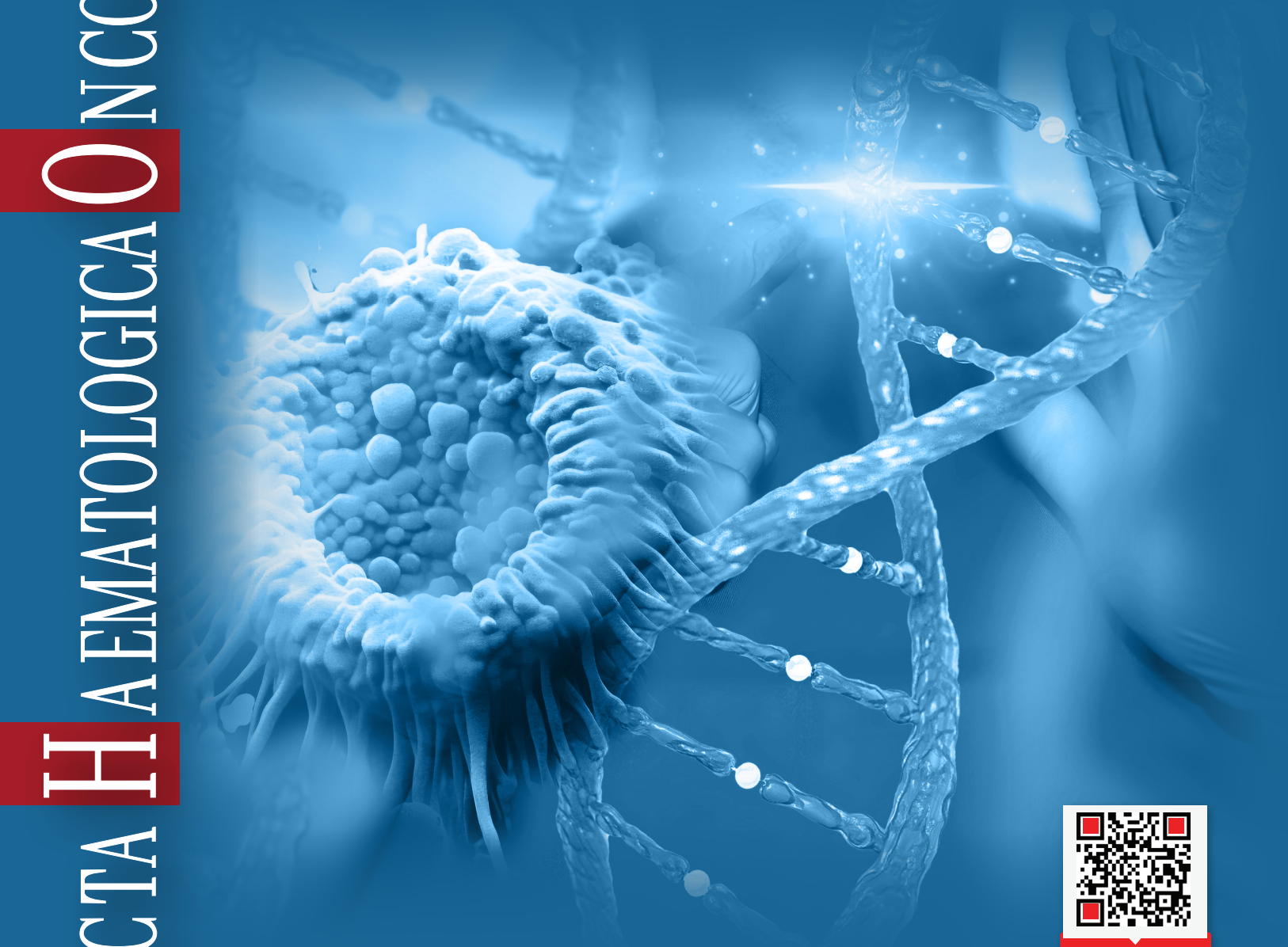
ACTA HAEMATOLOGICA ONCOLOGICA TURCICA

AHOT | Acta Haematologica Oncologica Turcica

E-ISSN: 3061-9947

2025

58 Volume | 3 Issue | December Month



galenos yayinevi

www.actaoncologicaturcica.com

AHOT | Acta Haematologica Oncologica Turcica

DECEMBER 2025

VOLUME 58 / ISSUE 3

EDITORIAL BOARD

AHOT | Acta Haematologica Oncologica Turcica

DECEMBER 2025

VOLUME 58 / ISSUE 3

Please refer to the journal's webpage (**https://www.actaoncologicaturcica.com/**) for "Journal Policy" and "Instructions to Authors".

The editorial and publication process of the Acta Haematologica Oncologica Turcica are shaped in accordance with the guidelines of the **ICMJE, WAME, CSE, COPE, EASE,** and **NISO**.

Acta Haematologica Oncologica Turcica is indexed in **Tübitak/Ulakbim TR Dizin, DOAJ, EBSCO, CNKI, SOBIAD, DRJI, WorldCat** and **ScienceGate**.

The journal is published online.

Owner: Fevzi Altuntaş on behalf of the Ankara Hematology Oncology Association

Responsible Manager: Assoc. Prof. Bahar UNCU ULU, MD

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1
34093 İstanbul, Türkiye
Phone: +90 (530) 177 30 97 / +90 (539) 307 32 03
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr
Publisher Certificate Number: 14521

Online Publishing Date: December 2025
E-ISSN: 3061-9947
International scientific journal published quarterly.

AHOT | Acta Haematologica Oncologica Turcica

DECEMBER 2025

VOLUME 58 / ISSUE 3

CONTENTS

Original Articles

AHOT | Acta Haematologica Oncologica Turcica

DECEMBER 2025

VOLUME 58 / ISSUE 3

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):156-161

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-2-1

Sentinel Lymph Node Study and Its Relationship with Molecular Profile in Breast Cancer After Neoadjuvant Therapy

Gülin Uçmak[1], Süleyman Aksu[1], Bedriye Büşra Demirel[1], Seda Gülbahar Ateş[2], Emine Benzer[3], Cihangir Özaslan[4]

[1]*University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Research and Training Hospital, Clinic of Nuclear Medicine, Ankara, Türkiye*
[2]*Gazi University Faculty of Medicine, Department of Nuclear Medicine, Ankara, Türkiye*
[3]*University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Research and Training Hospital, Clinic of Pathology, Ankara, Türkiye*
[4]*University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Research and Training Hospital, Clinic of Surgical Oncology, Ankara, Türkiye*

ABSTRACT

Aim: Lymphoscintigraphy (LS)/sentinel lymph node (SLN) study is one of the controversial issues in patients with breast cancer who have undergone neoadjuvant therapy (NAT). The aim of our study is to investigate the value of LS/SLN study after NAT and to evaluate its relationship with the molecular profile in breast cancer.

Methods: This retrospective study included 59 female patients diagnosed with breast cancer who received NAT. Tumor biopsy pathology results were recorded. Following NAT, LS was performed, and SLN biopsy (SLNB) was conducted intraoperatively using a dual-tracer technique (methylene blue and radionuclide). Axillary lymph node dissection (ALND) was performed in selected cases. Intraoperative frozen results and ALND findings were documented. Logistic regression analysis was performed to identify the factors predicting SLN positivity in the study.

Results: Among patients with SLN detected in the SLN study, 15 (28.8%) had positive results on intraoperative frozen pathology. A significant relationship was found between SLNB positivity and human epidermal growth factor receptor-2 (HER2) status (p=0.003). Both univariate and multivariate analyses conducted to identify the factors predicting SLNB positivity; HER2 negativity (p=0.004) was determined to be an independent risk factor for SLNB positivity. HER2 negativity increased the risk of SLNB positivity by a factor of 34.1.

Conclusion: In the HER2 negative group, LS/SLN study alone may be insufficient, and ALND should be considered in selected cases. On the other hand, it has been shown that LS/SLN study can avoid unnecessary ALND in patients with HER2 positivity.

Keywords: Breast cancer, sentinel lymph node, lymphoscintigraphy, neoadjuvant therapy

Introduction

The incidence of early-diagnosed breast cancer is increasing worldwide, including in our country, largely due to advances in diagnostic methods. According to 2020 data, breast cancer is the most commonly diagnosed cancer among women and ranks as the fifth cause of cancer-related deaths [1]. Breast cancer is a heterogeneous and complex disease, a spectrum of many subtypes with distinct molecular-biological features that lead to differences in response patterns to various treatment modalities [2]. For this reason, various diagnostic techniques and novel treatment modalities for breast cancer are continually being developed.

Locally advanced breast cancer (LABC) is a subset of breast cancer characterized by T3-4 tumours in the absence of distant metastasis with/without regional lymphadenopathy involvement [3]. Regional metastatic lymph nodes (MLN) are very important for the prognosis of LABC. In a retrospective study, it was demonstrated that LABC patients with 10 or more regional MLN have a poor prognosis [4].

Address for Correspondence: Süleyman Aksu MD, University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Research and Training Hospital, Clinic of Nuclear Medicine, Ankara, Türkiye

E-mail: suleymanaksu95@gmail.com **ORCID ID:** orcid.org/0000-0002-7146-393X

Received: 17.02.2025 **Accepted:** 19.08.2025 **Epub:** 09.09.2025 **Publication Date:** 12.12.2025

Cite this article as: Uçmak G, Aksu S, Demirel BB, Ateş SG, Benzer E, Özaslan C. Sentinel lymph node study and its relationship with molecular profile in breast cancer after neoadjuvant therapy. Acta Haematol Oncol Turc. 2025;58(3):156-161

Therefore, neoadjuvant therapy (NAT) in LABC aims to not only reduce the size of the primary tumor but also convert the regional lymph node-positive disease to a negative state. According to the current National Comprehensive Cancer Network guideline, sentinel lymph node biopsy (SLNB) may be considered after NAT for a selected group of patients. These are patients with clinically positive lymph nodes (cN+) at baseline that become cN0 after NAT. Highly selected patients with biopsy-proven axillary metastases, who convert to clinically node negative after preoperative systemic therapy, may undergo SLNB with removal of the clipped lymph node (category 2B recommendation) [5]. Although different treatment response rates are observed in tumors with different molecular profiles [estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and Ki-67 proliferation index], both in tumor and lymph nodes after NAT, an overall pathological complete response (pCR) rate of 40% has been observed [6,7]. In a study, it was found that patients with HER2 positive breast cancer exhibited the highest rates of breast-conserving surgery and pCR after NAT when compared to patients with other molecular profiles [8].

The sentinel lymph node (SLN) is defined as the first lymph node or group of nodes to which cancer cells are most likely to spread from the primary tumor via lymphatic channels [9]. SLNB is a reliable method for detecting metastatic disease in regional lymph nodes and avoiding patients the potential morbidity associated with axillary lymph node dissection (ALND). In the GANEA-2 study, breast cancer patients with negative SLN treated with NAT could safely be spared an unnecessary ALND after NAT with a low-risk of relapse [10]. SLNB can be effectively performed through a combination of preoperative lymphoscintigraphy (LS) and intraoperative methylene blue injection. The decision to proceed with ALND is typically based on frozen section results and intraoperative assessment [11].

Although it is known that tumors with different molecular profiles have varying rates of pCR after NAT, there is limited research on the role of SLN study and its relationship with the molecular profile after NAT. Our study aimed to investigate the role of LS/SLN study, and its relationship with the molecular profile in LABC patients after NAT.

Methods

This retrospective study was conducted according to the principles of the Declaration of Helsinki, and approval was obtained from the Ethics Committee of University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (decision no: 2022-02/46, date: 10.02.2022).

Fifty-nine female patients who were diagnosed with LABC through clinical-radiological and histopathological evaluations were included in this study.

The molecular profiles of all patients were documented and they underwent NAT (chemotherapy and/or immunotherapy) between January 2019 and December 2021. The demographic characteristics, clinical-radiological findings, results of axillary lymph node biopsy prior to NAT, and pre-NAT tumor biopsy pathology (grade, Ki-67 proliferation index, ER-PR, and HER2 expression status) of the patients were recorded. The patients received neoadjuvant treatment consisting of alkylating agents (cyclophosphamide), anthracycline derivatives (doxorubicin), and taxane group agents (paclitaxel or docetaxel) chemotherapeutic agents. In cases of HER2 positivity, monoclonal antibodies (trastuzumab or trastuzumab+pertuzumab) were added to the treatment regimen.

Lymphoscintigraphy

LS was performed in patients at least 2-3 hours before the surgery or on the day prior to the surgery to identify the SLN. For LS, 37 MBq of 99mTc-labeled nanocolloid was injected intradermally into four quadrants around the tumor or the periareolar area. Additionally, one deep injection was administered intraparenchymally or peritumorally. After the injections, dynamic imaging was performed in two planes (anterior and lateral) using a 64x64 matrix, with a frame duration of 30 seconds , and lasting a total of 15 minutes. Subsequently, static or planar imaging was performed in two planes using a 256x256 matrix. Additional images were acquired if necessary.

Sentinel Lymph Node Study

At the beginning of surgery, methylene blue was injected into the subareolar region. SLNB was performed using both methylene blue dye and a gamma probe. Excised SLNs were referred to the pathology department for frozen examination. Axillary dissection was performed in selected patients based on the MLN' frozen section examination results (SLNB positivity), in cases where the SLN could not be identified and/or intraoperative suspicion. Frozen/biopsy results, ALND outcomes, and pathological nodal (pN) stages of the patients were recorded.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). The conformity of numerical variables to the normal distribution was evaluated with the Kolmogorov-Smirnov test. Descriptive statistics in the analyses are presented as numbers and percentages; distribution statistics were expressed as mean, standard deviation, median, minimum, and maximum values. The Pearson chi-square test was used to evaluate whether there was a difference in clinicopathological features between SLNB positive/negative groups. The Youden index (sensitivity+specificity-1) was used in the receiver operating characteristic (ROC) analysis to determine the threshold value for primary tumor size and Ki-67 index. Logistic regression analysis was performed to determine the factors predicting SLNB positivity in the LS/SLN study. Logistic regression analysis was performed using the

forward method. An overall 5% type I error level was used to infer statistical significance ($p<0.05$).

Results

In our study, the mean age of the patients was 47.9±10.5 years (range 25-66). The clinical-pathological characteristics of the patients are presented in Table 1.

Eleven of the patients (18.6%) received injections around the lesion, while the remaining 48 (81.4%) received superficial and deep injections in the periareolar area. On LS, axillary drainage was observed in 34 patients (57.6%) on dynamic imaging. Focal activity involvement was observed in 40 patients (67.7%) in the early static images and in 12 patients (10.3%) in the late static images. SLN was detected in 52 (88.1%) patients, while it could not be identified in 7 (11.9%) patients either at LS or during operation. The average number of SLNs extracted was 4 (range 3-12). The SLNB results showed that 15 (28.8%) patients had positive SLN, while 37 (71.2%) patients had a negative SLN. ALND was performed in 31 (52.5%) patients. These were patients who had SLNB positivity or who could not have their SLN identified, or who had intraoperative suspicion. The rate of ALND was 23% in HER2 positive patients and 83% in ER positive/HER2 negative patients. Among the patients who underwent ALND, 19 (61.2%) had positive lymph nodes and 12 (38.8%) had negative lymph nodes. The pathological N stage was determined as pN0 in 35 (59.3%) patients, pN1 in 12 (20.3%) patients, pN2 in 10 (16.9%) patients, and pN3 in 2 (3.4%) patients.

A statistically significant association was found between SLNB positivity and HER2 receptor status ($p=0.003$). Among the 15 patients with positive SLN, only 1 (6.6%) patient was HER2 positive, 12 (80%) patients were ER positive/HER2 negative, and 2 (13.4%) patients were triple negative (Table 2).

ROC curve analysis revealed that Ki-67 proliferation indices have a statistically significant diagnostic value in predicting SLNB positivity (area under the curve: 0.650, 95% confidence interval: 0.486-0.815, $p=0.042$). A statistically significant difference was found between SLNB positivity and Ki-67 index with a cut-off of 55% ($p=0.034$) (Table 2). There was no statistically significant difference among SLNB positivity, tumor localization ($p=0.513$), tumor size ($p=0.404$), clinical axilla status ($p=0.097$), ER status ($p=0.198$), PR status ($p=0.070$), and tumor grade ($p=0.154$).

The results of the univariate and multivariate analyses conducted to identify factors predicting SLNB positivity are shown in Table 3. In the univariate analysis, a statistically

Table 1. Clinical-pathological feature

Clinical-pathological features			n (%)/Average±SD
Primary tumor size			29.6±12.2
	≤27 mm		33 (55.9%)
	>27 mm		26 (44.1%)
Localization of tumor	Upper-outer quadrant		35 (59.3%)
	Lower-outer quadrant		12 (20.2%)
	Upper-inner quadrant		5 (8.5%)
	Lower-inner quadrant		1 (1.7%)
	6 o'clock position		5 (8.5%)
	12 o'clock position		1 (1.7%)
Pathological features of primary tumors	Grade	1	3 (5.2%)
		2	16 (27.6%)
		3	39 (67.2%)
	ER positivity		45 (77.6%)
	PR positivity		38 (65.5%)
	HER2 positivity		21 (35.6%)
	Ki-67 proliferation index		45.5±23.7
		≤55%	40 (67.7%)
		>55%	19 (32.2%)
Axillary LN status before NAT	Clinical positive		19 (32.2%)
	Clinical negative-radiological positive		33 (56%)
	Clinical-radiological negative		7 (11.8%)
Result of axillary LN biopsy before NAT (n=20)	Positive		18 (90%)
	Negative		2 (10%)

n: The number of patients, LN: Lymph node, NAT: Neoadjuvant therapy, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor-2, SD: Standard deviation

significant association was found between SLNB positivity and patient age (p=0.027), HER2 negativity (p=0.013), and Ki-67 index ≤55% (p=0.047). Additionally, due to the p value of PR positivity being nearly 0.05, it was included in the multivariate analysis. In the multivariate analysis, advanced age, PR positivity, and HER2 negativity were identified as independent risk factors for predicting SLNB positivity. PR positivity increases the risk of SLNB positivity by approximately 6.6 times, while HER2 negativity increases the risk of SLNB positivity by 34.1 times (Table 3).

The result of ALND was positive in 6 of 7 patients whose SLN could not be detected in the SLN study. Moreover, the result of ALND was positive in 3 patients whose SLN biopsy was negative (false negative). In these 9 patients with positive ALND, it was found that 2 (22.2%) were HER2 positive, and 7 (77.8%) were ER positive/HER2 negative. In addition, 8 patients (88.8%) had a Ki-67 index ≤55%.

Discussion

The field of NAT in breast cancer is actively researched, and the literature is continually expanding with over 1,000 studies in the last year alone. As a result, our knowledge and insights about breast cancer are constantly evolving, with new discoveries and improvements being made every year. In certain cases of LABC, NAT can achieve pCR in the primary tumor and the axillary region. This successful response to NAT allows some patients to avoid ALND using the LS/SLN study and SLNB. However, in patients who do not achieve pCR, it can be challenging to detect the SLN and perform SLNB due to the potential blockage of lymphatic pathways by tumor cells. Although it is known that different pCR rates are seen in different molecular profiles after NAT, there are not enough published studies to provide definitive evidence regarding the relationship between the SLN study and the molecular profile.

Table 2. SLNB distribution according to HER2 receptor status and Ki-67 index

Negative		SLNB			p value (Chi-square test)
		Positive	Total		
HER2 receptor	Negative	18 (56.3%)	14 (43.7%)	32 (100%)	p=0.003
	Positive	19 (95%)	1 (5%)	20 (100%)	
Ki-67 index	≤55%	20 (60.6%)	13 (39.4%)	33 (100%)	p=0.034
	>55%	16 (88.9%)	2 (11.1%)	18 (100%)	

SLNB: Sentinel lymph node biopsy, HER2: Human epidermal growth factor receptor-2

Table 3. Factors predicting SLNB positivity in univariant and multivariant analysis

Clinical-pathological features	Univariant analysis			Multivariant analysis		
	OR	95% CI	p value	OR	95% CI	p value
Age	1.076	1.008-1.147	*p=0.027	1.087	1.007-1.172	p=0.032
Right/left breast			p=0.686			
Quadrant of the tumor			p=0.972			
Radiologic size of the tumor			p=0.302			
BIRADS			p=0.166			
Axillary LN status before NAT			p=0.999			
Results of axillary LN biopsy before NAT			p=0.999			
ER positivity			p=0.212			
PR positivity	3.579	0.861-14.871	**p=0.079	6.653	1.181-37.494	p=0.032
HER2 negativety	14.778	1.758-124.194	*p=0.013	34.154	3.171-367.832	p=0.04
Grade			p=0.261			
Ki-67 index (≤%55)	5.200	1.021-26.471	*p=0.047			
Pathologic size of the tumor, after NAT			p=0.825			

*p<0.05: Statistically significant parameters.
**It was included in the multivariate analysis because the p value was 0.05.
SLNB: Sentinel lymph node biopsy, OR: Odd ratio, CI: Confidence interval, BIRADS: Breast imaging reporting and data systems, LN: Lymph node, NAT: Neoadjuvant therapy, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epitelyal growth factor receptor-2

Furthermore, there are no recommendations associated with the molecular profile in the current SLNB guidelines. In our study, we investigated the value of LS/SLN study after NAT in breast cancer and its relationship with the molecular profile.

In breast cancer, several studies have demonstrated high rates of pCR in HER2 positive tumors after NAT, while lower pCR rates have been observed in ER positive/HER2 negative tumors [12-18]. In a recent study, the total pCR rate in HER2 positive and ER positive/HER2 negative profiles was 32.3% and 6.9%, respectively, while the ALND rate was 13.3% and 28.8%. The study concluded that SLNB can be performed before NAT in ER positive/HER2 negative tumors, while HER2 positive tumors show a good response to targeted therapy, and SLNB can be performed after NAT to avoid unnecessary morbidity associated with ALND [12]. In our study, we found that the rate of ALND was 23% in patients with HER2 positive breast cancer, while it was 83% in patients with ER positive/HER2 negative breast cancer. We observed low rates of pCR and high rates of SLNB positivity in the ER positive/HER2 negative patient group. Conversely, we found high rates of pCR and SLNB negativity in the HER2 positive patient group. Furthermore, it was noted that HER2 negativity increased the risk of SLNB positivity by approximately 34.1 times.

In our study, we found that PR positivity was an independent risk factor for SLNB positivity, increasing the likelihood of SLNB positivity by 6.6 times. Davey et al. [19] reported pCR rates of 10.1% in patients with a PR positive profile and 18% in patients with a PR negative profile. Other studies have also observed that PR negativity is associated with higher pCR rates in the ER positive/HER2 negative patient group [17,20,21]. In PR positivity, (considering the increased probability of SLNB positivity and the lower pCR rates after NAT), ALND should not be disregarded in selected patients.

In our study, we found a significant correlation between Ki-67 index ≤55% and SLNB positivity, with the majority of false negatives observed in patients with Ki-67 index ≤55%. Wang et al. [22] demonstrated that tumors with a high Ki-67 index (≥14%) had a higher likelihood of achieving pCR compared to those with a low Ki-67 index (<14%). In another study, Ki-67 index <50% predicted higher risk of residual lymph node disease [18]. Based on our study findings, it was suggested that an LS/SLN study should be performed in patients with a Ki-67 index ≤55%, and ALND should be considered even if SLNB results are negative.

In elderly patients, visualizing the SLN in LS can be challenging due to potentially slower lymphatic drainage. Studies have indeed demonstrated a lower rate of SLN detection in advanced-age patients [23-25]. In our study, we found that SLNB positivity increased with patients' age. Another study highlighted the importance of individualizing SLNB in patients over 70 years of age, considering that axillary staging may not always be beneficial for survival. However, if performed, SLNB can help improve local control, provide prognostic information, and guide decisions regarding adjuvant therapies like chemo-radiotherapy [26]. Therefore, even though it can be difficult to detect the SLN in advanced age patients, SLNB is believed to have potential benefits for patient management where it may impact survival outcomes.

Study Limitations

Several limitations should be considered in our study. First, this study was a retrospective analysis, and it was limited by the small size of our study population. Secondly, not perform axillary dissection to all patients. Thirdly, due to the absence of follow-up data for the patients, the long-term impact of the study remains unknown.

Conclusion

In conclusion, our study demonstrated that SLNB may not reduce the need for ALND in patients with an ER+/HER2 negative profile. However, in patients with a HER2 positive profile, SLNB can potentially reduce unnecessary ALND. Further prospective studies with larger patient populations can provide valuable insights into optimizing the use of SLNB in managing breast cancer patients undergoing NAT and its relationship with clinical, histopathological, and molecular profiles.

Ethics

Ethics Committee Approval: This retrospective study was conducted according to the principles of the Declaration of Helsinki, and approval was obtained from the Ethics Committee of University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (decision no: 2022-02/46, date: 10.02.2022).

Informed Consent: The study was retrospective, consent was not obtained from the patients.

Footnotes

Authorship Contributions

Design: G.U., B.B.D, Desing: G.U, B.B.D., Data Collection or Processing: S.A., Analysis or Interpretation: S.A., S.G.A., Literature Search: S.A., Writing: G.U., S.A., B.B.D., S.G.A., E.B., C.Ö.

Conflict of Interest: The authors declare that they have no conflict of interest.

