advantages of being convenient, non-invasive and reproducible, PNI pretreatment results were reported to be a useful prognostic indicator for locally advanced breast cancer patients receiving neoadjuvant chemotherapy and before surgery, and a promising biomarker for breast cancer in treatment strategy decisions.^{28,29}

In a previous study that demonstrated that PNI serves as a prognostic marker for the use of CDK4/6 inhibitors in HR-positive and HER2-negative breast cancer and that showed a survival benefit in the high PNI group, a cut-off value of 50.4 for PNI was adopted.³⁰ Most studies used different values in ROC curve analysis depending on the study design. In our study, instead of using this, we grouped the patients according to whether PNI increased or decreased during follow-up. Our aim was, first, to eliminate the false cut-off value that may be population-related and therefore not generalizable, and second, to reduce the possibility that patients with PNI below the cut-off point at baseline would remain below the cut-off point even if their values increased during follow-up. For all these reasons, this study is the first to examine changes in indices at 0-6 months and the correlation between PNI and SII in patients using CDK4/6 inhibitors. We aimed to determine whether the change at 3 months was persistent and whether the negative correlation between PNI and SII persisted. Another key difference is that all patients had newly diagnosed metastases and received CDK4/6 therapy at the primary care level. Chemotherapy or radiation therapy should not have affected blood parameters as measured by both PNI and SII. Studies showing that a decrease in PNI reduces survival have included patients from all treatment lines.³¹

In a recent study in patients with acute respiratory distress syndrome, ribociclib was shown to reduce neutrophilic inflammation with its PDE4 inhibitor effect and may be offered as a new treatment option in this group of patients.³² In another study, palbociclib was given to lupus-prone

mice, and the inflammatory response was examined, and it was shown that disease-related inflammatory effects were reduced with palbociclib, albeit differently in both genders.³³ Palbociclib, by suppressing immunoglobulin E-mediated mast cell activation both *in vitro* and *in vivo*, which it does through inhibition of mast cell degranulation, has been considered for development as a treatment for mast cell-mediated allergic diseases.³⁴ The anti-inflammatory activity of CDK4/6 inhibitors was also observed in our study, with a significant decrease in SII in the third and sixth months of treatment. Despite CDK4/6 inhibitor treatment, PFS is significantly lower in patients with increased SII during follow-up. A rapid increase in SII during the third month may necessitate earlier radiological evaluation or closer clinical monitoring before symptomatic progression occurs; therefore, caution is warranted.

Study Limitations

Our study has several limitations, including its retrospective design, the lack of a definitive cut-off value for SII and PNI, and the short median follow-up time for both PFS and OS; therefore, the results should be interpreted in light of these limitations. The relatively small number of patients was also a limitation. The p-values for changes in both PNI and SII in CDK4/6 inhibitor users (as a whole) and in patients treated with ribociclib and palbociclib are given separately. Our aim here was to demonstrate numerically that the trends among treatments were similar, even though we acknowledged that the sample was small. The results should be confirmed in larger sample sizes. Prospective studies with larger patient cohorts are needed in which these indices are calculated at regular intervals and the impact of changes on PFS is analyzed over the long term. However, this is the first study to analyze the combination of a CDK4/6 inhibitor and letrozole as first-line treatment and to calculate index changes at 3 and 6 months.

CONCLUSION

PNI is also important, as it indicates poor survival in patients with declining PNI during follow-up. SII, which does not require pathologic or genetic examination and is measured using routine blood parameters during follow-up may be a inexpensive and easily accessible prognostic indicator in metastatic hormone-positive and HER2-negative patients receiving first-line treatment with letrozole and a CDK4/6 inhibitor.

Ethics

Ethics Committee Approval: This study approved by the Necmettin Erbakan University, Non-Drug and Non-Medical Device Research Ethics Committee (approval number: 2024/4787, date: 02.02.2024).

Informed Consent: Due to the retrospective nature of the study, the requirement for informed consent was waived.

Footnotes

Authorship Contributions

Concept: A.O., Me.A., Design: A.O., Me.A., Data Collection or Processing: A.O., B.K., M.K.E., M.A., Me.A., Analysis or Interpretation: A.O., Me.A., Literature Search: A.O., Me.A., Writing: A.O., B.K., M.K.E., M.A., Me.A.

Conflict of Interest: Murat Araz Prof. MD is Associate Editor in Journal of Oncological Sciences. They had no involvement in the peer-review of this article and had no access to information regarding its peer-review.

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The Prognostic Value of Pretreatment Neutrophil-to-Lymphocyte Ratio, Platelet-to-Lymphocyte Ratio, and Systemic Immune-inflammation Index on Survival Outcomes in Non-metastatic Breast Cancer: A Retrospective Cohort Study

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ABSTRACT

Objective: Systemic inflammatory response contributes to tumor progression and may influence survival outcomes in breast cancer. Blood-based inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII), have been proposed as prognostic biomarkers. This study evaluated the prognostic value of pretreatment NLR, PLR, and SII across molecular subtypes of breast cancer.

Material and Methods: This retrospective cohort study included 190 patients with invasive breast cancer who were treated between February 2012 and April 2022. Pretreatment NLR, PLR, and SII were calculated from complete blood counts. Optimal cut-off values were determined using a 60 months landmark receiver operating characteristic analysis. Overall survival (OS) and disease-free survival (DFS) were analyzed using the Kaplan-Meier method, the log-rank test, and Cox regression. Subgroup analyses were performed according to molecular subtype.

Results: After a median follow-up of 58.8 months, 30 deaths (15.8%) and 44 DFS events (23.2%) occurred. Optimal cut-offs were 1.78 for NLR, 138.08 for PLR, and 449.55 for SII. In the overall cohort, elevated NLR was associated with numerically worse OS (hazard ratio [HR]=2.05; $p=0.096$) and DFS (HR: 1.78; $p=0.088$), although these associations did not reach statistical significance. In the triple-negative breast cancer subgroup, high NLR was associated with poorer OS (log-rank $p=0.019$) and DFS (log-rank $p=0.025$); however, ridge-penalized Cox estimates were imprecise [OS: HR=1.52, 95% confidence interval (CI)=0.42-5.57; DFS: HR=1.75, 95% CI=0.50-6.11]. Multivariate analysis identified Ki-67 $\geq 20\%$ and lymph node positivity as independent predictors of OS, while progesterone receptor positivity, Ki-67 $\geq 20\%$, and Stage III independently predicted DFS.

Conclusion: NLR was not independently prognostic in the overall cohort. Its association with survival outcomes in triple-negative breast cancer is preliminary and hypothesis-generating and requires validation in larger independent cohorts.

Keywords: Breast neoplasms; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; systemic immune-inflammation index; prognosis; triple-negative breast neoplasms

INTRODUCTION

Breast cancer remains the most commonly diagnosed malignancy and a leading cause of cancer-related mortality among women worldwide, accounting for approximately

2.3 million new cases and 666,000 deaths annually.¹ Despite significant advances in early detection, surgical techniques, and systemic therapies, considerable heterogeneity exists in clinical outcomes, even among patients with similar

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clinicopathological characteristics.² This underscores the critical need for readily accessible, cost-effective biomarkers that can refine prognostic stratification and guide therapeutic decision-making.

Over the past two decades, a growing body of evidence has established the tumor microenvironment and systemic inflammatory response as fundamental hallmarks of cancer.³ Chronic inflammation contributes to tumorigenesis, promotes angiogenesis, facilitates immune evasion, and enhances metastatic potential through a complex interplay of cytokines, chemokines, and immune cells.^{4,5} In breast cancer specifically, the inflammatory milieu has been linked to disease progression, treatment resistance, and poor survival outcomes.⁶

Peripheral blood-based inflammatory indices have attracted considerable attention as surrogate markers of the host systemic inflammatory response. The neutrophil-to-lymphocyte ratio (NLR), defined as the absolute neutrophil count divided by the absolute lymphocyte count, reflects the balance between pro-tumor neutrophil-mediated inflammation and anti-tumor lymphocyte-mediated immune surveillance.⁷ Elevated NLR has been associated with adverse outcomes in multiple solid tumors, including colorectal, lung, gastric, and hepatocellular carcinomas.^{8,9} In breast cancer, several meta-analyses have confirmed the prognostic relevance of NLR, although considerable heterogeneity exists regarding optimal cut-off values and the consistency of results across molecular subtypes.^{10,11}

The platelet-to-lymphocyte ratio (PLR) is another composite hematological index that integrates the protumorigenic role of platelets—which facilitate tumor cell dissemination through promotion of epithelial-mesenchymal transition and shielding of circulating tumor cells from immune clearance—with lymphocyte-mediated antitumor immunity.^{12,13} Elevated PLR has been reported as a negative prognostic factor in breast cancer, though its independent predictive value remains debated.¹⁴

More recently, the systemic immune-inflammation index (SII), calculated as $SII = \text{platelet count} \times \text{neutrophil count} / \text{lymphocyte count}$, has been proposed as a more comprehensive marker that simultaneously captures three key components of the immune-inflammatory axis.¹⁵ Initial studies in breast cancer have yielded promising but inconsistent results, with some demonstrating independent prognostic significance and others reporting only marginal associations.^{16,17}

Breast cancer is a biologically heterogeneous disease comprising distinct molecular subtypes—luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-

enriched, and triple-negative—each with unique biological behavior, therapeutic responsiveness, and prognosis.^{18,19} Triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression, remains the most clinically challenging subtype due to its aggressive behavior, limited targeted therapy options, and high relapse rates.²⁰ The role of systemic inflammation may be particularly relevant in TNBC, given the established immunogenicity and higher tumor-infiltrating lymphocyte content of this subtype.^{21,22} However, subtype-specific analyses of inflammatory markers remain scarce and warrant further investigation.

The aim of this study was to evaluate, in a cohort of breast cancer patients, the prognostic significance of pretreatment NLR, PLR, and SII for overall survival (OS) and disease-free survival (DFS), with specific emphasis on analyses stratified by molecular subtype. We hypothesized that inflammatory markers may exert differential prognostic effects across molecular subtypes, potentially carrying the greatest clinical utility in biologically aggressive subtypes such as TNBC.

MATERIAL AND METHODS

Study Design and Patient Selection

This retrospective cohort study was conducted at a single tertiary oncology center. The medical records of consecutive patients with histologically confirmed invasive breast cancer diagnosed between February 2012 and April 2022 were retrospectively reviewed. Patients were followed through November 2023, using hospital records and data from the national death registry. The data cut-off date for survival analyses was November 30, 2023. Of the 192 patients with available pretreatment complete blood count (CBC) data, two were subsequently excluded due to incomplete follow-up information, yielding a final analytic cohort of 190 patients. The study protocol was approved by the Necmettin Erbakan University Non-Drug and Non-Medical Device Research Ethics Committee (approval number: 2023/4610, date: 3.11.2023), in accordance with the Declaration of Helsinki, and the requirement for informed consent was waived due to the retrospective nature of the study.

Inclusion criteria were: (1) histologically confirmed invasive breast carcinoma; (2) availability of pretreatment CBC obtained within 7 days prior to the initiation of any anti-cancer treatment; (3) substantially complete clinicopathological and follow-up data, with isolated missing values for individual covariates permitted; and (4) no evidence of distant metastasis at initial diagnosis. Exclusion criteria included: (1) metastatic disease (Stage IV) at presentation; (2) concurrent hematological disorders; (3) active infection or systemic inflammatory conditions at the time of blood sampling; (4)

chronic use of corticosteroids or immunosuppressive agents; (5) prior history of another malignancy; and (6) incomplete medical records. After applying these criteria, 190 patients were included in the final analysis.

Data Collection

The following data were extracted from medical records: demographic characteristics [age, menopausal status, body mass index (BMI)]; histopathological features (histological type, grade, ER status, PR status, HER2 status, Ki-67 proliferation index); staging parameters [tumor size, lymph node status, TNM stage according to the American Joint Committee on Cancer (AJCC) 8th edition]; lymphovascular invasion (LVI); perineural invasion (PNI); treatment modalities (surgery, chemotherapy, radiotherapy, and/or hormonal therapy as clinically indicated, including neoadjuvant chemotherapy where applicable); and survival outcomes. For patients who received neoadjuvant chemotherapy, disease stage was recorded as the pre-treatment clinical stage according to the AJCC 8th edition; for patients who underwent primary surgery, disease stage was recorded using pathological staging.

Molecular subtype classification was performed based on immunohistochemical surrogates according to the St. Gallen International Expert Consensus:²³ luminal A (ER⁺ and/or PR⁺, HER2⁻, Ki-67 < 20%), luminal B (ER⁺ and/or PR⁺, HER2⁻ with Ki-67 ≥ 20%, or ER⁺ and/or PR⁺, HER2⁺), HER2-enriched (ER⁻, PR⁻, HER2⁺), and triple-negative (ER⁻, PR⁻, HER2⁻).

Inflammatory Marker Definitions

Pretreatment CBC parameters—including absolute neutrophil count, absolute lymphocyte count, and platelet count—were obtained from venous blood samples collected within 7 days prior to any anti-cancer treatment (surgery or neoadjuvant chemotherapy). The following inflammatory indices were calculated:

$NLR = \text{Absolute neutrophil count} / \text{absolute lymphocyte count}$

$PLR = \text{Platelet count} / \text{absolute lymphocyte count}$

$SII = \text{Platelet count} \times \text{neutrophil count} / \text{absolute lymphocyte count}$

The lymphocyte-to-monocyte ratio (LMR) could not be evaluated because monocyte counts were not routinely documented in the institutional records during the study period.

Survival Endpoints

The primary endpoints were OS and DFS. OS was defined as the time interval from the date of histological diagnosis to the date of death from any cause or to the date of last follow-up.

DFS was defined as the time from the date of diagnosis to the first documented locoregional recurrence, distant metastasis, or death from any cause. Patients who were alive and event-free at the last follow-up were censored on that date. The data cut-off date was November 30, 2023.

Statistical Analysis

Descriptive statistics were presented as the median with interquartile range (IQR) for continuous variables with a non-normal distribution, and as frequencies (percentages) for categorical variables. Optimal cut-off values for NLR, PLR, and SII were determined using 60 months landmark receiver operating characteristic (ROC) curve analysis with the Youden index ($J = \text{sensitivity} + \text{specificity} - 1$). The area under the curve (AUC) was calculated to assess discriminatory capacity. Specifically, a time-dependent ROC analysis using a 60 months (5 years) landmark time point was performed to define the binary outcome for cut-off determination: patients who experienced the event (death for OS; recurrence, metastasis, or death for DFS) before 60 months were classified as event-positive, whereas patients who remained alive and event-free at 60 months were classified as event-negative. Patients who were censored before the 60 months landmark (i.e., lost to follow-up without experiencing an event) were excluded from the ROC analysis because their 60 months survival status could not be determined. A landmark approach was chosen because it converts the time-to-event outcome into a well-defined binary status at a clinically meaningful horizon (5 years survival), allowing the application of conventional ROC methodology and the Youden index. Under this rule, 114 of the 190 patients contributed to the OS landmark ROC analysis (23 who died within 60 months and 91 known to be alive at the 60 months landmark), and 116 patients contributed to the DFS landmark ROC analysis (42 with a DFS event within 60 months and 74 known to be event-free at the landmark); the same patients contributed to all three marker analyses because NLR, PLR, and SII were available for the entire cohort. The cut-off values obtained in this landmark subset (NLR=1.78; PLR=138.08; SII=449.55) were subsequently applied unchanged to the complete cohort of 190 patients, and all Kaplan-Meier, log-rank, and Cox analyses reported below were performed.

Associations between dichotomized inflammatory markers and clinicopathological features were evaluated using the chi-square test or Fisher's exact test, as appropriate. Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test. Univariate and multivariate Cox proportional hazards regression analyses were performed to identify prognostic factors; variables with $p < 0.25$ in univariate analysis were entered into the multivariate model.

Specifically, multivariable Cox proportional hazards models were fitted using L2 (Ridge) penalization to improve the stability of coefficient estimates, given the relatively limited number of events; a fixed penalty of $\lambda=0.01$ was specified a priori (lifelines, CoxPHFitter, penalizer=0.01) and not tuned on the data. The hazard ratios (HRs), 95% confidence intervals (CIs) and p-values reported in Table 1 are the Wald-type quantities returned by this penalized fit: coefficients were obtained by maximizing the penalized log partial likelihood, standard errors were taken from the inverse of the negative Hessian at the penalized solution, confidence limits were calculated as coefficient $\pm 1.96 \times$ standard error and then exponentiated, and p-values were derived from the corresponding Wald z statistics. Because these standard errors are conditional on λ and are not adjusted for penalization, the intervals in Table 1 are reported as measures of the estimates' precision rather than intervals with exact nominal coverage. For the TNBC subgroup, in which the observed events were confined to the high-NLR group, the same implementation was used with a larger penalty (lifelines, CoxPHFitter, penalizer=0.5) to obtain finite parameter estimates under near-complete separation. This is an L2 (Ridge) penalty rather than Firth's penalized partial likelihood, which lifelines does not implement. Subgroup analyses were performed by molecular subtype. All statistical tests were two-sided; $p < 0.05$ was considered statistically significant. Statistical analyses were performed using Python (version 3.11) with the following packages: lifelines (v0.30.3), scikit-learn (v1.9.0), scipy (v1.14.1), pandas (v2.2.3), numpy (v1.26.4), and matplotlib (v3.9.2). This study was conducted and reported in accordance with the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines where applicable.²⁴

A complete-case analysis was employed; patients with missing values for specific covariates were excluded from the corresponding multivariable models.

In addition to the dichotomized analysis using ROC-derived cutoffs, inflammatory markers (NLR, PLR, SII) were also analyzed as continuous variables using Cox proportional hazards regression. HRs were reported both per unit increase and, after z-score standardization [mean=0, standard deviation (SD)=1], per one SD increase, to facilitate comparison across markers with different scales. Multivariate models were adjusted for patient age (continuous), Stage III disease, and Ki-67 status ($\geq 20\%$). To address the potential optimism bias inherent in ROC-derived cut-offs obtained from the same dataset, internal bootstrap validation was performed with 1000 resamples. In each iteration, the complete analytic pipeline was repeated within the resample: the 60 months landmark ROC analysis was re-run, a new optimal Youden cut-off was derived, the marker was dichotomized at that bootstrap-derived cut-off, and the Kaplan-Meier (log-rank) and Cox proportional hazards analyses were repeated. The bootstrap-derived cutoff, HR, and log-rank p-value were recorded at each iteration and summarized as medians with 2.5th-97.5th percentile intervals, together with the proportion of resamples yielding both a HR greater than 1 and a log-rank p-value below 0.05 (Supplementary Table S1).

RESULTS

Patient Characteristics

A total of 190 patients with non-metastatic invasive breast cancer who met the inclusion criteria were analyzed.

TABLE 1: Multivariate Cox proportional hazards regression analysis for OS and DFS.

Variable	OS model			DFS model		
	HR	95% CI	p-value	HR	95% CI	p-value
Ki-67 $\geq 20\%$	3.20	1.31-7.80	0.011	2.52	1.26-5.07	0.009
Lymph node positive ^a	3.09	1.07-8.93	0.037	—	—	—
Stage III disease	2.08	0.97-4.47	0.059	2.27	1.26-4.09	0.007
Neoadjuvant CT ^a	2.35	0.96-5.73	0.060	—	—	—
PR positive	0.41	0.14-1.16	0.094	0.33	0.15-0.76	0.009
High NLR (≥ 1.78)	1.92	0.83-4.46	0.128	1.76	0.88-3.54	0.113
ER positive	0.65	0.23-1.84	0.418	0.94	0.41-2.15	0.880
Grade 3 histology	0.90	0.40-2.03	0.802	1.22	0.63-2.35	0.559
High PLR (≥ 138.08) ^a	—	—	—	1.10	0.59-2.08	0.761

Variables with $p < 0.25$ in univariate analysis were entered into the multivariate model. Bold values indicate statistical significance ($p < 0.05$).

^a: Lymph node positivity and neoadjuvant chemotherapy were entered into the OS model only, and high PLR was entered into the DFS model only; each met the $p < 0.25$ threshold for one endpoint but not the other.