Financial Disclosure: The authors have not disclosed any funding.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209-249.
2. Yersal O, Barutca S. Biological subtypes of breast cancer: prognostic and therapeutic implications. World J Clin Oncol. 2014;5:412-424.
3. Garg PK, Prakash G. Current definition of locally advanced breast cancer. Curr Oncol. 2015;22:409-410.
4. Koca E, Kuzan TY, Dizdar O, et al. Outcomes of locally advanced breast cancer patients with ≥ 10 positive axillary lymph nodes. Med Oncol. 2013;30:615.
5. National Comprehensive Cancer Network. NCCN Clinical Breast Guidelines in Oncology. Breast Cancer. Version 4.2025. Available from: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

6. von Minckwitz G, Untch M, Blohmer JU, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol. 2012;30:1796-1804.
7. Giammarile F, Alazraki N, Aarsvold JN, et al. The EANM and SNMMI practice guideline for lymphoscintigraphy and sentinel node localization in breast cancer. Eur J Nucl Med Mol Imaging. 2013;40:1932-1947.
8. Boughey JC, McCall LM, Ballman KV, et al. Tumor biology correlates with rates of breast-conserving surgery and pathologic complete response after neoadjuvant chemotherapy for breast cancer: findings from the ACOSOG Z1071 (Alliance) Prospective Multicenter Clinical Trial. Ann Surg. 2014;260:608-616.
9. Ucmak Vural G, Demirel BB, Bozkurt MF, et al. Procedure guideline for lymphoscintigraphy and sentinel lymph node in breast cancer. Nucl Med Semin. 2020;6:321-338.
10. Classe JM, Loaec C, Gimbergues P, et al. Sentinel lymph node biopsy without axillary lymphadenectomy after neoadjuvant chemotherapy is accurate and safe for selected patients: the GANEA 2 study. Breast Cancer Res Treat. 2019;173:343-352.
11. Benson JR, della Rovere GQ; Axilla Management Consensus Group. Management of the axilla in women with breast cancer. Lancet Oncol. 2007;8:331-348.
12. Bi Z, Liu J, Chen P, et al. Neoadjuvant chemotherapy and timing of sentinel lymph node biopsy in different molecular subtypes of breast cancer with clinically negative axilla. Breast Cancer. 2019;26:373-377.
13. Blanco Sánchez A, Yébenes L, Berjón A, Hardisson D. Evaluation of pathological response to neoadjuvant chemotherapy in breast cancer: correlation with molecular phenotype. Rev Esp Patol. 2021;54:8-16.
14. Goel N, Yadegarynia S, Rodgers S, et al. Axillary response rates to neoadjuvant chemotherapy in breast cancer patients with advanced nodal disease. J Surg Oncol. 2021;124:25-32.
15. Al-Tweigeri T, Elshenawy M, Badran A, et al. Impact of pathologic complete response following neoadjuvant chemotherapy±trastuzumab in locally advanced breast cancer. J Oncol. 2021;2021:6639763.
16. Youssef MMG, Metwally AA, Manie TM. The implications of a pathological complete response of the primary tumour after neoadjuvant chemotherapy for breast cancer on axillary surgery. J Egypt Natl Canc Inst. 2021;33:5.
17. Ladak F, Chua N, Lesniak D, et al. Predictors of axillary node response in node-positive patients undergoing neoadjuvant chemotherapy for breast cancer. Can J Surg. 2022;65:89-96.
18. Clark BZ, Johnson RR, Berg WA, McAuliffe P, Bhargava R. Response in axillary lymph nodes to neoadjuvant chemotherapy for breast cancers: correlation with breast response, pathologic features, and accuracy of radioactive seed localization. Breast Cancer Res Treat. 2023;200:363-373.
19. Davey MG, Ryan ÉJ, Folan PJ, et al. The impact of progesterone receptor negativity on oncological outcomes in oestrogen-receptor-positive breast cancer. BJS Open. 2021;5:zrab040.
20. van Mackelenbergh MT, Denkert C, Nekljudova V, et al. Outcome after neoadjuvant chemotherapy in estrogen receptor-positive and progesterone receptor-negative breast cancer patients: a pooled analysis of individual patient data from ten prospectively randomized controlled neoadjuvant trials. Breast Cancer Res Treat. 2018;167:59-71.
21. Lips EH, Mulder L, de Ronde JJ, et al. Neoadjuvant chemotherapy in ER+ HER2- breast cancer: response prediction based on immunohistochemical and molecular characteristics. Breast Cancer Res Treat. 2012;131:827-836.
22. Wang Y, Li L, Liu X, et al. Treatment response correlation between primary tumor and axillary lymph nodes after neoadjuvant therapy in breast cancer: a retrospective study based on real-world data. Gland Surg. 2021;10:656-669.
23. McMasters KM, Tuttle TM, Carlson DJ, et al. Sentinel lymph node biopsy for breast cancer: a suitable alternative to routine axillary dissection in multi-institutional practice when optimal technique is used. J Clin Oncol. 2000;18:2560-2566.
24. Chakera AH, Friis E, Hesse U, Al-Suliman N, Zerahn B, Hesse B. Factors of importance for scintigraphic non-visualisation of sentinel nodes in breast cancer. Eur J Nucl Med Mol Imaging. 2005;32:286-293.
25. Chagpar AB, Martin RC, Scoggins CR, et al. Factors predicting failure to identify a sentinel lymph node in breast cancer. Surgery. 2005;138:56-63.
26. Blair SL, Tsai C, Tafra L. ASBRS Great Debate: Sentinel Node Biopsy in Patients Over 70 Years of Age. Ann Surg Oncol. 2018;25:2813-2817.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):162-168

DOI: 10.4274/ahot.galenos.2025.2025-4-5

Original Article

Investigation of $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ Regulatory T-cells and PD-1, PD-L1 mRNA Expression in the Bone Marrow in Newly Diagnosed Multiple Myeloma Patients

Deniz Ekinci[1], Aysun Özkan[2], Ozan Salim[3], Aslı Toylu[4], Melike Ulubahşi[3], Nurten Sayın Ekinci[4], Levent Ündar[3]

[1] *Akdeniz Univerversity Faculty of Medicine, Institute of Science, Department of Biology, Antalya, Türkiye*
[2] *Akdeniz University Faculty of Science, Department of Biology, Antalya, Türkiye*
[3] *Akdeniz University Faculty of Medicine, Department of Internal Medicine, Division of Hematology, Antalya, Türkiye*
[4] *Akdeniz University Faculty of Medicine, Department of Medical Genetics, Antalya, Türkiye*

ABSTRACT

Aim: Multiple myeloma (MM) is a cancer that plasma cells. Increased regulatory T-cells (Tregs) have been associated with poor clinical outcomes in both newly diagnosed and treated MM patients. Treg cells are a T-cell subgroup that suppresses immune response. Programmed cell death protein-1 (PD-1) signaling pathway plays an essential role in Treg function and development. In literature, there is insufficient knowledge about the distribution of Treg cells in the bone marrow microenvironment despite the presence of these clinical studies in MM. In this study, we aimed to investigate $PD\text{-}1^+$ Treg cell distribution and PD-1/PD-ligand 1 (PD-L1) gene expression levels in MM patients' bone marrow.

Methods: Twenty newly diagnosed patients with MM and 9 idiopathic thrombocytopenic purpura patients were included in the study. Cluster of differentiation 4 (CD4), CD25, FoxP3 and PD-1 (CD279) expressions were evaluated by flow cytometry, and messenger ribonucleic acid (mRNA) expression of PD-1 and PD-L1 genes were determined using the quantitative polymerase chain reaction method.

Results: In this study, no significant difference was found among $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ Treg cell levels, PD-1, and PD-L1 mRNA expression levels in the patient and control groups. There was no significant difference in Treg cell levels or in PD-1 and PD-L1 mRNA expression levels with respect to patient revised international staging system. There was no significant correlation between the expression of Treg cells, PD-1 mRNA, and PD-L1 mRNA levels ($p>0.05$). A strong correlation was found between PD-1 mRNA and PD-L1 mRNA levels ($p<0.0001$, $r=0.827$).

Conclusion: $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ Treg cell and PD-1, PD-L1 mRNA expression levels were found to be high in the patient group, although not significantly. We found a positive correlation between PD-1 and PD-L1 mRNA expression, supporting that PD-1/PD-L1 may be a prognostic biomarker.

Keywords: Biomarkers, clinical hematology, multiple myeloma, targeted therapy

Introduction

Multiple myeloma (MM) is the second most common widespread hematologic malignancy. There is a need for the development of new treatments for MM [1]. Single-agent activity of programmed cell death protein-1 (PD-1) and PD-ligand 1 (PD-L1) blockade is limited in MM patients. Strategies to expand MM-specific T-cells and combination therapies that involve T-cell activation through PD-1/PD-L1 blockade are still being investigated [2]. The PD-1 pathway stimulates helper T-cell populations toward regulatory T-cell (Treg) development. Treg cells express many checkpoint molecules and thus are a target for direct immune checkpoint inhibitors [3]. The number of clinical trials using inhibitors of PD-1 and its ligand PD-L1 is rapidly increasing in MM [4].

Address for Correspondence: Nurten Sayın Ekinci PhD, Akdeniz University Faculty of Medicine, Department of Medical Genetics, Antalya, Türkiye
E-mail: sayin.nurten@gmail.com **ORCID ID:** orcid.org/0000-0001-5010-7955

Received: 14.04.2025 **Accepted:** 20.08.2025 **Epub:** 09.09.2025 **Publication Date:** 12.12.2025

Cite this article as: Ekinci D, Özkan A, Salim O, et al. Investigation of $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ regulatory T-cells and PD-1, PD-L1 mRNA expression in the bone marrow in newly diagnosed multiple myeloma patients. Acta Haematol Oncol Turc. 2025;58(3):162-168

Tregs are a special T-cell subset that suppress the immune response, thereby maintaining self-tolerance and homeostasis. It has been shown that Tregs can inhibit cytokine production and T-cell proliferation and play a crucial role in preventing autoimmunity [5]. Since Sakaguchi's discovery in 1995 that the interleukin-2 (IL-2) receptor α chain [cluster of differentiation 25 (CD25)] is a phenotypic marker for $CD4^+$ Tregs, studies on Tregs have rapidly increased. Treg cells are primarily a subset of T cells that exhibit the FoxP3 "forkhead winged helix $P3^+$" $CD4^+$, $CD25^+$ phenotype. The primary function of these cells is not to generate an immune response to the individual's own self-antigens and to take part in maintaining and sustaining immune homeostasis [6-8].

There are two main views regarding the mechanism of action of Tregs. It is believed that in order for Tregs to exhibit their immune suppressive function, they either make cell-to-cell contact or affect distanT-cells by secreting cytokines such as IL-10 or transforming growth factor. The T-cell-inhibiting effects of PD-1 and CTLA-4 are well-known [9]. In cell-to-cell contact, Tregs have been shown to kill antigen-presenting cells or responding T-cells by using granzyme and perforin facilitated by CD39, CD73, and lymphocyte-activation gene-3. However, mechanisms of Treg action also involve cytokines such as transforming growth factor-β, IL-35, IL-10, and galectin-1, mediated by soluble factors or IL-2 deficiency [10-12].

The lack of studies on Treg cells and PD-1/PD-L1 messenger ribonucleic acid (mRNA) expression in the bone marrow microenvironment of MM patients has led us to conduct this study. In this study, $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ Treg cells and PD-1 and PD-L1 mRNA expression levels were determined in the bone marrow of newly diagnosed MM and idiopathic thrombocytopenic purpura (ITP) patients who applied to the Akdeniz University Hospital Adult Hematology Clinic. We believe that this and similar studies will contribute to identifying therapeutic targets in patients by measuring both PD-1 and PD-L1 mRNA expression levels and $PD\text{-}1^+$ Treg cells (%) in the bone marrow microenvironment.

Methods

Ethical approval was obtained from the Faculty of Medicine at Akdeniz University (decision no: 664, date: 14.12.2016,). The study was conducted at the Hematology Outpatient 'Clinic of Akdeniz University Faculty of Medicine' Hospital, with the necessary permissions obtained from the hospital administration, and in accordance with research and publication ethics. Following approval from the ethics committee, 37 patients who had presented with suspected MM or ITP and who had undergone bone marrow aspiration and bone marrow biopsy (BMA/BMB) at the the hematology outpatient clinic within one year were included. One patient who had undergone BMA/BMB for suspected ITP was later diagnosed with myelodysplastic syndrome, and seven patients who had undergone BMA/BMB for suspected MM were excluded from the analysis. They did not meet the criteria for MM diagnosis after BMA/BMB. The final analysis included 20 newly diagnosed MM patients and 9 ITP patients. ITP patients have a bone marrow cell distribution like healthy individuals, on whom a BMB can be performed. Since we could not perform this procedure on a healthy individual, a patient group with similar bone marrow cell distribution was chosen as the control. We believe that our study is the first to provide a new perspective on this subject, as no previous study has used ITP as a control group.

Patient age and gender were recorded. Data on hemoglobin (g/dL), leukocyte (number/mm^3), neutrophil (number/mm^3), lymphocyte (number/mm^3), platelet (number/mm^3), lactate dehydrogenase, total protein, albumin, creatinine, calcium, beta-2 microglobulin, immunoglobulin G (IgG), IgA, IgM, free kappa, and free lambda at the time of MM diagnosis were collected. Additionally, cytogenetic analysis data, including the MM FISH panel [t (14;16), del (17p), t (11;14), t (4;14), and del (13q)], were recorded. MM patients included in the study were staged according to the revised international staging system (R-ISS) and ISS based on the National Comprehensive Cancer Network guidelines and were also classified into myeloma subgroups (e.g., IgA Lambda, IgG Kappa) [13]. This cross-sectional case-control study was conducted in accordance with the Helsinki Declaration. Ethical approval was obtained from the Faculty of Medicine at Akdeniz University for this study (decision no: 664, date: 14.12.2016). Written consent was obtained from all patients.

Mononuclear Cell Separation

Samples from patients whose diagnosis was confirmed by flow cytometry and bone marrow smear examination were evaluated. During BMA, and biopsy, performed aseptically from the right iliac crest of patients with MM and ITP, 3 mL of bone marrow aspirate was taken. The aspirate was collected in a tube containing sodium heparin and another tube containing ethylenediamine tetraacetic acid in addition to routine procedures. After 3.5 mL of ficoll histopaque-1077 (Sigma-Aldrich) was added to the Falcon tubes, bone marrow diluted with PBS was slowly added at a ratio of 1:2. The sample was centrifuged for 30 minutes at 2000 rpm (800 g) and 20 °C, using a Beckman-Coulter centrifuge. Mononuclear cells were taken with a pipette. PBS was added and centrifuged. The cells obtained were diluted with Becton Dickinson (BD) Pharmingen Stain Buffer (FBS) to 10^6 cells per mL.

Flow Cytometry

Cell surface staining: Appropriate amounts of surface antibodies (CD4 and CD25 (20 µl), CD279 (PD-1) (5 µl), CD45 (3 µl)) and isotypes were pipetted into 12x75mm tubes. Allophycocyanin-H7-CD45 [catalog number (cat. no.) 560178], phycoerythrin -CD25 (cat. no. 555432), fluorescein isothiocyanate-CD4 (cat. no. 555346), Brilliant Violet 421-PD-1 (cat. no. 562516) - 100 µl of cells were added to each tube, vortexed, and incubated for 20 minutes in the dark at room temperature. Two mL wash buffer (FBS) was added. The sample was centrifuged at 250 x g for 10 minutes the supernatant was removed. To fix the cells, the cell pellet remaining in the tube was gently

resuspended and 2 mL 1x human FoxP3 buffer A was added on top. Two mL BD FBS, was added to the tubes, and the pellet was gently resuspended.

Intracellular staining: 0.5 mL 1x working solution human FoxP3 buffer C was added to permeabilize the cells. It was vortexed and incubated for 30 minutes at room temperature in the dark. FoxP3 antibody (20 µL) was added to the tubes and incubated for 30 minutes at room temperature in the dark. Alexa Fluor 647-FoxP3 (cat. no. 560045). The washing process was repeated with BD FBS, and after the supernatant was removed, the cell pellet remaining in the tubes was diluted with 400 µL wash buffer and analyzed as soon as possible. Treg cells were identified by flow cytometry as $CD4^+$, $CD25^+$, $FoxP3^+$, $PD\text{-}1^+$ based on their phenotypic properties [14]. During the reading, a minimum of 15,000 to 25,000 CD4 positive lymphocytes were counted. Samples were evaluated by flow cytometry (FACSCanto 2, CA) and analyzed using FACS Diva software [15].

Real-Time Polymerase Chain Reaction

Bone marrow samples were pelleted by centrifugation. Erythrocytes were removed using lysis buffer solution, and total RNA was obtained from the remaining nucleated cells by the silica column method (NucleoSpin® RNA Blood (MN-740200.50, Germany). RNA quality and quantity were measured spectrophotometrically with a Nanodrop (Nano). RNA samples were stored at -20 °C for cDNA synthesis. Before starting cDNA synthesis, all RNA samples were adjusted to 100 ng/µL. The EasyScript Plus TM cDNA Synthesis Kit, (ABM-G236, Canada) was used for cDNA synthesis. Before starting cDNA synthesis, all RNA samples were adjusted to 100 ng/µL. The EasyScript Plus TM cDNA Synthesis Kit, (ABM-G236, Canada) was used for cDNA synthesis. Total RNA samples that we kept at -20 °C were thawed at room temperature. Samples were İncubated at 25 °C for 10 minutes, at 40 °C for 50 minutes, and at 85 °C for 5 minutes in a thermal cycler, respectively, in accordance with the kit procedure. The melting curve was analyzed using LightCycler® 480 Software release 1.5.0. The cycle values of the target and reference genes were normalized using the REST© program, with the RPL13A gene used as a reference. PCR for all patients and controls was repeated twice. The primers were synthesized by Sentegen Biotechnology (Türkiye). The primers used for the PD-1 gene were FW: CCCTGGTGGTTGGTGTCGT and R: GCCTGGCTCCTATTGTCCCTC; while those for the PD-L1 (CD274) gene were FW: GGTGCCGACTACAAGCGAAT and R: ATGGTCACTGCTTGTCCAGATG [16].

Statistical Analysis

The data were analyzed utilizing Statistical Package for the Social Sciences statistics for Windows version 23 (IBM Corp., Armonk, NY, USA). In the within-group analysis, the chi-square test of independence was used to analyze the relationship between categorical variables, and the Mann-Whitney U and Kruskal-Wallis tests were used to compare continuous data. The correlation between continuous variables was evaluated using Spearman's rho correlation. A p value less than 0.05 was considered statistically significant.

Results

There is similarity in all individuals in the study in terms of age and biochemical parameters. This enables us to compare the Treg and PD-1/PD-L1 mRNA values obtained in the patient and control groups. It was observed that the female and male ratios were similar in the control and patient groups, and the study groups were homogeneous in terms of gender (p=0.53). The mean age of all individuals in the study was 60.59±8.62. It was observed that the mean ages were not significantly different between the control and patient groups (p=0.90).

It was observed that the MM subtypes were IgA K (Kappa) at 15%, IgA L (Lambda) at 10%, IgG K at 25%, and IgG L at 35%. The disease stage levels of the patients were stage 1 at 25%, stage 2 at 15%, and stage 3 at 60%. Five patients were R-ISS 1 (25%), seven patients were R-ISS 2 (35%), and eight patients were R-ISS 3 (40%) (Table 1).

In the study, there was no statistically significant difference in the levels of $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ Treg cells (%) (1.4-11.2%, 0.01-3.5%) between the patient and control groups (p=0.095) (Figure 1a). However, the cell percentage was higher in myeloma patients than in our control group. Relative PD-1 mRNA expression levels in the patient and control groups (0.1-6.62, 0.1-2.17) did not show a statistically significant difference (p=0.167) (Figure 1b). There was no statistically significant difference in the relative PD-L1 mRNA expression levels between the patient and control groups (0.13-37.77, 0.014-11.61) (p=0.69) (Figure 1c). The gating strategy for Tregs is shown in Figure 2. There was no statistically significant difference in the levels of $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ Treg cells (%) (p=0.68), PD-1 mRNA expression levels (p=0.59), and PD-L1 mRNA expression levels (p=0.7) between the groups according to R- of the patients. There was no significant correlation between the expression levels of Treg cells and the levels of PD-1 mRNA and PD-L1 mRNA (p>0.05). There was a strong correlation between PD-1 mRNA and PD-L1 mRNA levels in the control and patient groups (p<0.0001, r=0.827).

Discussion

In MM, as with other hematologic cancers, the relationship between Tregs and cancer has been questioned and has become an area of research. In recent years, the increase in clinical trials of PD-1 and PD-L1 inhibitors in hematologic cancers has led to more scrutiny of the relationship between MM and Tregs, and has also necessitated the development of new research strategies and parameters. In 2014, Braga et al. [15] investigated Tregs in the bone marrow of MM patients as potential biomarkers, therapeutic targets, and prognostic indicators in the local immune environment. Compared to controls, FoxP3 expression was found to be six-fold higher in MM patients. In another study conducted by D'Arena et al. [16] in 2016, the percentage and absolute number of circulating Tregs ($CD4^+$ $CD25^+$high CD127-low) of 39 untreated MM patients and 44 monoclonal gammopathy of undetermined significance (MGUS) patients were tested and compared.

Table 1. Experimental results of MM patients and controls

Patient	Gender	Age	ISS	R-ISS	PD-1⁺ Tregs(%)	PD-1 mRNA copy	PD-L1 mRNA copy
1	F	55	I	1	2.3	0.65	1.05
2	M	75	II	2	1.7	0.92	0.91
3	F	55	I	2	1.4	4.83	2.67
4	M	66	III	3	1.8	0.28	0.15
5	M	67	I	1	1.43	2.98	4.27
6	F	73	II	2	1.9	2.31	5.33
7	F	51	III	3	2.9	6.30	5.06
8	F	65	III	3	2.1	0.40	1.31
9	M	53	III	3	1.5	0.21	0.52
10	F	60	III	3	2.8	4.08	5.891
11	M	45	III	3	1.4	0.18	0.20
12	F	50	III	2	11.2	0.42	1.21
13	F	62	I	1	2.7	0.10	0.16
14	M	67	III	2	2.6	0.21	0.28
15	F	58	1	1	1.8	0.53	0.13
16	M	65	III	3	2.5	0.66	1.23
17	F	66	III	3	1.9	1.64	1.91
18	F	57	I	1	2.8	6.10	37.7
19	M	60	III	2	1.8	6.62	13.1
20	F	59	II	2	1.96	3.01	2.21
Control	**Gender**	**Age**					
1	F	61			1.1	0.29	0.01
2	F	55			0.01	1.06	0.88
3	M	81			0.26	0.19	0.31
4	F	64			0.7	0.28	0.73
5	F	58			0.01	0.48	2.46
6	M	40			2.9	0.58	2.86
7	M	61			3.5	0.81	3.84
8	F	68			0.01	2.17	11.6
9	F	60			1.3	0.10	0.42

F: Female, M: Male, R-ISS: Revised International Staging System, PD-1/PDL-1: Programmed cell death protein 1/death ligand 1, mRNA: Messenger ribonucleic acid