—: Not included in model. Complete-case analysis: OS model $n=183$ (30 deaths); DFS model $n=184$ (44 events). Models were fitted with L2 (Ridge) penalization ($\lambda=0.01$; lifelines, CoxPHFitter, penalizer=0.01). HRs are exponentiated penalized coefficients; 95% CIs and p-values are Wald-type quantities calculated from the standard errors of the same penalized fit (coefficient $\pm 1.96 \times$ standard error, exponentiated) and are therefore conditional on λ .

HR: Hazard ratio; CI: Confidence interval; CT: Chemotherapy; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; ER: Estrogen receptor; PR: Progesterone receptor OS: Overall survival; DFS: Disease-free survival.

TABLE 2: Baseline demographic, clinicopathological, and laboratory characteristics of the study cohort (n=190).

Variable	Value
Demographics	
Age (years), median (range)	51 (26-76)
<50 years	92 (48.4%)
≥50 years	98 (51.6%)
Menopausal status	
Premenopausal	93 (50.5%)
Postmenopausal	91 (49.5%)
BMI (kg/m ²), median (range)	29.4 (18.8-46.4)
Normal (<25)	38 (20.0%)
Overweight (25-29.9)	66 (34.7%)
Obese class I (30-34.9)	51 (26.8%)
Obese class II (35-39.9)	27 (14.2%)
Obese class III (≥40)	8 (4.2%)
Histopathological features	
Histological type	
Invasive ductal carcinoma	145 (78.0%)
Invasive lobular carcinoma	7 (3.8%)
Other	34 (18.3%)
Histological grade	
Grade 1	14 (7.8%)
Grade 2	116 (64.4%)
Grade 3	50 (27.8%)
Receptor status	
ER positive	131 (71.2%)
PR positive	114 (62.0%)
HER2 positive	40 (21.7%)
Ki-67 index (%), median (range)	20 (0-95)
Ki-67 <20%	80 (43.5%)
Ki-67 ≥20%	104 (56.5%)
Molecular subtype	
Luminal A	52 (28.3%)
Luminal B	81 (44.0%)
HER2-enriched	10 (5.4%)
Triple-negative (TNBC)	41 (22.3%)
Disease staging	
Stage I	25 (14.0%)
Stage IIA	45 (25.1%)
Stage IIB	50 (27.9%)
Stage IIIA	41 (22.9%)
Stage IIIB	4 (2.2%)
Stage IIIC	14 (7.8%)
Lymph node positive	125 (66.1%)
LVI positive	104 (55.9%)
PNI positive	70 (37.6%)

TABLE 2: Continued

Variable	Value
Treatment	
Neoadjuvant chemotherapy	46 (24.3%)
Inflammatory markers	
NLR, median (IQR)	2.06 (1.52-2.55)
PLR, median (IQR)	132.86 (104.68-169.40)
SII, median (IQR)	589.24 (449.66-811.76)
Survival outcomes	
Median follow-up (months)	58.8
Deaths (OS events)	30 (15.8%)
DFS events	44 (23.2%)

Data are presented as n (%) for categorical variables and as median (range) or median (IQR) for continuous variables. Percentages are calculated using the number of patients with an evaluable value for the variable concerned as the denominator (valid-case analysis). The same rule applies to Table 2, Table 3, and Section Patient Characteristics, and no imputation was performed. Evaluable patients per variable (missing): menopausal status, 184 (6); histological type, 186 (4); histological grade, 180 (10); ER/PR/HER2/Ki-67 status, 184 (6); molecular subtype, 184 (6); pathological stage, 179 (11); lymph node status, 189 (1); LVI/PNI, 186 (4); neoadjuvant chemotherapy, 189 (1). All remaining variables were evaluable in all 190 patients. Records that did not contain an interpretable value for a given variable were counted as missing for that variable only and were retained in the cohort for every other analysis. Of the 192 patients with available pretreatment CBC data, two were excluded due to incomplete follow-up information. Therefore, the final analytic cohort consisted of 190 patients, and all analyses were performed using this cohort. Molecular subtypes were classified based on IHC surrogates, in accordance with the St. Gallen International Expert Consensus.

BMI: Body mass index; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; TNBC: Triple-negative breast cancer; LVI: Lymphovascular invasion; PNI: Perineural invasion; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; IQR: Interquartile range; OS: Overall survival; DFS: Disease-free survival; IHC: Immunohistochemical; CBC: Complete blood count.

The baseline demographic, clinicopathological, and laboratory characteristics of the study cohort are summarized in Table 2. Unless otherwise stated, percentages are calculated using the number of patients with an evaluable value for the variable concerned as the denominator; the number of evaluable patients for each variable is listed in the footnote to Table 2; the same rule applies to Table 3.

The median age at diagnosis was 51 years (range: 26-76 years), with 98 patients (51.6%) aged ≥50 years. Menopausal status was almost equally distributed (premenopausal: 50.5%; postmenopausal: 49.5%). The median BMI was 29.4 kg/m² (range: 18.8-46.4), indicating that the study population was predominantly overweight or obese. The predominant histological type was invasive ductal carcinoma (78.0%). The majority of tumors were Grade 2 (64.4%). ER and PR positivity were detected in 71.2% and 62.0% of cases, respectively. HER2 overexpression was present in 21.7% of tumors. The median Ki-67 index was 20% (range: 0-95%), with 56.5% of patients having Ki-67 ≥20%.

TABLE 3: Association between dichotomized inflammatory markers and clinicopathological features.

Clinical feature	NLR high (≥1.78) n=121	NLR low (<1.78) n=69	p-value	PLR high (≥138.08) n=92	PLR low (<138.08) n=98	p-value	SII high (≥449.55) n=143	SII low (<449.55) n=47	p-value
Age ≥50 years	55/121 (45.5%)	43/69 (62.3%)	0.037	39/92 (42.4%)	59/98 (60.2%)	0.021	64/143 (44.8%)	34/47 (72.3%)	0.002
Postmenopausal	51/118 (43.2%)	40/66 (60.6%)	—	36/89 (40.4%)	55/95 (57.9%)	—	59/139 (42.4%)	32/45 (71.1%)	—
Grade 3	35/114 (30.7%)	15/66 (22.7%)	0.328	27/86 (31.4%)	23/94 (24.5%)	0.384	42/137 (30.7%)	8/43 (18.6%)	0.179
ER positive	77/118 (65.3%)	54/66 (81.8%)	0.027	59/89 (66.3%)	72/95 (75.8%)	0.208	96/140 (68.6%)	35/44 (79.5%)	0.226
PR positive	68/118 (57.6%)	46/66 (69.7%)	0.145	52/89 (58.4%)	62/95 (65.3%)	0.422	84/140 (60.0%)	30/44 (68.2%)	0.425
HER2 positive	23/118 (19.5%)	17/66 (25.8%)	0.423	22/89 (24.7%)	18/95 (18.9%)	0.441	28/140 (20.0%)	12/44 (27.3%)	0.418
Ki-67 ≥20%	65/117 (55.6%)	39/67 (58.2%)	0.846	52/89 (58.4%)	52/95 (54.7%)	0.722	76/140 (54.3%)	28/44 (63.6%)	0.359
LN positive	77/121 (63.6%)	48/68 (70.6%)	0.418	56/92 (60.9%)	69/97 (71.1%)	0.181	94/143 (65.7%)	31/46 (67.4%)	0.978
LVI positive	66/120 (55.0%)	38/66 (57.6%)	0.854	46/91 (50.5%)	58/95 (61.1%)	0.195	78/142 (54.9%)	26/44 (59.1%)	0.755
PNI positive	43/120 (35.8%)	27/66 (40.9%)	0.599	30/91 (33.0%)	40/95 (42.1%)	0.257	50/142 (35.2%)	20/44 (45.5%)	0.295
Stage III	39/112 (34.8%)	20/67 (29.9%)	0.603	28/84 (33.3%)	31/95 (32.6%)	1.000	48/135 (35.6%)	11/44 (25.0%)	0.267
Neoadjuvant CT	35/120 (29.2%)	11/69 (15.9%)	0.062	28/92 (30.4%)	18/97 (18.6%)	0.083	38/142 (26.8%)	8/47 (17.0%)	0.249

Inflammatory markers were dichotomized using optimal cut-off values derived from a 60 months landmark ROC analysis (NLR=1.78, PLR=138.08, and SII=449.55). All analyses were performed in the final study cohort of 190 patients. P-values were calculated using the chi-square test or Fisher's exact test, as appropriate. Bold p-values indicate statistical significance (p<0.05). Denominators are the numbers of patients with an evaluable value for the corresponding variable within each marker subgroup, applying the same valid-case rule used in Table 2 and Section Patient Characteristics; therefore, subgroup totals differ between rows according to variable-specific data availability. ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; LVI: Lymphovascular invasion; PNI: Perineural invasion; CT: Chemotherapy; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; ROC: Receiver operating characteristic.

Luminal B was the most prevalent molecular subtype (44.0%), followed by luminal A (28.3%), TNBC (22.3%), and HER2-enriched (5.4%). The majority presented with Stage II disease (53.1%), while 33.0% presented with Stage III disease. Lymph node involvement was present in 66.1% of patients. LVI and PNI were observed in 55.9% and 37.6%, respectively. Neoadjuvant chemotherapy was administered to 24.3% of patients. The median values of inflammatory markers were NLR 2.06 (IQR: 1.52-2.55), PLR 132.86 (IQR: 104.68-169.40), and SII 589.24 (IQR: 449.66-811.76). Patients were followed through November 2023. After a median follow-up of 58.8 months, 30 patients (15.8%) died, and 44 DFS events (23.2%) were recorded.

Determination of Optimal Cut-off Values

The 60 months landmark ROC analysis yielded the following optimal cut-off values: NLR=1.78, PLR=138.08, and SII=449.55 (Table 4). The AUC values for 5-year OS prediction were 0.566 (NLR), 0.560 (PLR), and 0.543 (SII). For 5 years DFS prediction, the AUC values were 0.581 (NLR), 0.605 (PLR), and 0.570 (SII).

These modest AUC values suggest limited discriminatory power at the individual level, which is consistent with the established role of inflammatory markers as supplementary rather than stand-alone prognostic tools.

Based on the optimal NLR cut-off of 1.78, 121 patients (63.7%) were classified into the high NLR group (≥1.78) and 69 patients (36.3%) into the low NLR group (<1.78). Using the PLR cut-off of 138.08, 92 patients (48.4%) had high PLR, and 98 patients (51.6%) had low PLR. The SII cut-off of 449.55 categorized 143 patients (75.3%) as high SII and 47 (24.7%) as low SII.

Association Between Inflammatory Markers and Clinicopathological Features

The relationships between dichotomized inflammatory markers and clinicopathological features are presented in Table 3. High NLR (≥1.78) was significantly associated with younger age (<50 years) (p=0.037) and ER negativity (p=0.027). High PLR (≥138.08) was significantly associated with younger age (p=0.021). High SII (≥449.55) was significantly

TABLE 4: ROC analysis results and Kaplan-Meier survival estimates stratified by optimal inflammatory marker cut-offs.

Marker	Cut-off	AUC (OS)	AUC (DFS)	Group	n (events)	5 years OS (%)	5 years DFS (%)	p-value (OS)	p-value (DFS)
NLR	1.78	0.566	0.581	High (≥ 1.78)	121 (23 OS/32 DFS)	79.4	65.9	0.089	0.084
				Low (< 1.78)	69 (7 OS/12 DFS)	93.8	83.1		
PLR	138.08	0.560	0.605	High (≥ 138.08)	92 (16 OS/24 DFS)	79.4	65.4	0.323	0.149
				Low (< 138.08)	98 (14 OS/20 DFS)	89.1	78.1		
SII	449.55	0.543	0.570	High (≥ 449.55)	143 (22 OS/32 DFS)	82.4	71.5	0.871	0.917
				Low (< 449.55)	47 (8 OS/12 DFS)	90.9	75.1		

Optimal cut-off values were determined by 60 months landmark ROC analysis using the Youden index. Five years survival rates were estimated by the Kaplan-Meier method. P-values were calculated using the log-rank test.
AUC: Area under the curve; OS: Overall survival; DFS: Disease-free survival; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; ROC: Receiver operating characteristic.

associated with older age (≥ 50 years) ($p=0.002$). None of the inflammatory markers showed significant associations with tumor grade, lymph node status, disease stage, LVI, or PNI.

Kaplan-Meier Survival Analysis

Kaplan-Meier survival curves stratified by inflammatory marker cut-offs are summarized in Table 4. For OS, patients with high NLR had a 5 years OS rate of 79.4%, compared with 93.8% in the low NLR group (log-rank $p=0.089$). High PLR was associated with a 5 years OS rate of 79.4% vs. 89.1% for low PLR ($p=0.323$). SII showed no meaningful separation between the survival curves (high SII: 82.4% vs. low SII: 90.9%; $p=0.871$). For DFS, patients with high NLR had a 5 years DFS rate of 65.9%, compared with 83.1% in the low NLR group (log-rank $p=0.084$). NLR consistently demonstrated the strongest prognostic signal among the three inflammatory markers (Figures 1 and 2).

Univariate Cox Regression Analysis

The results of univariate Cox proportional hazards regression for OS and DFS are presented in Table 5. For OS, the following variables were identified as significant prognostic factors ($p<0.05$): Ki-67 $\geq 20\%$ (HR: 3.63; 95% CI: 1.48-8.88; $p=0.005$), PR positivity (HR: 0.30; 95% CI: 0.14-0.63; $p=0.002$), ER positivity (HR: 0.33; 95% CI: 0.16-0.67; $p=0.002$), Stage III disease (HR: 2.76; 95% CI: 1.34-5.69; $p=0.006$), lymph node positivity (HR: 2.90; 95% CI: 1.01-8.33; $p=0.048$), and Grade 3 histology (HR: 2.30; 95% CI: 1.08-4.87; $p=0.030$). High NLR was associated with a numerically inferior OS (HR: 2.05; 95% CI: 0.88-4.79; $p=0.096$), although this did not reach statistical significance. For DFS, significant prognostic factors included PR positivity, ER positivity, Ki-67 $\geq 20\%$, Stage III disease, and Grade 3 histology. High NLR was associated with a numerically elevated risk of DFS (HR: 1.78; 95% CI: 0.92-3.46; $p=0.088$), which did not reach statistical significance. PLR and SII did not reach statistical significance for either endpoint (Figure 3).

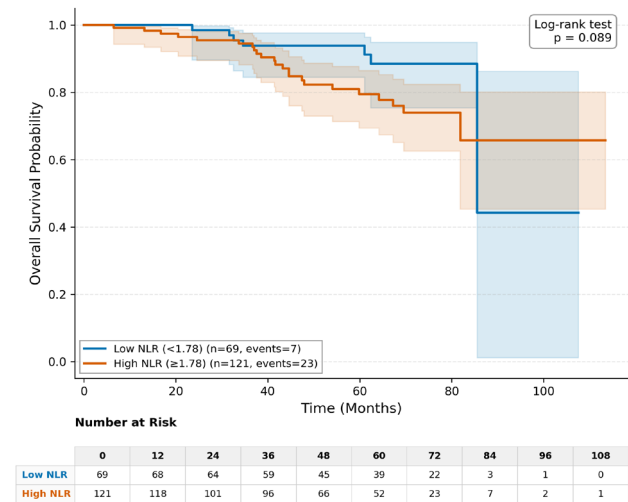


FIGURE 1: Kaplan-Meier overall survival curves according to pretreatment NLR. Patients were stratified into low NLR (<1.78 ; $n=69$) and high NLR (≥ 1.78 ; $n=121$) groups. The p-value was obtained from the log-rank test.
NLR: Neutrophil-to-lymphocyte ratio

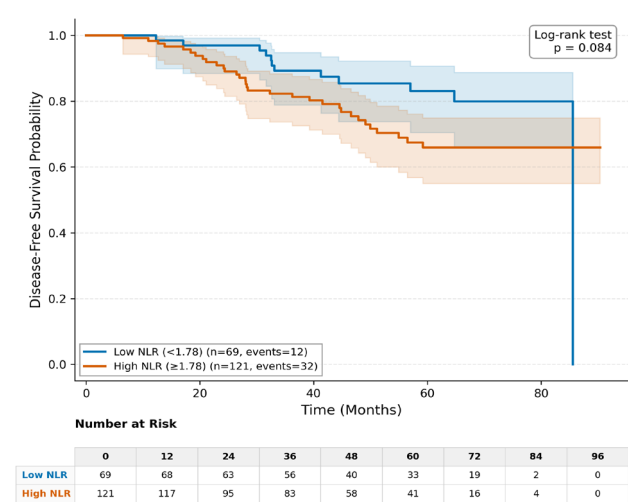


FIGURE 2: Kaplan-Meier disease-free survival curves according to pretreatment NLR. Patients were stratified into low NLR (<1.78 ; $n=69$) and high NLR (≥ 1.78 ; $n=121$) groups. The p-value was obtained from the log-rank test.
NLR: Neutrophil-to-lymphocyte ratio

TABLE 5: Univariate Cox proportional hazards regression analysis for overall survival and disease-free survival.

Variable	Overall survival			Disease-free survival		
	HR	95% CI	p-value	HR	95% CI	p-value
Age ≥50 years	1.29	0.62-2.69	0.492	0.94	0.51-1.70	0.826
Postmenopausal status	1.10	0.59-2.05	0.758	0.87	0.51-1.48	0.610
Grade 3 histology	2.30	1.08-4.87	0.030	2.14	1.13-4.04	0.019
ER positive	0.33	0.16-0.67	0.002	0.36	0.20-0.66	<0.001
PR positive	0.30	0.14-0.63	0.002	0.30	0.16-0.55	<0.001
HER2 positive	0.94	0.40-2.20	0.884	0.79	0.37-1.65	0.524
Ki-67 ≥20%	3.63	1.48-8.88	0.005	2.91	1.47-5.77	0.002
Lymph node positive	2.90	1.01-8.33	0.048	1.43	0.71-2.91	0.320
LVI positive	1.55	0.71-3.42	0.274	1.32	0.71-2.48	0.380
PNI positive	1.12	0.53-2.37	0.758	1.29	0.70-2.37	0.408
Neoadjuvant CT	1.73	0.74-4.06	0.206	1.47	0.70-3.08	0.310
Stage III disease	2.76	1.34-5.69	0.006	2.17	1.20-3.93	0.011
High NLR (≥1.78)	2.05	0.88-4.79	0.096	1.78	0.92-3.46	0.088
High PLR (≥138.08)	1.44	0.70-2.95	0.326	1.54	0.85-2.80	0.152
High SII (≥449.55)	1.07	0.48-2.41	0.870	1.04	0.53-2.01	0.917

Bold values indicate statistical significance (p<0.05).

HR: Hazard ratio; CI: Confidence interval; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; LVI: Lymphovascular invasion; PNI: Perineural invasion; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index.

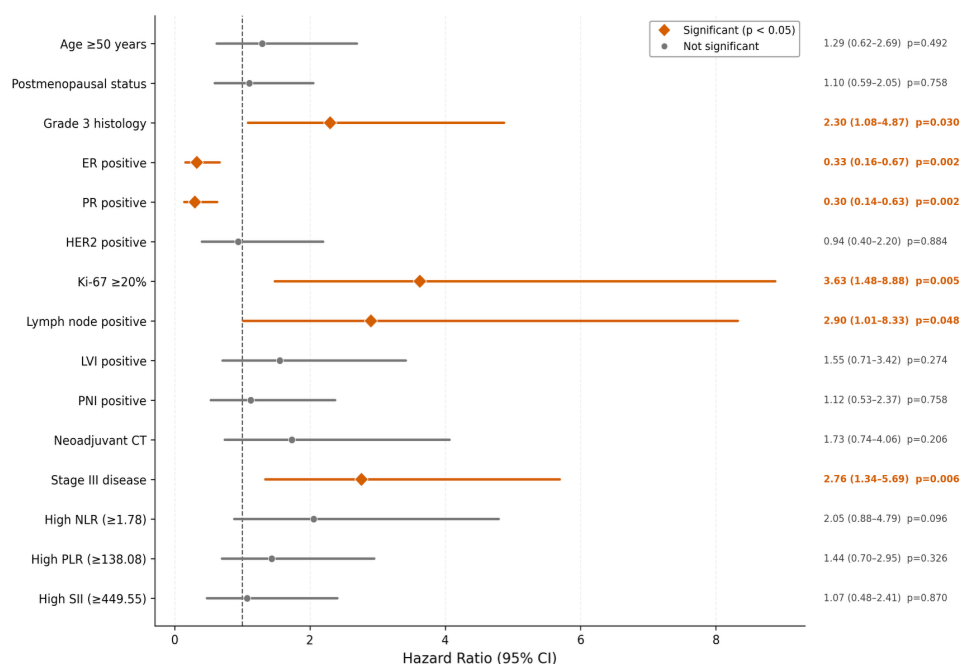


FIGURE 3: Forest plot of univariate Cox regression analysis for OS. HR with 95% CI are shown for each clinical and laboratory variable. The vertical dashed line represents HR: 1.0 (no effect).