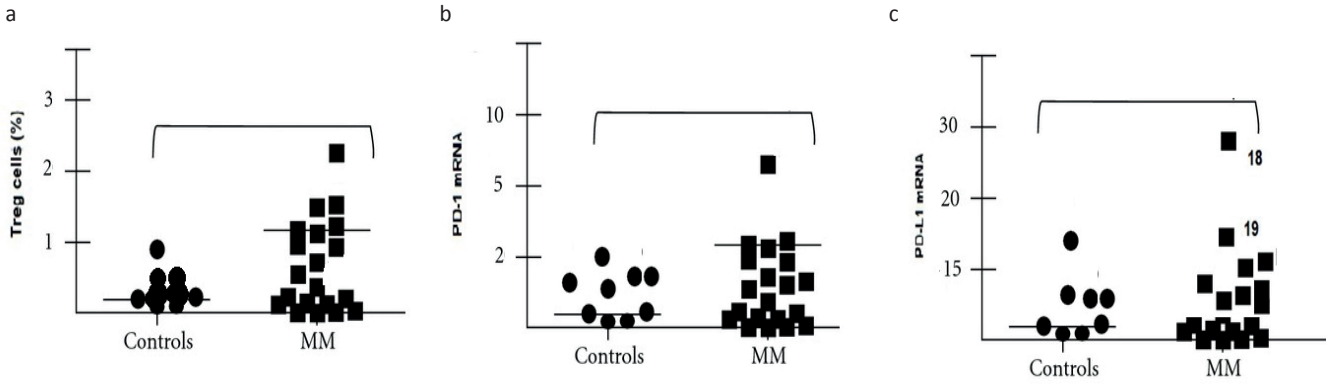
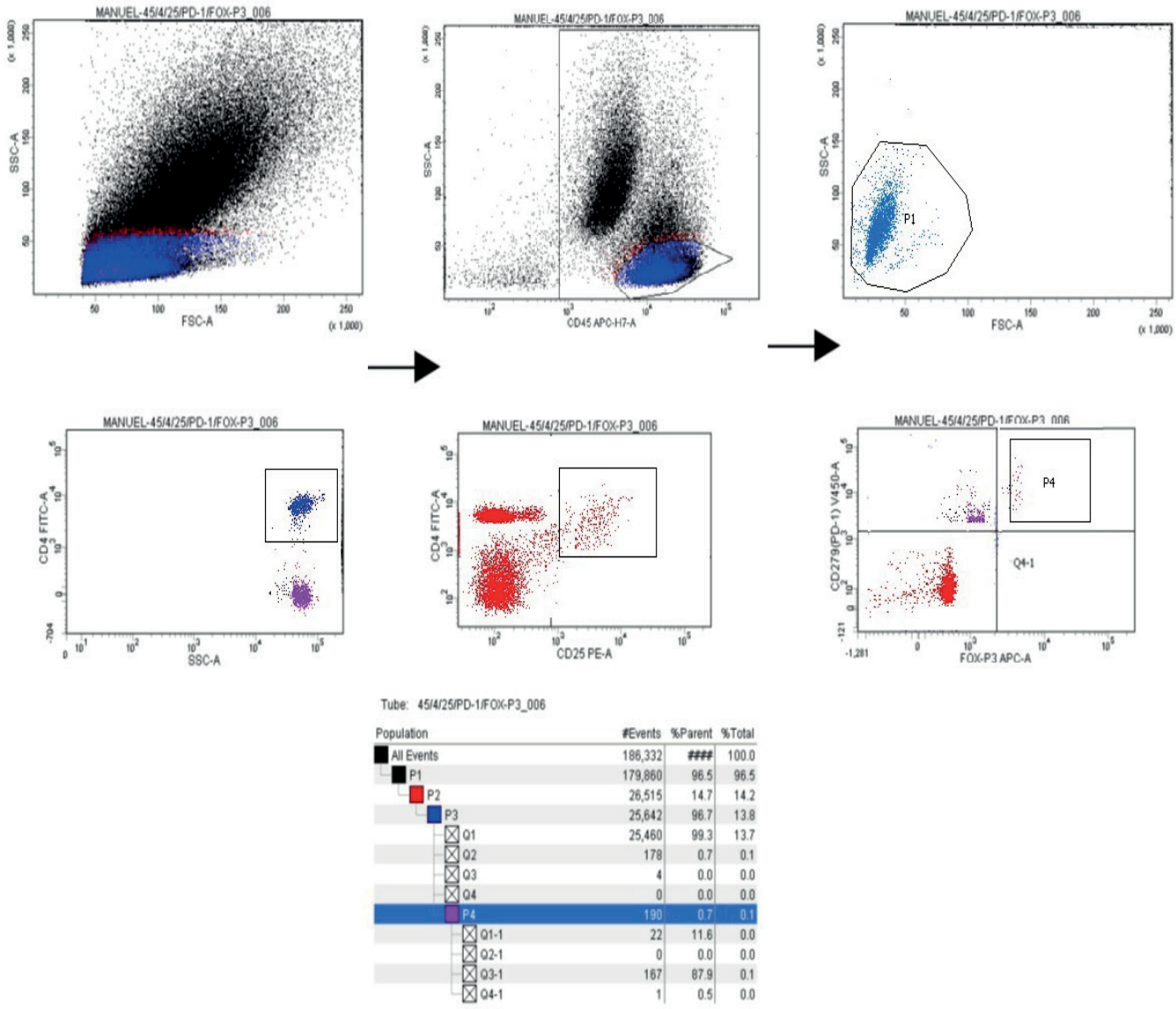


Figure 1. (a,b,c). Treg and PD-1 PD-L1 expression
MM: Multiple myeloma, PD: Programmed cell death protein 1, PD-L1: Programmed cell death-ligand 1, mRNA: Messenger ribonucleic acid



Tube: 45/4/25/PD-1/FOX-P3_006

Population	#Events	%Parent	%Total
All Events	186,332	####	100.0
P1	179,860	96.5	96.5
P2	26,515	14.7	14.2
P3	25,642	96.7	13.8
Q1	25,460	99.3	13.7
Q2	178	0.7	0.1
Q3	4	0.0	0.0
Q4	0	0.0	0.0
P4	190	0.7	0.1
Q1-1	22	11.6	0.0
Q2-1	0	0.0	0.0
Q3-1	167	87.9	0.1
Q4-1	1	0.5	0.0

Figure 2. Gating strategy for Tregs
Fox: Forkhead box, PD: Programmed cell death

Twenty healthy individuals were tested as a control group. However, there is a significant disagreement in the literature regarding the numbers and functions of Tregs in MM.

The average percentage of circulating Tregs was found to be 2.1±1.0% (0.75-6.1%) in MM patients, 2.1±0.9% (0.3-4.4%) in MGUS patients, and 1.5±0.4% (0.9-2.1%) in controls [16,17]. Prabhala et al. [18] found lower Treg cell numbers compared to the control group. In contrast, Beyer et al. [19] showed the presence of strong inhibitory function in MGUS and MM with high Treg cell numbers. A recent study reported that regulatory T-cells (Tregs) were more abundant in the bone marrow in patients with MM [20]. Our study supports this result. We found that PD-1+ Treg cell percentage was higher in myeloma patients than in our control group. However, there was no statistically significant difference in the levels of $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ Treg cells (%) (1.4-11.2%, 0.01-3.5%) between the patient and control groups. In another study conducted on peripheral blood lymphocytes in ITP patients, the $CD4^+$ CD25 high $FoxP3^+$ Treg cell rate was found to be 9.69±3.70% [21]. In our study, unlike this study, 0.01±3.5% was measured in lymphocytes obtained from bone marrow, not from peripheral blood, and this difference in percentage occurred because PD-1 expression was also included [21]. The differences in Treg function and numbers in these studies may be due to differences in purification techniques and evaluations. Although peripheral blood is typically used in such studies, we used bone marrow material, which is more difficult to obtain.

Clinical studies have been conducted on PD-1 and PD-L1 inhibitors for hematological malignancies. Badros et al.

[22] published the results of their combination study of pembrolizumab, low-dose dexamethasone, and pomalidomide in relapsed/refractory MM. In this study, PD-L1 expression was evaluated as positive or negative using immunohistochemical staining of bone marrow biopsies in 29 patients, and it was found to be correlated with treatment response. In analyses of pre-treatment bone marrow samples, there is increased expression of PD-L1 in responding patients, independent of PD-1 expression. In a recent study conducted, it was found that low-dose, single-fraction radiotherapy in combination with pembrolizumab was safe and induced an early response. They stated that larger studies were needed to confirm their findings [23].

The number of studies that have investigated the correlation of Treg cell levels with PD-1 mRNA and PD-L1 mRNA levels is limited in the literature. However, in our study, no positive or negative relationship was found between Treg cell expression and PD-L1 and PD-1 mRNA levels. A recent review reported that studies with proteasome inhibitors and monoclonal antibodies are ongoing, and long-term analyses are awaited [24]. Studies like ours contribute to expanding the knowledge base and accelerating clinical studies.

Study Limitations

Limitations of this study include the selection of bone marrow, which is not as easily accessible as peripheral blood, this may have limited both the small patient population and our comparison group.

Conclusion

Although not statistically significant, $CD4^+CD25^+FoxP3^+PD\text{-}1^+$ Treg cell percentages and PD-1, PD-L1 gene expression distributions differed in our patient control groups. We found both higher cell percentages and higher gene expressions in our patient group compared to controls. There was no significant relationship between Treg cell expression and both PD-1 mRNA and PD-L1 mRNA levels. However, a strong correlation was found between PD-1 mRNA and PD-L1 mRNA levels. This strong relationship supports the use of blockade points in the treatment of myeloma patients. In addition, selecting bone marrow, which is difficult to access, as the study material and including ITP patients as the control group increases the specificity of our study and its contribution to science.

In the coming years, our study should be supported by further studies with a larger number of patients to identify who will benefit from treatment strategies targeting Treg cells and the PD-1/PD-L1 pathway.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Faculty of Medicine at Akdeniz University (decision no: 664, date: 14.12.2016). The study was conducted at the Hematology Outpatient "Clinic of Akdeniz University Faculty of Medicine" Hospital, with the necessary permissions obtained from the hospital administration, and in accordance with research and publication ethics.

Informed Consent: Written consent was obtained from all patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: O,S,. L.Ü., Concept: D.E., A.Ö., A.T., N.S.E., Design: D.E., A.Ö., N.S.E., L.Ü., Data Collection or Processing: D.E., O.S., A.T., M.U., N.S.E., Analysis or Interpretation: D.E., A.Ö., O.S., A.T., M.U., N.S.E., L.Ü., Literature Search: D.E., A.Ö., N.S.E., L.Ü., Writing: D.E., A.Ö., A.T., N.S.E., L.Ü.

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

Financial Disclosure: This study was supported by the Akdeniz University Scientific Research Projects Management Unit (Project No: FDK-2017-2382).

References

1. Wu C, Zhang L, Brockman QR, Zhan F, Chen L. Chimeric antigen receptor T cell therapies for multiple myeloma. J Hematol Oncol. 2019;12:120.
2. Rosenblatt J, Avigan D. Targeting the PD-1/PD-L1 axis in multiple myeloma: a dream or a reality? Blood. 2017;129:275-279.
3. Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors in cancer therapy: a focus on T-regulatory cells. Immunol Cell Biol. 2018;96:21-33.
4. Oliva S, Troia R, D'Agostino M, Boccadoro M, Gay F. Promises and pitfalls in the use of PD-1/PD-L1 inhibitors in multiple myeloma. Front Immunol. 2018;9:2749.
5. Zhao H, Liao X, Kang Y. Tregs: where we are and what comes next? Front Immunol. 2017;8:1578.
6. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155:1151-1164.
7. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299:1057-1061.
8. O'garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nature Med. 2004;10:801-805.
9. Miyara M, Sakaguchi S. Natural regulatory T cells: mechanisms of suppression. Trends Mol Med. 2007;13:108-116.
10. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. Nat Immunol. 2008;9:239-244.
11. Bardel E, Larousserie F, Charlot-Rabiega P, Coulomb-L'Herminé A, Devergne O. Human CD4+ CD25+ Foxp3+ regulatory T cells do not constitutively express IL-35. J Immunol. 2008;181:6898-905.
12. Shevach EM. Mechanisms of Foxp3+ T regulatory cell-mediated suppression. Immunity. 2009;30:636-645.
13. Palumbo A, Avet-Loiseau H, Oliva S, et al. Revised international staging system for multiple myeloma: a report from International Myeloma Working Group. J Clin Oncol. 2015;33:2863-2869.
14. Hariyanto AD, Permata TBM, Gondhowiardjo SA. Role of CD4+ CD25+ FOXP3+ TReg cells on tumor immunity. Immunol Med. 2022;45:94-107.
15. Braga WMT, da Silva BR, de Carvalho AC, et al. FOXP3 and CTLA4 overexpression in multiple myeloma bone marrow as a sign of accumulation of CD4+ T regulatory cells. Cancer Immunol Immunother. 2014;63:1189-1197.
16. D'Arena G, Rossi G, Laurenti L, et al. Circulating regulatory T-cells in monoclonal gammopathies of uncertain significance and multiple myeloma: in search of a role. J Immunol Res. 2016;2016:9271469.

17. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29:e45.
18. Prabhala RH, Neri P, Bae JE, et al. Dysfunctional T regulatory cells in multiple myeloma. Blood. 2006;107:301-304.
19. Beyer M, Kochanek M, Giese T, et al. In vivo peripheral expansion of naive CD4+CD25high FoxP3+ regulatory T cells in patients with multiple myeloma. Blood. 2006;107:3940-3949.
20. Alrasheed N, Lee L, Ghorani E, et al. Marrow-Infiltrating regulatory T cells correlate with the presence of dysfunctional CD4+PD-1+ cells and inferior survival in patients with newly diagnosed multiple myeloma. Clin Cancer Res. 2020;26:3443-3454.
21. Akyol Erikçi A, Karagöz B, Bilgi O. Regulatory T cells in patients with idiopathic thrombocytopenic purpura. Turk J Hematol. 2016;33:153-155.
22. Badros A, Hyjek E, Ma N, et al. Pembrolizumab, pomalidomide, and low-dose dexamethasone for relapsed/refractory multiple myeloma. Blood. 2017;130:1189-1197.
23. Khan MK, Nasti TH, Qian JY, et al. Pembrolizumab and low-dose, single-fraction radiotherapy for patients with relapsed or refractory multiple myeloma: a prospective, single-centre, single-group, open-label, phase 2 pilot trial in the USA. Lancet Haematol. 2024;11:e510-e520.
24. Chakrabarti R, Siegel D, Biran B. The evolving role of checkpoint inhibitors in multiple myeloma. Clinical Lymphoma Myeloma Leuk. 2025;25:96-108.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):169-176

DOI: 10.4274/ahot.galenos.2025.2025-7-6

Original Article

Correlation of Ga-68 PSMA PET/CT Positivity with Gleason Scores and Serum PSA Levels in Initial Staging of Prostate Adenocarcinoma, and the Contribution of PET/CT Imaging to Patient Management

İpek Öztürk[1], Gülin Uçmak[2], Bedriye Büşra Demirel[2], Semra Demirtaş Şenlik[3], Hülya Efetürk[4], Aykut Kürşat Fidan[5], Halil Başar[6]

[1]*University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of Nuclear Medicine, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye, Dr. Abdurrahman Yurtarslan Ankara Oncology Training and Research Hospital, Clinic of Nuclear Medicine, Ankara, Türkiye*

[3]*University of Health Sciences Türkiye, Etlik City Hospital, Clinic of Nuclear Medicine, Ankara, Türkiye*

[4]*Dr. Burhan Nalbantoğlu State Hospital, Clinic of Nuclear Medicine, Nicosia, Northern Cyprus*

[5]*Gazi State Hospital, Clinic of Nuclear Medicine, Samsun, Türkiye*

[6]*University of Health Sciences Türkiye, Dr. Abdurrahman Yurtarslan Ankara Oncology Training and Research Hospital, Clinic of Urology, Ankara, Türkiye*

ABSTRACT

Aim: Prostate cancer is the second most frequently diagnosed malignancy and the fifth leading cause of cancer-related mortality worldwide. Accurate staging at diagnosis is crucial for guiding treatment decisions. However, conventional imaging methods have limited sensitivity, especially for detecting early metastatic disease. Gallium-68 (Ga-68) prostate-specific membrane antigen (PSMA) positron emission tomography/computed tomography (PET/CT), a molecular imaging modality targeting PSMA, provides high sensitivity and specificity by visualizing PSMA expression in both primary and metastatic lesions. This study aimed to evaluate the correlation of serum prostate-specific antigen (PSA) levels and Gleason grade groups with primary lesion standardized uptake value maximum (SUV_{max}), and to assess the predictive value of PSMA expression for extraprostatic involvement in patients undergoing initial staging for prostate adenocarcinoma.

Methods: This retrospective study included 37 patients with biopsy-proven prostate adenocarcinoma who underwent Ga-68 PSMA PET/CT for initial staging. Correlations between primary tumor SUV_{max}, serum PSA levels, and Gleason grade groups were analyzed. The predictive value of SUV_{max} for extraprostatic metastases was assessed. Additionally, associations between PSA, Gleason grade groups, and the presence of lymph node or bone metastases were evaluated. In 33 patients, Ga-68 PSMA PET/CT findings were compared with concurrent bone scintigraphy. A subgroup underwent dual-phase PET/CT imaging to assess changes in delayed SUV_{max} in relation to PSA and Gleason grade groups.

Results: SUV_{max} of the primary tumor showed significant positive correlations with PSA levels ($p<0.001$) and Gleason grade groups ($p=0.005$). Gleason grade group was significantly associated with lymph node metastasis ($p=0.04$), but not with bone metastasis. A SUV_{max} threshold of 8.97 predicted extraprostatic disease with 81.25% sensitivity and 66.67% specificity. No significant association was found between PSA levels and lymphatic or osseous metastases. PSMA PET/CT outperformed bone scintigraphy in 76% of cases with suspected skeletal lesions. Although dual-phase imaging revealed no additional lesions, delayed SUV_{max} values remained significantly correlated with PSA and Gleason grade groups ($p=0.002$ and $p=0.013$, respectively).

Conclusion: Ga-68 PSMA PET/CT demonstrates high diagnostic performance in the staging of prostate adenocarcinoma and correlates significantly with key prognostic factors. It offers superior accuracy compared to bone scintigraphy and may guide personalized management strategies. While dual-phase imaging adds limited incremental value, it may be beneficial in selected cases. Further prospective studies are warranted to validate these findings.

Keywords: Clinical oncology, gallium-68, oncology, positron emission tomography computed tomography, prostate cancer, prostatic neoplasms

Address for Correspondence: İpek Öztürk MD, University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of Nuclear Medicine, İstanbul, Türkiye

E-mail: ipekkerimel@gmail.com **ORCID ID:** orcid.org/0000-0003-4183-5824

Received: 29.07.2025 **Accepted:** 08.09.2025 **Epub:** 23.09.2025 **Publication Date:** 12.12.2025

Cite this article as: Öztürk İ, Uçmak G, Demirel BB, et al. Correlation of Ga-68 PSMA PET/CT positivity with Gleason Scores and serum PSA levels in initial staging of prostate adenocarcinoma, and the contribution of PET/CT imaging to patient management. Acta Heamatol Oncol Turc. 2025;58(3):169-176

Introduction

Prostate cancer is one of the most frequently diagnosed malignancies and remains a significant cause of morbidity and mortality among men worldwide. According to GLOBOCAN 2018 data, it is the second most common cancer and the fifth leading cause of cancer-related deaths in men globally [1]. Both the European Association of Urology (EAU) and the National Comprehensive Cancer Network emphasize the necessity of accurate risk stratification and staging for optimal treatment planning [2].

In intermediate- and high-risk patients, multiparametric magnetic resonance imaging (mpMRI), computed tomography (CT), and bone scintigraphy are traditionally used for initial staging. However, these conventional imaging techniques show significant limitations, particularly in identifying lymph node involvement and early metastatic spread. The 2024 EAU guidelines now recommend the use of prostate-specific membrane antigen (PSMA) positron emission tomography/CT (PET/CT) as the preferred imaging modality for staging high-risk and selected intermediate-risk prostate cancer cases, owing to its superior diagnostic performance in detecting both nodal and distant metastases [2].

A meta-analysis by Hövels et al. [3] reported that CT and MRI exhibit relatively low sensitivity (approximately 42%) for lymph node staging, despite showing reasonable specificity (around 82%). In contrast, gallium-68 (Ga-68) PSMA PET/CT, a molecular imaging modality targeting PSMA, has emerged as a highly sensitive and specific tool for detecting both locoregional and systemic spread of disease. The proPSMA randomized trial further confirmed that Ga-68 PSMA PET/CT demonstrates significantly higher diagnostic accuracy (92% vs. 65%) than conventional imaging and leads to more frequent and impactful management changes [4].

Given this context, the present study aims to assess the clinical utility of Ga-68 PSMA PET/CT in the initial staging of biopsy-proven intermediate- to high-risk prostate cancer. Specifically, we evaluate the correlations between PSMA PET/CT findings and key prognostic markers, including serum prostate-specific antigen (PSA) levels, Gleason Grade Groups, and metastatic involvement. Furthermore, the potential added value of dual-phase PET/CT imaging in a selected subgroup of patients is also investigated.

Methods

This retrospective study included 37 patients with biopsy-confirmed prostate adenocarcinoma who underwent Ga-68 PSMA PET/CT for initial staging. Ethical approval was received from the University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Education and Research Institute Institutional Review Board (approval no: 9, date: 01.06.2017). Demographic data, histopathological characteristics (Gleason score/Grade Group), serum PSA levels (measured within one month prior to imaging), clinical and radiological findings, and treatment details were retrieved from the hospital information system.

Ga-68 PSMA- Inhibitor & Therapy (I&T) was synthesized using a scintomics gallium radiopharmaceuticals module with a germanium-68/Ga-68 (Ge-68/Ga-68) generator (iThemba Labs, South Africa) and reagents from ABX (Germany). Radiolabeling efficiency and radiochemical purity were assessed using radio-thin layer chromatography and radio- high performance liquid chromatography, with a final purity of ≥95%.

All patients received 1.8-2.2 MBq/kg of Ga-68 PSMA-I&T via intravenous injection. Oral hydration with 1-1.5 liters of water containing contrast agent was administered approximately 30 minutes prior to injection. PET/CT imaging was performed approximately 60 minutes post-injection using a Siemens Biograph TruePoint 6 PET/CT scanner, covering the area from the vertex to mid-thigh. When the initial scan showed equivocal pelvic findings, typically due to urinary bladder tracer activity obscuring the prostate bed or subcentimetric nodal uptake, a 120-min delayed pelvic acquisition was obtained to improve lesion conspicuity.

Low-dose CT scans were acquired for attenuation correction and anatomical localization. PET/CT images were reconstructed in axial, coronal, and sagittal planes using a Siemens Leonardo workstation. Two experienced nuclear medicine physicians performed image interpretation. Lesions showing focal or heterogeneous uptake distinguishable from physiological background were evaluated in correlation with CT. Volumes of interest (VOIs) were semi-automatically delineated based on standardized uptake value (SUV) thresholds and manually adjusted to exclude adjacent normal tissues. The SUV maximum (SUV_{max}) was recorded for each VOI.

The SUV_{max} of the primary prostate lesion was measured on early-phase PET/CT images. The presence of lymph node and bone metastases was also assessed. In 33 patients, bone scintigraphy was performed within one week of PET/CT, and findings were compared for concordance.

A subgroup of patients underwent dual-phase Ga-68 PSMA PET/CT with delayed-phase images acquired at 120 minutes post-injection. SUV_{max} values from early and delayed phases were compared, and their correlations with serum PSA levels and Gleason Grade groups were analyzed.

All statistical analyses were conducted using IBM Statistical Package for the Social Sciences statistics version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as number (n), percentage (%), mean ± standard deviation ($\bar{x}$±SD), or median with interquartile range, as appropriate. The distribution of continuous variables was evaluated using the Shapiro-Wilk test and Q-Q plots.

For correlation analyses, Pearson's correlation coefficient was used for normally distributed variables, while Spearman's rank correlation was applied for non-normally distributed data. Independent samples t-test and Mann-Whitney U test were used to compare two groups, depending on the distribution. One-way ANOVA or Kruskal-Wallis tests were used for comparisons among more than two groups. Categorical variables were analyzed using Pearson's chi-square or Fisher's exact test, as appropriate.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of SUV_{max} in predicting extraprostatic disease (lymph node and/or bone metastases). The optimal cut-off value was determined using the Youden index, and corresponding sensitivity and specificity values were calculated. A p value <0.05 was considered statistically significant.

Results

A total of 37 patients who underwent Ga-68 PSMA PET/CT for primary staging due to elevated serum PSA levels and/or suspicious radiologic or scintigraphic findings were retrospectively evaluated. The mean age was 65.6 years (range: 44-84). According to biopsy results, Gleason Grade group distribution was as follows: 3 patients (8.1%) in group 1, 10 (27.0%) in group 2, 4 (10.8%) in group 3, 8 (21.6%) in group 4, and 12 (32.4%) in group 5. The mean ± SD serum PSA level was 29.88±34.40 ng/mL.

In all patients, Ga-68 PSMA PET/CT demonstrated focal or heterogeneous an increase in PSMA uptake in the prostate gland, corresponding to the primary tumor. The mean SUV_{max} of the primary lesions was 11.84±8.14 (range: 3.64-35.61) (Table 1). A statistically significant positive correlation was found between SUV_{max} and serum PSA levels (p<0.001, r=0.572), as well as between SUV_{max} and Gleason Grade group (p=0.005, r=0.449) (Table 2).

Lymph Node Metastases

Lymph node involvement consistent with prostate cancer metastasis was identified in 15 of 37 patients (40.5%), with localization confined to the pelvic region in 10 patients and involving both pelvic and extrapelvic regions in 5. One patient exhibited lymphadenopathy with low PSMA expression and atypical imaging features; further workup confirmed a diagnosis of chronic lymphocytic leukemia/lymphoma.

Among patients with nodal metastases, three had Gleason Grade group 2, six had group 4, and six had group 5 disease. A statistically significant association was found between Gleason Grade group and nodal metastasis (p=0.04). However, no significant relationship was observed between Gleason Grade group and the distribution pattern of nodal disease (pelvic vs. pelvic + extrapelvic) (p=0.16). Additionally, PSA levels were not significantly associated with nodal metastasis (p=0.19). The smallest PSMA-avid lymph node measured 5 mm in diameter.

Following the study, we were able to assess the surgical outcomes of 6 patients who underwent radical prostatectomy and lymph node dissection. Among these, lymph node metastasis was histopathologically confirmed in 2 patients, who showed lymph node involvement on PET/CT. In the remaining 4 patients, no lymph node involvement was observed on PET/CT, and histopathological examination also ruled out metastasis. In these 6 patients, PET/CT findings were in complete concordance with histopathological results; however, the sample size was relatively small for a robust comparative analysis.

Bone Metastases and Scintigraphy Comparison

Bone metastases were detected in 7 patients (18.9%), with a mean SUV_{max} of 10.61±6.77. In 4 additional patients (10.8%), sclerotic bone lesions were observed on CT without corresponding PSMA uptake. These lesions were deemed indeterminate, given that certain intensely sclerotic metastases may be PSMA-negative. Further evaluation via other imaging modalities or follow-up was recommended. No significant correlation was found between the presence of bone metastases and Gleason grade or PSA levels.