HR: Hazard ratio; CI: Confidence interval; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; OS: Overall survival; CT: Chemotherapy; HER2: Human epidermal growth factor receptor 2; ER: Estrogen receptor; PR: Progesterone receptor; LVI: Lymphovascular invasion; PNI: Perineural invasion

The ROC curves for the inflammatory markers predicting OS and DFS are presented in Figure 4.

Multivariate Cox Regression Analysis

Variables with $p < 0.25$ in univariate analysis were included in the multivariate model. The results are presented in Table 1. For OS, two variables retained independent prognostic significance: Ki-67 $\geq 20\%$ (HR: 3.20; 95% CI: 1.31-7.80; $p=0.011$) and lymph node positivity (HR: 3.09; 95% CI: 1.07-8.93; $p=0.037$). Stage III disease (HR: 2.08; 95% CI: 0.97-4.47; $p=0.059$) and neoadjuvant chemotherapy (HR: 2.35; 95% CI: 0.96-5.73; $p=0.060$) were associated with effects of comparable magnitude, which narrowly missed statistical significance. PR positivity showed a numerically protective effect that did not reach statistical significance (HR: 0.41; 95% CI: 0.14-1.16; $p=0.094$). High NLR was not independently associated with OS (HR: 1.92; 95% CI: 0.83-4.46; $p=0.128$), though the direction and magnitude of the HR remained clinically noteworthy.

For DFS, three independent prognostic factors were identified: PR positivity (HR: 0.33; 95% CI: 0.15-0.76; $p=0.009$) as a protective factor, Ki-67 $\geq 20\%$ (HR: 2.52; 95% CI: 1.26-5.07; $p=0.009$), and Stage III disease (HR: 2.27; 95% CI: 1.26-4.09; $p=0.007$) as independent risk factors. High NLR was not statistically significant for DFS (HR: 1.76; 95% CI: 0.88-3.54; $p=0.113$), although the direction and magnitude of the HR remained consistent with the univariate estimate.

Subgroup Analysis by Molecular Subtype

To evaluate whether the prognostic significance of inflammatory markers differed across molecular subtypes, subgroup survival analyses were performed (Table 6).

The most striking finding was observed in the TNBC subgroup ($n=41$). Among TNBC patients, elevated NLR (≥ 1.78) was significantly associated with both inferior OS ($p=0.019$) and inferior DFS ($p=0.025$). In the high NLR TNBC group ($n=29$), 12 OS events and 15 DFS events were observed, compared with 1 OS event and 2 DFS events among the 12 patients with low NLR. PLR did not show significant prognostic value in the TNBC subgroup for OS ($p=0.446$) and DFS ($p=0.340$). Because the observed survival events in the TNBC subgroup were concentrated within the high-NLR group, standard Cox regression could not be reliably estimated because of near-complete separation; ridge-penalized Cox regression (L2 penalty, $\lambda=0.5$; lifelines CoxPHFitter) was therefore applied, yielding a HR of 1.52 (95% CI: 0.42-5.57) for OS and 1.75 (95% CI: 0.50-6.11) for DFS. Given the very small subgroup size and the limited number of events, these effect estimates are unstable and should be interpreted with considerable caution.

In contrast, NLR showed no prognostic significance in the luminal B subgroup (OS: $p=0.506$; DFS: $p=0.416$) or the luminal A subgroup (DFS: $p=0.560$). PLR and SII similarly failed to achieve significance in the luminal subtypes (all $p > 0.30$). The HER2-enriched subgroup ($n=10$) was considered too small for meaningful survival analysis. These findings demonstrate a subtype-specific prognostic role of NLR, with its predictive value confined primarily to the TNBC.

Continuous Variable Analysis and Internal Validation

When analyzed as a continuous variable, NLR was not significantly associated with OS (HR: 0.984; 95% CI: 0.729-1.328; $p=0.915$) or DFS (HR: 1.117; 95% CI: 0.880-1.418; $p=0.362$).

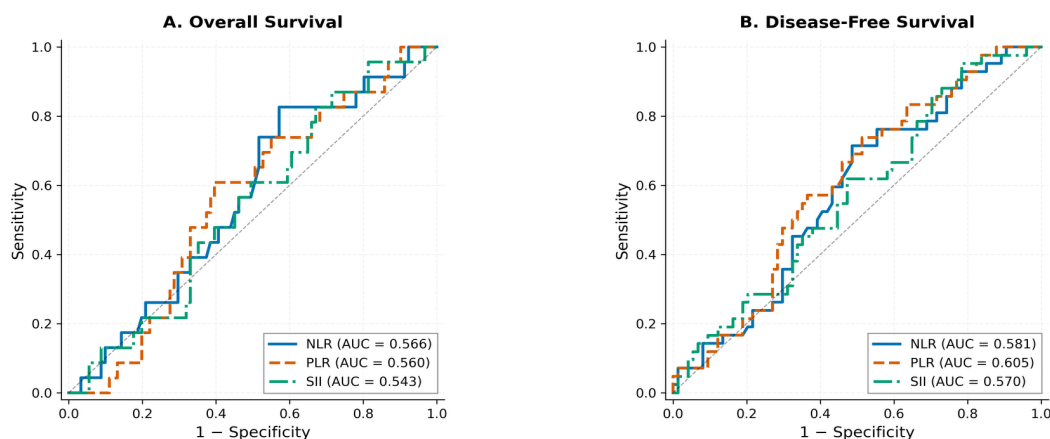


FIGURE 4: ROC curves for NLR, PLR, and SII in predicting OS (A) and DFS (B). AUC values are shown for each marker. OS AUCs: NLR=0.566, PLR=0.560, SII=0.543; DFS AUCs: NLR=0.581, PLR=0.605, SII=0.570.

ROC: Receiver operating characteristic; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; OS: Overall survival; DFS: Disease-free survival; AUC: Area under the curve

TABLE 6: Subgroup analysis: Kaplan-Meier survival estimates by molecular subtype.

Molecular subtype	Marker	Endpoint	High group n (events)	Low group n (events)	Log-rank p-value
Luminal A (n=52)	NLR	DFS	26 (2)	26 (3)	0.560
	PLR	DFS	22 (2)	30 (3)	0.920
	SII	DFS	48 (5)	4 (0)	0.480
Luminal B (n=81)	NLR	OS	44 (8)	37 (5)	0.506
	NLR	DFS	44 (12)	37 (8)	0.416
	PLR	OS	37 (7)	44 (6)	0.410
	PLR	DFS	37 (10)	44 (10)	0.398
	SII	OS	73 (12)	8 (1)	0.817
	SII	DFS	73 (19)	8 (1)	0.387
TNBC (n=41)	NLR	OS	29 (12)	12 (1)	0.019
	NLR	DFS	29 (15)	12 (2)	0.025
	PLR	OS	24 (8)	17 (5)	0.446
	PLR	DFS	24 (11)	17 (6)	0.340

Kaplan-Meier log-rank test results for inflammatory markers stratified by molecular subtype. The HER2-enriched subgroup (n=10) was considered too small for meaningful survival analysis and was excluded. Bold values indicate statistical significance ($p < 0.05$). For the luminal A subgroup, only DFS comparisons are presented, because the number of deaths observed in this subgroup was too small for a meaningful OS comparison. For SII, the cut-off value classified the majority of patients within each subtype as high-SII, resulting in low-SII comparison groups that included only 4 patients (luminal A) and 8 patients (luminal B). Consequently, the SII rows are based on markedly unbalanced groups and should be regarded as exploratory. SII comparisons are not presented for the TNBC subgroup because the low-SII group was too small for meaningful comparison.

NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; OS: Overall survival; DFS: Disease-free survival; HER2: Human epidermal growth factor receptor 2; TNBC: Triple-negative breast cancer.

For a one-SD increase, the HRs were 0.98 (OS) and 1.13 (DFS) for NLR; 0.94 (OS) and 1.06 (DFS) for PLR; and 0.97 (OS) and 1.08 (DFS) for SII. After adjustment for age, stage, and Ki-67 status, NLR retained a per-SD HR of 1.06 (95% CI: 0.76-1.48; $p=0.728$) for OS. Bootstrap internal validation of the complete cut-off selection procedure (1000 resamples), in which the optimal cut-off was re-derived in every resample, yielded median NLR cutoffs of 1.78 for OS (95% percentile interval, 1.29-2.41) and 1.95 for DFS (95% percentile interval, 1.29-2.30), which were close to the primary estimates. The median bootstrap HRs were 2.25 (95% percentile interval, 1.04-8.24) for OS and 2.15 (95% percentile interval, 1.28-5.99) for DFS, with a HR greater than 1 in 98.1% of resamples for OS and in 100% of resamples for DFS and a log-rank p -value below 0.05 in 47.7% of resamples for OS and in 62.9% of resamples for DFS. These findings indicate sampling variability in the ROC-derived cut-off values and should be interpreted with appropriate caution (Supplementary Table S1). Overall, the continuous-variable analyses should be interpreted as complementary to the ROC-derived categorical analyses rather than as confirmatory.

DISCUSSION

The present study, with a median follow-up of 58.8 months, evaluated the prognostic significance of three pretreatment inflammatory indices—NLR, PLR, and SII—in 190 patients with non-metastatic breast cancer. The principal findings are as follows: (1) NLR showed a consistent direction of

association with both OS and DFS in the overall cohort, although statistical significance was not reached; (2) Ki-67 $\geq 20\%$ was confirmed as an independent risk factor for both OS and DFS, lymph node positivity for OS and Stage III disease for DFS, while PR positivity was independently protective for DFS; (3) NLR was a statistically significant prognostic marker in the TNBC subgroup for both OS and DFS but not in Luminal subtypes, indicating a subtype-specific effect; and (4) PLR and SII did not achieve significant prognostic value in this cohort.

NLR as a Prognostic Marker

In the overall cohort, NLR showed the strongest prognostic signal among the three inflammatory markers evaluated, with HRs of 2.05 for OS and 1.78 for DFS in univariate analysis; however, statistical significance was not achieved ($p=0.096$ and $p=0.088$; respectively). This finding is consistent with a meta-analysis by Ethier et al.¹⁰, which included 15 studies and 8,563 patients and reported that elevated NLR was associated with worse OS (HR: 2.56; 95% CI: 1.96-3.35) and DFS (HR: 1.74; 95% CI: 1.47-2.07). However, the same meta-analysis highlighted substantial heterogeneity in cut-off values (ranging from 1.9 to 5.0), which may explain the variable significance across individual studies.

Our NLR cut-off of 1.78, derived from ROC analysis, is comparable to the cut-off of 1.34 reported by Cho et al.²⁵ in a cohort of 661 Korean breast cancer patients. The modest AUC of 0.566 observed in our study is also consistent with their

finding (AUC=0.58), reinforcing the notion that NLR alone has limited discriminatory capacity but may serve as a useful adjunct within a broader prognostic framework.

Subtype-specific Prognostic Significance of NLR in TNBC

A notable hypothesis-generating observation in our study was an apparent association between elevated NLR and inferior survival outcomes in the TNBC subgroup (OS: $p=0.019$; DFS: $p=0.025$); no such association was observed in luminal subtypes. This subtype-specific pattern has several important implications. From a biological perspective, TNBC is characterized by higher mutational burden, greater genomic instability, and a more immunogenic tumor microenvironment compared to hormone receptor-positive subtypes.^{20,21} Neutrophils, which constitute the numerator of NLR, promote tumor progression through the release of reactive oxygen species, matrix metalloproteinases, and vascular endothelial growth factor, while also suppressing T-cell function.^{26,27} An elevated NLR, therefore, reflects a pro-tumorigenic, immunosuppressive systemic state that may disproportionately impact outcomes in an inherently immunogenic subtype, such as TNBC.

This finding aligns with the study by Pistelli et al.²⁸, who reported that NLR >3 was independently associated with inferior DFS in early TNBC (HR: 5.15; 95% CI: 1.11-23.88; $p=0.03$). Similarly, Jia et al.²⁹ found that elevated NLR was associated with worse OS and DFS in the overall breast cancer cohort and in the TNBC subgroup, but not in the other molecular subtypes. This observation is particularly relevant given the recognized molecular heterogeneity within TNBC itself.³⁰ The clinical relevance of this finding is substantial: TNBC lacks established therapeutic targets; NLR—a universally accessible, inexpensive marker derived from routine blood tests—warrants further investigation as a potential supplementary prognostic tool; its role in informing clinical decision-making remains to be established through prospective validation. Immunotherapy-based regimens have shown particular promise in TNBC.³¹ It must be emphasized, however, that the TNBC subgroup was small and included few events, resulting in near-complete separation, necessitating ridge-penalized (L2) Cox regression to obtain finite parameter estimates; consequently, these findings should be regarded as hypothesis-generating and require validation in larger, prospective TNBC cohorts.

PLR and SII: Limited Prognostic Value

In contrast to NLR, neither PLR nor SII demonstrated statistically significant prognostic value in this cohort. PLR showed a modest, non-significant trend for DFS (HR: 1.54; $p=0.152$),

but not for OS ($p=0.326$). This contrasts with the findings of Cho et al.²⁵, who identified PLR as an independent predictor of both disease-specific survival and DFS, with PLR >185.5 conferring a substantially increased risk (HR: 3.23 for DSS). Their larger sample size ($n=661$) provided greater statistical power to detect associations, and they used a considerably higher PLR cut-off (185.5 vs. 138.08 in our study). SII, despite its theoretical advantage of integrating three hematological components, failed to show any prognostic signal in our cohort (OS: $p=0.871$; DFS: $p=0.917$) (Supplementary Figures S1-S4). A possible explanation is that adding platelet count to the SII formula may introduce noise rather than signal in this patient population.

Independent Prognostic Factors

The multivariate analysis confirmed Ki-67 $\geq 20\%$ and lymph node positivity as independent predictors of OS, and PR positivity, Ki-67 $\geq 20\%$, and Stage III disease as independent predictors of DFS. Stage III disease showed an effect of comparable magnitude on OS; this effect narrowly missed conventional significance (HR: 2.08; $p=0.059$). These findings are well-established in the breast cancer prognostic literature.^{32,33} Ki-67, a nuclear protein associated with cellular proliferation, has been consistently identified as a prognostic marker across molecular subtypes and is incorporated into clinical decision-making frameworks, including the St. Gallen Consensus.²³

The protective effect of PR positivity on DFS (HR: 0.33; $p=0.009$) is consistent with published data indicating that PR expression carries independent prognostic significance. PR-positive tumors are generally more differentiated, have lower proliferative activity, and are more responsive to endocrine therapy.³⁴ The observation that PR did not achieve independent significance for OS (HR: 0.41; $p=0.094$) may reflect the limited number of OS events ($n=30$) and may warrant validation in larger cohorts.

Associations Between Inflammatory Markers and Clinical Characteristics

Our study revealed that elevated NLR and PLR were significantly associated with age <50 years, whereas elevated SII was associated with older age. The association between high NLR and ER negativity ($p=0.027$) is biologically plausible, as ER-negative tumors—particularly TNBC—tend to elicit a stronger inflammatory response.³⁵ None of the inflammatory markers showed significant associations with established adverse clinicopathological features such as lymph node metastasis, advanced stage, or LVI, suggesting that these markers reflect a distinct biological dimension independent of traditional prognostic factors.

Study Limitations

This study has several strengths. First, it simultaneously evaluated three inflammatory indices and their association with survival outcomes in a homogeneous, non-metastatic breast cancer cohort. Second, the subgroup analysis by molecular subtype adds clinically relevant information to the existing literature. Third, a penalized Cox regression approach mitigated overfitting concerns inherent to studies with a limited number of events. Fourth, the optimism associated with the data-driven selection of cut-off values was quantified using a bootstrap procedure that re-derived the cut-off in each resample.

However, several limitations must be acknowledged. First, the retrospective single-center design introduces potential selection bias and limits generalizability. Second, the relatively modest sample size ($n=190$) with 30 OS events and 44 DFS events restricts statistical power. Third, the TNBC subgroup comprised only 41 patients, and these findings require validation in larger, multicenter cohorts. Fourth, LMR could not be assessed due to the absence of monocyte count data. Fifth, other established inflammatory markers, such as C-reactive protein and albumin levels, were not available for analysis. Sixth, the association between NLR and OS/DFS in TNBC did not undergo formal multivariate adjustment within the subgroup due to limited events and should therefore be interpreted as hypothesis-generating. Additionally, the relatively small number of events (30 deaths for OS and 44 events for DFS), relative to the number of covariates included in the multivariable models may limit the stability of the regression estimates; therefore, these findings should be interpreted with caution. The late portions of the Kaplan-Meier survival curves should be interpreted with caution, as the number of patients at risk decreases over time and CIs widen accordingly. The imbalance in SII subgroup sizes (high SII, $n=143$ vs. low SII, $n=47$) may have reduced statistical power for detecting differences, particularly in subgroup analyses, and this should be considered when interpreting SII-related findings. ER and PR status are biologically correlated; some multicollinearity between these covariates in the multivariable models may have influenced the precision of individual coefficient estimates. Although inclusion criteria required substantially complete clinicopathological data, some variables had missing values (as detailed in Table 2), and complete-case analysis may have introduced selection bias if the data were not missing completely at random. Multiple comparisons were performed to analyze associations between inflammatory marker groups and clinicopathological variables (Table 3) without formal adjustment for multiplicity. Therefore, some statistically significant associations may have arisen by chance; these findings should be interpreted with caution and confirmed in independent studies.

To address the concern of optimism bias inherent in using data-derived cut-offs for prognosis, we performed three complementary analyses. First, inflammatory markers were analyzed as continuous variables in Cox regression models, making the analyses independent of cut-off selection. Analysis of continuous variables did not confirm the direction or magnitude of the associations observed using dichotomized variables. Therefore, these analyses should be considered complementary rather than confirmatory. Second, bootstrap internal validation with 1000 resamples was performed in a manner that incorporated the cut-off selection step itself, with the landmark ROC analysis, the derivation of the optimal Youden cut-off, and the subsequent Kaplan-Meier and Cox analyses repeated within each resample. Third, this procedure yielded a median bootstrap NLR cut-off of 1.78 for OS, corresponding to the value identified in the primary analysis, with a 95% percentile interval of 1.29-2.41; the direction of the association was consistent across resamples (HR greater than 1 in 98.1%), whereas the log-rank p -value was below 0.05 in 47.7% of resamples, indicating sampling variability and uncertainty regarding the precision of the cut-off. Nevertheless, these cut-offs should be considered hypothesis-generating and require external validation in independent cohorts before clinical implementation. Additionally, the landmark ROC approach excluded patients censored before 60 months, such that the cut-offs were derived from 114 patients for OS and 116 patients for DFS, and then applied to the complete cohort of 190. Although the median follow-up of 58.8 months meant that the majority of patients contributed to this analysis, the reduced effective sample size for cut-off determination means that the thresholds reflect only the subset of patients whose 60 months status was observable. An inverse-probability-of-censoring-weighted time-dependent ROC analysis would retain all 190 patients by weighting for censoring and would represent a methodologically attractive alternative. However, it was outside the predefined analysis plan of the present study and therefore was not undertaken; we regard it as the preferred approach for future validation work.

CONCLUSION

In this retrospective analysis of 190 non-metastatic breast cancer patients, pretreatment NLR showed a consistent direction of association with both OS and DFS in the overall cohort, although statistical significance was not reached. Of particular interest, NLR showed a preliminary, hypothesis-generating association with survival outcomes specifically in TNBC, while no such association was observed in luminal subtypes; however, given the small TNBC subgroup and the few events, this observation requires confirmation.

This subtype-specific finding suggests that the prognostic relevance of systemic inflammation in breast cancer is modulated by the molecular context of the tumor.

Ki-67 proliferation index, lymph node status, and disease stage were confirmed as the strongest independent predictors of survival, with PR positivity serving as a protective factor for DFS. Inflammatory markers such as NLR may serve as readily accessible, cost-effective supplementary prognostic tools, particularly in TNBC, where conventional hormone receptor-based stratification is unavailable. Prospective, multicenter studies with larger TNBC subgroups are warranted to validate these findings and explore whether inflammatory indices may have a supplementary role in the prognostic assessment of TNBC.

Ethics

Ethics Committee Approval: The study protocol was approved by the Necmettin Erbakan University Non-Drug and Non-Medical Device Research Ethics Committee (approval number: 2023/4610, date: 3.11.2023).

Informed Consent: The requirement for written informed consent was waived due to the retrospective design of the study.

Footnotes

Authorship Contributions

Concept: E.H., Me.A., Design: E.H., Me.A., Data Collection or Processing: E.H., M.K.E., M.A., Analysis or Interpretation: E.H., Me.A., Literature Search: E.H., M.K.E., Writing: E.H.

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

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Supplementary Figures S1-S4: <https://d2v96fxpocvxx.cloudfront.net/a5223c9c-50f0-490d-b293-6a74c2af8d3b/content-images/b3a65465-2546-438d-8b81-41ce32cfead0.pdf>

Supplementary Table S1: <https://d2v96fxpocvxx.cloudfront.net/a5223c9c-50f0-490d-b293-6a74c2af8d3b/content-images/463ea7c8-82fc-4690-af93-6a2dea780f0e.pdf>

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Clinical Predictors of Progression-free Survival in Patients with Metastatic Castration-resistant Prostate Cancer Treated with Lu-177 PSMA: A Real-world Study

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ABSTRACT

Objective: Accurate pretreatment risk assessment remains an important challenge for patients with metastatic castration-resistant prostate cancer (mCRPC) undergoing lutetium-177-labeled prostate-specific membrane antigen (Lu-177 PSMA) radioligand therapy, highlighting the need for reliable prognostic markers.

Material and Methods: We retrospectively analyzed 52 consecutive patients with mCRPC who underwent Lu-177 PSMA radioligand therapy at a single tertiary referral center. Baseline demographic, laboratory, and imaging characteristics were recorded before treatment initiation. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method, and baseline factors associated with PFS were examined using Cox proportional hazards regression models.

Results: After a median follow-up of 14.0 months, the median PFS and OS were 8.34 and 16.99 months, respectively. Disease progression occurred in 48 patients (92.3%), while 42 (80.8%) died during follow-up. Elevated baseline prostate-specific antigen (PSA) levels and the presence of visceral metastases were associated with significantly shorter PFS. In multivariable analysis, baseline PSA [adjusted hazard ratio (HR): 1.446; 95% confidence interval (CI): 1.204-1.738; $p < 0.001$] and visceral metastasis (adjusted HR: 2.441; 95% CI: 1.049-5.682; $p = 0.038$) remained independently associated with disease progression. Baseline maximum standardized uptake value (SUV_{max}) and mean SUV did not demonstrate significant prognostic value.

Conclusion: Baseline PSA and visceral metastasis were independently associated with shorter PFS in patients with mCRPC treated with Lu-177 PSMA, whereas conventional semiquantitative PSMA positron emission tomography/computed tomography parameters were not. These findings suggest that routinely available baseline clinical characteristics may help identify patients at increased risk of early progression prior to radioligand therapy.

Keywords: Metastatic castration-resistant prostate cancer; Lu-177 PSMA; prostate-specific antigen; progression-free survival; visceral metastasis

INTRODUCTION

Metastatic castration-resistant prostate cancer (mCRPC) represents the terminal stage of prostate cancer progression and continues to impose a substantial clinical burden despite remarkable advances in systemic therapy. The introduction of androgen receptor signaling inhibitors, taxane-based

chemotherapy, PARP inhibitors in selected patients, and prostate-specific membrane antigen (PSMA)-targeted radioligand therapy has significantly prolonged survival. Nevertheless, disease progression remains inevitable for most patients, highlighting the importance of reliable prognostic markers that may facilitate individualized treatment planning and optimize therapeutic sequencing.^{1,2}

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Among recently introduced treatment strategies, lutetium-177-labeled prostate-specific membrane antigen (Lu-177 PSMA) radioligand therapy has become an established option for patients with PSMA-positive mCRPC. Its clinical benefit was demonstrated in the VISION and TheraP trials, which showed improvements in progression-free survival (PFS), overall survival (OS), and patient-reported outcomes compared with standard therapeutic approaches. Consequently, Lu-177 PSMA has been incorporated into contemporary international treatment guidelines for appropriately selected patients with advanced disease.³⁻⁶

Although many patients derive meaningful benefit from Lu-177 PSMA therapy, treatment outcomes remain heterogeneous. Some individuals achieve durable disease control, whereas others experience rapid progression despite adequate PSMA uptake on baseline positron emission tomography/computed tomography (PET/CT) imaging. This variability has increased interest in identifying pretreatment factors that may predict clinical outcomes and support risk stratification before therapy initiation.⁷⁻⁹

Previous studies have identified several baseline clinical characteristics, including serum prostate-specific antigen (PSA), disease burden, visceral metastatic involvement, and performance status, as potential prognostic indicators in patients undergoing Lu-177 PSMA therapy. However, the reported prognostic impact of these variables has not been entirely consistent, likely reflecting differences in patient characteristics, treatment sequences, and study methodology across published cohorts.⁹⁻¹¹

Baseline PSMA PET/CT is indispensable for confirming sufficient PSMA expression and determining eligibility for radioligand therapy. In addition to its role in patient selection, semiquantitative imaging parameters such as maximum standardized uptake value (SUV_{max}) and mean SUV (SUV_{mean}) have been investigated as possible prognostic biomarkers. Nevertheless, existing evidence remains conflicting, and it is uncertain whether these PET-derived parameters provide prognostic information beyond that offered by routinely available clinical variables.¹²⁻¹⁴

Real-world investigations provide valuable complementary evidence to randomized clinical trials by reflecting the diversity of patients encountered in routine oncology practice. Therefore, the present study aimed to evaluate baseline clinical and imaging factors associated with PFS in patients with mCRPC treated with Lu-177 PSMA in a real-world setting, with particular emphasis on comparing the prognostic contributions of conventional clinical variables and semiquantitative PSMA PET/CT parameters.

MATERIAL AND METHODS

Study Design and Patient Population

This retrospective observational study was conducted at a single tertiary referral center and included consecutive patients with mCRPC who underwent Lu-177 PSMA radioligand therapy at Pamukkale University between May 2018 and April 2025. Survival follow-up was updated through May 2026. Patients were eligible if they had histologically confirmed prostate adenocarcinoma, metastatic castration-resistant disease, a baseline PSMA PET/CT examination performed before treatment initiation, and complete clinical follow-up data. Individuals lacking pretreatment imaging or essential clinical information were excluded from the analysis.

The study protocol was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval number: E.895531, date: 01.07.2026) and carried out in accordance with the ethical principles of the Declaration of Helsinki. The requirement for written informed consent was waived due to the retrospective design of the study.

Clinical information was retrieved from electronic medical records, including demographic characteristics, Gleason score, serum PSA level at baseline, interval from initial diagnosis to Lu-177 PSMA therapy, previous systemic treatments, metastatic disease sites, and the total number of treatment cycles administered.

PSMA PET/CT Assessment

Pretreatment PSMA PET/CT examinations obtained before the first Lu-177 PSMA administration were reviewed. The semiquantitative imaging variables analyzed were the SUV_{max} and SUV_{mean} , both recorded on the baseline scan. SUV_{max} and SUV_{mean} were measured in the PSMA-avid lesion with the highest tracer uptake on the baseline PET/CT examination, according to the institutional imaging protocol.

Follow-up and Study Endpoints

Patients were monitored from the initiation of Lu-177 PSMA therapy until radiological or clinical progression, death, or the last available follow-up. PFS was defined as the interval between the first treatment cycle and documented disease progression or death from any cause, whichever occurred first. OS was calculated from the first administration of Lu-177 PSMA to death from any cause or to the date of the last follow-up for surviving patients. Median follow-up was calculated from the date of the first Lu-177 PSMA administration to the date of the last available follow-up or death.

Statistical Analysis

Continuous variables are presented as medians with interquartile ranges (IQRs), whereas categorical variables are reported as frequencies and percentages. Survival distributions were estimated using the Kaplan-Meier method and compared with the log-rank test. Potential prognostic factors associated with PFS were initially assessed using univariable Cox proportional hazards regression. Variables considered clinically relevant, together with those demonstrating a p-value below 0.10 in univariable analyses, were subsequently entered into a multivariable Cox regression model. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated, and statistical significance was defined as a two-sided p-value below 0.05. All statistical analyses were performed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). Baseline PSA was entered into the Cox proportional hazards regression model as a continuous variable. Variables included in the multivariable model were selected based on clinical relevance and on a univariable p-value <0.10.

RESULTS

Patient Characteristics

A total of 52 patients with mCRPC who were treated with Lu-177 PSMA therapy were included. The median age was 64.5 years (IQR: 58.8-71.3), and the median interval from initial diagnosis to Lu-177 therapy was 37.0 months (IQR: 20.3-77.3). The median baseline PSA level was 78.07 ng/mL (IQR: 21.43-226.50), and patients received a median of 3 Lu-177 cycles (IQR: 2-6). Most patients had Gleason scores of 9-10 (63.5%). Bone metastases were present in all patients, whereas lymph node and visceral metastases were observed in 55.8% and 23.1% of patients, respectively. The median baseline SUV_{max} and SUV_{mean} values were 19.46 (IQR: 13.39-36.67) and 8.25 (IQR: 5.98-14.04), respectively (Table 1).

Survival Outcomes

After a median follow-up of 14.0 months, 48 patients (92.3%) experienced disease progression. The median PFS was 8.34 months, whereas the median OS was 16.99 months. During follow-up, 42 patients (80.8%) died (Table 2).

Prognostic Analyses

Patients with baseline PSA values above the cohort median (>78.07 ng/mL) had significantly shorter PFS than patients with lower PSA values (median PFS: 4.96 vs. 14.29 months; log-rank $p < 0.001$) (Figure 1). Likewise, patients with visceral metastases had significantly shorter PFS compared with those without visceral involvement (median PFS: 4.17 vs. 11.43 months; log-rank $p < 0.001$) (Figure 2).

TABLE 1: Baseline demographic, clinical, treatment, and PSMA PET/CT characteristics of patients (n=52).

Variable	Overall (n=52)
Age, years, median (IQR)	64.5 (58.8-71.3)
Time from initial diagnosis to Lu-177 therapy, months, median (IQR)	37.0 (20.3-77.3)
Baseline PSA before Lu-177 (ng/mL), median (IQR)	78.07 (21.43-226.50)
Gleason score	
≤7	11 (21.2%)
8	8 (15.4%)
9-10	33 (63.5%)
Number of Lu-177 cycles, median (IQR)	3 (2-6)
Treatment history before Lu-177 PSMA therapy	
ADT only	5 (9.6%)
ADT + taxane chemotherapy	25 (48.1%)
ADT + radiotherapy	4 (7.7%)
ADT + taxane chemotherapy + radiotherapy	17 (32.7%)
Taxane chemotherapy only	1 (1.9%)
Metastatic sites before Lu-177	
Bone metastasis	52 (100%)
Lymph node metastasis	29 (55.8%)
Visceral metastasis	12 (23.1%)
Baseline PSMA PET/CT parameters	
SUV_{max} , median (IQR)	19.46 (13.39-36.67)
SUV_{mean} , median (IQR)	8.25 (5.98-14.04)
ADT: Androgen deprivation therapy; IQR: Interquartile range; PET/CT: Positron emission tomography/computed tomography; PSA: Prostate-specific antigen; PSMA: Prostate-specific membrane antigen; SUV: Standardized uptake value; Lu: Lutetium.	

TABLE 2: Survival outcomes of patients treated with Lu-177 PSMA (n=52).

Outcome	Value
Median follow-up, months	14.0
Median PFS, months	8.34
PFS events, n (%)	48 (92.3)
Median OS, months	16.99
Deaths, n (%)	42 (80.8)
Median follow-up was calculated from the initiation of Lu-177 PSMA therapy to the last follow-up or death. OS: Overall survival; PFS: Progression-free survival; PSMA: Prostate-specific membrane antigen; Lu: Lutetium.	

In contrast, no significant differences in PFS were observed based on baseline SUV_{max} (8.34 vs. 7.82 months; log-rank $p = 0.931$) or baseline SUV_{mean} (7.72 vs. 8.61 months; log-rank $p = 0.612$) (Table 3).

In the univariable Cox regression analysis, baseline PSA (HR: 1.524; 95% CI: 1.272-1.825; $p < 0.001$) and visceral metastasis

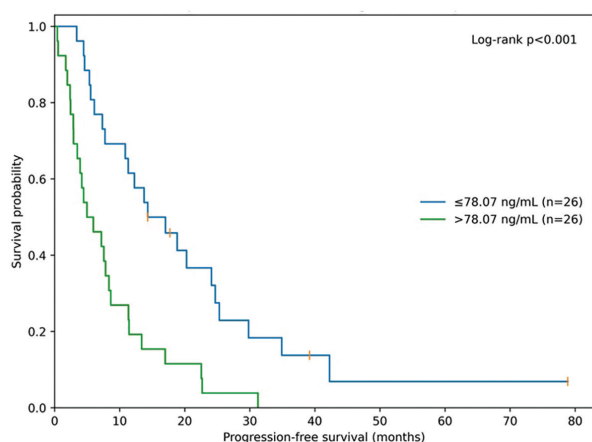


FIGURE 1: Kaplan-Meier curve for PFS according to baseline PSA.

PSA: Prostate-specific antigen; PFS: Progression-free survival

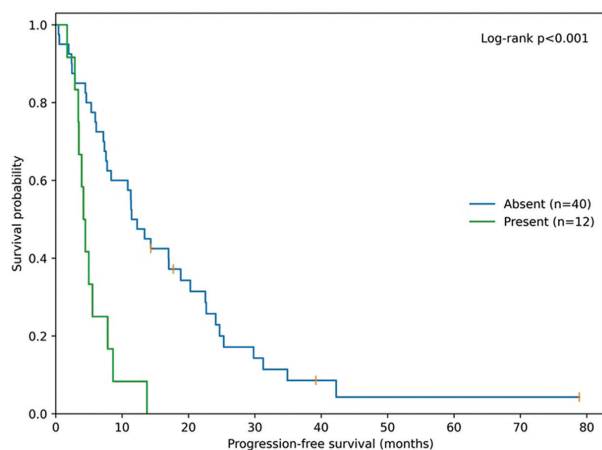


FIGURE 2: Kaplan-Meier curve for PFS according to visceral metastasis.

PFS: Progression-free survival

TABLE 3: Kaplan-Meier and log-rank comparisons for PFS.			
Variable	Group	Median PFS (months)	Log-rank p-value
Baseline PSA	≤78.07 ng/mL	14.29	<0.001
	>78.07 ng/mL	4.96	
Visceral metastasis	Absent	11.43	<0.001
	Present	4.17	
SUV _{max}	≤19.46	8.34	0.931
	>19.46	7.82	
SUV _{mean}	≤8.25	7.72	0.612
	>8.25	8.61	

Bold values indicate statistically significant results (p<0.05).
PSA: Prostate-specific antigen; PFS: Progression-free survival; SUV: Standardized uptake value.

(HR: 3.815; 95% CI: 1.827-7.966; p<0.001) were significantly associated with shorter PFS. Age, time from diagnosis to Lu-177 therapy, Gleason score, lymph node metastasis, SUV_{max} and SUV_{mean} were not significantly associated with PFS (Table 4).

In the multivariable model, baseline PSA remained independently associated with shorter PFS (adjusted HR: 1.446; 95% CI: 1.204-1.738; p<0.001). Visceral metastasis also remained independently associated with inferior PFS (adjusted HR: 2.441; 95% CI: 1.049-5.682; p=0.038). Neither age nor baseline SUV_{max} retained statistical significance (Table 5).

TABLE 4: Univariable Cox regression analysis for progression-free survival.

Variable	n	HR	95% CI	p-value
Age (per 1-year increase)	52	1.025	0.990-1.062	0.165
Time from initial diagnosis to Lu-177 therapy (per 1-month increase)	50	0.999	0.992-1.006	0.748
Baseline PSA (per doubling, log2-transformed)	52	1.524	1.272-1.825	<0.001
Gleason score ≥9 (vs. ≤8)	52	1.514	0.832-2.756	0.175
Visceral metastasis (yes vs. no)	52	3.815	1.827-7.966	<0.001
Lymph node metastasis (yes vs. no)	52	1.353	0.759-2.414	0.305
SUV _{max} (per 1-unit increase)	52	0.999	0.987-1.010	0.832
SUV _{mean} (per 1-unit increase)	52	0.989	0.952-1.028	0.583

Bold values indicate statistically significant results (p<0.05). Baseline PSA was log2-transformed; therefore, the HR represents the change in progression risk for each doubling of PSA.

CI: Confidence interval; HR: Hazard ratio; PSA: Prostate-specific antigen; SUV: Standardized uptake value; Lu: Lutetium.

TABLE 5: Multivariable Cox regression analysis for progression-free survival.

Variable	Adjusted HR	95% CI	p-value
Age (per 1-year increase)	1.033	0.997-1.070	0.072
Baseline PSA (per doubling, log2-transformed)	1.446	1.204-1.738	<0.001
Visceral metastasis (yes vs. no)	2.441	1.049-5.682	0.038
SUV _{max} (per 1-unit increase)	0.990	0.977-1.004	0.164

Bold values indicate statistically significant results (p<0.05).

CI: Confidence interval; HR: Hazard ratio; PSA: Prostate-specific antigen; SUV: Standardized uptake value.

DISCUSSION

The present study explored baseline predictors of PFS in a real-world cohort of patients with mCRPC treated with Lu-177 PSMA radioligand therapy. Two principal observations emerged from our analysis. First, elevated pretreatment PSA levels and visceral metastatic disease were consistently associated with an increased risk of progression. Second, these associations remained significant after multivariable adjustment, whereas conventional semiquantitative PSMA PET/CT parameters, including SUV_{max} and SUV_{mean} , did not provide independent prognostic information.

The survival outcomes observed in this cohort were comparable with those reported in major prospective trials and real-world studies evaluating Lu-177 PSMA therapy. Median PFS and OS were similar to the ranges described in the VISION trial and subsequent observational series, supporting the representativeness of our patient population.^{3,4,9,15} Although randomized clinical trials have established the efficacy of Lu-177 PSMA, observational studies remain essential because they reflect the broader spectrum of patients encountered in routine oncology practice. Consequently, real-world evidence plays an important role in evaluating prognostic factors that may influence treatment outcomes outside the controlled setting of clinical trials.

Among all baseline variables evaluated, serum PSA demonstrated the strongest association with disease progression. Patients with higher pre-treatment PSA concentrations had significantly shorter PFS, and this relationship persisted after adjustment for potential confounding variables. Elevated PSA is widely regarded as a surrogate marker of tumor burden and biological aggressiveness, which may partly explain the observed association. Previous investigations have similarly identified pretreatment PSA as an adverse prognostic factor in patients receiving Lu-177 PSMA, although the magnitude of its impact has varied among published cohorts because of differences in study design, patient selection, and therapeutic sequencing.^{7,9-11} Our findings support the potential clinical usefulness of baseline PSA as a simple and readily available biomarker that may assist pretreatment risk assessment.