Thirty-three patients underwent concurrent bone scintigraphy. Among these, 6 patients (18%) showed no scintigraphic evidence of metastases, 2 (6%) showed definite metastases, and 25 (76%) had indeterminate findings. PSMA PET/CT was concordant with bone scintigraphy in only 8 cases (24%), specifically in patients with either negative or definitively positive scans.

In the subgroup with indeterminate scintigraphy (n=25), 20 patients (80%) had no PSMA-avid lesions, attributed to benign causes such as degenerative changes. In the remaining 5 patients (20%), PSMA-avid lesions confirmed metastatic involvement, thereby clarifying the diagnosis.

Table 1. Patient characteristics and imaging findings

Parameter	Value
Gleason Grade group (Gleason score)	
- 1 (3+3)	3 patients (8.1%)
- 2 (3+4)	10 patients (27%)
- 3 (4+3)	4 patients (10.8%)
- 4 (4+4, 3+5, 5+3)	8 patients (21.6%)
- 5 (4+5, 5+4, 5+5)	12 patients (32.4%)
Age (years)	65.62±8.12
Serum PSA (ng/mL)	29.88±34.40
Gleason Grade group (median)	4 (z: 2-5)
- primary lesion	11.84±8.14
- bone metastasis	10.61±6.77
- lymph node metastasis	6.25 (3.64-9.16)

*Data are presented as median (interquartile range) or mean ± standard deviation. Percentages may not total 100% because of rounding.
PSA: Prostate-specific antigen, SUV_{max}: Standardized uptake value maximum

Table 2. Statistically significant correlations and metastatic findings

Parameter	Value
Total number of patients	37
Patients with lymph node metastases	15 (40.5%)
Patients with bone metastases	7 (18.9%)
Correlation: PSA vs. of primary tumor	r=0.572, p<0.001
Correlation: Gleason Grade group vs.	r=0.449, p=0.005
Correlation: Gleason Grade group vs. LN metastasis	p=0.04, Cramér's V=0.51

PSA: Prostate-specific antigen, : Standardized uptake value maximum, LN: Lymph Node

Correlation with SUV_{max} and Disease Extent

Among all patients, 16 (43.2%) were found to have extraprostatic disease (nodal and/or bone metastases). A statistically significant correlation was observed between primary tumor SUV_{max} and the presence of metastasis (p=0.04). ROC curve analysis identified a cut-off SUV_{max} value of 8.97 for predicting extraprostatic involvement, with a sensitivity of 81.3% and specificity of 66.7% (Figure 1, Table 3). In the remaining 21 patients, no extraprostatic spread was detected, and these patients underwent curative treatments accordingly. Patients with advanced disease were managed with systemic therapies. Below, Case 1 and Case 2 are presented as two patient examples, highlighting the importance of Ga-68 PSMA PET/CT in treatment planning (Case 1, 2)

Dual-Phase Imaging

Dual-phase PSMA PET/CT imaging was performed in 27 patients to evaluate pelvic lymph nodes with subcentimetric size or equivocal uptake. No additional lesions were identified on delayed images. However, PSMA-avid lymph nodes were better visualized on delayed scans.

When comparing SUV_{max} between early and delayed phases, delayed SUV_{max} remained significantly correlated with PSA levels (p=0.002) and Gleason Grade group (p=0.013), similar to early-phase values. Although dual-phase imaging did not reveal additional lesions, it provided improved lesion conspicuity in selected cases and may be helpful in patients with high PSA levels or equivocal findings on standard imaging.

Discussion

This retrospective study assessed 37 patients with biopsy-proven intermediate- to high-risk prostate adenocarcinoma who underwent Ga-68 PSMA PET/CT for initial staging. The aim was to evaluate the correlation between imaging findings and key clinical parameters including PSA levels, Gleason Grade group, and presence of metastasis.

Based on previous studies in the literature, Silver et al. [5] demonstrated that PSMA expression is absent in benign prostatic epithelium and significantly elevated in high-grade prostate carcinomas, highlighting its potential as a molecular target for imaging and therapy. Building upon this, Perner et al. [6] reported a strong correlation between PSMA overexpression and adverse pathological features such as poor differentiation, tumor progression, and biochemical recurrence, further supporting its prognostic relevance. Fendler et al. [7] evaluated the accuracy of 68Ga-PSMA PET/CT in localizing intraprostatic tumor lesions and reported significantly higher SUV_{max} values in histopathologically confirmed tumor-positive segments compared to negative ones (mean 11.8 vs. 4.9; p<0.001), with a high positive predictive value (97%) and notable accuracy in the study. Consistent with these findings, our study demonstrated that all 37 patients exhibited visually discernible PSMA uptake in the primary prostate tumor, regardless of Gleason score or PSA level, further supporting the reliability of 68Ga-PSMA PET/CT in identifying the intraprostatic tumor site with high sensitivity, even in early-stage or multifocal disease presentations.

In our study, a statistically significant positive correlation was observed between the SUV_{max} of the primary tumor and both serum PSA levels (p<0.001) and Gleason Grade group (p=0.005). The mean SUV_{max} of the primary lesion was 11.84±8.14. These findings underscore the potential utility of SUV_{max} as a non-invasive surrogate biomarker reflecting tumor aggressiveness. Similarly, Kwan et al. [8] demonstrated that intraprostatic SUV_{max} values derived from Ga-68 PSMA PET/CT strongly correlated with International Society of Urological Pathology Grade groups, particularly in identifying Grade group 5 lesions. A SUV_{max} threshold >10 was associated with a 2.3-fold increased risk for high-grade disease, reinforcing its diagnostic value for risk stratification. Also, Uprimny et al. [9] reported significantly higher SUV_{max} values in primary tumors of patients with elevated PSA and higher Gleason scores, supporting the use of SUV_{max} values in initial staging and prognostication.

In a retrospective study by Zhou et al. [10], ^{68}Ga-PSMA PET/CT demonstrated superior detection rates compared to mpMRI in high-risk prostate cancer (97.0% vs. 87.9%, p< 0.05). Conversely, mpMRI showed higher sensitivity in low- and

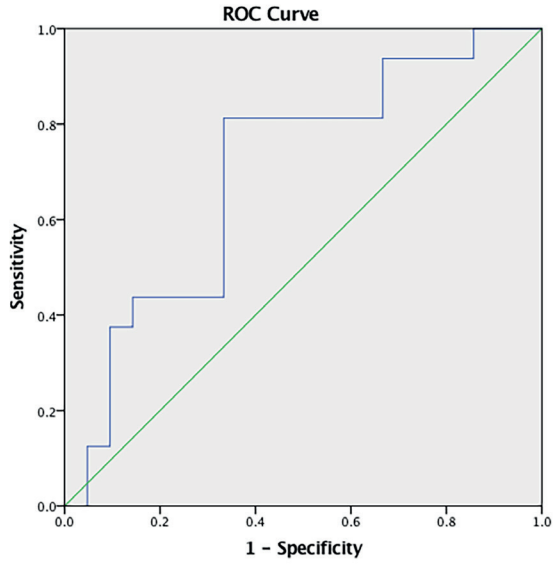
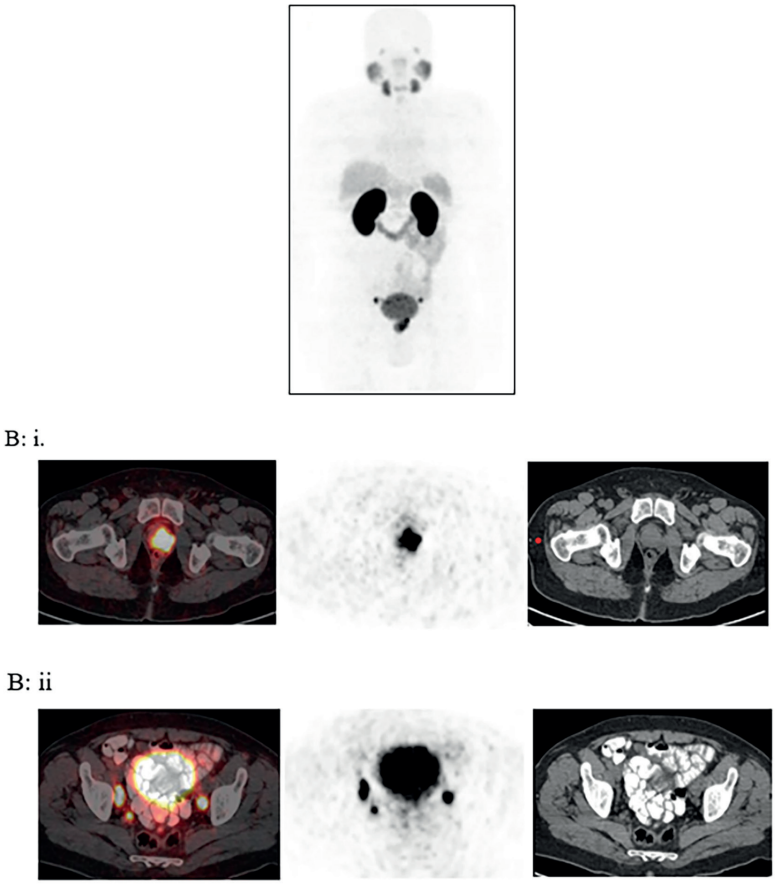


Figure 1. ROC Analysis ROC curve demonstrating the relationship between of the primary lesion and extraprostatic spread (lymph node and/or bone metastases)
ROC: Receiver operating characteristic, SUV_{max}: Standardized uptake value maximum

Table 3. Optimal cut-off for predicting extraprostatic disease

Parameter	AUC (95% CI)	Optimal cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)	p value
	0.70 (0.53-0.87)	8.97	81.3 (57.0-93.4)	66.7 (45.4-82.8)	0.04

AUC: Area under the curve, : Standardized uptake value maximum, CI: Confidence interval



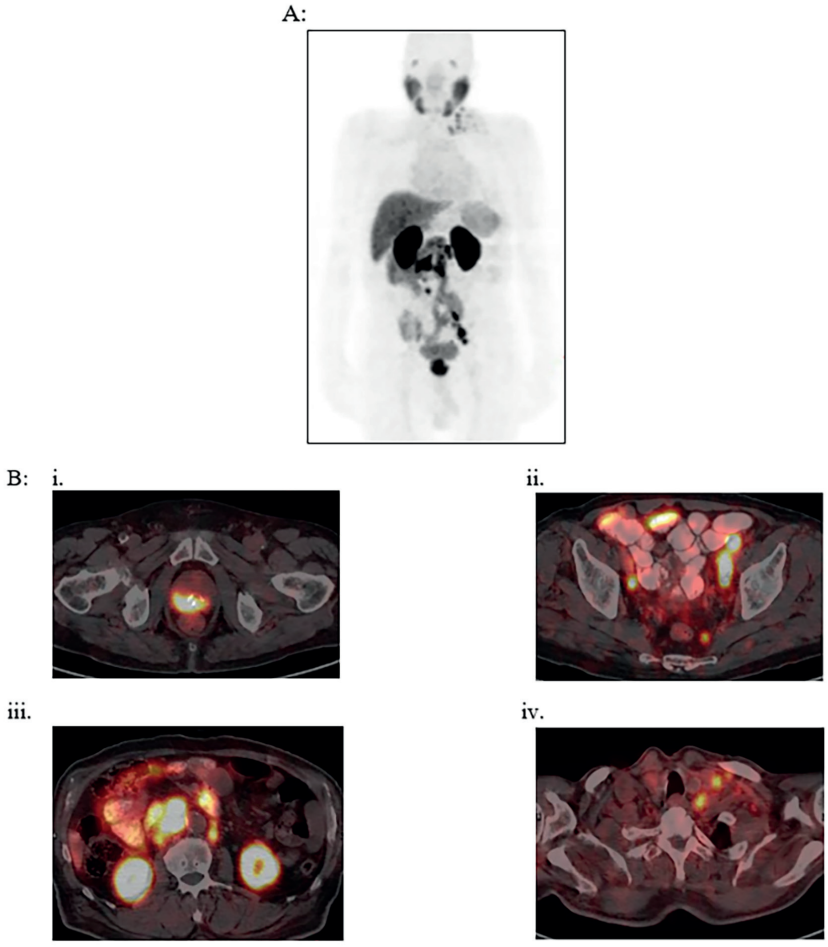
Case 1. A fifty-five-year-old male patient was diagnosed with prostate adenocarcinoma (Gleason Grade group 5; Gleason score 5+5) based on TRUS-guided biopsy. Due to elevated PSA level (19.07 ng/mL) and suspicious pelvic lymph nodes detected on MRI, the patient was referred for Ga-68 PSMA PET/CT for disease staging.
Figure A shows the MIP image demonstrating intense PSMA uptake in the prostate gland and pelvic lymph nodes.
Figure B(i) displays axial PET/CT images of the primary prostate tumor with a maximum SUV of 29.55.
Figure B(ii) shows bilateral external iliac and obturator lymph nodes with pathological PSMA uptake; the smallest node measured 5 mm (range: 5-15 mm).
Following PET/CT, the patient underwent radical prostatectomy and pelvic lymph node dissection. Histopathological evaluation confirmed lymph node metastases. Postoperative pelvic radiotherapy was administered. At 9 months post-treatment, the patient is disease-free with undetectable PSA levels (<0.003 ng/mL).
ROC: Receiver operating characteristic, : Standardized uptake value maximum, TRUS: Transrectal ultrasound, PSA: Prostate-specific antigen, MRI: Magnetic resonance imaging, Ga-68: Gallium-68, PSMA: Prostate-specific membrane antigen, PET/CT: Positron emission tomography/computed tomography, MIP: Maximum intensity projection

intermediate-risk cases (85.7% vs. 60.0%, p<0.05), particularly in younger patients and those with lower PSA levels. Notably, PSMA PET/CT positivity in lower-risk groups was more likely in patients aged ≥62.5 years and with PSA ≥9.4 ng/mL. These findings highlight the complementary roles of PSMA PET/CT and mpMRI across different risk categories. Although some prior studies have reported variability in the correlation between PET parameters and histopathological grades, our data support a robust association between PSMA expression level (SUV_{max}) and tumor differentiation. Nevertheless, further validation in prospective multicenter cohorts is warranted.

The proPSMA trial by Hofman et al. [4] showed that PSMA PET/CT outperformed conventional imaging in detecting nodal and distant metastases, with an accuracy of 92%, sensitivity of 85%, and specificity of 98%, compared to 65%, 38%, and 91%, respectively, for conventional imaging. Additionally, a meta-analysis by Hövels et al. [3] demonstrated limited sensitivity for both CT (42%) and MRI (39%) in detecting nodal involvement, despite relatively high specificity (~82%), highlighting the diagnostic gap that molecular imaging can address [3].

In a retrospective study of 50 intermediate- to high-risk prostate cancer patients, Spena et al. [11] evaluated Ga-68 PSMA PET/CT in patients undergoing radical prostatectomy and extended pelvic lymph node dissection, reporting a specificity of 88.1% but limited sensitivity of 25% for nodal metastasis. Furthermore, they noted a positive correlation between the SUV_{max} of the primary lesion and baseline PSA levels and tumor burden, suggesting its dual role in local and systemic assessment [11].

In a multicentre study, Öbek et al. [12] evaluated the diagnostic performance of ^{68}Ga-PSMA PET/CT for primary lymph node



Case 2. A seventy-four-year-old male patient was diagnosed with prostate adenocarcinoma, Gleason Grade group 4 (Gleason score 5+3), based on TRUS-guided biopsy. Due to elevated serum PSA levels (168 ng/mL), a Ga-68 PSMA PET/CT scan was performed for staging purposes.
A: MIP image showing increased tracer uptake in the prostate gland, as well as in abdominopelvic and left supra-infraclavicular lymph nodes.
B: PET/CT images demonstrating pathological PSMA uptake in the primary tumor within the prostate gland (i), and in metastatic lymph nodes in the pelvic (ii), abdominal (iii), and left supra-/infraclavicular (iv) regions.
The diagnosis of metastasis in the supraclavicular lymph nodes was histopathologically confirmed. The patient was diagnosed with metastatic prostate carcinoma with widespread lymph node involvement, and hormone therapy was initiated.
Ga-68: Gallium-68, PSMA: Prostate-specific membrane antigen, PET/CT: Positron emission tomography/computed tomography, MIP: Maximum intensity projection, TRUS: Transrectal ultrasound, PSA: Prostate-specific antigen

staging in high- and very high-risk prostate cancer. PSMA PET/CT demonstrated higher specificity (86%) and accuracy (76%) than conventional imaging, with improved values in patients undergoing extensive lymphadenectomy (accuracy: 81%). Kappa analysis showed better concordance with histopathology compared to morphological imaging, (0.41 vs. 0.18) and supports PSMA PET/CT as a superior non-invasive tool, although extended surgical dissection remains the gold standard for nodal staging [12].

Corona-Montes et al. [13] assessed the accuracy of ^{68}Ga-PSMA PET/CT for primary lymph node staging in high-risk prostate cancer using extended pelvic lymphadenectomy as the reference. In a small cohort of 17 patients, PSMA PET/CT demonstrated high specificity (92.3%) and negative predictive value (92.3%), with an overall diagnostic accuracy of 88.2%. Although sensitivity was lower (75%), the findings support the potential of PSMA PET/CT to reliably exclude nodal metastases preoperatively, suggesting it may help avoid unnecessary lymphadenectomy in selected patients [13].

In a large multicenter phase 3 trial, Hope et al. [14] assessed the diagnostic performance of ^{68}Ga-PSMA-11 PET/CT for detecting pelvic lymph node metastases in intermediate- to high-risk prostate cancer. Among 277 patients who underwent radical prostatectomy with lymph node dissection, PSMA PET/CT demonstrated high specificity (95%) but limited sensitivity (40%) for pelvic nodal disease. Despite its high negative predictive value (81%), the relatively low sensitivity highlights its limitations in detecting small-volume metastases, reinforcing that a negative PSMA PET result should not preclude pelvic lymph node dissection in surgical candidates [14].

Jochumsen and Bouchelouche [15] provided an updated overview of PSMA PET/CT for primary staging of prostate

cancer, highlighting its superior sensitivity and specificity for metastatic detection compared to conventional imaging. When combined with multiparametric MRI, PSMA PET/CT enhances assessment of extracapsular extension and seminal vesicle invasion, and may serve as a second-line modality in patients with inconclusive MRI or negative biopsies. Notably, PSMA PET/CT alters clinical management in approximately 25% of cases. However, its impact on long-term outcomes remains uncertain, underscoring the need for prospective validation.

In our study, lymph node metastases were detected in 40.5% of patients and were significantly associated with Gleason Grade group (p=0.04). Among the 37 patients included in our primary staging cohort, lymph node metastases were detected in 15 individuals. Of these, ten had metastatic involvement limited to the pelvic region, whereas five patients exhibited both pelvic and extrapelvic nodal metastases. The identification of extrapelvic spread led to significant modifications in treatment planning. The smallest lymph node demonstrating PSMA uptake measured 5 mm in short-axis diameter (range: 5-20 mm). Notably, the majority of these lymph nodes were below the threshold for pathological enlargement, rendering them unlikely to be identified with conventional radiological modalities. These findings highlight the added diagnostic value of PSMA PET/CT in detecting subclinical nodal metastases that may be missed on anatomical imaging alone.

In our study, bone metastases were identified in seven patients. Among patients who underwent concurrent conventional bone scintigraphy and Ga-68 PSMA PET/CT, PSMA PET/CT successfully characterized suspicious lesions in 76% of cases initially considered equivocal for metastasis on scintigraphy. This imaging modality demonstrated a significant clinical impact by contributing to more accurate disease staging and informing subsequent modifications in therapeutic management. Zhao et al. [16] conducted a meta-analysis comparing PSMA PET/CT with conventional bone scintigraphy and demonstrated superior sensitivity (98% vs. 83%) and specificity (97% vs. 68%) for PSMA PET/CT in detecting osseous involvement. Mainta et al. [17] reported that combining PSMA-RADS and PROMISE criteria in interpretation significantly reduced equivocal bone lesions and increased diagnostic accuracy, particularly in the pelvis and ribs, underscoring the value of structured reporting systems.

Some sclerotic lesions in our cohort showed no PSMA uptake and were deemed benign, highlighting the potential for false negatives. This is in line with findings by Afshar-Oromieh et al., [18] who found that although PSMA PET/CT achieves excellent tumor-to-background ratios and high lesion detectability (up to 100% in patients with PSA >2.2 ng/mL), variants such as dedifferentiated or neuroendocrine tumors may demonstrate low uptake. In our study, Ga-68 PSMA PET/CT resolved indeterminate findings in 76% of patients who also underwent bone scintigraphy, further supporting its clinical advantage.

In our cohort, an SUV_{max} cutoff of 8.97 yielded 81.3% sensitivity and 66.7% specificity for predicting extraprostatic extension. These results are comparable to those of Koerber et al., [19] who reported a threshold of 11.9 with sensitivity and specificity values of 76% and 58.4%, respectively, indicating that SUV_{max} may help stratify local invasion risk preoperatively.

Dual-phase imaging was performed in 27 patients. A significant correlation was again observed between delayed-phase SUV_{max} values and both PSA (p=0.002), and Gleason Grade group (p=0.013), although no additional lesions were identified. Derlin et al. [20] demonstrated that delayed imaging following diuretic administration can enhance lesion visualization in the prostate region by minimizing urinary tracer interference, particularly aiding pelvic lymph node assessment. Intraprostatic uptake patterns in our cohort ranged from heterogeneous to focally intense. This has previously been described by Afshar-Oromieh et al., [18] who reported strong tumor contrast and progressive lesion conspicuity in delayed imaging phases. Thus, while our findings suggest limited added diagnostic value in this population, tailored imaging protocols may offer benefits in specific clinical scenarios.

Tsechelidis and Vrachimis [21] highlighted in their review, that PSMA PET/CT detects more extensive disease than conventional modalities, and may significantly impact clinical management, with the potential to upstage more than 50% of patients initially classified as M0. Their conclusions support its growing role as a new standard in the staging of high-risk prostate cancer [21].Von Stauffenberg et al. [22] highlighted the transformative role of PSMA-PET in prostate cancer imaging, with superior sensitivity and specificity over conventional modalities. Its clinical utility spans initial staging of intermediate- to high-risk cases, detection of biochemical recurrence, and evaluation of metastatic disease. The use of targeted radiotracers enables detection of small-volume metastases, facilitating both diagnostic precision and personalized therapeutic strategies such as radioligand therapy. While its integration into guidelines has improved care pathways, prospective outcome data remain limited [22].

In our study, PSMA PET/CT was superior to bone scintigraphy in skeletal evaluation, particularly in cases with indeterminate findings, and demonstrated the ability to detect subcentimetric lymph node metastases undetectable by conventional imaging. Although dual-phase imaging did not yield additional lesions, delayed-phase SUV_{max} values remained consistent with early-phase measurements and may provide added value in selected clinical scenarios.

Given its capacity to provide both anatomical and functional information, PSMA PET/CT contributes meaningfully to initial staging, treatment planning, and prognostic assessment. Our findings support its incorporation into clinical algorithms for individualized management of prostate cancer patients. This study is limited by its retrospective nature, small sample size, and partial histopathological verification. Nonetheless, the findings offer valuable insights for hypothesis generation and warrant further prospective validation. Further large-scale prospective studies are warranted to refine the prognostic thresholds for optimal clinical decision-making.

Study Limitations

This study has several limitations. First, its retrospective design may have introduced selection bias and limited the

completeness of clinical data. Second, the relatively small sample size (n=37) may reduce the statistical power and limit the generalizability of the results. Third, histopathological confirmation of lymph node metastases was available in only a limited number of patients, potentially affecting the assessment of imaging accuracy. Lastly, the number of patients who underwent dual-phase imaging was low, making it difficult to draw definitive conclusions about the added value of delayed-phase acquisition. Further prospective, multicenter studies with larger cohorts and histopathological validation are needed to confirm these findings.

Conclusion

Ga-68 PSMA PET/CT demonstrates high diagnostic accuracy in the staging of intermediate to high-risk prostate cancer and shows significant correlation with key clinical and pathological prognostic markers, including serum PSA levels and Gleason Grade group. The SUV_{max} of the primary tumor was significantly associated with both the presence of extraprostatic disease and overall tumor aggressiveness, highlighting its potential as a non-invasive imaging biomarker for risk stratification.

Ethics

Ethics Committee Approval: Ethical approval was received from the University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Education and Research Institute Institutional Review Board (approval no: 9, date: 01.06.2017).

Informed Consent: This retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.Ö., G.U., B.B.D., S.D.Ş., H.E., A.K.F., H.B., Concept: İ.Ö., G.U., B.B.D., S.D.Ş., H.B., Design: İ.Ö., G.U., B.B.D., H.B., Data Collection or Processing: İ.Ö., G.U., B.B.D., S.D.Ş., H.E., A.K.F., Analysis or Interpretation: İ.Ö., G.U., B.B.D., S.D.Ş., H.E., A.K.F., Literature Search: İ.Ö., G.U., H.B., Writing: İ.Ö.

Conflict of Interest: No conflict of interest was declared by theauthors.