Our results also extend previous observations regarding PSA-based prognostication. Whereas Soydal et al. demonstrated that PSA response after treatment was associated with improved clinical outcomes, the present study focused exclusively on pretreatment characteristics. The independent association between baseline PSA and PFS suggests that serum PSA may provide prognostic information before therapy is initiated, complementing its established role as a marker of treatment response.¹⁶

Visceral metastasis was also independently associated with shorter PFS. Patients with visceral involvement experienced substantially shorter PFS than those without visceral disease. This finding is consistent with the recognized adverse biological behavior of visceral metastatic spread in mCRPC and agrees with previous reports identifying visceral involvement as an indicator of poor prognosis in patients undergoing Lu-177 PSMA therapy.^{7,8,11} The persistence of this association after multivariable adjustment suggests its potential clinical relevance during pretreatment evaluation.

Unlike the clinical variables evaluated in this study, neither baseline SUV_{max} nor SUV_{mean} was independently associated with PFS. Although PSMA PET/CT is indispensable for confirming adequate PSMA expression and selecting candidates for radioligand therapy, the prognostic relevance of conventional semiquantitative uptake parameters remains uncertain. Previous studies have produced inconsistent findings, with some reporting a relationship between higher tracer uptake and favorable treatment outcomes, whereas others failed to demonstrate an independent association after adjustment for established clinical variables.^{10,12,13} Differences in patient selection, imaging protocols, lesion measurement strategies, treatment timing, and study endpoints likely contribute to these conflicting observations. In this cohort, conventional SUV-based measurements alone were not significantly associated with PFS.

Several aspects of the present study strengthen its clinical relevance. All patients were treated at a single tertiary referral center following a relatively consistent therapeutic approach, and baseline PSMA PET/CT scans were available for every patient before treatment initiation. In addition, the concordance between the Kaplan-Meier survival analyses and the Cox regression models supports the robustness of the observed associations for baseline PSA and visceral metastatic disease.

Study Limitations

The findings of this study should, however, be interpreted in light of several limitations. The single-center setting may limit generalizability, and residual confounding by unmeasured clinical factors cannot be excluded. No internal or external validation was performed; therefore, the multivariable findings should be considered exploratory. The retrospective design introduces the possibility of selection bias, and the relatively limited sample size may have reduced the statistical power to detect weaker associations. Although multivariable analysis was performed, the number of available events limited the complexity of the regression model and precluded inclusion of a larger set of covariates. Furthermore, only conventional semiquantitative PET parameters (SUV_{max} and

SUV_{mean}) were evaluated. Because SUV_{max} and SUV_{mean} were derived from a single target lesion, they may not reflect whole-body tumor burden. More comprehensive volumetric biomarkers, including PSMA-derived tumor volume and total lesion PSMA, were not available; they may provide additional prognostic information. Treatment-related variables, such as PSA response during therapy and serial imaging assessments, were intentionally excluded because the primary objective was to identify prognostic factors available before the initiation of Lu-177 PSMA radioligand therapy. In addition, heterogeneity in post-progression treatments may have influenced OS. Furthermore, SUV measurements may be affected by differences in image acquisition and reconstruction protocols, which may limit the generalizability of PET-derived parameters.

CONCLUSION

Pre-treatment PSA level and the presence of visceral metastases were independently associated with shorter PFS in patients with mCRPC receiving Lu-177 PSMA radioligand therapy. By contrast, baseline SUV_{max} and SUV_{mean} were not significantly associated with PFS. These exploratory findings suggest that simple clinical variables available before treatment initiation may contribute to risk stratification. Prospective multicenter studies incorporating larger patient populations and advanced PSMA PET-derived volumetric biomarkers are warranted to further validate these observations.

Ethics

Ethics Committee Approval: The study protocol was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval number: E.895531, date: 01.07.2026)

Informed Consent: The requirement for written informed consent was waived due to the retrospective design of the study.

Footnotes

Authorship Contributions

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

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Prognostic Value of Inflammation-based Biomarkers in Patients with *De Novo* Metastatic Biliary Tract Cancer

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ABSTRACT

Objective: Biliary tract cancers (BTCs) are highly aggressive malignancies associated with poor clinical outcomes; metastatic disease is already present at diagnosis in many patients. We investigated whether baseline inflammation-based biomarkers could provide prognostic information in patients with *de novo* metastatic BTC.

Material and Methods: This retrospective study included 70 patients with BTC who were treated with initial chemotherapy for *de novo* metastatic disease between January 2013 and December 2024. Clinical features and laboratory parameters at the time of metastatic diagnosis were obtained. Inflammation-based biomarkers, namely neutrophil-to-lymphocyte ratio, systemic immune-inflammation index, hemoglobin-albumin-lymphocyte-platelet (HALP) score, lactate dehydrogenase, and C-reactive protein (CRP), were evaluated. Overall survival (OS) was used as the primary outcome to evaluate the prognostic significance of baseline inflammation-based biomarkers.

Results: The cohort was followed for a median of 10.8 months, during which the median progression-free survival was 6.6 months [95% confidence interval (CI): 5.1-8.1] and the median OS was 10.8 months (95% CI: 7.4-14.1). In multivariable analysis, elevated CRP [hazard ratio (HR): 9.187; $p < 0.001$], Eastern Cooperative Oncology Group performance status (ECOG-PS) 2 (HR: 2.439; $p = 0.022$), and low HALP score (HR: 3.091; $p < 0.001$) were independently associated with worse OS. Stratification by HALP score demonstrated a significant difference in survival, with median OS of 18.6 months among patients with high HALP, compared with 5.9 months among those with low HALP.

Conclusion: Poor ECOG-PS, elevated CRP, and low HALP score were independently related to worse survival in *de novo* metastatic BTC. These widely accessible and inexpensive parameters may provide a practical approach for identifying high-risk patients at baseline and improving prognostic stratification in routine clinical practice.

Keywords: Biliary tract cancer; prognosis; inflammation-based biomarkers; HALP score; C-reactive protein; overall survival

INTRODUCTION

Biliary tract cancers (BTCs), primarily cholangiocarcinoma and gallbladder carcinoma, are relatively rare malignancies associated with poor clinical outcomes. Although BTC is relatively uncommon, the incidence of this disease, particularly intrahepatic cholangiocarcinoma, has increased over time. Because early-stage BTC often progresses with subtle and non-specific clinical manifestations, many patients are diagnosed with metastatic disease at presentation, rendering curative treatment infeasible.^{1,2}

For patients diagnosed with *de novo* metastatic BTC, systemic chemotherapy remains the cornerstone of therapeutic management. For patients with advanced BTC, gemcitabine-based platinum combinations have remained the cornerstone of first-line systemic treatment for many years. More recently, the incorporation of immunotherapy into frontline treatment has led to incremental improvements in survival outcomes. Despite these therapeutic advances, the prognosis remains poor, with median overall survival (OS) rarely exceeding one year in real-world settings. Given the substantial clinical heterogeneity among patients, identifying readily available

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prognostic biomarkers may improve baseline risk assessment and help identify those with an unfavorable prognosis.^{3,4}

Accumulating evidence indicates that the crosstalk between tumor cells and the host inflammatory environment contributes to tumor growth, angiogenesis, and metastatic dissemination. In addition, systemic inflammatory responses contribute to immune dysregulation, malnutrition, and cancer cachexia, all of which may adversely affect treatment tolerance and survival outcomes. Over the past decade, readily available laboratory-derived inflammatory markers have increasingly been investigated as inexpensive tools for prognostic assessment in multiple solid tumors. The neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SII), hemoglobin-albumin-lymphocyte-platelet (HALP) score, C-reactive protein (CRP), and lactate dehydrogenase (LDH) are all easily obtainable and may provide clinically relevant prognostic information.⁴⁻⁷

Inflammatory biomarkers are correlated with unfavorable survival across various gastrointestinal malignancies, particularly pancreatic, colorectal, and hepatobiliary cancers.^{7,8} In BTC, the prognostic significance of inflammation-based biomarkers remains limited and heterogeneous, particularly in real-world cohorts comprising patients with newly diagnosed metastatic BTC who started systemic treatment in the first-line setting. Although interest in these biomarkers has increased in recent years, their prognostic role in this particular clinical context has not been fully established.^{5,9-11}

We assessed, in patients with *de novo* metastatic BTC who initiated first-line systemic chemotherapy, the prognostic utility of baseline inflammation-based biomarkers, including NLR, SII, HALP, CRP, and LDH, measured at the time of metastatic diagnosis, with respect to OS.

MATERIAL AND METHODS

Study Cohort

The retrospective analysis included patients with *de novo* metastatic BTC who received first-line systemic chemotherapy between January 2013 and December 2024. Baseline demographic, clinical, and laboratory data at the time of metastatic diagnosis were extracted from institutional medical records.

The study included adults (≥ 18 years) with histologically confirmed BTC, including cholangiocarcinoma and gallbladder carcinoma, who had radiologically confirmed *de novo* metastatic disease at initial diagnosis and initiated first-line chemotherapy.

Patients with recurrent metastatic disease following prior curative-intent treatment, non-metastatic disease, active

systemic infection at baseline, or patients with an Eastern Cooperative Oncology Group performance status (ECOG-PS) ≥ 3 were considered ineligible. Tumor burden was assessed radiologically at baseline and during follow-up by serial computed tomography (CT) or positron emission tomography/CT examinations. Tumor response was evaluated using RECIST v1.1.

OS was the primary study endpoint, defined as the time from the date of metastatic diagnosis to death from any cause or to last follow-up. Progression-free survival (PFS) was defined as the time from initiation of first-line systemic treatment to the date of radiological or clinical disease progression, death from any cause, or the final follow-up, whichever came first. The study was conducted in accordance with the Declaration of Helsinki and was approved by the University of Health Sciences Türkiye, Haydarpaşa Numune Research and Training Hospital Non-Interventional Clinical Research Ethics Committee (approval number: HNEAH-GOEAK/KK/2026/97, date: 28.04.2026).

Inflammation-based Biomarkers

Baseline laboratory parameters obtained within 30 days before the initiation of first-line chemotherapy were used to calculate inflammation-based biomarkers. When multiple measurements were available within this period, the measurement closest to treatment initiation was selected. NLR was determined as the absolute neutrophil count divided by the absolute lymphocyte count. SII was derived as platelet count $\times$ neutrophil count / lymphocyte count, whereas HALP was calculated as hemoglobin $\times$ albumin $\times$ lymphocyte count / platelet count. Baseline CRP (mg/L) and LDH (U/L) levels were also analyzed as individual inflammatory biomarkers. Lymphocyte, neutrophil, and platelet counts were expressed as $\times 10^3/\mu\text{L}$, whereas hemoglobin and albumin levels were reported in g/dL.

Statistical Analysis

Data analyses were conducted using the IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequencies and percentages, whereas continuous variables were summarized as mean $\pm$ standard deviation or median (range), as appropriate. Data distribution was assessed using the Kolmogorov-Smirnov test. Comparisons between categorical variables were performed using the Pearson's chi-square (χ^2) test. Continuous laboratory variables, including NLR, SII, HALP, CRP, and LDH, were dichotomized at their median values. Carcinoembryonic antigen and carbohydrate antigen 19-9 were categorized as normal or abnormal based on institutional laboratory reference ranges. OS was estimated using the Kaplan-Meier

method and compared between groups using the log-rank test. Factors associated with OS were evaluated using univariate and multivariable Cox proportional hazards regression models. Variables with $p < 0.10$ in univariate analysis were added to the multivariable model. A two-sided p -value < 0.05 was considered significant.

RESULTS

The cohort included 70 patients with *de novo* metastatic BTC. The median follow-up duration was 10.8 months (5.8-19.1 months). Men accounted for 52.9% of the study population, whereas women comprised 47.1%, with a mean age of 61.9 ± 9.9 years. The majority of tumors were cholangiocarcinomas (80%), while gallbladder carcinomas accounted for 20% of cases. A favorable performance status was observed in most patients: 70% had an ECOG-PS of 0-1, and 30% had an ECOG-PS of 2. The highest frequency of metastatic involvement was observed in the liver (70%), followed by peritoneal metastases (22.9%), bone metastases (22.9%), and lung metastases (18.6%). Most patients (60%) had metastatic involvement of a single organ, whereas 40% had metastases involving more than one organ. Regarding treatment for metastatic disease, cisplatin plus gemcitabine was the most frequently administered first-line regimen (58.6%), whereas 41.4% of patients received other chemotherapy regimens [gemcitabine 18 patients (25.7%), carboplatin + gemcitabine 3 patients (4.3%), capecitabine 3 patients (4.3%), FOLFOX (leucovorin, 5-fluorouracil, and oxaliplatin) 2 patients (2.9%), gemcitabine + capecitabine 1 patient (1.4%), and gemcitabine + oxaliplatin 2 patients (2.9%)]. Table 1 presents the baseline clinicopathological data.

Partial responses were identified in 15 patients (21.4%), stable disease was observed in 27 patients (38.5%), and progressive disease was observed in 28 patients (40.0%); no complete responses were recorded in first-line treatment. The objective response rate and disease control rate were 21.4% and 59.9%, respectively. Median PFS was 6.6 months [95% confidence interval (CI): 5.1-8.1], with 6- and 12-month PFS estimates of 58.6% and 17.1%, respectively. Median OS was 10.8 months (95% CI: 7.4-14.1); estimated OS at 6, 12, and 24 months was 72.2%, 46.5%, and 16.7%, respectively. Severe treatment-related toxicities (grade ≥ 3) during first-line therapy were observed in 8 patients (11.4%). Of the study population, 29 patients (41.4%) received second-line systemic treatment, whereas only 13 (18.6%) received third-line therapy. In addition, 32 patients (45.7%) underwent palliative radiotherapy at any time during the disease course for symptom control or management of metastatic complications.

Baseline laboratory parameters and inflammation-based biomarkers at the time of metastatic diagnosis

are summarized in Table 2. In univariate Cox regression analysis, cholangiocarcinoma histology, ECOG-PS 2, presence of liver metastases, low HALP score (≤ 22.2), high SII (> 1060), high NLR (> 3.9), and elevated CRP (> 25 mg/L) were significantly associated with worse OS (Table 3). ECOG-PS 2 [hazard ratio (HR): 2.439; $p = 0.022$], low HALP score (HR: 3.091; $p < 0.001$), elevated CRP (HR: 9.187; $p < 0.001$) remained independent predictors of worse OS in multivariable analysis (Table 3).

Using the median HALP score (22.2) as the cut-off value, 35 patients (50%) were stratified into the low HALP group (≤ 22.2),

TABLE 1: Baseline patient characteristics.

Age (years, mean $\pm$ SD)	61.94 $\pm$ 9.91
Age	
≥ 65	34 (48.6%)
< 65	36 (51.4%)
Sex	
Male	37 (52.9%)
Female	33 (47.1%)
BMI (kg/m ² , mean $\pm$ SD)	27.37 $\pm$ 5.48
Primary site	
Cholangiocarcinoma	56 (80%)
Gallbladder	14 (20%)
ECOG-PS	
0-1	49 (70%)
2	21 (30%)
Liver metastases	
Yes	49 (70%)
No	21 (30%)
Lung metastases	
Yes	13 (18.6%)
No	57 (81.4%)
Bone metastases	
Yes	16 (22.9%)
No	54 (77.1%)
Peritoneal metastases	
Yes	16 (23%)
No	54 (77%)
Metastatic organ site	
1	42 (60%)
> 1	28 (40%)
First line treatment regimen	
Cisplatin + gemcitabine	41 (58.6%)
Others	29 (41.4%)
ECOG PS: Eastern Cooperative Oncology Group performance status; SD: Standard deviation; BMI: Body mass index.	

while the remaining 35 patients (50%) were categorized as the high HALP group (>22.2). Median OS was markedly longer in the high HALP cohort than in the low HALP cohort. Median OS was 18.6 months (95% CI: 12.9-24.3) in the high HALP cohort versus 5.9 months (95% CI: 3.9-8.0) in the low

HALP cohort ($p<0.001$). One- and two-year OS estimates in the high HALP cohort were 77.2% and 30%, respectively; in the low HALP cohort they were 15% and 3%. Figure 1 shows the Kaplan-Meier survival curves.

TABLE 2: Laboratory parameters and inflammation-based biomarkers at metastatic diagnosis.

Variables (unit)	Median	Minimum	Maximum
CEA (ng/mL)	4	1	1573
CA 19-9 (U/mL)	65	2	160500
LDH (U/L)	281	120	1639
CRP (mg/L)	25.5	1	139
Neutrophil ($10^3/\mu\text{L}$)	5580	1100	33000
Hemoglobin (g/dL)	11.75	7.30	16
Albumin (g/dL)	3.60	2.00	5.00
Lymphocyte ($10^3/\mu\text{L}$)	1550	410	5800
Platelet ($10^3/\mu\text{L}$)	270000	97000	841000
NLR	3.90	0.74	19.41
SII	1059	123.9	11619.08
HALP score	22.17	2.33	120.93

CRP: C-reactive protein; CEA: Carcinoembryonic antigen; LDH: Lactate dehydrogenase; CA 19-9: Cancer antigen 19-9; NLR: Neutrophil-to-lymphocyte ratio; HALP: Hemoglobin-albumin-lymphocyte-platelet; SII: Systemic immune-inflammation index.

TABLE 3: Univariate and multivariate Cox proportional analyses for overall survival.

Variables	Univariable analysis	p-value	Multivariable analysis	p-value
	HR (95% CI)		HR (95% CI)	
Male gender	1.238 (0.759-2.021)	$p=0.391$	-	-
Age	1.016 (0.991- 1.041)	$p=0.209$	-	-
Age ≥ 65 years	1.499 (0.916-2.454)	$p=0.107$	-	-
ECOG-PS 2	9.145 (4.524-18.485)	$p<0.001$	2.439 (1.068-5.574)	$p=0.022$
BMI ≥ 25 kg/m ²	1.458 (0.868-2.448)	$p=0.154$	-	-
Cholangiocarcinoma	1.920 (1.008-3.660)	$p=0.047$	1.213 (0.578-2.546)	$p=0.667$
Liver metastases	2.468 (1.348-4.518)	$p<0.001$	1.318 (0.691-2.472)	$p=0.415$
Lung metastases	1.430 (0.749-2.730)	$p=0.279$	-	-
Peritoneum metastases	1.141 (0.626-2.080)	$p=0.666$	-	-
Bone metastases	1.183 (0.651-2.150)	$p=0.580$	-	-
Metastatic organ site >1	1.378 (0.813-2.336)	$p=0.234$	-	-
First line treatment regimen others	1.148 (0.681-1.935)	$p=0.603$	-	-
CEA (abnormal)	1.446 (0.871-2.400)	$p=0.152$	-	-
CA 19-9 (abnormal)	1.479 (0.884- 2.476)	$p=0.136$	-	-
HALP score ≤ 22.2	3.862 (2.289-6.515)	$p<0.001$	3.091 (1.723-5.542)	$p<0.001$
SII >1060	1.896 (1.155-3.111)	$p=0.011$	1.298 (0.743-2.265)	$p=0.359$
NLR >3.9	2.525 (1.369-3.700)	$p=0.001$	0.814 (0.407-1.601)	$p=0.714$
CRP >25	14.92 (6.01-37.07)	$p<0.001$	9.187 (3.358-25.135)	$p<0.001$
LDH >281	1.543 (0.940-2.532)	$p=0.087$	1.062 (0.602-1.876)	$p=0.956$

Statistically significant p-values are shown in bold.

BMI: Body mass index; ECOG-PS: Eastern Cooperative Oncology Group performance status CRP: C-reactive protein; CEA: Carcinoembryonic antigen; LDH: Lactate dehydrogenase; CA 19-9: Cancer antigen 19-9; NLR: Neutrophil-to-lymphocyte ratio; HALP: Hemoglobin-albumin-lymphocyte-platelet; SII: Systemic immune-inflammation index.

CEA levels ≤ 5 ng/mL and CA 19-9 levels ≤ 37 U/mL were considered normal; values above these cut-offs were considered abnormal.

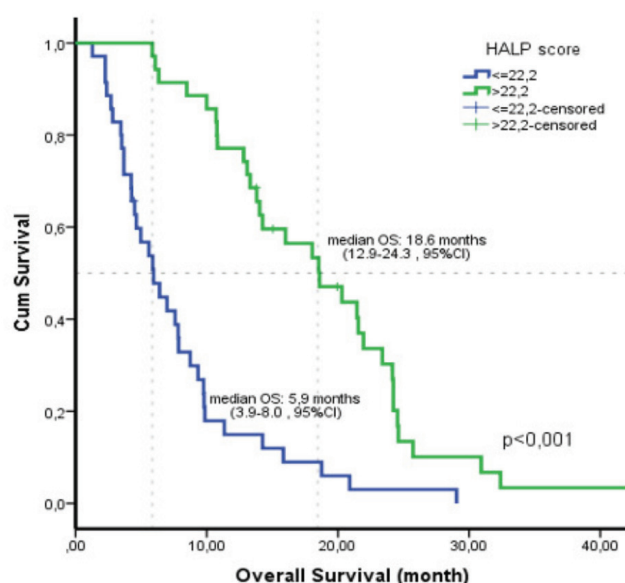


FIGURE 1: Kaplan-Meier analysis of overall survival stratified by baseline HALP score (≤ 22.2 vs. > 22.2).

HALP: Hemoglobin-albumin-lymphocyte-platelet; OS: Overall survival; CI: Confidence interval

DISCUSSION

This retrospective study identified baseline inflammation-based biomarkers and clinical characteristics as predictors of OS among patients with *de novo* metastatic BTC. Poor ECOG-PS, elevated CRP levels, and a low HALP score independently predicted poorer OS.

The survival outcomes observed in our cohort were comparable to those reported in previous real-world studies and landmark clinical trials. Median OS and PFS of 10.8 and 6.6 months closely resembled those reported in the landmark ABC-02 trial¹², in which first-line gemcitabine plus cisplatin resulted in median survival times of 11.7 months for OS and 8.0 months for PFS in patients with advanced BTC. More recently, the TOPAZ-1 trial¹³ showed that the addition of durvalumab to gemcitabine-cisplatin modestly improved survival, establishing chemoimmunotherapy as the preferred first-line treatment. Despite these therapeutic advances, survival outcomes remain poor and highly heterogeneous, highlighting the need for readily available prognostic biomarkers to improve baseline risk stratification.

Performance status is one of the most well-established prognostic factors in advanced BTC. In the present study, an ECOG-PS of 2 was associated with more than twice the risk of death compared with ECOG-PS 0-1 patients (HR: 2.44; $p=0.022$) and remained independently associated with worse OS in the multivariable model. Similar observations have

been noted by Kim et al.¹⁴, Bridgewater et al.¹⁵, and Valle et al.¹², all of which demonstrated the adverse prognostic impact of poor performance status in advanced BTC.

Among inflammation-based biomarkers, CRP was the most robust independent prognostic factor in the study population, and remained independently associated with worse OS after multivariable adjustment (HR: 9.19; 95% CI: 3.36-25.14; $p<0.001$). CRP is a well-established marker of systemic inflammation and reflects tumor-associated cytokine activation, particularly interleukin-6-mediated signaling, which has been implicated in tumor progression, cancer cachexia, and immune dysregulation. Previous studies in gastrointestinal malignancies have consistently demonstrated the adverse prognostic impact of elevated CRP, and our findings further support its prognostic value in patients with *de novo* metastatic BTC.^{8,16,17}

The HALP score demonstrated meaningful prognostic utility in our cohort. Patients with a low HALP score experienced markedly worse OS than those with a high HALP score, with an approximately threefold higher risk of death in the multivariable analysis. As a composite index integrating nutritional and inflammatory status, the HALP score reflects several biological processes associated with cancer progression. Its individual components—including anemia, hypoalbuminemia, lymphopenia, and thrombocytosis—have each been associated with impaired nutritional status, systemic inflammation, immune dysfunction, and tumor progression. The combined assessment of these parameters may therefore explain the strong prognostic discrimination observed in our cohort. Although evidence in BTC remains limited, previous studies in gastric, colorectal, pancreatic, and hepatocellular cancers have also demonstrated the prognostic utility of the HALP score.¹⁸⁻²¹

While NLR and SII were significant predictors in univariate analysis, their association with survival did not persist after multivariable adjustment. This finding suggests that their prognostic value may not be independent of other established clinical and inflammation-based prognostic factors. Similar inconsistencies have been described in previous BTC studies, where the prognostic impact of NLR and SII varied according to patient population, disease stage, and treatment setting.^{4,9,11,22,23} Our findings further support the notion that these biomarkers should be interpreted in conjunction with other established prognostic factors rather than in isolation.

Study Limitations

Several methodological limitations should be acknowledged. The retrospective nature of the analysis and the modest number of included patients may restrict the broader applicability of the results. Although most patients received

first-line cisplatin plus gemcitabine, heterogeneity in treatment regimens among the remaining patients may have influenced survival outcomes. Moreover, no patients received immune checkpoint inhibitors because these agents were not reimbursed during the study period; therefore, our findings primarily reflect the pre-immunotherapy era. Accordingly, the generalizability of our findings to current clinical practice, where chemoimmunotherapy is increasingly used in the first-line setting, may be limited and should be interpreted with caution. Finally, longitudinal changes in inflammation-based biomarkers during treatment were not evaluated; these changes may provide additional prognostic information. Prospective studies are warranted to validate these findings. Nevertheless, the present findings support the prognostic relevance of clinical and inflammatory parameters in *de novo* metastatic BTC. As readily available, inexpensive biomarkers, CRP, HALP, and ECOG-PS may facilitate baseline risk stratification and identify patients at higher risk in routine clinical practice. Prospective studies should evaluate whether these parameters can be integrated into routine clinical practice to support individualized treatment decisions.

CONCLUSION

Our findings suggest that poor ECOG performance status, elevated CRP, and a low HALP score are independent predictors of adverse survival in *de novo* metastatic BTC. Because these parameters are inexpensive and routinely available, they may serve as practical tools for baseline risk assessment and prognostic stratification in clinical practice. Prospective validations in larger cohorts are needed before their widespread clinical implementation.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, Haydarpaşa Numune Research and Training Hospital Non-Interventional Clinical Research Ethics Committee (approval number: HNEAH-GOEAK/KK/2026/97, date: 28.04.2026).

Informed Consent: Due to the retrospective nature of the study, the requirement for informed consent was waived.

Footnotes

Authorship Contributions

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

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Pretreatment Hemoglobin, Albumin, Lymphocyte, and Platelet Score Predicts Pathological Response and Postoperative Pathological Findings in Locally Advanced Gastric Cancer

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ABSTRACT

Objective: The hemoglobin, albumin, lymphocyte, and platelet (HALP) score has been extensively studied as a prognostic biomarker in various malignancies; however, its predictive value for pathological response to neoadjuvant chemotherapy remains unclear. This study aimed to evaluate the association between the pretreatment HALP score and tumor regression grade (TRG) in patients with locally advanced gastric cancer (LAGC).

Material and Methods: This retrospective single-center study included 65 patients with histologically confirmed LAGC who received neoadjuvant fluorouracil, leucovorin, oxaliplatin, and docetaxel chemotherapy followed by curative-intent surgery. The HALP score was calculated using baseline laboratory parameters obtained prior to treatment initiation. Pathological response was assessed using the Mandard TRG system. Receiver operating characteristic (ROC) analysis was used to determine the optimal HALP cut-off value. Logistic regression analysis was performed to identify predictors of treatment response.

Results: Among the 65 patients, 34 (52.3%) were classified as good responders and 31 (47.7%) as poor responders. The HALP score was significantly higher in good responders (46.3 ± 25.1 vs. 30.8 ± 20.0 ; $p=0.006$). ROC analysis demonstrated moderate discriminatory ability [area under the curve: 0.700; 95% confidence interval (CI): 0.570-0.830; $p=0.006$], with an optimal cut-off value of 38.6. Low HALP scores were significantly associated with poor response ($p=0.001$) and with more advanced pathological stages. In logistic regression analysis, HALP was the only significant predictor of treatment response (odds ratio: 0.16; 95% CI: 0.05-0.49; $p=0.001$).

Conclusion: The pretreatment HALP score is significantly associated with pathological response to neoadjuvant chemotherapy in LAGC. As a simple and accessible biomarker, HALP may aid in pre-treatment risk stratification and treatment decision-making. Prospective validation is warranted.

Keywords: Gastric cancer; neoadjuvant therapy; tumor regression grade; biomarkers; inflammation; nutritional status

INTRODUCTION

Gastric cancer (GC) remains the fifth most commonly diagnosed malignancy worldwide and the fifth leading cause of cancer-related mortality, with more than 968,000 new cases and approximately 660,000 deaths reported in 2022.¹ A substantial proportion of patients present with locally advanced disease at diagnosis, contributing to the persistently

poor prognosis of gastric adenocarcinoma. Although the addition of perioperative systemic therapy to curative-intent gastrectomy has significantly improved survival outcomes, treatment response remains markedly heterogeneous among patients.²

Perioperative chemotherapy has long been a cornerstone of standard treatment for locally advanced GC (LAGC). In recent

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years, the incorporation of immune checkpoint inhibitors into perioperative treatment strategies has been associated with improved pathological response rates and event-free survival.³⁻⁵ However, the integration of chemoimmunotherapy into routine clinical practice varies by country, reimbursement policy, and patient subgroup. Therefore, chemotherapy-based neoadjuvant approaches continue to play an important role in many clinical settings. Patients achieving pathological complete response after neoadjuvant therapy have consistently demonstrated significantly better survival outcomes compared with those with partial or no response.⁶ Accordingly, pathological response following neoadjuvant therapy is considered not only a treatment endpoint but also a strong prognostic indicator.

Despite its clinical importance, reliable, easily accessible, and cost-effective biomarkers that predict which patients are more likely to benefit from neoadjuvant treatment remain limited in routine clinical practice. In recent years, the impact of nutritional status and systemic inflammation on cancer biology, treatment response, and survival outcomes has become increasingly recognized.⁷ In this context, the hemoglobin, albumin, lymphocyte, and platelet (HALP) score has been proposed as a composite biomarker reflecting the host's immunonutritional status. The HALP score was first introduced by Chen et al.⁸ as a novel prognostic index for predicting postoperative survival in patients with GC. Subsequently, a large meta-analysis demonstrated that a low pretreatment HALP score was consistently associated with poorer survival outcomes across multiple cancer types.⁹

However, the existing literature on the HALP score has largely focused on its prognostic value in relation to survival outcomes, while its potential role as a predictor of treatment response has not been sufficiently investigated. In particular, data evaluating the association between HALP and tumor regression grade (TRG), an objective and standardized measure of pathological response following neoadjuvant therapy, remain scarce.¹⁰⁻¹² Given that TRG not only reflects treatment efficacy but is also closely associated with long-term oncological outcomes, identifying biomarkers that predict TRG prior to treatment is of considerable clinical relevance.

In the present study, we aimed to evaluate the predictive value of the baseline HALP score for pathological response to neoadjuvant fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT) chemotherapy in patients with LAGC. Specifically, we investigated the association of HALP with TRG and with postoperative pathological features to determine whether HALP may serve as a practical tool for pre-treatment risk stratification.

MATERIAL AND METHODS

Study Design and Patient Population

This retrospective single-center study included 65 patients with histologically confirmed locally advanced gastric adenocarcinoma who received four cycles of neoadjuvant FLOT chemotherapy at a tertiary referral center and subsequently underwent curative-intent gastrectomy between November 2023 and March 2025.

Locally advanced stage was defined as clinical stage $\geq$ cT2 and/or positive regional lymph nodes (cN+) without evidence of distant metastasis, according to the 8th edition of the American Joint Committee on Cancer staging system. Clinical staging was established using contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging of the chest, abdomen, and pelvis and endoscopic ultrasonography. Diagnostic staging laparoscopy with peritoneal cytology was not routinely performed before initiation of neoadjuvant chemotherapy. Treatment decisions were based on conventional clinical staging using contrast-enhanced imaging and endoscopic ultrasonography.

Patients whose baseline CT images were not available at the time of diagnosis, whose imaging quality was not adequate for staging evaluation, or in whom metastatic disease was detected intraoperatively were excluded from the study.

Patients were included if postoperative pathology reports, including TRG, were available for evaluation. Surgical procedures were performed at either University of Health Sciences Türkiye, İzmir City Hospital or external centers, provided that complete postoperative pathology reports were accessible for review.

Patients were excluded if baseline laboratory parameters required for HALP score calculation were not available within 7 days before initiation of neoadjuvant chemotherapy, if postoperative pathological response data were not available, or if they did not complete the planned four cycles of neoadjuvant FLOT chemotherapy prior to surgery.

The present study is a predefined subgroup analysis of patients receiving neoadjuvant FLOT chemotherapy within a larger retrospective cohort of patients with LAGC treated at our institution.

Ethical Approval

The study was approved by the University of Health Sciences Türkiye, İzmir City Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 2025/297, date: 18.06.2025) and was conducted in accordance with the Declaration of Helsinki. Due to the retrospective nature of the study, the requirement for individual informed consent was

waived. However, written informed consent for the use of clinical and pathological data for research purposes had been obtained from patients at the time of treatment according to institutional policy.

Treatment Protocol

All patients received four cycles of neoadjuvant FLOT chemotherapy prior to surgery. The FLOT regimen consisted of FLOT administered according to standard institutional protocols. Curative-intent gastrectomy with appropriate lymph node dissection was performed following completion of neoadjuvant treatment.

Laboratory Parameters and HALP Score Calculation

Baseline laboratory parameters used to calculate the HALP score were obtained within 7 days prior to the initiation of the first cycle of neoadjuvant chemotherapy. Laboratory values obtained after initiation of chemotherapy were not considered for analysis.

The HALP score was calculated using the following formula:

$$\text{HALP} = [\text{hemoglobin (g/L)} \times \text{albumin (g/L)} \times \text{lymphocyte count} (\times 10^9/\text{L})] / \text{platelet count} (\times 10^9/\text{L})$$

Pathological Response Assessment

Pathological response to neoadjuvant treatment was evaluated using the Mandard TRG system. TRG 1 indicates complete regression with no residual tumor cells; TRG 2 indicates rare residual tumor cells scattered through fibrosis; TRG 3 indicates residual tumor cells with fibrosis still predominating; TRG 4 indicates residual tumor outgrowing fibrosis; and TRG 5 indicates absence of regressive changes. For response analysis, patients were dichotomized into good responders (TRG 1-3) and poor responders (TRG 4-5) consistent with previously published studies evaluating pathological response following neoadjuvant therapy in GC.

Data Collection

Clinical, laboratory, and pathological data were retrospectively retrieved from electronic medical records. Collected variables included demographic characteristics, clinicopathological features, baseline laboratory parameters prior to neoadjuvant chemotherapy, treatment details, and postoperative pathological response findings.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA).

Continuous variables were expressed as mean $\pm$ standard deviation and median (interquartile range, if appropriate), while categorical variables were presented as frequencies

and percentages. The normality of continuous variables was assessed using the Shapiro-Wilk test. As most continuous variables were not normally distributed, comparisons between groups were performed using the Mann-Whitney U test. Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate.

The predictive performance of the HALP score for treatment response was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) and the corresponding 95% confidence intervals (CIs) were calculated. The optimal cut-off value for the HALP score was determined using the Youden index.

Patients were categorized into two groups based on the optimal HALP cut-off value, and subgroup comparisons were performed accordingly. Associations between HALP groups and clinicopathological variables, including TRG, ypT stage, ypN stage, lymphovascular invasion, and perineural invasion, were analyzed.

To identify predictors of treatment response, univariable logistic regression analysis was performed. Variables with potential clinical relevance, including HALP score, clinical T stage, clinical nodal status, histological subtype, and signet-ring cell component, were included in the model. Odds ratios (ORs) and 95% CIs were calculated. To assess the internal robustness of the logistic regression model, bootstrap resampling with 1,000 samples was performed and bias-corrected and accelerated (BCa) 95% CIs were calculated. A two-sided p-value of <0.05 was considered statistically significant.

RESULTS

A total of 65 patients with LAGC were included in the analysis, of whom 34 (52.3%) were classified as good responders (TRG 1-3) and 31 (47.7%) as poor responders (TRG 4-5). The median age of the study population was 63 years, and 66.2% of patients were male.

Baseline clinicopathological characteristics according to treatment response are presented in Table 1. No significant differences were observed between good and poor responders for age ($p=0.400$), gender ($p=0.796$), clinical T stage ($p=0.084$), clinical nodal status ($p=0.123$), clinical stage ($p=0.268$), histological subtype ($p=0.379$), signet-ring cell component ($p=0.116$), or mismatch repair status ($p=0.146$). However, the HALP score was significantly higher in good responders compared to poor responders (46.3 ± 25.1 vs. 30.8 ± 20.0 ; $p=0.006$).

ROC analysis demonstrated that the HALP score had a moderate ability to discriminate between good and poor responders, with an AUC of 0.700 (95% CI: 0.570-0.830;

TABLE 1: Baseline clinicopathological characteristics according to treatment response.				
Variables	All (n=65)	Good responders (n=34)	Poor responders (n=31)	p-value
Age, mean $\pm$ SD (median)	61.10 $\pm$ 9.95 (63)	60.08 $\pm$ 10.24 (61.5)	62.22 $\pm$ 9.67 (65)	0.400
Gender				0.796
Female	22 (33.8)	12 (35.3)	10 (32.2)	
Male	43 (66.2)	22 (64.7)	21 (67.8)	
cT Stage				0.084
cT2	10 (15.4)	8 (23.5)	2 (6.5)	
cT3	38 (58.5)	16 (47.1)	22 (71.0)	
cT4	17 (26.2)	10 (29.4)	7 (22.6)	
cN Stage				0.123
cN0	12 (18.5)	5 (14.7)	7 (22.6)	
cN1	31 (47.7)	21 (61.8)	10 (32.3)	
cN2	16 (24.6)	6 (17.6)	10 (32.3)	
cN3	6 (9.2)	2 (5.9)	4 (12.9)	
c-Stage				0.268
Stage 2	19 (29.2)	12 (35.3)	7 (22.6)	
Stage 3	33 (50.8)	14 (41.2)	19 (61.3)	
Stage IVa	13 (20.0)	8 (23.5)	5 (16.1)	
Tumor location				0.455
Gastric	57 (87.7)	28 (82.4)	29 (93.5)	
GEJ	8 (12.3)	6 (17.6)	2 (6.5)	
Histological subtype				0.379*
Adenocarcinoma	49 (75.4)	28 (82.4)	21 (67.7)	
Signet ring cell carcinoma	9 (13.8)	4 (11.8)	5 (16.1)	
Others	7 (10.8)	2 (5.9)	5 (16.1)	
Signet-ring cell component				0.116
Present	25 (38.5)	10 (29.4)	15 (48.4)	
Absent	40 (61.5)	24 (70.6)	16 (51.6)	
Mismatch repair status				0.146*
MSS	58 (89.2)	28 (82.4)	30 (96.8)	
Unknown	7 (10.8)	6 (17.6)	1 (3.2)	
HALP score, mean $\pm$ SD (median)	38.9 $\pm$ 23.9 (33.1)	46.3 $\pm$ 25.1 (40.6)	30.8 $\pm$ 20.0 (25.5)	0.006
Data are presented as mean $\pm$ standard deviation (median) or n (%). Continuous variables (age and HALP score) were compared using the Mann-Whitney U test. Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate.				
* Fisher's exact test was used where appropriate. GEJ: Gastroesophageal junction; SD: Standard deviation; MSS: Microsatellite stable; HALP: Hemoglobin, albumin, lymphocyte, and platelet.				

$p=0.006$) (Figure 1). The optimal cut-off value of 38.6 yielded a sensitivity of 64.7%, specificity of 77.4%, positive predictive value of 75.9%, negative predictive value of 66.7%, and an overall diagnostic accuracy of 70.8%.

Patients were subsequently stratified according to the HALP cut-off value (Table 2). A significantly higher proportion of good responders was observed in the high HALP group than in the low HALP group (75.9% vs. 33.3%; $p=0.001$). In addition, patients with low HALP scores had significantly

more advanced pathological tumor stage (ypT3-4: 83.3% vs. 48.3%; $p=0.003$) and nodal stage ($p=0.003$). A trend toward increased lymphovascular invasion was observed in the low HALP group, although this did not reach statistical significance ($p=0.054$). No significant differences were found in terms of type of surgery, extent of lymph node dissection, resection margin status, or perineural invasion.