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

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Mottet N, van den Bergh RCN, Briers E, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer-2020 update. Part 1: screening, diagnosis, and local treatment with curative intent. Eur Urol. 2021;79:243-262.
3. Hövels AM, Heesakkers RA, Adang EM, et al. The diagnostic accuracy of CT and MRI in the staging of pelvic lymph nodes in patients with prostate cancer: a meta-analysis. Clin Radiol. 2008;63:387-395.
4. Hofman MS, Lawrentschuk N, Francis RJ, et al. Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): a prospective, randomised, multicentre study. Lancet. 2020;395:1208-1216.
5. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C. Prostate-specific membrane antigen expression in normal and malignant human tissues. Clin Cancer Res. 1997;3:81-5.
6. Perner S, Hofer MD, Kim R, et al. Prostate-specific membrane antigen expression as a predictor of prostate cancer progression. Hum Pathol. 2007;38:696-701.
7. Fendler WP, Schmidt DF, Wenter V, et al. 68Ga-PSMA PET/CT detects the location and extent of primary prostate cancer. J Nucl Med. 2016;57:1720-1725.
8. Kwan TN, Spremo S, Teh AYM, McHarg D, Thangasamy I, Woo HH. Performance of Ga-68 PSMA PET/CT for diagnosis and grading of local prostate cancer. Prostate Int. 2021;9:107-112.
9. Uprimny C, Kroiss AS, Decristoforo C, et al. ^{68}Ga-PSMA-11 PET/CT in primary staging of prostate cancer: PSA and Gleason score predict the intensity of tracer accumulation in the primary tumour. Eur J Nucl Med Mol Imaging. 2017;44:941-949.
10. Zhou C, Tang Y, Deng Z. et al. Comparison of 68Ga-PSMA PET/CT and multiparametric MRI for the detection of low- and intermediate-risk prostate cancer. EJNMMI Res. 2022;12:10.
11. Spena G, Moretti TB, Dávila FS, et al. Ga68-PSMA PET for lymph node staging in intermediate and high-risk prostate cancer patients undergoing robotic assisted radical prostatectomy. Minerva Urol Nephrol. 2024;76:467-473.
12. Öbek C, Doğanca T, Demirci E, et al. The accuracy of ^{68}Ga-PSMA PET/CT in primary lymph node staging in high-risk prostate cancer. Eur J Nucl Med Mol Imaging. 2017;44:1806-1812.
13. Corona-Montes VE, González-Cuenca E, Fernández-Noyola G, et al. Primary lymph-node staging with ^{68}Ga-PSMA PET in high-risk prostate cancer: pathologic correlation with extended pelvic lymphadenectomy specimens. Urol Oncol. 2021;39:494.e1-494.e6.
14. Hope TA, Eiber M, Armstrong WR, et al. Diagnostic accuracy of 68Ga-PSMA-11 PET for pelvic nodal metastasis detection prior to radical prostatectomy and pelvic lymph node dissection: a multicenter prospective phase 3 imaging trial. JAMA Oncol. 2021;7:1635-1642.
15. Jochumsen MR, Bouchelouche K. PSMA PET/CT for primary staging of prostate cancer - an updated overview. Semin Nucl Med. 2024;54:39-45.
16. Zhao R, Li Y, Nie L, Qin K, Zhang H, Shi H. The meta-analysis of the effect of 68Ga-PSMA-PET/CT diagnosis of prostatic cancer compared with bone scan. Medicine (Baltimore). 2021;100:e25417.
17. Mainta IC, Neroladaki A, Wolf NB, et al. [^{68}Ga]Ga-PSMA-11 PET and prostate cancer bone metastases: diagnostic performance of available standardized criteria. J Nucl Med. 2024;65:1376-1382.
18. Afshar-Oromieh A, Malcher A, Eder M, et al. PET imaging with a [68Ga] gallium-labelled PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of tumour lesions. Eur J Nucl Med Mol Imaging. 2013;40:486-95.
19. Koerber SA, Boesch J, Kratochwil C, et al. Predicting the risk of metastases by PSMA-PET/CT-evaluation of 335 men with treatment-naïve prostate carcinoma. Cancers (Basel). 2021;13:1508.
20. Derlin T, Weiberg D, von Klot C, et al. ^{68}Ga-PSMA I&T PET/CT for assessment of prostate cancer: evaluation of image quality after forced diuresis and delayed imaging. Eur Radiol. 2016;26:4345-4353.
21. Tsechelidis I, Vrachimis A. PSMA PET in imaging prostate cancer. Front Oncol. 2022;12:831429.
22. von Stauffenberg F, Poyet C, Beintner-Skawran S, Maurer A, Schmid FA. Current clinical applications of PSMA-PET for prostate cancer diagnosis, staging, and treatment. Cancers (Basel). 2024;16:4263.

AHOT | Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):177-182

DOI: 10.4274/ahot.galenos.2025.2025-7-1

Original Article

Prognostic Value of the Hemoglobin Albumin Lymphocyte Platelet Score in Extrapulmonary Neuroendocrine Carcinomas

Perihan Perkin, Serhat Sekmek, Mehmet Akif Parlar, Doğan Bayram, Mirmehdi Mehdiyev, Şebnem Yücel, Fahriye Tuğba Köş

Ankara Bilkent City Hospital, Clinic of Medical Oncology, Ankara, Türkiye

ABSTRACT

Aim: Extrapulmonary neuroendocrine carcinomas (EPNECs) are rare and aggressive malignancies characterized by poor prognosis. The hemoglobin, albumin, lymphocyte, platelet (HALP) score is an immunonutritional index that has shown prognostic value in various cancers. However, its significance in EPNECs remains unclear.

Methods: This retrospective study included 48 patients with metastatic EPNECs treated at a single institution between 2018 and 2023. Baseline HALP scores were calculated from laboratory values obtained before systemic treatment. Patients were categorized into low (≤19.54) and high (>19.54) HALP groups based on receiver operating characteristic curve analysis. Associations between HALP score and survival outcomes, overall survival (OS) and progression-free survival (PFS), were assessed using Kaplan-Meier and Cox regression methods.

Results: The median OS was 7.8 months. Although the HALP score was not a statistically significant predictor of OS (p=0.072), patients with low HALP scores demonstrated a numerical trend toward shorter survival. Elevated lactate dehydrogenase (LDH) levels and absence of chemotherapy were significantly associated with poorer OS. Chemotherapy was confirmed as an independent prognostic factor in multivariate analysis (p=0.001). No significant association was found between HALP scores and PFS.

Conclusion: While the HALP score did not show statistically significant prognostic impact in this cohort, the observed trends suggest potential clinical relevance. Further prospective studies are needed to validate its utility in EPNEC and to explore whether immunonutritional factors may inform treatment decisions.

Keywords: Extrapulmonary neuroendocrine carcinomas, HALP score, inflammatory markers, predictive biomarkers

Introduction

Neuroendocrine carcinomas are a group of poorly differentiated, biologically aggressive malignancies believed to originate from neuroendocrine cells located in endocrine glands, within glandular structures, or scattered among epithelial tissues. These tumors can arise in various anatomical locations, with the lungs being the most common site.

When these carcinomas develop outside the pulmonary system, they are referred to as extrapulmonary neuroendocrine carcinomas (EPNECs), most frequently found in the gastroenteropancreatic tract, followed by the urinary and gynecological systems [1,2]. In certain cases, the primary tumor remains undetected despite extensive diagnostic workup, and such cases are categorized as unknown primary tumors.

EPNECs are known for their aggressive clinical course, often marked by early and extensive metastasis. Although a minority of patients with localized disease may achieve durable remission through intensive multimodal therapy, recurrence rates remain high, and the overall prognosis is generally poor, with five-year survival reported to be below 15% [3]. The hemoglobin, albumin, lymphocyte, platelet (HALP) score is a composite biomarker derived from routine hematological and biochemical parameters, and has emerged as a prognostic indicator in various malignancies.

Address for Correspondence: Perihan Perkin MD, Ankara Bilkent City Hospital, Clinic of Medical Oncology, Ankara, Türkiye

E-mail: perihanperkin@gmail.com **ORCID ID:** orcid.org/0000-0003-3994-815X

Received: 09.07.2025 **Accepted:** 10.09.2025 **Epub:** 23.09.2025 **Publication Date:** 12.12.2025

Cite this article as: Perkin P, Sekmek S, Parlar MA, et al. Prognostic value of the hemoglobin albumin lymphocyte platelet score in extrapulmonary neuroendocrine carcinomas. Acta Haematol Oncol Turc. 2025;58(3):177-182

Anemia in cancer can be driven by chronic inflammation or iron deficiency related to blood loss and malnutrition. Hypoalbuminemia, similarly, often reflects poor nutritional and inflammatory status and has been linked to worse outcomes in several cancer types [4,5]. These alterations are also commonly seen in cancer cachexia, a multifactorial syndrome characterized by progressive skeletal muscle loss [6].

Lymphocytes play a key role in anti-tumor immunity, and lymphopenia has been associated with worse survival in studies evaluating ratios such as the platelet-to-lymphocyte ratio and the neutrophil-to-lymphocyte ratio [7,8].

On the other hand, platelets contribute to tumor progression by promoting angiogenesis through vascular endothelial growth factor release and by shielding tumor cells from immune surveillance. Thrombocytosis, a marker of systemic inflammation, has been shown to correlate with poor prognosis in many cancers [9,10].

First introduced by Chen et al. [11] in gastric cancer, the HALP score is calculated using the formula:

[hemoglobin (g/L) × albumin (g/L) × lymphocyte count (/L)] / platelet count (/L), and integrates immune and nutritional markers to reflect systemic host status. Since then, its prognostic utility has been explored in various malignancies.

While several studies have reported the prognostic value of the HALP score in small cell lung cancer (SCLC) patients receiving first-line etoposide-based chemotherapy, there remains a lack of evidence regarding its role in EPNECs. Therefore, this study aims to investigate the prognostic significance of the HALP score in this rare and heterogeneous tumor group.

Methods

This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Ethics Committee of Ankara Bilkent City Hospital (1st Medical Research Scientific and Ethics Review Board) (decision no: TABED 1-24-777, date: 04.12.2024).

This retrospective cohort study included 48 adult patients diagnosed with EPNEC at Ankara Bilkent City Hospital between March 2018 and October 2024. Eligibility criteria comprised age ≥18 years, histopathological confirmation of neuroendocrine carcinoma, and presence of metastatic disease at the time of diagnosis. Patients were excluded if they had incomplete medical records, non-metastatic disease, pulmonary-origin NEC, or were younger than 18 years. Data collected for analysis included demographic variables (age and sex), clinical characteristics (Eastern Cooperative Oncology Group [ECOG] performance status), pathological features (histological subtype: small cell, large cell, or mixed type), primary tumor site (colorectal, pancreatic, gastric, or unknown origin), Ki-67 proliferation index, and laboratory parameters : hemoglobin, albumin, lymphocyte count, and platelet count. Additional variables included lactate dehydrogenase (LDH) levels and systemic treatment status, specifically whether patients received chemotherapy.

The HALP score, reflecting the patient's immune and nutritional status, was calculated using the following formula:

HALP = Hemoglobin (g/L) × Albumin (g/L) × Lymphocyte count (/L) ÷ Platelet count (/L)

Values used for this calculation were obtained from blood tests performed prior to the initiation of chemotherapy to ensure assessment of baseline conditions.

To determine the optimal HALP threshold for prognostic classification, a receiver operating characteristic (ROC) curve analysis was conducted. The analysis identified 19.54 as the cut-off value (area under curve: 0.676, sensitivity: 75%, specificity: 58.3%, p=0.07). Based on this threshold, patients were categorized into two groups: low HALP (≤19.54) and high HALP (>19.54). Similarly, LDH levels were classified as ≤246 IU/L or >246 IU/L, based on the upper limit of normal in our institution. The Ki-67 index was dichotomized at the median value (85%) into ≤85% and >85%.

Endpoints

The primary endpoints were overall survival (OS), defined as the time from diagnosis to death from any cause, and progression-free survival (PFS), defined as the time from diagnosis to disease progression or death from any cause.

Statistical Analysis

Descriptive statistics were used to summarize baseline characteristics. Group comparisons were performed using the chi-square or Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. The optimal HALP cut-off value was determined by ROC analysis. Kaplan-Meier survival analysis was used to estimate OS and PFS, and survival differences between groups were evaluated using the Log-Rank Test. Univariate and multivariate analyses were conducted using Cox proportional hazards regression to identify independent prognostic factors. Results were reported as hazard ratios with corresponding 95% confidence intervals, and statistical significance was defined as a two-tailed p value <0.05.

Results

Patient Characteristics

A total of 48 patients diagnosed with EPNECs were enrolled in this study. The median age was 60 years, ranging from 20 to 83. Males comprised the majority of the cohort (77.1%, n=37). Most patients (87.5%, n=42) had an ECOG performance status of 2 or below. Regarding histological subtypes, small cell NEC was the predominant form (81.3%, n=39), followed by mixed NEC types (14.6%, n=7) and large cell NEC (4.2%, n=2). The most common primary tumor sites were colorectal (20.8%, n=10), unknown sites (20.8%, n=10), gastric (14.6%, n=7), and pancreas (12.5%, n=6). LDH levels were elevated (>246 IU/L) in 64.6% (n=31) of patients (Table 1).

HALP Groups and Baseline Characteristics

Patients were divided into low HALP (≤19.54, n=24) and high HALP (>19.54, n=24) groups. Comparison of baseline features revealed that elevated LDH levels were significantly more common in the low HALP group (83.3%) compared to the high HALP group (45.8%) (p=0.006). No statistically significant differences were detected between the two groups concerning age, sex, ECOG status, histological subtype, or Ki-67 proliferation index (Table 2).

Overall Survival (OS)

The median OS for the entire cohort was 7.8 months (range 0.09-10.48 months). Univariate analysis identified elevated LDH levels (≥246 IU/L) as significantly associated with decreased OS (p=0.023). Although the HALP score demonstrated a trend toward predicting OS, this association did not reach statistical significance (p=0.072). These results should therefore be interpreted with caution. (Figure 1).

Patients who received chemotherapy had significantly better OS than those who did not (p<0.001). Multivariate analysis identified chemotherapy as an independent prognostic factor for OS (p=0.001), while LDH levels approached statistical significance (p=0.082) (Table 3).

Table 1. Clinical, pathological and laboratory characteristics of the patients

Variables	n (%)
Age, years, median	60 (20-83)
<60 years	22 (45.8%)
≥60 years	26 (54.2%)
Sex	
Male	37 (77.1%)
Female	11 (22.9%)
ECOG performance status	
0	2 (4.2%)
1	25 (52.1%)
2	15 (31.3%)
3	5 (10.4%)
4	1 (2.1%)
Histological type	
Small cell NEC	39 (81.3%)
Large cell NEC	2 (4.2%)
Mix (NEC + other component)	7 (14.6%)
Primary tumour site	
Colorectal	10 (20.8%)
Unknown	10 (20.8%)
Pancreas	6 (12.5%)
Gastric	7 (14.6%)
Other	15 (31.3%)
Ki-67	
≤85%	18 (43.9%)
>85%	23 (56.1%)
LDH	
≤246	17 (35.4%)
>246	31 (64.6%)

ECOG: Eastern Cooperative Oncology Group, NEC: Neuroendocrine carcinoma, LDH: Lactate dehydrogenase

Progression-Free Survival (PFS)

The median PFS for the cohort was not reported in detail due to the limited number of progression events. Univariate analysis showed that receiving chemotherapy was the only factor significantly associated with longer PFS (p<0.001). Consequently, multivariate analysis was not performed for PFS (Table 4, Figure 2).

Response Rates

The overall response rate did not show significant associations with any clinical or laboratory variables, including HALP scores. However, when considering the disease control rate (DCR), patients with mixed histological subtypes demonstrated a significantly higher DCR compared to other subtypes (p=0.028). No other variables were found to be significantly associated with DCR.

Discussion

This study aimed to evaluate the prognostic significance of the HALP score in patients with EPNECs, a rare and biologically aggressive tumor group. Although our findings did not demonstrate statistically significant associations, the observed trends suggest that lower HALP scores may be linked to inferior clinical outcomes. This aligns with existing literature highlighting the prognostic value of immunonutritional markers in oncology.

The prognostic utility of the HALP score has been extensively studied in various cancers. For instance, Shen et al. [12] demonstrated that a low HALP score before first-line treatment with etoposide was independently associated with shorter PFS in patients with SCLC, who are 65 years or older, a pulmonary NEC subtype. Similarly, Yang et al. [13] identified the HALP score as a significant prognostic factor for OS in SCLC patients undergoing chemotherapy, highlighting its role as a reliable biomarker in this patient population.

In comparison to SCLC, studies focusing on HALP scores in EPNECs are scarce. Our findings contribute to filling this gap by exploring the potential role of HALP scores in a distinct,

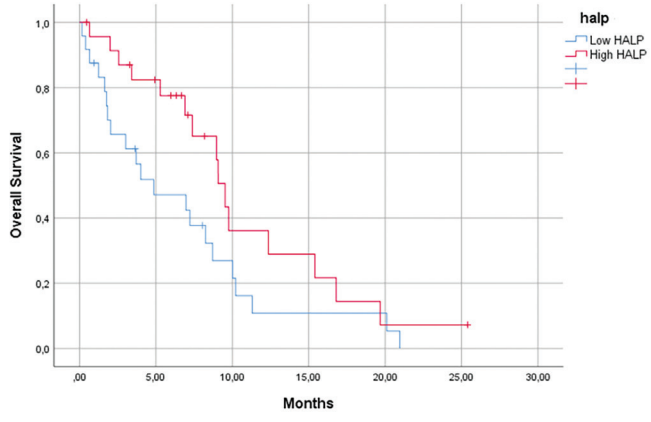


Figure 1. The Kaplan-Meier curves of overall survival for HALP
HALP: Hemoglobin, albumin,lymphocyte, platelet

non-pulmonary NEC cohort. While SCLC and EPNECs share significant biological characteristics, including their aggressive clinical course, their prognosis and therapeutic strategies differ in important ways. Both malignancies are typically treated with etoposide and platinum-based chemotherapy regimens, and demonstrate rapid progression if left untreated. However, subtle differences in biological behavior and treatment outcomes between the two entities warrant further investigation [2,14,15].

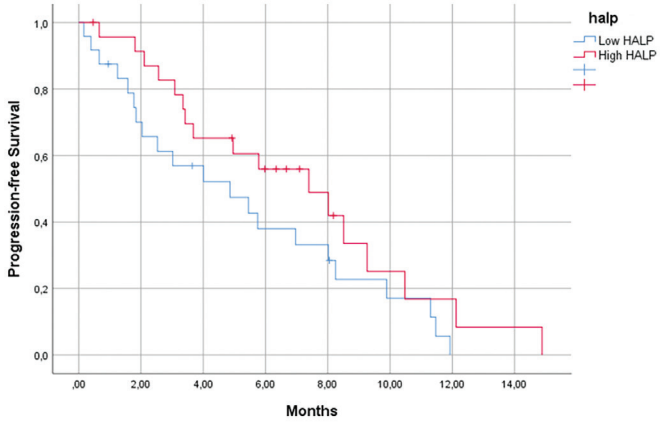


Figure 2. The Kaplan-Meier curves of progression free survival for HALP

HALP: Hemoglobin, albumin,lymphocyte, platelet

Table 2. The features of HALP groups

Variables	Low HALP (n: 24)	High HALP (n: 24)	p value
Age, years, median			
≤60 years	8 (33.3%)	14 (58.3%)	0.081
>60 years	16 (66.7%)	10 (41.7%)	
Sex			
Male	17 (70.8%)	20 (83.3%)	0.300
Female	7 (29.2%)	4 (16.7%)	
ECOG performance status			
0-1	11 (45.8%)	16 (66.7%)	0.130
2-4	13 (54.2%)	8 (33.3%)	
Histological type			
Small cell NEC	21 (87.5%)	18 (75.0%)	0.144
Large cell NEC	1 (4.2%)	1 (4.2%)	
Mix (NEC + other component)	2 (8.3%)	5 (20.8%)	
Ki-67			
≤85%	7 (35.0%)	11 (52.4%)	0.261
>85%	13 (65.0%)	10 (47.6%)	
LDH			
<246	4 (16.7%)	13 (54.2%)	**0.006**
≥246	20 (83.3%)	11 (45.8%)	
Chemotherapy			
Yes	21 (87.5%)	2 (8.3%)	0.637
No	3 (12.5%)	22 (91.7%)	

HALP: Hemoglobin, albumin, lymphocyte, platelet, ECOG: Eastern Cooperative Oncology Group, LDH: Lactate dehydrogenase, NEC: Neuroendocrine carcinomas

In our study, the median OS of patients with EPNEC was notably shorter compared to reported outcomes in SCLC, highlighting the aggressive nature of this disease. Consistent with the literature, EPNECs are reported to have a worse prognosis and a poorer response to chemotherapy than SCLC. Tumor localization patterns in our cohort were also consistent with those reported in the literature, with the majority of cases originating in the gastrointestinal tract, followed by unknown primary sites [2]. These findings align with previously documented trends, providing a solid basis for investigating the prognostic role of HALP scores in EPNEC patients. Further research is needed to better understand the interplay of these clinical parameters and their impact on patient outcomes.

Beyond NEC, the HALP score has shown consistent prognostic value across a range of malignancies. A recent meta-analysis by Farag et al. [16] reviewed the prognostic ability of HALP scores in different cancer types and emphasized its robust association with outcomes such as PFS and OS across various solid tumors. Another meta-analysis by Xu et al. [17] involving 13, 110 patients reinforced these findings, highlighting the HALP score's utility as a simple yet effective biomarker for cancer prognosis. These meta-analyses provide strong evidence for incorporating the HALP score into clinical practice, given its ability to reflect both inflammatory and nutritional states, which are critical determinants of cancer progression and treatment response.

Various studies have demonstrated the potential of the HALP score as an immunonutritional biomarker, showing its association with prognosis in a variety of cancers [16,17]. A low HALP score has been linked to poorer survival outcomes, suggesting that it may serve as a valuable prognostic indicator. However, the clinical applicability of the HALP score remains an area of active exploration. Specifically, while a lower HALP score is often indicative of worse prognosis, it raises the question of whether immunonutritional interventions could positively impact survival outcomes in these patients. Given the association between low HALP scores and poorer survival, incorporating routine nutritional and immunological assessments into clinical practice may help identify high-risk patients. Furthermore, targeted nutritional interventions, such as high-protein diets or supplementation with immune-modulating nutrients, could be explored as part of a comprehensive treatment strategy.

Although no significant differences were observed in our study between HALP groups and age or gender, the differences in cutoff values across studies may be related to variations in age-related factors, such as the decline in hemoglobin and albumin levels with age [18,19]. This can influence the prognostic value of the HALP score. Additionally, other variables such as gender, which may influence immune and inflammatory responses, could further affect the prognostic accuracy of the HALP score. Some studies have noted variations in baseline HALP scores between males and females. However, even when adjustments are made for sex, HALP has been shown to retain its prognostic significance. This suggests that while

gender-related differences in HALP scores may exist, they do not substantially impact their reliability as a biomarker [16].

The discrepancies in cutoff values may stem from variations in calculation methods, such as X-tile or other approaches, as well as differences in patient demographics and clinical parameters [20]. This highlights the critical need for standardization in HALP score calculations. Future studies should focus on how these variables impact the interpretation and utility of HALP scores across different cancer populations.

Although our study's findings did not reach statistical significance, which is likely because of the limited sample size, the numerical trend observed, aligns with these prior studies. The HALP score's numerical association with survival in EPNEC patients suggests that it may serve as a useful tool for risk stratification. Nevertheless, this numerical trend should be interpreted cautiously, particularly in light of the limited sample size. However, larger, prospective studies are needed to confirm these observations and establish definitive clinical guidelines.

Table 3. Prognostic factors of overall survival in patients

Univariate analysis	
Variables	**p value**
Age, ≤60 years vs >60 years	0.438
Sex, male vs female	0.411
ECOG performance status, 0-1 vs 2-4	0.287
Histological type, small cell NEC, large cell NEC vs mix (NEC + other component)	0.657
Ki-67, ≤85% vs >85%	0.966
LDH, <246 vs ≥246	**0.023**
HALP, high vs low Chemotherapy, yes vs no	0.072 **<0.001**
Multivariate analysis	
Variables	**p value**
LDH, <246 vs ≥246	0.082
Chemotherapy, yes vs no	**0.001**

ECOG: Eastern Cooperative Oncology Group, LDH: Lactate dehydrogenase, NEC: Neuroendocrine carcinomas, HALP: Hemoglobin, albumin,lymphocyte, platelet

Table 4. Prognostic factors of progression-free survival in patients

Univariate analysis	
Variables	**p value**
Age, ≤60 years vs >60 years	0.363
Sex, male vs female	0.402
ECOG performance status, 0-1 vs 2-4	0.089
Histological type, small cell NEC, large cell NEC vs mix (NEC + other component)	0.667
Ki-67, ≤85% vs >85%	0.826
LDH, <246 vs ≥246	0.247
HALP, high vs low Chemotherapy, yes vs no	0.124 **<0.001**

ECOG: Eastern Cooperative Oncology Group, LDH: Lactate dehydrogenase, NEC: Neuroendocrine carcinomas, HALP: Hemoglobin, albumin, lymphocyte, platelet

Study Limitations

The retrospective nature of our study and the relatively small cohort size are notable limitations, potentially impacting the statistical power of our analyses. In particular, the limited sample size may have increased the risk of type 2 error and reduced the generalizability of our results to broader EPNEC populations. Despite these limitations, the comprehensive evaluation of patient records and the focus on a rare cancer type are strengths that enhance the relevance of our findings. Furthermore, the uniformity in treatment protocols, with all patients receiving platinum plus etoposide-based chemotherapy at first line, minimizes potential confounding effects related to variability in treatment approaches.