In univariable logistic regression analysis, the HALP score was the only variable significantly associated with treatment

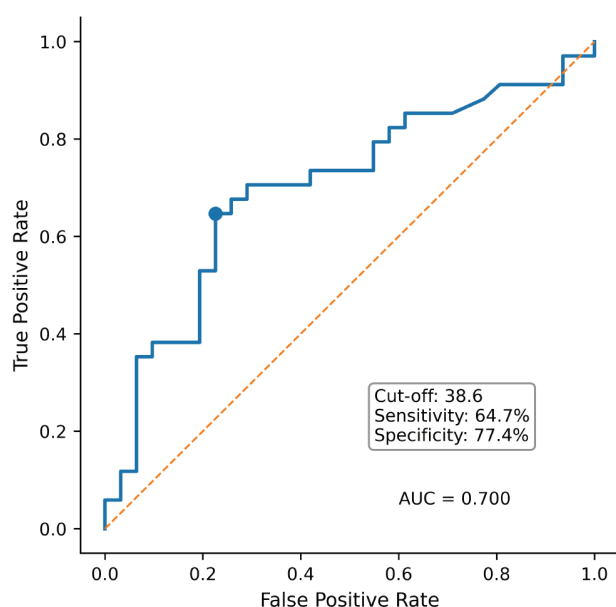


FIGURE 1. ROC curve of the HALP score for predicting treatment response based on TRG. The AUC was 0.700 (95% CI: 0.570-0.830; $p=0.006$). The optimal cut-off value (38.6) yielded a sensitivity of 64.7%, a specificity of 77.4%, a positive predictive value of 75.9%, a negative predictive value of 66.7%, and an overall diagnostic accuracy of 70.8%.

TRG: Tumor regression grade; AUC: Area under the curve; CI: Confidence interval; ROC: Receiver operating characteristic; HALP: Hemoglobin, albumin, lymphocyte, and platelet

response (Table 3). Patients with higher HALP scores had a significantly lower likelihood of poor response (OR: 0.16; 95% CI: 0.05-0.49; $p=0.001$). Other clinicopathological variables, including clinical T stage ($p=0.105$), clinical nodal status ($p=0.414$), histological subtype ($p=0.345$), and signet-ring cell component ($p=0.119$), were not significantly associated with treatment response. Internal validation using bootstrap resampling (1,000 samples) confirmed the robustness of the logistic regression model. The association between the HALP score and treatment response remained statistically significant after bootstrap validation (bootstrap coefficient: 1.838; bias: 0.066; BCa 95% CI: 0.802-3.173; $p=0.001$).

DISCUSSION

In the present study, we investigated the association between the HALP score, calculated at diagnosis, and pathological response to neoadjuvant chemotherapy in patients with LAGC. Our findings demonstrated that patients who did not respond to neoadjuvant treatment had significantly lower HALP scores and that the HALP score showed moderate discriminatory ability between good and poor responders. In addition, using an optimal cut-off value of 38.6 derived from ROC analysis, the HALP score effectively stratified patients by treatment response. Notably, in logistic regression analysis,

TABLE 2: Association between HALP score and clinicopathological features.

	HALP score <38.6 (n=36)	HALP score ≥38.6 (n=29)	p-value
TRG			0.001
Good response	12 (33.3)	22 (75.9)	
Poor response	24 (66.7)	7 (24.1)	
Type of surgery			0.156*
Subtotal gastrectomy	9 (25)	3 (10.3)	
Total gastrectomy	27 (75)	25 (86.2)	
Esophagectomy	0	1 (3.5)	
Extent of lymph node dissection			1.000
D1	2 (5.6)	2 (6.9)	
D2	34 (94.4)	27 (93.1)	
Resection margin status			0.447*
R0 (negative margin)	31 (86.1)	27 (93.1)	
R1 (microscopic residual disease)	5 (13.9)	2 (6.9)	
ypT stage			0.003
T0-2	6 (16.7)	15 (51.7)	
T3-4	30 (83.3)	14 (48.3)	
ypN stage			0.003*
N0	7 (19.4)	13 (44.8)	
N1	2 (5.6)	4 (13.8)	
N2	9 (25)	9 (31.0)	
N3	18 (50)	3 (10.3)	
Lymphovascular invasion			0.054
Present	22 (61.1)	9 (31.0)	
Absent	14 (38.9)	20 (69.0)	
Perineural invasion			0.283
Present	18 (50)	9 (31.0)	
Absent	18 (50)	20 (69.0)	

Data are presented as n (%). Comparisons between groups were performed using the χ^2 test or Fisher's exact test, as appropriate. The HALP cut-off value (38.6) was determined using receiver operating characteristic (ROC) curve analysis and the Youden index. TRG was assessed according to the Mandard classification, and patients were categorized as good responders (TRG 1-3) or poor responders (TRG 4-5).

ypT and ypN stages represent postoperative pathological tumor and nodal stages following neoadjuvant chemotherapy.

* Fisher's exact test was used where appropriate. TRG: Tumor regression grade; HALP: Hemoglobin, albumin, lymphocyte, and platelet.

the HALP score emerged as the only variable significantly associated with treatment response, highlighting its potential role as an independent predictive biomarker. These findings suggest that patients with low HALP scores may represent a subgroup at increased risk of poor pathological response and may therefore warrant closer clinical assessment. Prospective studies are needed to determine whether the HALP score could be used to guide individualized treatment strategies.

TABLE 3: Univariable logistic regression analysis for predictors of treatment response.			
Variable	OR	95% CI	p-value
HALP (<38.6 vs. ≥38.6)	0.159	0.052-0.492	0.001
HALP (continuous)	0.969	0.946-0.993	0.013
cT stage	1.427	0.921-2.211	0.105
cN stage	1.117	0.862-1.449	0.414
Signet-ring component	2.248	0.832-6.076	0.119
Histological subtype	1.401	0.701-2.800	0.345
OR: Odds ratio; CI: Confidence interval; HALP: Hemoglobin, albumin, lymphocyte, and platelet.			

Most of the existing literature on the HALP score has focused on postoperative survival outcomes rather than on treatment response. Indeed, previous studies and meta-analyses have consistently demonstrated that a low HALP score is associated with worse overall survival across multiple cancer types, including GC.^{8,10} However, data evaluating the relationship between HALP and pathological response to neoadjuvant therapy remain limited, particularly in patients with LAGC.^{11,12}

One of the distinguishing features of our study is the use of TRG as the primary endpoint. TRG represents an objective and standardized measure of pathological response following neoadjuvant therapy and has been shown to correlate strongly with both disease-free and overall survival.¹³ By directly evaluating the association between HALP and TRG, our study provides novel insight into the predictive role of HALP beyond its established prognostic significance. Furthermore, we demonstrated that a low HALP score was associated not only with poor treatment response but also with more advanced postoperative pathological features, including higher ypT and ypN stages, thereby supporting its biological relevance in tumor aggressiveness and treatment resistance.

Systemic inflammation and nutritional status are increasingly recognized as key determinants of tumor biology, treatment response, and survival in GC. Various inflammatory and nutritional indices, including the Glasgow prognostic score, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, prognostic nutritional index, and controlling nutritional status score, have been extensively investigated as prognostic markers.¹⁴⁻¹⁶ These indices provide indirect insight into host-tumor interactions by reflecting the balance between systemic inflammation and nutritional reserve.

The HALP score, integrating hemoglobin, albumin, lymphocyte count, and platelet count into a single composite index, captures multiple aspects of host immunonutritional status.⁸⁻¹⁰ Each of these components has well-established biological relevance in GC. Anemia and hypoalbuminemia reflect poor nutritional and systemic status and have been

associated with worse clinical outcomes.¹⁷⁻¹⁹ Lymphocytes play a central role in antitumor immunity and have been linked to both pathological response and survival in patients receiving neoadjuvant therapy.^{20,21} In contrast, platelets contribute to tumor progression and angiogenesis through the release of growth factors (GFs) such as transforming GF- β and platelet-derived GF, and thrombocytosis has been reported as an adverse prognostic factor.²²⁻²⁴ Therefore, a low HALP score may reflect an unfavorable host environment characterized by impaired immune surveillance, systemic inflammation, and poor nutritional status, which, in turn, may reduce sensitivity to cytotoxic therapy.

Another important issue in the literature is the variability in HALP cut-off values across studies. Reported cut-off values in GC range widely from 35.3 to 56.8, with a median of approximately 46.05.^{10,25-28} This variability likely reflects differences in patient populations, disease stages, and treatment settings. In our study, the optimal cut-off value of 38.6, determined by ROC analysis, falls within this range and appears to be clinically meaningful in distinguishing treatment response groups. This finding further supports the context-dependent nature of HALP and highlights the importance of cohort-specific validation.

Although studies specifically evaluating the predictive role of HALP in neoadjuvant settings are limited, available data are consistent with our findings. In a multicenter retrospective study by Köşeci et al.¹¹, a HALP cut-off of 28.9 was associated with significantly higher pathological response rates in patients receiving neoadjuvant FLOT chemotherapy, and a higher HALP score increased the likelihood of response by approximately 6.5-fold. In contrast, our study was conducted in a single-center cohort in which all patients received a standardized four-cycle neoadjuvant FLOT regimen and in which pathological response was assessed using the Mandard TRG system. Similarly, a recent single-center study reported that the HALP score was independently associated with neoadjuvant chemotherapy response in gastric and gastroesophageal junction adenocarcinoma.¹² In addition, we demonstrated that the HALP score was associated not only with pathological response but also with postoperative pathological stage (ypT and ypN). Our findings are consistent with these studies and further extend the evidence by demonstrating a clear association of HALP with TRG-defined response and with postoperative pathological characteristics.

Taken together, our results suggest that the HALP score, a simple and readily available biomarker, may serve as a practical tool for predicting response to neoadjuvant chemotherapy in patients with LAGC. However, given the variability in cut-off values and the influence of comorbid conditions on HALP

components, this index should not be used in isolation but rather be interpreted in conjunction with clinical staging and other pathological parameters. Prospective multicenter studies are needed to validate these findings and establish standardized cut-off values for broader clinical application.

Study Limitations

This study has several limitations. First, its retrospective single-center design may have introduced selection bias and may have limited the generalizability of the findings. Second, although bootstrap resampling supported the robustness of the model, the relatively small sample size remains a limitation. In addition, the HALP cut-off value was derived from the same study cohort and was not externally validated. Therefore, some degree of overfitting cannot be excluded, and the proposed cut-off should be interpreted with caution until confirmed in larger independent cohorts. Third, diagnostic staging laparoscopy was not routinely performed before neoadjuvant chemotherapy. As a result, occult peritoneal metastases could not be completely excluded and may have influenced treatment completion and pathological response in a small number of patients. In addition, a small proportion of patients had an unknown MMR status, which may have affected the interpretation of treatment response. Finally, important factors that may influence the HALP score, such as body mass index, baseline weight loss or cachexia, comorbidities, and other conditions affecting nutritional or inflammatory status, were not systematically evaluated. Despite these limitations, all patients received the same standardized four-cycle FLOT regimen, and pathological response was assessed using the Mandard TRG system, resulting in a relatively homogeneous study population.

CONCLUSION

In conclusion, our study demonstrates that the HALP score, a simple and readily accessible immunonutritional biomarker, is significantly associated with the pathological response to neoadjuvant chemotherapy in patients with LAGC. A low HALP score was associated with poor treatment response and more advanced postoperative pathological features, including higher ypT and ypN stages. Notably, the HALP score emerged as the only significant predictor of treatment response in logistic regression analysis.

Unlike most previous studies that have primarily focused on survival outcomes, our findings highlight the potential predictive value of the HALP score for TRG, an objective and clinically meaningful endpoint of neoadjuvant treatment efficacy. These results suggest that the HALP score may serve as a practical tool for pre-treatment risk stratification and help identify patients less likely to benefit from standard

neoadjuvant chemotherapy. Prospective multicenter studies are warranted to further validate these findings.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, İzmir City Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 2025/297, date: 18.06.2025) and was conducted in accordance with the Declaration of Helsinki.

Informed Consent: Due to the retrospective nature of the study, the requirement for individual informed consent was waived.

Footnotes

Author Contributions

Surgical and Medical Practices: Ö.K., A.A., Concept: Ö.K., H.T., Ö.Ö., Design: Ö.K., D.G., H.T., Ö.Ö., Data Collection or Processing: Ö.K., G.G., Analysis or Interpretation: Ö.K., D.G., Literature Search: Ö.K., Writing: Ö.K., G.G.

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Fourteen Years of Continuous Erlotinib Therapy in EGFR-mutant NSCLC: A Case Report of Isolated Cranial Progression Managed with Local Treatment

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ABSTRACT

Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have markedly improved outcomes in EGFR-mutant non-small cell lung cancer. However, prolonged use beyond 10 years, particularly in cases of isolated central nervous system (CNS) progression, has rarely been reported. We report the case of a 53-year-old male with stage IIA lung adenocarcinoma who underwent a right lower lobectomy and received adjuvant chemotherapy in 2011. One year after the initial diagnosis, cranial metastases were detected on neuroimaging. In light of this disease progression, molecular analysis for an EGFR mutation was performed using the primary tumor tissue sample. The analysis revealed an EGFR exon 19 deletion; erlotinib therapy (150 mg/day) was initiated after CNS metastases were detected. During a 14-year follow-up period, the patient experienced isolated intracranial progression, which was managed successfully with three courses of Gamma Knife radiosurgery (2013, 2019, 2023). During follow-up, liquid biopsies at each progression showed no resistance-associated EGFR mutations. Instead of altering the systemic therapy, local treatment was administered as an interim measure, while erlotinib was continued without interruption throughout the period. Notably, no evidence of extracranial disease was observed at any point. The patient has received uninterrupted erlotinib therapy for 14 years. While intracranial progressions were effectively managed with local treatments such as Gamma Knife radiosurgery, no evidence of extracranial (systemic) disease was observed throughout the follow-up period. As of the most recent assessment, the patient remains in remission, with stable disease on imaging and preserved performance status while receiving ongoing erlotinib treatment. This case highlights the potential for exceptionally durable disease control with long-term EGFR-TKI therapy in selected patients, especially when CNS-limited progression is effectively managed through local interventions. It also underscores the importance of individualized pharmacologic management and continuous reassessment in targeted therapy.

Keywords: Erlotinib; long-term survival; oligometastatic disease; EGFR-mutant NSCLC; Gamma Knife radiosurgery

INTRODUCTION

Globally, lung cancer is the most commonly diagnosed cancer type and the leading cause of cancer-related death.¹ Comprising about 85% of all diagnosed lung cancers, non-small cell lung cancer (NSCLC) represents the predominant form of the disease.² In NSCLC, treatment decisions are largely guided by molecular profiling of the tumor. Key actionable genetic alterations that influence targeted therapy selection include epidermal growth factor receptor (EGFR) mutations, *ALK* and *ROS1* rearrangements, *BRAF* V600E mutation, *KRAS*

G12C mutation, *MET* exon 14 skipping mutations, as well as *RET* and *NTRK* gene fusions.³ Activating mutations in the *EGFR* gene have been reported in approximately 16.6% of NSCLC cases.⁴ In NSCLC, EGFR mutations represent a distinct molecular subtype that predicts responsiveness to EGFR tyrosine kinase inhibitors (TKIs). Compared to conventional platinum-based chemotherapy, EGFR-TKIs significantly improve objective response rates, progression-free survival and quality of life in patients harboring sensitizing EGFR mutations, such as exon 19 deletions and *L858R* point mutations.⁵

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EGFR-TKIs are classified into three generations based on their mechanism of action. First-generation agents such as gefitinib and erlotinib reversibly bind to the adenosine triphosphate-binding site of the mutant EGFR. Second-generation TKIs, including afatinib and dacomitinib, irreversibly inhibit EGFR and other members of the ErbB family (e.g., *HER2*), providing broader and more durable inhibition. Third-generation TKIs, such as osimertinib, selectively target mutant EGFR, including the *T790M* resistance mutation. Although osimertinib is now the category 1 preferred first-line treatment,³ erlotinib was widely used for many years as a first-line option. In patients with sensitizing EGFR mutations, erlotinib demonstrated a median overall survival (OS) of approximately 22-24 months.⁶⁻⁹

Although the OS for patients with EGFR-mutant lung adenocarcinoma treated with erlotinib is around 2 years,^{7,8} some individuals can exhibit exceptional long-term responses and remain highly responsive to treatment. Here, we present a case of a patient with intracranial metastatic EGFR-mutant lung adenocarcinoma who has remained responsive for 14 years, with intermittent intracranial progressions effectively managed by local therapies.

CASE REPORT

A 53-year-old male patient, a heavy smoker, presented in January 2011 with a lesion in the right lower lobe of the lung. During the initial evaluation, a resectable mass was detected in the lung. The patient subsequently underwent a right lower lobectomy and mediastinal lymph node dissection. Pathological examination revealed a non-mucinous lung adenocarcinoma staged as pT1N1M0 (7th edition, Stage IIA). From April 26, 2011, to August 10, 2011, the patient received adjuvant chemotherapy with a cisplatin-vinorelbine regimen. After completing chemotherapy, the patient was placed on routine surveillance, which included contrast-enhanced thoracic and abdominal computed tomography every 3-6 months.

In September 2012, the patient developed new-onset headaches and focal neurological symptoms, which prompted further neuroimaging. Cranial imaging revealed multiple metastatic lesions; the largest measured approximately 12 mm and was located in the right cerebellar hemisphere. The patient subsequently underwent whole-brain radiotherapy. At the same time, molecular analysis of the primary tumor identified an EGFR exon 19 deletion by real-time polymerase chain reaction assay, prompting initiation of erlotinib therapy at a daily dose of 150 mg.

The patient was then followed with cranial magnetic resonance imaging (MRI) and thoracic and abdominal

computed tomography at 3-4 months intervals to evaluate brain metastases. While the disease remained stable during follow-up, a cranial MRI performed in September 2013 demonstrated an increase in the size of the lesion in the right half of the pons. The patient was therefore considered to have disease progression and subsequently underwent Gamma Knife radiosurgery. Erlotinib treatment was continued thereafter.

After the Gamma Knife procedure, the metastatic brain lesions in various regions remained stable until November 2017, when MRI showed new suspicious findings. These included gliotic lesions, with faint contrast enhancement and slight enlargement, in the right cerebellar hemisphere and the left posterior trigone. The patient was closely monitored until November 2019, when MRI revealed a partial increase in the size of metastatic nodules in the right pons, right cerebellar hemisphere, and left parietal lobe. A second Gamma Knife procedure was performed on November 19, 2019. Erlotinib treatment was continued at the same dose.

During follow-up from 2019 to 2023, the patient had stable intracranial disease. In early 2023, an increase in the size of the metastatic lesion in the left parietal region was noted, indicating progression. A third Gamma Knife procedure was performed in April 2023.

During follow-up, EGFR mutation analysis was repeatedly performed using peripheral blood-based liquid biopsy (circulating tumor deoxyribonucleic acid) at each episode of disease progression; no resistance-associated mutations, including EGFR *T790M*, were detected. Therefore, the patient has been on uninterrupted erlotinib therapy for 14 years. While intracranial progressions were managed with local treatments such as Gamma Knife radiosurgery, no evidence of extracranial (systemic) disease was observed throughout the entire follow-up period (Figures 1 and 2).

DISCUSSION

Advanced NSCLC is typically associated with an unfavorable prognosis, especially once metastatic or with brain involvement. Outcomes have substantially improved with the emergence of EGFR-TKIs for the subset of patients with EGFR-mutant tumors. Long-term survivors (beyond 5 years) remain relatively rare—one analysis estimated that only ~14.6% of EGFR-mutant advanced NSCLC patients remain progression-free at 5 years on TKI therapy.¹⁰ Our case, now 14 years in remission on continuous erlotinib, is an extraordinary example of durable disease control that far exceeds typical outcomes.