Our study supports the potential utility of the HALP score as a prognostic marker in EPNECs. While the association observed in this cohort was not statistically significant, the numerical trend aligns with existing evidence from other cancer types and NEC subtypes. The HALP score's ease of measurement and cost-effectiveness make it an attractive biomarker for routine clinical use. Further research, particularly in the form of large-scale, prospective studies, is warranted to confirm these findings and explore the HALP score's role in guiding individualized treatment strategies.

Conclusion

Although the HALP score was not significantly associated with OS or PFS in our study, its correlation with LDH levels and observed trends suggest potential prognostic implications that deserve further exploration. Elevated LDH levels, a known marker of tumor burden and metabolic stress, were associated with worse survival outcomes. Furthermore, the absence of chemotherapy was another critical factor linked to poor prognosis. These findings underscore the importance of comprehensive metabolic and treatment-based assessments in the management of patients with extrapulmonary NEC. Future research should focus on validating these observations and exploring the potential benefits of interventions, such as immunonutritional support, tailored to specific metabolic profiles.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Ethics Committee of Ankara Bilkent City Hospital (1st Medical Research Scientific and Ethics Review Board) (decision no: TABED 1-24-777, date: 04.12.2024).

Informed Consent: Informed consent was waived due to the use of anonymized clinical data.

Footnotes

Authorship Contributions

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

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

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

References

1. Robinson MD, Livesey D, Hubner RA, Valle JW, McNamara MG. Future therapeutic strategies in the treatment of extrapulmonary neuroendocrine carcinoma: a review. Ther Adv Med Oncol. 2023;15:17588359231156870.
2. Dasari A, Mehta K, Byers LA, Sorbye H, Yao JC. Comparative study of lung and extrapulmonary poorly differentiated neuroendocrine carcinomas: a SEER database analysis of 162,983 cases. Cancer. 2018;124:807-815.
3. Walenkamp AM, Sonke GS, Sleijfer DT. Clinical and therapeutic aspects of extrapulmonary small cell carcinoma. Cancer Treat Rev. 2009;35:228-236.
4. Yamamoto T, Kawada K, Obama K. Inflammation-related biomarkers for the prediction of prognosis in colorectal cancer patients. Int J Mol Sci. 2021;22:8002.
5. Jiang Z, Li Y, Han G, et al. [Association of serum albumin level with clinicopathologic features and prognosis in colon cancer]. Zhonghua Wei Chang Wai Ke Za Zhi. 2016;19:80-83.
6. Sadeghi M, Keshavarz-Fathi M, Baracos V, Arends J, Mahmoudi M, Rezaei N. Cancer cachexia: diagnosis, assessment, and treatment. Crit Rev Oncol Hematol. 2018;127:91-104.
7. Catal O, Ozer B, Sit M. Prediction of lymph node metastasis in colon cancer via platelet to lymphocyte ratio and platelet count. J Coll Physicians Surg Pak. 2020;30:250-253.
8. Ethier JL, Desautels D, Templeton A, Shah PS, Amir E. Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis. Breast Cancer Res. 2017;19:2.
9. Palacios-Acedo AL, Mège D, Crescence L, Dignat-George F, Dubois C, Panicot-Dubois L. Platelets, thrombo-inflammation, and cancer: collaborating with the enemy. Front Immunol. 2019;10:1805.
10. Menter DG, Tucker SC, Kopetz S, Sood AK, Crissman JD, Honn KV. Platelets and cancer: a casual or causal relationship: revisited. Cancer Metastasis Rev. 2014;33:231-269.
11. Chen XL, Xue L, Wang W, et al. Prognostic significance of the combination of preoperative hemoglobin, albumin, lymphocyte and platelet in patients with gastric carcinoma: a retrospective cohort study. Oncotarget. 2015;6:41370-41382.
12. Shen XB, Zhang YX, Wang W, Pan YY. The hemoglobin, albumin, lymphocyte, and platelet (HALP) score in patients with small cell lung cancer before first-line treatment with etoposide and progression-free survival. Med Sci Monit. 2019;25:5630-5639.
13. Yang N, Han X, Yu J, Shu W, Qiu F, Han J. Hemoglobin, albumin, lymphocyte, and platelet score and neutrophil-to-lymphocyte ratio are novel significant prognostic factors for patients with small-cell lung cancer undergoing chemotherapy. J Cancer Res Ther. 2020 Sep;16(5):1134-1139.
14. Terashima T, Morizane C, Hiraoka N, et al. Comparison of chemotherapeutic treatment outcomes of advanced extrapulmonary neuroendocrine carcinomas and advanced small-cell lung carcinoma. Neuroendocrinology. 2012;96:324-332.
15. Stumpo S, Formelli MG, Persano I, et al. Extrapulmonary neuroendocrine carcinomas: current management and future perspectives. J Clin Med. 2023;12:7715.
16. Farag CM, Antar R, Akosman S, Ng M, Whalen MJ. What is hemoglobin, albumin, lymphocyte, platelet (HALP) score? A comprehensive literature review of HALP's prognostic ability in different cancer types. Oncotarget. 2023;14:153-172.
17. Xu H, Zheng X, Ai J, Yang L. Hemoglobin, albumin, lymphocyte, and platelet (HALP) score and cancer prognosis: a systematic review and meta-analysis of 13,110 patients. Int Immunopharmacol. 2023;114:109496.
18. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and management. Blood. 2018;131:505-514
19. Gom I, Fukushima H, Shiraki M, et al. Relationship between serum albumin level and aging in community-dwelling self-supported elderly population. J Nutr Sci Vitaminol (Tokyo). 2007;53:37-42.
20. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. Clin Cancer Res. 2004;10:7252-7259.

AHOT | Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):183-189

DOI: 10.4274/ahot.galenos.2025.2025-6-8

Original Article

Risk-based Evaluation of Adjuvant Chemotherapy in Stage I Epithelial Ovarian Cancer: A Retrospective Cohort Study

İsmet Seven, Selin Aktürk Esen, Semanur Özsan Çelebi, Efnan Algın, Öznur Bal, Doğan Uncu

Ankara Bilkent City Hospital, Clinic of Medical Oncology, Ankara, Türkiye

ABSTRACT

Aim: This study aims to assess the clinical utility of adjuvant chemotherapy in patients diagnosed with International Federation of Gynecology and Obstetrics (FIGO) stage I epithelial ovarian cancer (EOC), focusing on its impact on recurrence, disease-free survival (DFS), and overall survival (OS) based on patient risk stratification.

Methods: A retrospective cohort of 67 patients with FIGO stage I EOC treated at a single tertiary center between January 2006 and February 2024 was analyzed. Demographic, clinical, and pathological characteristics were reviewed. DFS and OS were evaluated using Kaplan-Meier analysis, log-rank tests, and Cox proportional hazards modeling.

Results: Among patients with low-risk stage I disease, the 10-year DFS and OS were both 100%. High-risk patients exhibited lower survival outcomes with DFS at 78% and OS at 92%. Interestingly, 10-year survival was marginally reduced among individuals who underwent adjuvant chemotherapy compared to those who were only observed. Variation in the number of administered chemotherapy cycles showed no statistically significant impact on survival rates.

Conclusion: These findings underscore the importance of risk-based therapeutic decision-making in stage I EOC. While low-risk patients may not benefit from adjuvant chemotherapy, high-risk individuals may still require tailored interventions.

Keywords: Adjuvant therapy, stage 1 epithelial ovarian cancer, chemotherapy, survival, recurrence

Introduction

Epithelial ovarian cancer (EOC) stands out as the deadliest form of gynecological malignancy. Even with the development of targeted treatments like poly (adenosine diphosphate-ribose) polymerase inhibitors, the majority of EOC is still identified at advanced stages [International Federation of Gynecology and Obstetrics (FIGO) III/IV], which adversely affects survival rates over time [1]. Earlier diagnosis has led to more cases being found at FIGO stages I-II, where five-year survival typically surpasses 70%. Still, recurrence rates within these early stages remain variable, ranging from 10% to 50% [2,3].

Management of FIGO stage I EOC typically involves total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH-BSO), lymph node dissection, omentectomy, and sampling of peritoneal fluid and tissues [4]. Precise surgical staging plays a crucial role both in diagnosis and in guiding decisions regarding additional systemic treatment [5]. Clinical studies including International Collaborative Ovarian Neoplasm trial (ICON1) and Adjuvant Chemotherapy in Ovarian Neoplasm trial (ACTION) have shown that platinum-based adjuvant chemotherapy may enhance survival, particularly for high-risk subgroups [6-8]. The necessity of such therapy in low-risk, comprehensively staged individuals remains a subject of debate.

This study investigates whether adjuvant chemotherapy provides a survival advantage in stage I EOC patients, particularly focusing on its relevance in low-risk cases.

Methods

A total of 67 FIGO stage I EOC cases treated between January 2006 and February 2023 at a single tertiary center were retrospectively reviewed. The study received ethical approval

Address for Correspondence: İsmet Seven MD, Ankara Bilkent City Hospital, Clinic of Medical Oncology, Ankara, Türkiye

E-mail: sevenismet84@gmail.com **ORCID ID:** orcid.org/0009-0001-4706-0495

Received: 07.08.2025 **Accepted:** 24.09.2025 **Epub:** 03.10.2025 **Publication Date:** 12.12.2025

Cite this article as: Seven İ, Aktürk Esen S, Özsan Çelebi S, Algın E, Bal Ö, Uncu D. Risk-based evaluation of adjuvant chemotherapy in stage I epithelial ovarian cancer: a retrospective cohort study. Acta Haematol Oncol Turc. 2025;58(3):183-189

from the Ethics Committee of Ankara Bilkent City Hospital (decision no: TABED 1-25-1055, date: 26.02.2025). Due to its retrospective nature, informed consent was not required.

Eligible participants were women aged 18 years or older with histologically confirmed stage IA, IB, or IC EOC, or including subtypes IC1, IC2, and IC3. A minimum clinical follow-up of 12 months was required for inclusion to ensure reliable outcome evaluation [9]. Patients were excluded if they had FIGO stage II or higher disease, non-epithelial tumors, borderline histologies, or insufficient follow-up documentation.

Data extracted from medical records included patient demographics, tumor characteristics (e.g., grade, histology), menopausal status, treatment modalities, surgical procedures, recurrence, and survival outcomes. Every patient received comprehensive staging surgery, encompassing TAH-BSO, lymph node excision, omentectomy, and sampling of the peritoneum [10]. Surgical re-staging was performed in cases of incidental diagnosis.

In our cohort, all patients underwent TAH+BSO, which aligns with our institution's standard practice for earlystage EOC. This surgical strategy was selected to minimize the presence of microscopic residual disease and to avert understaging-an issue reported in up to one-third of cases when less comprehensive procedures are performed [9,11]. Fertility-sparing surgery may be considered for young, well-selected patients; however, none of the individuals in our series met the established criteria: specifically, high-risk features such as grade 3 histology or unfavorable histologic subtypes were exclusionary. Consequently, we adopted a uniform standard radical surgical approach to ensure accurate staging and oncologic safety.

For the purposes of this study, risk classification was performed using established pathological and clinical parameters. Patients were considered to have low-risk disease if they were diagnosed with FIGO stage IA or IB tumors of grade 1-2 histology without the presence of clear cell subtype. High-risk disease was defined as FIGO stage IC (IC1-IC3), grade 3 tumors, or any case of clear cell carcinoma, irrespective of tumor grade [6,9,12,13]. Therapeutic decisions were tailored according to this categorization. Women in the low-risk group were usually managed with surveillance following complete surgical staging, whereas those in the high-risk category were more frequently offered adjuvant chemotherapy. This risk-based allocation of therapy reflects evidence from large randomized clinical trials, including ICON1 and ACTION [6], subsequent analyses by the Gynecologic Oncology Group [12,13], and the recommendations of the European Society for Medical Oncology – European Society of Gynaecological Oncology consensus panel [9].

Chemotherapy regimens administered in the adjuvant setting were selected in accordance with internationally accepted oncologic protocols and tailored to individual clinical circumstances. Typically, paclitaxel (175 mg/m²) was delivered via a 3-hour intravenous (IV) infusion, followed by a 1-hour carboplatin infusion at an area under the curve of 5-6. This combination was delivered on day one of a 21-day cycle [14].

In specific clinical contexts, alternative chemotherapy schedules were employed. The FOLinic acid (leucovorin) + Fluorouracil (5-FU) + OXaliplatin (FOLFOX) regimen included oxaliplatin (85 mg/m² IV) and leucovorin (400 mg/m² IV), accompanied by 5-FU delivered initially as a 400 mg/m² bolus, followed by a continuous infusion of 2400 mg/m² over 46 hours. This protocol was implemented on days 1 and 14 of a 28-day cycle [15]. Additionally, the XELoda (capecitabine) + OXaliplatin (XELOX) regimen, consisting of IV oxaliplatin (130 mg/m² on day 1) and oral capecitabine (2000 mg/m²/day in two divided doses for 14 days), was administered every 21 days as another treatment alternativean alternative treatment [16].

The primary endpoints of this study were disease-free survival (DFS) and overall survival (OS). DFS was defined as the time interval from the initial diagnosis of FIGO stage I EOC until either the first documented recurrence or the last date the patient was known to be alive. OS was calculated from the date of diagnosis to either the date of death or the most recent follow-up.

Statistical Analysis

All statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics version 25.0. The Kolmogorov-Smirnov test was applied to assess the distribution characteristics of continuous variables. Descriptive statistics, including median, minimum, and maximum values, were used to summarize patient demographics and clinical data.

Kaplan-Meier analysis was used to determine survival, while intergroup comparisons were conducted using the Log-Rank test. To identify independent prognostic factors, variables showing significance in univariate analysis were subsequently included in a multivariate Cox proportional hazards model. Additionally, receiver operating characteristic curve analysis was performed to determine optimal cut-off values for selected parameters. A two-sided p value less than 0.05 was considered statistically significant in all analyses.

Results

A total of 67 patients diagnosed with FIGO stage I EOC were included in this study. The median age at diagnosis was 53 years. Preoperative and postoperative serum cancer antigen-125 levels had median values of 74 and 8, respectively. Most participants had an ECOG performance status of 0 or 1, reflecting favorable baseline health. While 15 patients were premenopausal, 52 were postmenopausal at diagnosis. Abdominal pain was the predominant initial symptom, followed by abdominal distension.

Histopathological subtypes included high-grade serous carcinoma (n=24), mucinous carcinoma (n=14), endometrioid carcinoma (n=13), clear cell carcinoma (n=8), and low-grade serous carcinoma (n=8). FIGO sub-staging revealed that 36 patients were classified as stage IA, 5 as stage IB, 9 as stage IC1, and 17 as stage IC2. Based on predefined clinicopathological criteria, 48 patients were categorized as high-risk and 19 as

low-risk. Tumor grading was available in 52 cases: 16 patients had grade 1 tumors, 7 had grade 2 tumors, and 29 had grade 3 tumors. In all patients, complete tumor resection was achieved with no residual macroscopic disease (Table 1).

Among all participants, 50 patients (74.6%) received adjuvant chemotherapy, while 17 (25.4%) did not undergo additional systemic treatment. The most frequently used chemotherapy regimen was carboplatin-paclitaxel, administered in 48 patients (71.6%). FOLFOX and XELOX regimens were each administered to one patient. Regarding treatment duration, 28 patients (56.0%) received six cycles of chemotherapy, 18 patients (36.0%) received four cycles, and four patients (8.0%) completed three cycles.

Adverse events associated with chemotherapy were routinely monitored during treatment and follow-up. Although mild to moderate side effects such as nausea, vomiting, and neutropenia were observed in several cases, no patients experienced grade 3 or 4 toxicities. Peripheral neuropathy, primarily related to paclitaxel use, was the most commonly reported persistent adverse effect, affecting 13 patients (26.0%).

Disease recurrence occurred in four patients (6.0%). In the relapse setting, three patients received a rechallenge with carboplatin-paclitaxel, and one patient was treated with liposomal doxorubicin (Table 2).

Table 1. Baseline demographic and clinicopathological characteristics of the study population

		Mean (±SD)	Median (min.-max.)	n (%)
Age at diagnosis		53 (10)		
preopCA125			74 (11-3396)	
postopCA125			8 (1-107)	
ECOG PS	0			31 (46.3)
	1			32 (47.8)
	2			4 (6)
Menopausal	Premenopausal			15 (22.4)
	Postmenopausal			52 (77.6)
Symptom at admission	Painless hematuria			4 (6)
	Abdominal pain			43 (64.2)
	Urination symptoms (dysuria, nocturia...)			2 (3)
	Flank pain			2 (3)
	Incidental			4 (6)
	Vaginal bleeding			3 (4.5)
	Abdominal swelling			9 (13.4)
Pathological subtype	High grade serous carcinoma			24 (35.8)
	Endometriod carcinoma			13 (19.4)
	Clear cell carcinoma			8 (11.9)
	Mucinous carcinoma			14 (20.9)
	Low grade serous carcinoma			8 (11.9)
FIGO stage	1A			36 (53.7)
	1B			5 (7.5)
	IC1			9 (13.4)
	IC2			17 (25.4)
Risk	High risk early stage			48 (71.6)
	Low risk early stage			19 (28.4)
Grade	Unknown			15 (22.4)
	1			16 (23.9)
	2			7 (10.4)
	3			29 (43.3)
Residue	No			67 (100)

preopCA125: Preoperative cancer antigen 125, postop CA125: Postoperative cancer antigen 125, ECOG PS: Eastern Cooperative Oncology Group Performance Status, SD: Standard deviation, min.-max.: Minimum-maximum, FIGO: Federation of Gynecology and Obstetrics

The 10-year median DFS (mDFS) and median OS (mOS) for the entire cohort were 84% and 94%, respectively (Figures 1A, 1B). When stratified by risk status, the 10-year mDFS was 78% for high-risk patients and 100% for low-risk patients (p=0.140, Figure 2A), while the corresponding mOS rates were 92% and 100% (p=0.366, Figure 2B).

In patients who received adjuvant chemotherapy, the 10-year mDFS and mOS were 81% and 93%, respectively. Among those who did not receive adjuvant chemotherapy, both mDFS and mOS were 100% (p=0.253, p=0.452, respectively; Figures 3A, 3B).

When comparing chemotherapy cycles, patients who received six cycles had a 10-year mDFS and mOS of 73% and 92%, respectively. These outcomes were slightly lower than those observed in patients who received three to four cycles (mDFS 95%, mOS 95%), though the differences were not statistically significant (p=0.391, p=0.776; Figures 4A, 4B).

Discussion

EOC remains one of the most frequently encountered gynecologic malignancies. Although only approximately 20% of patients present with early-stage disease, outcomes in these cases are generally favorable, with reported 5-year survival rates ranging from 80% to 93% [17]. Nonetheless, the presence of high-risk features is associated with a significantly increased likelihood of recurrence high-risk factors in early-stage EOC are commonly defined as grade 3 histology, clear cell carcinoma of any grade, and FIGO stage IC or II disease [13]. These clinical variables have been adopted as inclusion criteria in randomized trials evaluating adjuvant chemotherapy and are often used to guide clinical decision-making outside of trial settings, particularly in patients whose 5-year DFS falls between 40% and 80% depending on their risk profile [12,18,19].

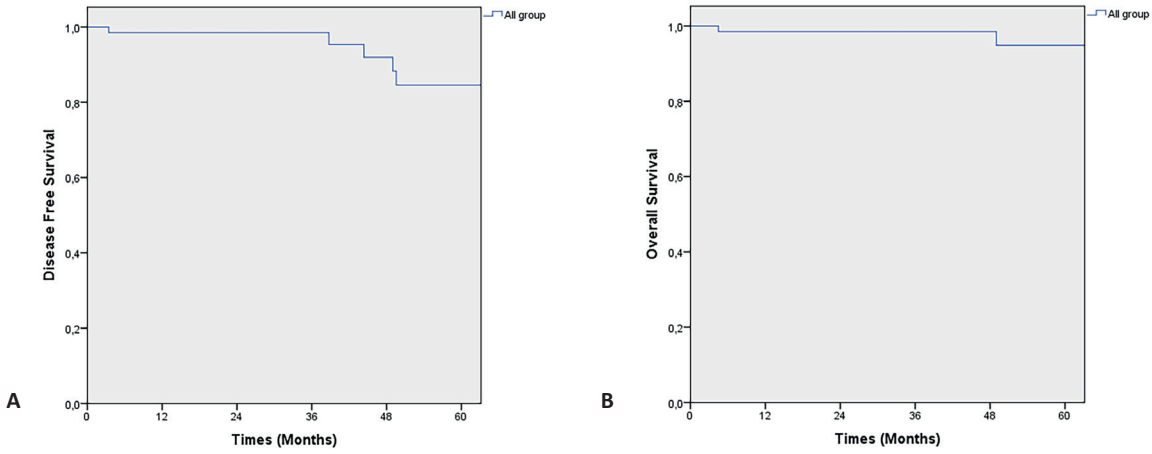


Figure 1. The 10-year median disease-free survival rate is 84% (A), and the 10-year median overall survival rate is 94% (B) for all patients

Table 2. Details of adjuvant chemotherapy, treatment-related toxicities, and relapse patterns in the study cohort

		n (%)	Column
Adjuvant therapy	Yes	50 (74.6)	74.6%
	No	17 (25.4)	25.4%
Adjuvant chemotherapy regimen	No	17 (25.4)	25.4%
	Carboplatin and paclitaxel	48 (71.6)	71.6%
	FOLFOX	1 (1.5)	1.5%
	XELOX	1 (1.5)	1.5%
Number of chemotherapy cycles	3	4 (8)	8.0%
	4	18 (36)	36.0%
	6	28 (56)	56.0%
Chemotherapy adverse effects	No	37 (74)	74.0%
	Neuropathy	13 (26)	26.0%
Relapse	Yes	4 (6)	6.0%
	No	63 (94)	94.0%
The regimen for relapses	No	63 (94)	94.0%
	Carboplatin and paclitaxel	3 (4.5)	3.0%
	Liposomal doxorubicin	1 (1.5)	1.5%

FOLFOX: FOLinic acid (leucovorin) + Fluorouracil (5-FU) + Oxaliplatin, XELOX: XELoda (capecitabine) + OXaliplatin

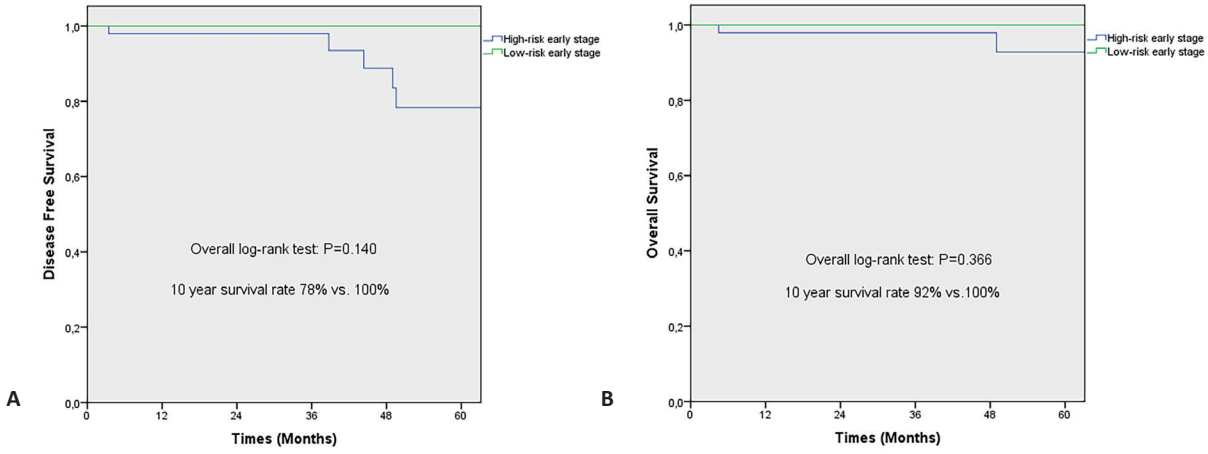


Figure 2. The 10-year median disease-free survival rate is 78% for high-risk and 100% for low-risk early-stage cases (p=0.140) (A), and the 10-year median overall survival rate is 92% and 100%, respectively, for high-risk and low-risk groups (p=0.366) (B)

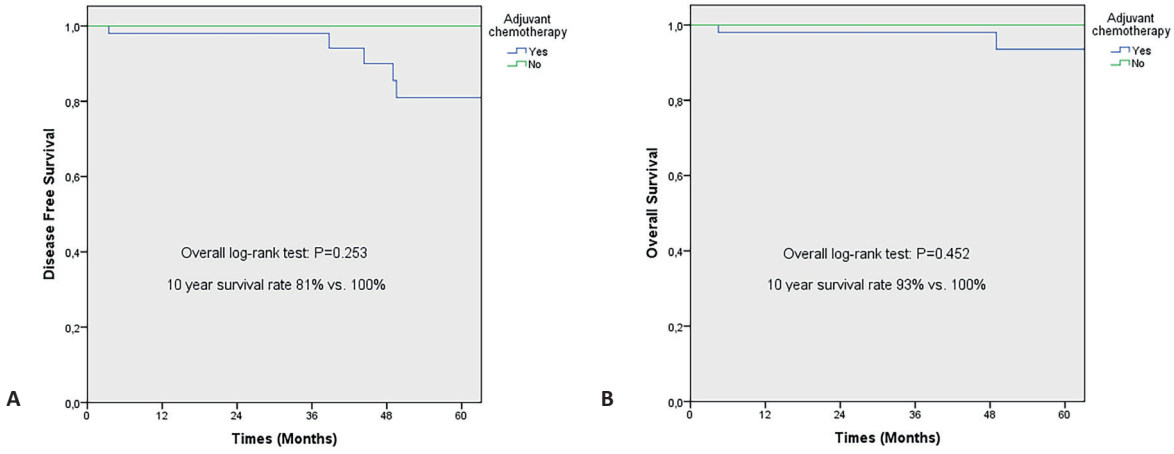


Figure 3. The 10-year median disease-free survival rates for patients with and without adjuvant chemotherapy were 81% and 100%, respectively (p=0.253) (A), while the 10-year median overall survival rates were 93% and 100%, respectively (p=0.452) (B)

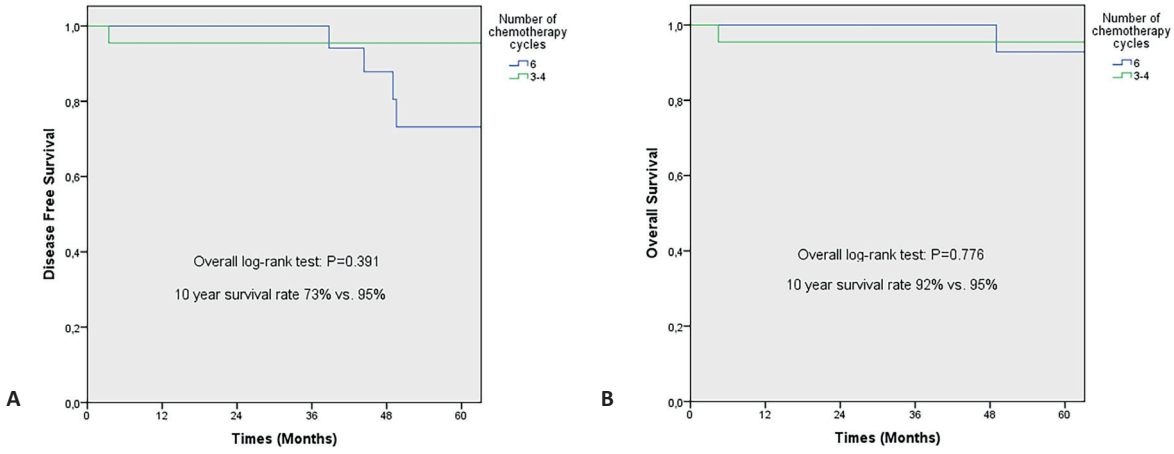


Figure 4. The 10-year median disease-free survival rates were 73% for patients who received six cycles of chemotherapy and 95% for those who received three or four cycles (p=0.391) (A). The corresponding 10-year median overall survival rates were 92% and 95%, respectively (p=0.776) (B)

In contrast, patients with low-grade tumors confined to one or both ovaries (FIGO stage IA or IB) typically demonstrate excellent long-term outcomes, with 5-year survival rates exceeding 90% [19,20]. In our cohort, low-risk patients demonstrated excellent long-term outcomes, achieving 100% 10-year DFS and OS DFS and OS, whereas high-risk patients had lower survival rates of 78% and 92%, respectively. Although this trend suggests a prognostic impact of risk stratification, the differences did not reach statistical significance, which may be attributed to the limited sample size and low recurrence rates.

The potential benefit of adjuvant chemotherapy in early-stage EOC has been explored in two meta-analyses. The first meta-analysis included 13 randomized phase III trials conducted between 1965 and 2004 of which only 8 exclusively enrolled patients with stage I disease [21]. The combined data demonstrated that patients who received adjuvant chemotherapy had significantly improved DFS and OS, particularly with platinum-based regimens. The second meta-analysis, which pooled data from five randomized trials involving 1277 patients published between 1990 and 2003, further supported the survival benefit of adjuvant chemotherapy in high-risk patients [22]. However, among those who had undergone complete surgical resection, the survival advantage of chemotherapy was less apparent. The benefit was largely restricted to patients with residual disease postoperatively, while patients with low-risk features derived minimal, if any, survival gain from systemic therapy [22]. Interestingly, patients who received adjuvant chemotherapy—most of whom were categorized as high-risk—demonstrated slightly worse survival outcomes compared to those who did not receive systemic treatment. Specifically, 10-year mDFS and mOS were 81% and 93%, respectively, among chemotherapy recipients, whereas both metrics were 100% in the observation group. This seemingly paradoxical finding likely reflects the higher baseline risk in the chemotherapy cohort rather than an adverse effect of the treatment itself.

Our analysis revealed no significant survival advantage associated with the number of chemotherapy cycles administered; patients who received six cycles had lower 10-year mDFS and mOS rates (73% and 92%, respectively) compared to those who received three or four cycles (95% for both). This observation aligns with the findings of the Gynecologic Oncology Group 157 study, which demonstrated that extending adjuvant chemotherapy from three to six cycles in high-risk early-stage EOC patients did not improve OS and instead led to increased toxicity without significantly reducing recurrence rates [23]. Paclitaxel plus carboplatin, a platinum-based duo, is recommended based on indirect evidence of significant benefit when utilized as adjuvant treatment for more progressed stages of the disease [24].

This study has several noteworthy strengths. Firstly, it presents a long-term follow-up of up to 10 years, which allows for robust evaluation of DFS and OS in a real-world cohort of patients with stage I EOC. The inclusion of both low- and high-risk patient subgroups provides valuable insight into risk-stratified treatment outcomes. Moreover, the study applied comprehensive surgical staging and utilized standardized chemotherapy regimens in accordance with international guidelines, ensuring consistency in therapeutic exposure.

In our cohort, the concept of complete resection referred to the surgical removal of all gross disease with pathologically negative margins. Even in patients with early-stage presentation, this principle was followed to minimize the risk of occult microscopic foci and to provide accurate staging information. Therefore, TAH+BSO was consistently performed, aiming to achieve both oncological safety and a uniform treatment approach. Contemporary evidence confirms that maximal cytoreduction, when no macroscopic disease is left behind, translates into improved survival outcomes and remains a fundamental goal in EOC surgery [25,26].

Study Limitations

A few limitations need to be acknowledged. The retrospective, single-center nature of the study introduces potential biases, particularly in treatment selection and risk categorization. The relatively small sample size, especially in the low-risk subgroup, may limit the statistical power to detect significant differences between treatment groups. Additionally, the absence of molecular or genomic profiling restricts the ability to correlate outcomes with underlying tumor biology. Despite these constraints, the study contributes meaningful data on the nuanced decision-making process surrounding adjuvant chemotherapy in stage I EOC and underscores the importance of individualized therapeutic strategies. Future studies should confirm current findings through larger, prospective trials. Integrating genomic profiling and patient-reported outcomes could enhance understanding of recurrence risks and guide more personalized, tolerable treatment strategies in stage I EOC.

Conclusion

This study reinforces the critical role of risk stratification in guiding adjuvant treatment decisions for patients with stage I EOC. Our findings suggest that patients classified as low-risk achieve excellent long-term survival outcomes without the need for additional systemic therapy. Conversely, individuals with high-risk features exhibit less favorable survival, underscoring the potential need for more intensive or personalized interventions. Notably, the number of adjuvant chemotherapy cycles did not significantly impact survival outcomes, indicating that extended treatment may not confer additional benefit in this setting. These insights advocate for a tailored approach to adjuvant therapy, balancing efficacy with potential toxicity. Future studies incorporating genomic and molecular profiling may further refine treatment strategies and support more individualized care.

Ethics

Ethics Committee Approval: The study received ethical approval from the Ethics Committee of Ankara Bilkent City Hospital (decision no: TABED 1-25-1055, date: 26.02.2025). **Informed Consent:** Due to its retrospective nature, informed consent was not required.

Footnotes

Authorship Contributions

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

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

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

References

1. Sambasivan S. Epithelial ovarian cancer: review article. Cancer Treat Res Commun. 2022;33:100629.
2. Wei W, Li N, Sun Y, Li B, Xu L, Wu L. Clinical outcome and prognostic factors of patients with early-stage epithelial ovarian cancer. Oncotarget. 2017;8:23862-23870.
3. Collinson F, Qian W, Fossati R, et al. Optimal treatment of early-stage ovarian cancer. Ann Oncol. 2014;25:1165-1171.
4. Garcia-Soto AE, Boren T, Wingo SN, Heffernen T, Miller DS. Is comprehensive surgical staging needed for thorough evaluation of early-stage ovarian carcinoma? Am J Obstet Gynecol. 2012;206:242.e1-5.
5. Ditto A, Martinelli F, Reato C, et al. Systematic para-aortic and pelvic lymphadenectomy in early stage epithelial ovarian cancer: a prospective study. Ann Surg Oncol. 2012;19:3849-3855.
6. Colombo N, Guthrie D, Chiari S, et al. International collaborative ovarian neoplasm trial 1: a randomized trial of adjuvant chemotherapy in women with early-stage ovarian cancer. J Natl Cancer Inst. 2003;95:125-132.
7. Trimbos JB, Parmar M, Vergote I, et al. International collaborative ovarian neoplasm trial 1 and adjuvant chemotherapy in ovarian neoplasm trial: two parallel randomized phase III trials of adjuvant chemotherapy in patients with early-stage ovarian carcinoma. J Natl Cancer Inst. 2003;95:105-112.
8. Trimbos JB, Vergote I, Bolis G, et al. Impact of adjuvant chemotherapy and surgical staging in early-stage ovarian carcinoma: European Organisation for Research and Treatment of Cancer-adjuvant chemotherapy in ovarian neoplasm trial. J Natl Cancer Inst. 2003;95:113-125.
9. Colombo N, Sessa C, du Bois A, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease†. Ann Oncol. 2019;30:672-705.
10. Harter P, Gnauert K, Hils R, et al. Pattern and clinical predictors of lymph node metastases in epithelial ovarian cancer. Int J Gynecol Cancer. 2007;17:1238-1244.
11. Fruscio R, Ceppi L, Corso S, et al. Long-term results of fertility-sparing treatment compared with standard radical surgery for early-stage epithelial ovarian cancer. Br J Cancer. 2016;115:641-648.
12. Chan JK, Tian C, Monk BJ, et al. Prognostic factors for high-risk early-stage epithelial ovarian cancer: a Gynecologic Oncology Group study. Cancer. 2008;112:2202-2210.
13. Chan JK, Tian C, Teoh D, et al. Survival after recurrence in early-stage high-risk epithelial ovarian cancer: a Gynecologic Oncology Group study. Gynecol Oncol. 2010;116:307-311.
14. Ozols RF, Bundy BN, Greer BE, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. J Clin Oncol. 2003;21:3194-200.
15. André T, Boni C, Navarro M, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol. 2009;27:3109-3116.
16. Cassidy J, Clarke S, Díaz-Rubio E, et al. XELOX vs FOLFOX-4 as first-line therapy for metastatic colorectal cancer: NO16966 updated results. Br J Cancer. 2011;105:58-64.
17. Berek JS, Renz M, Kehoe S, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. Int J Gynaecol Obstet. 2021;155:61-85.
18. Vergote IB, Vergote-De Vos LN, Abeler VM, et al. Randomized trial comparing cisplatin with radioactive phosphorus or whole-abdomen irradiation as adjuvant treatment of ovarian cancer. Cancer. 1992;69:741-749.
19. Ahmed FY, Wiltshaw E, A'Hern RP, et al. Natural history and prognosis of untreated stage I epithelial ovarian carcinoma. J Clin Oncol. 1996;14:2968-2975.
20. Young RC, Walton LA, Ellenberg SS, et al. Adjuvant therapy in stage I and stage II epithelial ovarian cancer. Results of two prospective randomized trials. N Engl J Med. 1990;322:1021-1027.
21. Elit L, Chambers A, Fyles A, Covens A, Carey M, Fung MF. Systematic review of adjuvant care for women with stage I ovarian carcinoma. Cancer. 2004;101:1926-1935.
22. Lawrie TA, Winter-Roach BA, Heus P, Kitchener HC. Adjuvant (post-surgery) chemotherapy for early stage epithelial ovarian cancer. Cochrane Database Syst Rev. 2015;2015:CD004706.
23. Bell J, Brady MF, Young RC, et al. Randomized phase III trial of three versus six cycles of adjuvant carboplatin and paclitaxel in early stage epithelial ovarian carcinoma: a Gynecologic Oncology Group study. Gynecol Oncol. 2006;102:432-439.
24. Adams G, Zekri J, Wong H, Walking J, Green JA. Platinum-based adjuvant chemotherapy for early-stage epithelial ovarian cancer: single or combination chemotherapy? BJOG. 2010;117:1459-1467.
25. Porter JM, McFarlane I, Bartos C, et al. The survival benefit associated with complete macroscopic resection in epithelial ovarian cancer is histotype specific. JNCI Cancer Spectr. 2024;8:pkae049.
26. Taylor KN, Li A, Manuel M, Rimel BJ, Kim KH. The association of black race with receipt of hysterectomy and survival in low-risk endometrial cancer. Gynecol Oncol. 2023;175:156-162.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):190-195

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-7-7

Frequency of NPM1 Mutation and Its Impact on Prognosis in Cytogenetically Normal AML Patients: Hyperleukocytosis Remains an Unfavorable Prognostic Marker Among NPM1 Positive Patients

Mehmet Sezgin Pepeler[1], Zübeyde Nur Özkurt[2], Ayşe Kaya[3], Sanem Gökçen[2], Asuman Sungurluoğlu[4], Handan Kayhan[2], Zeynep Arzu Yeğin[2], Münci Yağcı[2]

[1]*Ankara Bilkent City Hospital, Clinic of Hematology, Ankara, Türkiye*
[2]*Gazi University Faculty of Medicine, Department of Adult Hematology, Ankara, Türkiye*
[3]*University of Health Sciences Türkiye, Kocaeli City Hospital, Department of Adult Hematology, Kocaeli, Türkiye*
[4]*Ankara University Faculty of Medicine, Department of Medical Biology, Ankara, Türkiye*

ABSTRACT

Aim: Nucleophosmin 1 (NPM1) mutation is the most frequent genetic abnormality that points to a favorable prognosis in patients with cytogenetically normal acute myeloid leukemia (CN-AML). NPM1 mutation rate has not been documented yet in Turkish patients. We aimed to investigate NPM1 frequency and its prognostic impact.

Methods: A total of 160 patients diagnosed with CN-AML were enrolled in this study retrospectively. Clinical and laboratory data, as well as results from polymerase chain reaction tests for NPM1 and FMS-like tyrosine kinase 3 (FLT3) mutations, were analyzed.

Results: The NPM1 mutation was found in 35 (21.9%) patients. Patients were followed for a median of 16.5 (0.5-132.0) months. Patients with NPM1 mutation were characterized by a higher leukocyte count (p=0.03). Primary refractory disease (p=0.02) and early mortality (p=0.02) were lower in patients with NPM1 mutation positivity. Relapse-free survival (70.9% vs. 41.8%, p=0.03) and overall survival (OS) (65.5%vs. 47.0%, p=0.04) were higher in NMP1 mutation positive patients. In NPM1 positive group, relapse free survival (21.4%vs. 81.5%, p=0.01) and OS (22.9%vs. 70.0%, p=0.03) were lower in patients with hyperleukocytosis (p<0.05).

Conclusion: NPM1 mutation frequency was lower in Turkish CN-AML patients than western populations. NPM1 mutation status is related to good prognosis. However, we showed that hyperleukocytosis is a poor prognostic marker for NMP1-positive, FLT3-negative CN-AML patients.

Keywords: NMP1, FLT3-ITD, hyperleukocytosis, prognosis, acute myeloid leukemia

Introduction

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults. The disease prognosis is heterogeneous due to various mutations at cytogenetic and molecular levels. Genetic properties are known as one of the most important prognostic markers that determines consolidation therapy modality in AML. Almost half of the patients with AML have a normal karyotype [cytogenetically normal (CN)-AML], which is considered an intermediate risk disease. This subgroup shows quite heterogeneous prognoses [1,2]. Several studies reported that a positive nucleophosmin 1 (NPM1) mutation and a negative FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) status are usually associated with more favorable outcomes in CN-AML [3-6].

The NPM1 mutation is the most commonly identified genetic abnormality in AML. NPM1 frequency has been reported as 41-66% in Europe and the USA [7-9]. However, the frequency

Address for Correspondence: Mehmet Sezgin Pepeler MD, Ankara Bilkent City Hospital, Clinic of Hematology, Ankara, Türkiye
E-mail: drsezgin44@gmail.com **ORCID ID:** orcid.org/0000-0002-2762-8573
Received: 24.07.2025 **Accepted:** 24.09.2025 **Epub:** 07.10.2025 **Publication Date:** 12.12.2025

Cite this article as: Pepeler MS, Özkurt ZN, Kaya A, et al. Frequency of NPM1 mutation and its impact on prognosis in cytogenetically normal AML patients: hyperleukocytosis remains an unfavorable prognostic marker among NPM1 positive patients. Acta Haematol Oncol Turc. 2025;58(3):190-195

of NPM1 mutation and the impact of these mutations on prognosis have not been documented yet in the Turkish population with CN-AML. We aimed to investigate the NPM1 frequency and factors contributing to survival in Turkish patients with NPM1-mutated AML.

Methods

This is a single-center database retrospective cohort study. A total of 160 patients diagnosed with CN-AML from January 2007 to December 2018 [median age: 51.5 (18-91) years; male/female: 94/66] were enrolled in this study consecutively. Demographic information, complete blood count, percentage of blast at bone marrow aspiration, flow cytometry, conventional cytogenetic, and polymerase chain reaction (PCR) test results were obtained retrospectively. All of the patients had NPM1 data, but only 95 patients had FLT3-ITD test results.

We recorded follow-up data, including chemotherapy/transplantation information and response assessment. The induction regimen was cytarabine and idarubicin (3+7). Hypomethylating agents were used for elderly patients. Response assessment was performed at day 28 of intensive induction chemotherapy, at the end of 4th course of hypomethylating agents treatment. Fludarabine, cytarabine, granulocyte colony-stimulating factor (FLAG-Ida), regimen was used for primary refractory patients. High-dose cytarabine (HDAC), FLAG-Ida, hypomethylating agents, autologous stem cell transplantation (ASCT), or allogeneic stem cell transplantation (allo-SCT) were used as consolidation treatment in patients who obtained a complete response. The study was approved by the Clinical Research Ethics Committee of Keçiören Training and Research Hospital (decision no: 958, date: 14.10.2015). All patients signed informed consent forms and agreed to the use of their clinical data in medical research.

Detection of NPM1 Mutation

Detection of NPM1 mutation: Total ribonucleic acid (RNA) was isolated from bone marrow samples using the QIAamp RNA Blood Mini Kit (QIAGEN, Hilden, Germany). RNA integrity was assessed using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and samples with adequate quality, (260/280 ratio >1.8), were processed for complementary deoxyribonucleic acid (cDNA) synthesis. Total RNA from each sample was reverse-transcribed into cDNA using Ipsogen® RT kit (QIAGEN) according to the manufacturer's protocol. Real-time quantitative PCR (RQ-PCR) assay for detection and quantification of NPM1 type A mutation (the most common variant, representing ~75-80% of all NPM1 mutations) was performed using NPM1 mut A MutaQuant Kit (IPSOGEN, Marseille, France) on QIAGEN's Rotor-Gene Q real-time PCR cycler. The assay targets the 4-base pair insertion Timine, Sitozin, Timine, Guanin in exon 12 of the *NPM1* gene, which creates a frameshift mutation leading to aberrant cytoplasmic localization of the NPM1 protein. Each reaction was performed in duplicate with appropriate positive and negative controls. A sample was considered NPM1-mutated if the mutation was detected in both replicates with adequate amplification curves.

Quality control measures included: Use of abelson murine leukemia viral oncogene homologous 1 as a housekeeping gene for RNA quality assessment. Negative controls (water blanks) in each run. "Positive controls with known NPM1 mutations." Assessment of PCR efficiency and linearity.

Detection of FLT3 Mutations

Genomic DNA was extracted from bone marrow samples by the EZ1 Advanced XL System, QIAGEN. ITD mutation and tyrosine kinase domain (TKD) mutation (D835) of *FLT3* gene were identified using the LeukoStrat® CDx FLT3 mutation Assay Kit, INVIVOSCRIBE. DNA was amplified via PCR according to the manufacturer's instructions, and the size of the ITD PCR product was determined by the FlashGel™ System, Lonza. However, the FLT3 TKD PCR product was digested with EcoRV, and the presence of the mutation was also assessed using the FlashGel™ System, Lonza. A product measured at 327±1 bp was identified as wild-type for FLT3-ITD mutations, while alleles that contain ITD mutations produced a product that exceeds 327±1 bp. For FLT3-TKD mutations, wild-type alleles of the *FLT3* gene yield digestion products of 79±1 bp whereas mutant alleles yield products of 125±1 bp or 127±1 bp from the original undigested amplicon product of 145±1 bp or 147±1 bp [10].

Statistical Analysis

Continuous and categorical variables were compared with the Mann-Whitney U test and chi-squared test, respectively. Kaplan-Meier analysis was used for survival analysis SPSS 16.0 program was used for statistical analysis (SPSS Inc, Chicago, IL, USA). $P<0.05$ was considered to be statistically significant.

Results

NPM1 mutation was found in 35 (21.9%) patients. Patients' characteristics were similar between NPM1 positive and negative groups with respect to age, gender, French–American–British classification, hemoglobin levels, platelet counts, and percentage of bone marrow blast ($p>0.05$) (Table 1). Median white blood cell (WBC) count was found to be significantly higher in patients with NMP1 mutation-positive than in mutation-negative patients [62.8 (12.6-182.3) vs. 31.4 (1.2-69.7)] ($p=0.03$) (Table 1). The percentage of patients with hyperleukocytosis at the time of diagnosis was more frequent in the NPM1 mutation positive group (34.2% vs. 6.6%, $p=0.02$). Among patients who had an FLT3-ITD test result (n=95), FLT3-ITD was positive in 4 (14.8%) patients in the NPM1 positive group (n=27) and in 18 patients (26.6%) in the NPM1 negative group (n=68). FLT3-ITD positivity was not significantly different between NPM1 positive, and negative, patients ($p>0.05$).

Patients were followed median 16.5 (0.5-132.0) months. Primary refractory disease was found more frequently in NPM1 mutation negative patients compared to NPM1 mutation positive patients (22.4% vs. 5.7%, $p=0.02$). Early mortality

Table 1. Patient's characteristics respect to NPM1 positive and negative patients

	NPM1 positive n=35 (21.9%)	NPM1 negative n=125 (78.1)	p
Gender (male/female) n (%)	20/15 (57.1/42.8)	77/48 (61.6/38.4)	NS>0.05
Age (years)	50.0 (20-90)	44.0 (19-91)	NS
Hemoglobin (g/dL)	8.9 (5.7-14.0)	8.1 (4.1-13.2)	NS>0.05
WBC (/mm^3)	62.8 (12.6-182.3)	31.4 (1.2-119.7)	0.03
Hyperleukocytosis (WBC≥100000/mm^3) n (%)	12 (34.2)	10 (6.6)	0.02
Platelet (/mm^3)	71000 (3900-284000)	51650 (3000-85000)	NS>0.05
Blast in bone marrow (%)	90 (22-100)	90 (22-100)	NS>0.05
FAB classification (n/%) M0 M1 M2 M4 M5 M6	 3 (8.6) 5 (14.2) 6 (17.1) 14 (40.0) 6 (17.1) 1 (2.8)	 24 (19.2) 35 (28.0) 19 (15.2) 39 (31.2) 6 (4.8) 2 (1.6)	NS
FLT3 positivity (n/%)	4 (14.8)	18 (26.6)	NS>0.05
Early mortality (within first month)	1 (2.8%)	15 (12.0%)	0.03
Refractory to first line chemotherapy	2 (5.7%)	28(22.4%)	0.02
Consolidation chemotherapy/ASCT	n=34 28 (82.4)	n=95 46 (55.8)	0.02
Consolidation allo-SCT	6 (17.6)	42 (44.2)	0.003

NPM1: Nucleophosmin 1, FLT3: FMS-like tyrosine kinase 3, ASCT: Autologous stem cell transplantation, Allo-SCT: Allogeneic stem cell transplantation, WBC: White blood cell, FAB: French–American–British

within the first month was higher in the NPM1 negative group compared to the NPM1 positive group (12.0% vs. 2.8%) (p=0.02). Consolidation procedures included chemotherapy, including high-dose cytarabine, FLAG-Ida, or hypomethylating agents, in 65 patients, and ASCT in 16 patients and allogeneic stem cell transplantation in 48 patients at first complete remission. Allo-SCT was a more frequent consolidation approach in the NPM1 mutation negative group. ASCT or chemotherapy was preferred in the NPM1 mutation positive group (p=0.02) (Table 1).