This case is particularly noteworthy given the presence of multiple adverse prognostic factors. The patient had a

significant smoking history, which is associated with poorer outcomes in EGFR-mutant metastatic NSCLC, nearly doubling the odds of 5 year mortality. Additionally, the development of brain metastases, another established negative prognostic factor, is typically associated with a 3-5-fold increased risk of death. Long-term survivors in large cohorts are more often never-smokers without central nervous system (CNS) involvement, making the prolonged remission observed in our patient especially unusual.¹¹

Comparable cases of exceptionally long survival with first-generation EGFR-TKIs are rare but have been reported. Go et al.¹² described a patient with EGFR exon 21 L858R-mutant lung adenocarcinoma who survived 24 years and remained on gefitinib for over 13 years. As in our case, postoperative

recurrence was managed with continuous EGFR-TKI therapy and local treatment of limited metastatic sites, resulting in sustained systemic disease control. However, whereas their patient had an exon 21 L858R mutation and received gefitinib amid prior chemotherapy and treatment interruptions, our patient harbored an exon 19 deletion and experienced 14 years of uninterrupted erlotinib therapy without resistance mutations, including *T790M*; progression was confined to intracranial oligometastases treated by repeated Gamma Knife radiosurgery. These observations support the role of sustained EGFR-TKI therapy combined with local ablative treatment in selected patients with oligometastatic or oligoprogressive EGFR-mutated NSCLC.

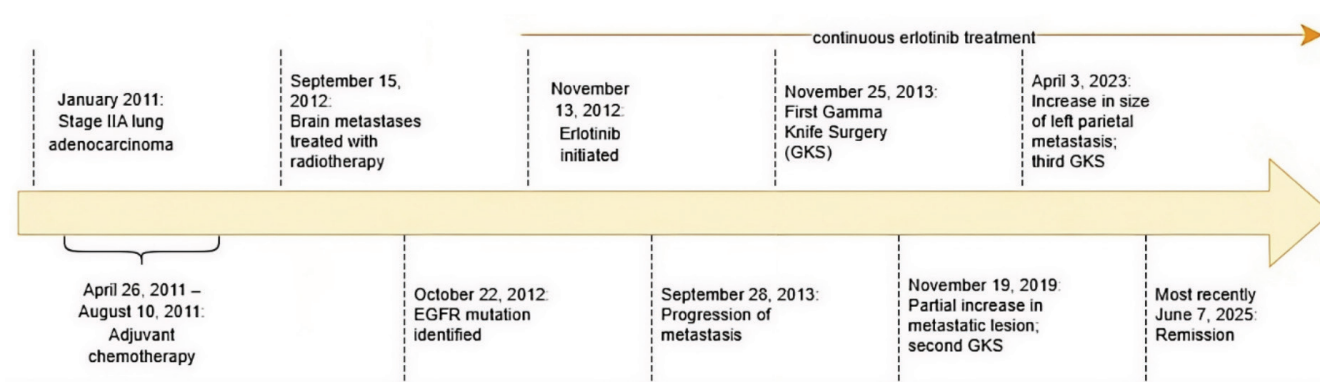


FIGURE 1. The treatment course of the patient.

EGFR: Epidermal growth factor receptor

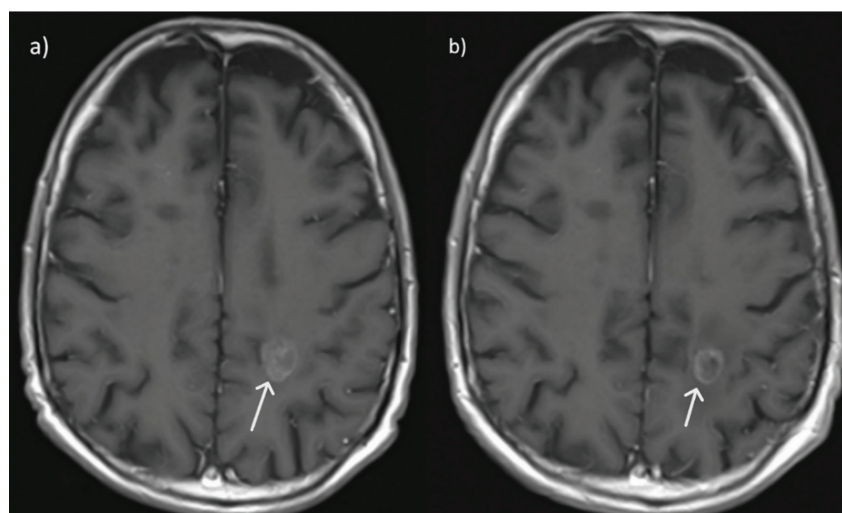


FIGURE 2. Pre- and post-treatment MRI images of the largest lesion among the patient's multiple metastases prior to the second Gamma Knife surgery in 2019 are shown a) the progressed hemorrhagic metastasis at the supraventricular level in the deep left parietal white matter (measuring 18×14 mm) is observed before the second GKS treatment in 2019. b) After the GKS treatment a regression in size has been observed (measuring 16×12 mm) of the previously mentioned metastasis accompanied by minimal residual signal changes in the peripheral edema region.

MRI: Magnetic resonance imaging; GKS: Gamma Knife surgery

Similar long-term survivors with EGFR exon 19 deletion treated with erlotinib have been reported in the literature. For example, Rab et al.¹³ described prolonged survival under erlotinib therapy; however, progression in their case was largely associated with treatment non-compliance and predominantly involved pulmonary disease, whereas our patient remained on uninterrupted erlotinib for 14 years and developed isolated intracranial oligoprogression successfully managed with repeated Gamma Knife radiosurgery. Likewise, Alkassis et al.¹⁰ reported exceptionally prolonged survival with first-generation EGFR-TKI therapy in a patient with an exon 19 deletion, although their patient achieved complete remission without CNS involvement. In contrast, our case demonstrates sustained long-term survival despite recurrent intracranial oligoprogression, highlighting the potential effectiveness of continued EGFR-TKI therapy combined with local treatment strategies.

Unusually long-term responses to erlotinib have also been reported outside the classical indications. Vatu et al.¹⁴ described prolonged disease stability exceeding eight years in a patient with CNS-metastatic stage IV NSCLC treated with second-line erlotinib, despite an unknown EGFR mutation status. While our patient's confirmed EGFR mutation provides a clear biological explanation for treatment sensitivity, such cases suggest that prolonged benefit from EGFR-directed therapy may occur in select non-classical settings. Additionally, both our case and the report by Vatu et al.¹⁴ describe well-managed brain oligometastases treated with Gamma Knife radiosurgery alongside continued EGFR-TKI treatment. In both cases, durable intracranial control was achieved while systemic disease remained stable.

Collectively, these reports place our patient among the longest-documented responders to EGFR-TKI therapy.

Although newer-generation TKIs such as osimertinib are now preferred due to superior CNS penetration,¹⁵ these agents were not available at the time of this patient's intracranial progression. The successful long-term outcome achieved with continued erlotinib and localized radiotherapy highlights the clinical value of maintaining effective TKIs when systemic disease remains controlled, even in the presence of CNS progression.

An additional distinctive feature of this case is the management of intracranial oligoprogression. Rather than switching systemic therapy, each episode of brain metastasis was treated with stereotactic radiosurgery, while erlotinib was continued. This strategy aligns with emerging evidence supporting the continuation of EGFR-TKIs beyond oligoprogression in combination with local ablative therapies.^{16,17} In a large cohort of EGFR-mutant NSCLC patients

treated with first-line TKIs, continuation of EGFR-TKI beyond oligoprogression together with local therapy was associated with prolonged survival, with a median OS of 37.4 months.¹⁷ Similarly, a prospective phase II study demonstrated that stereotactic radiosurgery for oligoprogressive lesions allowed continued erlotinib therapy, achieving a median progression-free survival of 6 months from the time of radiosurgery.¹⁸ In this context, our patient's prolonged systemic disease control on erlotinib, combined with repeated effective local treatment of CNS lesions, illustrates how sustained targeted therapy with focused eradication of resistant intracranial clones can delay changes in systemic therapy and contribute to exceptional long-term survival.

CONCLUSION

This case contributes to the limited but growing body of evidence that a subset of EGFR-mutant NSCLC can be managed as a chronic disease. The exceptional duration of response observed here likely reflects sustained EGFR dependence, uninterrupted TKI therapy with excellent adherence, absence of resistance-associated mutations, including *T790M*, and aggressive local management of CNS oligoprogression. While most patients ultimately develop resistance, understanding why a minority experiences extraordinarily prolonged responses remains an important area for future research. Additionally, defining the optimal duration of EGFR-TKI therapy in such exceptional responders represents an unresolved clinical challenge, as evidence guiding treatment discontinuation strategies is currently lacking.

Ethics

Informed Consent: Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.E., A.Ö., Concept: H.A., İ.E., A.Ö., Data Collection or Processing: H.A., Literature Search: H.A., Writing: H.A., İ.E.

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Dual ALK/ROS1 Positivity in Lung Adenocarcinoma: A Case of Complete Response to Lorlatinib

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ABSTRACT

Objective: Anaplastic lymphoma kinase (ALK) and ROS proto-oncogene 1 (ROS1) rearrangements are unique, mutually exclusive mutations that drive non-small cell lung cancer. Concurrent immunohistochemical positivity for both markers is exceedingly rare and may lead to diagnostic and therapeutic uncertainty, particularly when molecular confirmation is lacking.

Case Presentation: A 54-year-old woman presented with a solitary brain metastasis and underwent surgical resection, followed by whole-brain radiotherapy with a focal boost. Histopathology confirmed metastatic lung adenocarcinoma. Baseline staging with positron emission tomography-computed tomography (PET-CT) demonstrated a 20×19- mm hypermetabolic lingular lung nodule, mediastinal lymphadenopathy, and multiple sclerotic bone lesions. Immunohistochemistry of the tumor showed strong ALK (D5F3) and ROS1 (D4D6) expression, whereas deoxyribonucleic acid-based next-generation sequencing did not detect actionable rearrangements. Based on the immunophenotype, lorlatinib was initiated as first-line therapy. Serial PET-CT and brain magnetic resonance imaging demonstrated complete metabolic and radiologic remission of both intracranial and systemic disease, which has been sustained for nine months.

Conclusion: This case illustrates a rare discordance between immunohistochemical and molecular findings. In this individual patient, treatment with lorlatinib coincided with sustained systemic and intracranial disease control. These observations should be interpreted cautiously, but may provide practical insights for similar complex clinical scenarios.

Keywords: Lung adenocarcinoma; ALK; ROS1; lorlatinib

INTRODUCTION

Ranked as the second most commonly diagnosed cancer globally, lung cancer follows prostate cancer in men and breast cancer in women, and remains the top cause of cancer-related fatalities.¹ Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases. The identification of tumor-specific changes in cancer-related oncogenes, along with the introduction of techniques such as genetic sequencing, has enhanced our understanding of the etiopathogenesis of the disease and facilitated the development of targeted treatments.²

NSCLC is a prime example of precision oncology. Mutations in anaplastic lymphoma kinase (ALK) and ROS proto-oncogene

1 (ROS1) rearrangements characterize unique oncogenic pathways that are susceptible to targeting with tyrosine kinase inhibitors (TKIs), such as crizotinib and lorlatinib.³ Although these alterations are relatively rare, appearing in approximately 4% and 2% of lung adenocarcinomas, respectively, they define clinically significant molecular subgroups with important therapeutic consequences.^{4,5}

ALK and ROS1 are often grouped together as key oncogenic drivers in lung cancer because of their similar evolutionary origins, structural likeness, and comparable activation processes through gene rearrangements.⁶ The first targeted agent, crizotinib,⁷ was initially developed as a MET inhibitor but later demonstrated remarkable clinical efficacy in ALK- and ROS1-positive tumors. Because resistance to crizotinib

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inevitably develops, second-generation inhibitors—ceritinib, alectinib (which lacks anti-ROS1 activity), and brigatinib—have been introduced and have significantly improved patient survival. More recently, lorlatinib has been developed as the most thorough and selective inhibitor in the ALK/ROS1 inhibitor category.⁸ Preclinical studies have demonstrated that lorlatinib exhibits highly targeted activity against these two tyrosine kinases and effectively reduces off-target toxicity. At the same time, it maintains a wide range of effectiveness against secondary resistance mutations and achieves remarkable penetration through the blood-brain barrier.⁹

The Ventana immunohistochemistry (IHC) platform has been approved by the United States (US) Food and Drug Administration for the detection of ALK and ROS1 expression. However, the US National Comprehensive Cancer Network guidelines recommend fluorescence *in situ* hybridization (FISH) as the “gold standard” method for NSCLC.¹⁰

These rearrangements are mutually exclusive and represent distinct molecular events in tumor development.¹¹ Nonetheless, there have been infrequent accounts of ALK and ROS1 proteins being co-expressed through IHC without the presence of dual fusion events in molecular testing.^{4,6} Such discrepancies might result from antibody cross-reactivity, variations within the tumor itself, or fusion partners that remain undetected by current sequencing methods because of their low threshold.⁶ In this report, we present a rare case of a patient with metastatic lung adenocarcinoma who exhibited both ALK and ROS1 positivity, yet showed negative results on next-generation sequencing (NGS) and experienced an extended complete response (CR) to lorlatinib.

CASE PRESENTATION

A 54-year-old woman with no history of smoking presented with progressive headaches and visual disturbances. Magnetic resonance imaging (MRI) of the brain revealed multiple contrast-enhancing intracranial lesions, including a dominant left parietal mass that prompted surgical resection at Ordu University Training and Research Hospital. Histopathological analysis revealed an adenocarcinoma, indicating metastatic cancer originating in the lungs (OEAH-B24-13161).

Staging positron emission tomography-computed tomography (PET-CT) demonstrated a 20×19 mm hypermetabolic nodule in the lingular segment of the left upper lobe, which was considered the primary lung lesion. Mildly fluorodeoxyglucose (FDG)-avid subaortic and left hilar lymph nodes were also present, with the largest measuring approximately 2 cm. In addition, several skeletal sites—including the humeral heads, scapula, T11 and L1 vertebrae,

and the left femoral head—showed low-grade FDG uptake (standardized uptake value: 3,3) consistent with suspected bone metastases.

Immunohistochemical analysis was performed on formalin-fixed paraffin-embedded tumor tissue using the Ventana platform. The tumor showed diffuse, strong (3+) cytoplasmic staining for TTF-1 and napsin A, supporting a pulmonary origin. Both ALK (clone D5F3) and ROS1 (clone D4D6) demonstrated diffuse, strong cytoplasmic positivity in the majority of tumor cells (>80%), with appropriate internal positive controls.

Molecular profiling was conducted using a deoxyribonucleic acid (DNA)-based targeted NGS panel covering common actionable alterations, including rearrangements of ALK and ROS1, and alterations in *EGFR*, *KRAS*, *BRAF*, *MET*, *RET*, and *NTRK*. No gene fusions or pathogenic mutations were detected. The estimated tumor cellularity was approximately 40%.

Additional orthogonal confirmation using ribonucleic acid (RNA)-based fusion assays or FISH could not be performed because of limited residual tissue. Although re-biopsy was discussed, the patient preferred to avoid additional invasive procedures. This should be acknowledged as a limitation of this case.

Based on the strong and diffuse ALK and ROS1 immunoreactivity and the overall clinicoradiologic profile, first-line systemic therapy with lorlatinib (100 mg once daily) was initiated, together with denosumab for bone protection.

Prior to systemic therapy, the patient had undergone surgical resection of the brain metastasis, followed by whole-brain radiotherapy (30 Gy in 10 fractions), with a focal boost to metastatic nodules (up to 38 Gy).

Treatment response was prospectively monitored every three months using contrast-enhanced brain MRI and whole-body FDG PET-CT. Baseline target lesions included the pulmonary nodule in the lingular segment of the left upper lobe (20×19 mm), subaortic and left hilar lymph nodes, and multiple metabolically active skeletal lesions (humeral heads, scapula, thoracolumbar vertebrae, and left femoral head).

At the first evaluation (3 months), PET-CT demonstrated complete metabolic resolution of all extracranial lesions, while brain MRI showed no residual or recurrent enhancing intracranial disease. Serial imaging at 6 and 9 months confirmed a sustained complete radiologic and metabolic response without evidence of progression.

The treatment was well tolerated, with only grade 1 hyperlipidemia and no grade ≥3 adverse events. Written informed consent was obtained from the patient for the

publication of this case report and any accompanying clinical information and images.

DISCUSSION

This case describes an uncommon diagnostic and therapeutic scenario: concurrent ALK and ROS1 positivity by IHC in a metastatic lung adenocarcinoma despite negative DNA-based NGS, followed by a sustained clinical response to lorlatinib. Although ALK and ROS1 rearrangements are typically considered mutually exclusive driver events, discordant results between protein expression and molecular testing have occasionally been reported.

IHC remains a practical and widely used screening method for both ALK and ROS1. However, interpretation can be challenging. False-positive staining may occur due to antibody cross-reactivity, background mucin, or non-specific cytoplasmic staining, whereas false negatives may result from low protein expression or technical factors. For this reason, current guidelines recommend confirmatory testing with molecular techniques when results are unexpected or clinically discordant.^{12,13}

Discordance between IHC and DNA-based NGS is increasingly recognized. DNA panels may fail to detect complex or rare fusion partners, intronic breakpoints, or low-abundance transcripts. Several studies have shown that RNA-based fusion assays can identify rearrangements missed by DNA sequencing and may provide higher sensitivity for gene fusion detection.¹⁴ A recent study by Ilić et al.¹⁵ found that RNA-based NGS outperformed both IHC and FISH in identifying ALK rearrangements, effectively clarifying numerous cases previously deemed negative by DNA-based methods. In this scenario, our case showing double ALK/ROS1 immunopositivity responding to lorlatinib despite negative NGS findings highlights the potential for biologically significant but latent fusions or protein-level activation that may not be detectable by standard DNA-NGS methods. Therefore, negative DNA-based results do not definitively rule out biologically relevant oncogenic activation.

In routine clinical practice, when strong and diffuse IHC positivity is observed but DNA-based testing is negative, additional steps are often considered. These include reassessment of tumor cellularity, repeat sampling when feasible, FISH, or RNA-based fusion analysis. In the present case, orthogonal testing could not be performed due to limited residual tissue and the patient's preference to avoid further invasive procedures, which represents a limitation of this report.¹⁶

Despite the absence of molecular confirmation, our patient experienced a durable systemic and intracranial response to

lorlatinib. Lorlatinib is known for its potent activity against both ALK and ROS1 alterations, and for its excellent central nervous system (CNS) penetration.⁹ While a single case cannot establish predictive value, this observation suggests that strong protein-level expression may, in selected situations, still reflect a therapeutically relevant target.¹⁷

Lorlatinib is a multitargeted TKI that effectively penetrates the brain and demonstrates strong activity against ALK and ROS1 rearrangements, including those with resistance mutations.^{18,19} In this scenario, the effectiveness of the drug, even without detectable fusion, reinforces the idea that activation at the protein level, rather than just gene rearrangement, can initiate oncogenesis.

The sustained response lasting over nine months (CR) aligns with the results of the CROWN and PROFILE 1014 studies, highlighting lorlatinib's effectiveness in managing both CNS-dominant and systemic conditions.⁹

Dual ALK/ROS1 expression remains exceedingly rare, with only a limited number of cases described in the literature. Reported outcomes have been heterogeneous, and standardized management strategies are lacking.¹⁵⁻¹⁷ Our findings should therefore be interpreted cautiously, but they may contribute to the growing recognition that multimodal diagnostic approaches—integrating histology, IHC, molecular testing, and clinical behavior—are essential for individualized treatment decisions.

CONCLUSION

Although dual ALK and ROS1 positivity in lung adenocarcinoma is rare, this case illustrates the potential clinical relevance of discordant immunohistochemical and molecular findings. In this patient, lorlatinib was associated with sustained systemic and intracranial disease control despite the absence of molecularly confirmed gene fusions. These observations should be interpreted cautiously, but may provide practical insights for managing similar complex clinical scenarios.

Ethics

Informed Consent: Written informed consent was obtained from the patient for the publication of this case report and any accompanying clinical information and images.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.T., Concept: A.T., Design: A.T., Data Collection or Processing: A.T., Analysis or Interpretation: N.Ö., Literature Search: N.Ö., Writing: N.Ö.

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