When the FLT3-ITD positive patients are excluded from survival analysis, relapse free survival (RFS) [70.9% vs. 41.8%, p=0.03, confidence interval (CI): 52.0-75.5, standard error (SE): 5.9] and overall survival (OS) (65.5% vs. 47.0%, p=0.04, CI: 52.6-76.2, SE: 6.0) were lower in the NPM1 mutation positive group compared to the negative group (Figures 1, 2). Among NPM1 positive patients, RFS (21.4% vs. 81.5%, p=0.01, CI:35.6-54.3 SE:4.7) and OS (22.9% vs. 70.0%, p=0.03, CI:37.7-54.9, SE: 4.3) probabilities were significantly lower in patients WBC >100,000/mm^3 compared to <100,000/mm^3, respectively (Figures 3, 4).

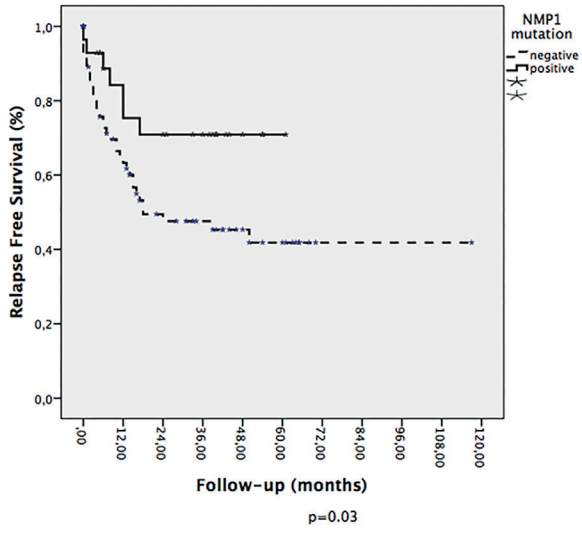


Figure 1. Relapse-free survival in NPM1 positive and negative patients
NPM1: Nucleophosmin 1

Discussion

NPM1 mutation is found in AML frequently, but some studies lack information regarding the NPM1 frequency in CN-AML due to a heterogeneous patient population, including mixed karyotype features or translocations. Almost all of the studies from the USA and Europe reported NPM1 frequency of 41-68%, and FLT3-ITD frequency has been reported: 20-38% of patients with CN-AML [11-14]. These data were obtained generally from phase I/II studies about AML genetic features and prognosis. On the other hand, lower NPM1 mutation

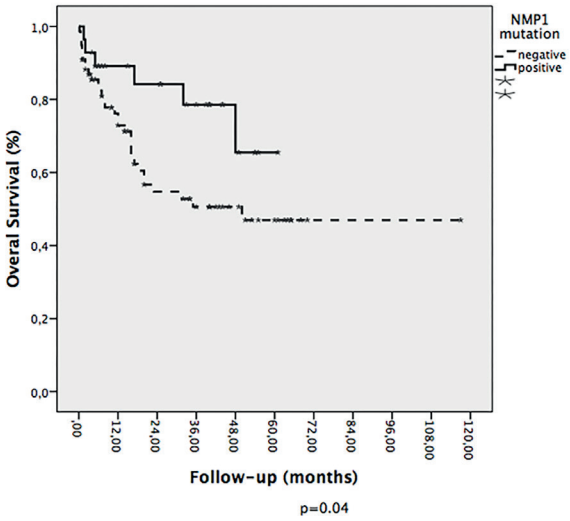


Figure 2. Overall survival in NPM1 positive and negative patients
NPM1: Nucleophosmin 1

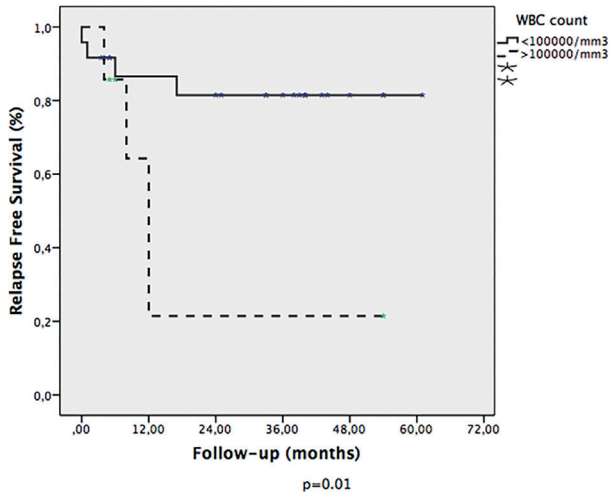


Figure 3. Relapse-free survivals according to WBC count in patients with NPM1 mutation
WBC: White blood cell, NPM1: Nucleophosmin 1

frequency was reported as 28.3% in the Egyptian [15], 27.7% in the Bulgarian [16], 24.7% in the Brazilian [17], 20.8% in the Iranian [18], and 17.1% in the Indian [19] CN-AML population. We found that 21.9% of CN-AML patients aged over 18 had mutated NPM1. This situation could indicate that the mutation in question may be influenced by ethnicity and geographic origin. However, it could be argued that limitations in the evaluation and laboratory testing (such as isolation issues or not performing the test on the same kit) may also play a role in this situation.

In some studies, NPM1 mutation has been reported more frequently in females and correlated with older age [20]. We did not confirm these findings. among NPM1 positive and negative patients, gender and median age were similar. As

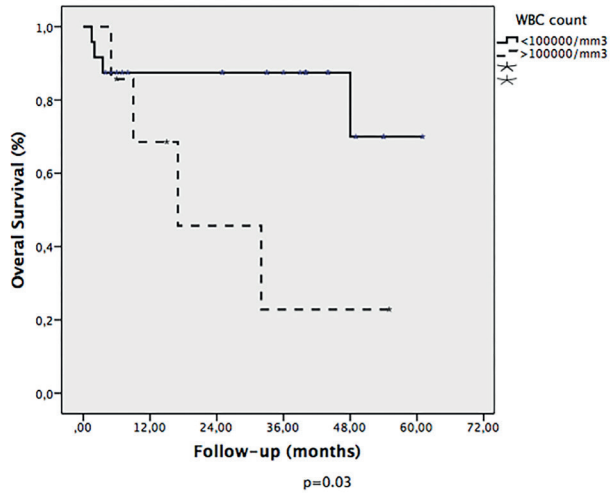


Figure 4. Overall survival according to WBC counts in patients with NPM1 mutation
WBC: White blood cell, NPM1: Nucleophosmin 1

seen in the literature, leucocyte count was higher in NPM1 positive patients; thrombocyte count and hemoglobin levels were not different in this study.

Among CN-AML patients, NPM1 mutation positive, patients reached complete response more frequently at the end of the first induction regimen compared to mutation negative, patients. In addition, the early mortality rate within the first month was lower in NPM1 mutation-positive patients. When the FLT3 positive patients were excluded from the survival analysis, both RFS and OS were higher in NPM1 positive patients than negative ones. These data confirmed those previously reported [4-6,21]. In our study, NPM1-positive patients were divided into two groups according to whether their leukocyte count was higher than or lower than 100,000/mm^3. Patients with higher leukocyte counts had significantly lower RFS and OS probabilities. Higher leukocyte count was known as a classical negative prognostic factor, but novel European LeukemiaNet (ELN) pretreatment risk stratification is now only based on cytogenetic and mutational status [22]. According to ELN, CN-AML with NPM1 mutation without FLT3-ITD has a good prognosis and is referred to allo-SCT at relapse. However, Bazarbachi et al. [23], reported that survival outcomes are better when patients with NPM1 mutation are transplanted in first complete remission (CR1) versus second CR2. Tien et al. [24] reported that hyperleukocytosis, defined as >50,000/mm^3, was an independent prognostic factor for poor outcomes in distinct genetic alterations and CN-AML patients. The authors pointed out allo-SCT in first CR, ameliorated the negative impact of hyperleukocytosis on survival. A higher leucocyte count may already be an important poor prognostic marker besides the cytogenetic markers.

The molecular processes underlying hyperleukocytosis remain unclear. However, we do know that there are interactions between leukaemic blasts and endothelial cells that lead to leukostasis. Leukemic blasts interact with endothelial

cells via cell adhesion molecules, such as various members of the selectin family. These molecules are upregulated by inflammatory cytokines released by the leukemic blasts themselves [25]. To understand the molecular processes contributing to hyperleukocytosis in AML, a METASCAPE analysis, and Gene Set Enrichment Analysis were conducted. The analyses revealed several gene sets that exhibited a negative association with hyperleukocytosis, including cytokine-cytokine receptor interaction, extracellular matrix-receptor interaction, cell cycle regulation, DNA replication, cell adhesion molecules, cytokine signaling pathway, adhesion signaling pathway, and epithelial mesenchymal transition. The correlation between hyperleucostasis and gene mutations, particularly those of FLT3 and DNA (Cytosine-5)-methyltransferase 3 alpha, suggests the importance of these mutations in hyperleucostasis development and progression in AML. Further research is needed to elucidate the underlying mechanisms and explore targeted therapies for patients with these specific genetic markers [26].

Study Limitations

The limitations of the study include the small number of NMP1 mutation positive cases and the short follow-up period.

Conclusion

In conclusion, this is the first report on the frequency and clinical characteristics of NPM1 mutation in the CN-AML Turkish population. We found that the NPM1 mutation frequency is 21.9% in the Turkish CN-AML population. NPM1 mutation status related with good prognosis. However, we showed that the hyperleukocytosis is a poor prognostic marker for NMP1 positive, FLT3 negative CN-AML patients. We may refer to allo SCT as first CR for NMP1 positive, FLT3 negative CN-AML patients if they have hyperleukocytosis at the time of diagnosis. These results should be confirmed by prospective randomized clinical trials.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Keçiören Training and Research Hospital (decision no: 958, date: 14.10.2015). **Informed Consent:** All patients signed informed consent forms and agreed to the use of their clinical data in medical research.

Footnotes

Authorship Contributions

Concept: M.S.P., Z.N.Ö., S.G., H.K., Design: A.S., M.Y., Data Collection or Processing: M.S.P., Z.N.Ö., A.K., S.G., H.K., Z.A.Y., M.Y., Analysis or Interpretation: H.K., Z.N.Ö., A.S., H.K., M.Y., Literature Search: M.S.P., A.K., H.K., Z.N.Ö., Writing: M.S.P., Z.N.Ö., A.K., A.S., M.Y.

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

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

References

1. Fröhling S, Scholl C, Gilliland DG, Levine RL. Genetics of myeloid malignancies: pathogenetic and clinical implications. J Clin Oncol. 2005;23:6285-6295.
2. Döhner K, Döhner H. Molecular characterization of acute myeloid leukemia. Haematologica. 2008;93:976-982.
3. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002;99:4326-4335.
4. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366:1079-1089.
5. Schlenk RF, Döhner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358:1909-1918.
6. Haferlach C, Mecucci C, Schnittger S, et al. AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. Blood. 2009;114:3024-3032.
7. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. Blood. 2007;109:874-885.
8. Schneider F, Hoster E, Schneider S, et al. Age-dependent frequencies of NPM1 mutations and FLT3-ITD in patients with normal karyotype AML (NK-AML). Ann Hematol. 2012;91:9-18.
9. Port M, Böttcher M, Thol F, et al. Prognostic significance of FLT3 internal tandem duplication, nucleophosmin 1, and CEBPA gene mutations for acute myeloid leukemia patients with normal karyotype and younger than 60 years: a systematic review and meta-analysis. Ann Hematol. 2014;93:1279-1286.
10. Murphy KM, Levis M, Hafez MJ, et al. Detection of FLT3 internal tandem duplication and D835 mutations by a multiplex polymerase chain reaction and capillary electrophoresis assay. J Mol Diagn. 2003;5:96-102.
11. Langer C, Marcucci G, Holland KB, et al. Prognostic importance of MN1 transcript levels, and biologic insights from MN1-associated gene and microRNA expression signatures in cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. J Clin Oncol. 2009;27:3198-3204.
12. Langer C, Radmacher MD, Ruppert AS, et al. High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: a cancer and leukemia group B (CALGB) study. Blood. 2008;111:5371-5379.
13. Paschka P, Marcucci G, Ruppert AS, et al. Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. J Clin Oncol. 2008;26:4595-4602.
14. Virappane P, Gale R, Hills R, et al. Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. J Clin Oncol. 2008;26:5429-5435.
15. Sofan MA, Elmasry S, Salem DA, Bazid MM. NPM1 gene mutation in Egyptian patients with cytogenetically normal acute myeloid leukemia. Clin Lab. 2014;60:1813-1822.
16. Balatzenko G, Spassov B, Stoyanov N, et al. NPM1 gene type A mutation in Bulgarian adults with acute myeloid leukemia: a single-institution study. Turk J Haematol. 2014;31:40-48.
17. Cruz NG, Ribeiro AF, Glória AB, et al. Characterization of NPM1, FLT3, and IDH1 mutations in adult patients with acute myeloid leukemia: a Brazilian cohort study. Leuk Lymphoma. 2016;57:2901-2904.
18. Rezaei N, Arandi N, Valibeigi B, Haghpanah S, Khansalar M, Ramzi M. FMS-like tyrosine kinase 3 (FLT3) and nucleophosmin 1 (NPM1) in Iranian adult acute myeloid leukemia patients with normal karyotypes: mutation

status and clinical and laboratory characteristics. Turk J Haematol. 2017;34:300-306.

19. Dunna NR, Rajappa S, Digumarti R, et al. Fms like tyrosine kinase (FLT3) and nucleophosmin 1 (NPM1) mutations in de novo normal karyotype acute myeloid leukemia (AML). Asian Pac J Cancer Prev. 2010;11:1811-1816.
20. Marshall RC, Tlagadi A, Bronze M, et al. Lower frequency of NPM1 and FLT3-ITD mutations in a South African adult de novo AML cohort. Int J Lab Hematol. 2014;36:656-664.
21. Colombo E, Alcalay M, Pelicci PG. Nucleophosmin and its complex network: a possible therapeutic target in hematological diseases. Oncogene. 2011;30:2595-2609.
22. Estey EH. Acute myeloid leukemia: 2019 update on risk-stratification and management. Am J Hematol. 2018;93:1267-1291.
23. Bazarbachi A, Labopin M, Kharfan-Dabaja MA, et al. Allogeneic hematopoietic cell transplantation in acute myeloid leukemia with normal karyotype and isolated nucleophosmin-1 (NPM1) mutation: outcome strongly correlates with disease status. Haematologica. 2016;101:e34-e37.
24. Tien FM, Hou HA, Tsai CH, et al. Hyperleukocytosis is associated with distinct genetic alterations and is an independent poor-risk factor in de novo acute myeloid leukemia patients. Eur J Haematol. 2018;101:86-94.
25. Bewersdorf JP, Zeidan AM. Hyperleukocytosis and leukostasis in acute myeloid leukemia: can a better understanding of the underlying molecular pathophysiology lead to novel treatments? Cells. 2020;9:2310.
26. Lyu G, Lyu W. Complete elimination of hyperleukocytosis risk in AML through early high-quality disease remission. Front. Oncol. 2014;14:1412583.

AHOT | Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):196-201

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-5-1

Assessment of Healthy Lifestyle Habits Among Cancer Patients Before Diagnosis

Umut Tahsin Mafratoğlu[1], Didem Şener Dede[2], Meryem Didem Göktaş[3], Senagül Ergiden[4]

[1]*Ankara Bilkent City Hospital, Clinic of Plastic, Reconstructive and Aesthetic Surgery, Ankara, Türkiye*
[2]*Ankara Yıldırım Beyazıt University Faculty of Medicine, Department of Clinical Internal Medicine, Ankara, Türkiye*
[3]*Ankara Bilkent City Hospital, Clinic of Clinical İnternal Medicine, Ankara, Türkiye*
[4]*Rize Fındıklı State Hospital, Clinic of Emergency Medicine, Rize, Türkiye*

ABSTRACT

Aim: Cancer is one of the biggest problems of this era and is progressing with increasing momentum. Understanding the etiology of cancer is as crucial as its treatment. Numerous sources emphasize that modifiable factors constitute the largest proportion of cancer causes. This study aimed to evaluate the lifestyle habits of cancer patients and identify potential new modifiable risk factors for cancer.

Methods: A cross-sectional survey study was conducted. A total of 199 individuals aged 18 years and older, with a histologically confirmed cancer diagnosis, regardless of cancer type or stage, participated in the study.

Results: The median age of the cancer patients included in the study was 60 years (range: 20-86). A significant majority, 175 (87.9% of respondents), reported not engaging in regular exercise, whereas 24 (12.1% of respondents) reported that they exercised regularly. Furthermore, 139 (69.8%) patients worked in sedentary jobs. It was observed that a large proportion of patients [126 (63%)] had the habit of eating or drinking ≤4 hours before sleep. A statistically significant association was found between marital status and cancer stage, as 30 (82%) of unmarried patients were in the advanced stage of cancer (p<0.001). The use of electromagnetic radiation-emitting devices, such as smartphones, Wi-Fi, and televisions, was significantly higher among college or university graduates [n=87 (82%)] compared to other groups (p<0.001).

Conclusion: Advances in modern medicine have led to significant progress in cancer treatments; however, the disease still maintains its frightening image due to high mortality rates. Therefore, raising awareness of modifiable causes of cancer, such as lifestyle habits, is essential.

Keywords: Cancer risk factors, lifestyle habits, primary prevention of cancer

Introduction

Cancer is one of the most diagnosed and the deadliest diseases. About 10 million deaths and 20 million new cases are reported worldwide each year. Unfortunately, the number of deaths is predicted to increase dramatically and will result in approximately double the current number in 20 years [1]. Therefore, hundreds of studies have been conducted to elucidate its etiology. Often, cancer development is considered multifactorial. It is usually caused by environmental factors and lifestyle choices, and just a small percentage of cases are associated with inherited genetic mutations [2,3]. Almost all risk factors of cancer are lifestyle-related, including smoking, diet, alcohol, sun exposure, obesity, physical inactivity, radiation, stress, etc. [4]. They are also modifiable risk factors. Among them, the strongest known risk factors are smoking and diet [5-8]. Based on these studies and similarities, we thought that primary prevention for cancer was essential, so we wanted to focus on modifiable factors. We hypothesize that cancer is more strongly associated with modifiable risk factors than previously thought, many of which are still unknown and not discussed. It is also, not fully known how to protect

Address for Correspondence: Didem Şener Dede Asst. MD, Ankara Yıldırım Beyazıt University Faculty of Medicine, Department of Clinical Internal Medicine, Ankara, Türkiye
E-mail: ddmsener@yahoo.com **ORCID ID:** orcid.org/0000-0002-9756-5942

Received: 09.05.2025 **Accepted:** 02.10.2025 **Epub:** 13.10.2025 **Publication Date:** 12.12.2025

Cite this article as: Mafratoğlu UT, Şener Dede D, Göktaş MD, Ergiden S. Assessment of healthy lifestyle habits among cancer patients before diagnosis. Acta Haematol Oncol Turc. 2025;58(3):196-201

oneself from known risk factors. The aim of our study was to evaluate the frequency of some lifestyle behaviors that may be related to cancer development and that are often ignored or normalized among cancer patients. Cancer requires more primary prevention than other diseases, because once the diagnosis is made, both the disease and its treatment lead to devastating consequences [9-11].

Methods

An observational single-center cross-sectional survey study was performed to investigate how lifestyle behaviors may affect cancer development. A total of 199 patients with histologically confirmed cancer were included. Data were obtained through a self-administered questionnaire completed by 199 patients from outpatient and inpatient units. The inclusion criteria were the following: [i] cancer diagnosis at any time in life, [ii] volunteering to participate, [iii] age ≥18 years. The exclusion criteria were the following: [i] too ill to understand the questions. The survey, composed of sixty-seven questions exploring at least ten dimensions, was designed after an extensive literature review. Questions aim to examine sociodemographic factors, environmental exposures, occupational history, exercise pattern, exercise environment, the relationship between exercise and food, timing of food intake, radiation exposures, daily water consumption, smoking and alcohol use, maternal birth age, breastfeeding duration, sleep duration, and quality, siesta frequency, tea and coffee consumption, UV exposure,screen-light exposure, rotation night shift work, mood pattern. Questions include multiple-choice, as well as free-text options. All the behaviors we investigated were related to the patients' lives before they were diagnosed with cancer. To improve the accuracy of the questionnaire, the researcher conducted face-to-face interviews with patients and gave them an opportunity to ask questions if there was anything they could not understand. The study garnered ethical approval, ensuring all participants fully understood its purpose. Personal permission was thoughtfully obtained from each individual, underscoring our commitment to transparency and respect throughout the research process. Ethical approval for the study was obtained from the Non-invasive Clinical Research Ethics Committee of Ankara City Hospital and was conducted in accordance with the Declaration of Helsinki (decision no: E2-22-2782, date: 09.11.2022).

Statistical Analysis

Descriptive statistical methods (number, mean, median, percentage, frequency) were used to evaluate the study data. The Pearson chi-square was used to compare the qualitative variables. The statistical significance level was set at 0.05 in all analyses. Statistical Package for the Social Sciences version 18.0, developed by IBM (Chicago, USA), was used to analyze the data.

Artificial Intelligence Declaration

No artificial intelligence tools were used in writing the article. All responsibility belongs to the authors of the article.

Results

199 patients were included in the study. The median age of patients was 60 (minimum 20-maximum 86). One hundred and six (53.3 %) patients were male and 93 (46.7%) were female. Fif-teen (7.5%) were single and 160 (80.4%) were married, and 22 (11%) were divorced or widowed. Other demographic parameters were also specified. Table 1 summarizes the patients' demographic status.

Types of cancer were ranked as, lung cancer 39 (19.6%), genitourinary system cancer 29 (14.6%), breast cancer 23 (11.6%) and colorectal cancer 14 (7%). Ninety-four (47.2%) patients had advanced cancer, and 76 (38.2%) patients had early stage cancer. Ninety-six (49.2%) patients reported a family history of cancer in first, second, or third-degree relatives. The majority of cancer patients participating in the study, 145 (73%), were breastfed for more than 6 months. Table 2 summarizes cancer, patients, and family characteristics.

According to the smoking status, patients were grouped as 98 (49.2%) smokers, 96 (48.2%) non-smokers. Forty-two (21.1%)

Table 1. Demographic status

	Median (min-max)	Number of patients (n)	Percentage (%)
Age, years			
	60 (20-86)		
Gender			
Female		93	46.7
Male		106	53.3
Marital status			
Single		15	7.5
Married		160	80.4
Divorced/widowed		22	11
Education			
Illiterate		18	9
Less than college		142	71.4
College/university		35	17.6
Number of children			
	3 (0-11)		
Never have		15	7.5
3 children or less		123	61.8
4 or more		41	20.5
Job			
Civil servant		29	14.6
Worker		38	19.1
Others		48	24.1
Salary (per month) (5500 TL = minimum wage)			
Less than 5500 TL		56	28.1
5500 TL		79	39.1
More than 5500 TL		50	25T

TL: Turkish lira, min-max: Minimum-maximum

patients quit smoking after being diagnosed and 157 (78.9%) continued to smoke. According to exercise status, 24 (12.1 %) patients engaged in regular exercise, while 175 (87.9%) of patients did not exercise. In this study population, 85 (42%) of the patients consumed water in plastic containers, while 113 (57%) consumed water from glass bottles or by purification. One hundred and thirty-nine (69.8%) cancer patients participating in the study work in sedentary conditions, whereas 51 (25.6%) of them work in non-sedentary conditions.

It was observed that 40 (20.6%) of cancer patients slept less than 6 hours a day and their sleep was frequently interrupted. Most of the patients [126 (63%)] eat something within ≤4 hours before going to sleep; some of them [68 (34%)] do not eat. Most of patients [146 (73%)] used smartphones or watched television as a last activity before sleep. In this study population, 137 (67%) patients have more than 2 hours of daily screen exposure time, including blue light. Mood status in cancer patients over the last few years before cancer diagnosis was categorized as follows: 83 (41.7%) of patients were generally stressed, 54 (27.1%) patients were generally happy, 19 (9.5%) of patients were generally upset, and 38 (19.1%) of patients had a non-dominant mood. Table 3 summarizes lifestyle behaviors.

Table 2. Cancer patients and family characteristics

	Number of patients (n)	Percentage (%)
Cancer type		
Lung	39	19.6
Breast	23	11.6
Gus	29	14.6
Colorectal	14	7
Other	39	19.6
Stages		
Early stage	76	38.2
Advanced stage	94	47.2
Family cancer history		
No	103	51.8
Yes	96	48.2
Maternal age at birth		
≤40 years	183	92
>40 years	7	3.5
Breastfeeding duration		
None	9	4.5
Less than 6 months	29	14.6
More than 6 months	145	72.9
Which child is she/he in the family		
First child	51	25.6
Second or third child	87	43.7
Fourth or more	57	28.6

There was statistical significance between marital status and disease stage. 30 (82%) of unmarried patients were in the advanced stage of the disease (p<0.001). It was observed that

Table 3. Lifestyle behaviors

	Number of patients (n)	Percentage (%)
Smoking use		
Yes	98	49.2
Never	96	48.2
Cessation of smoking after diagnosed (How many years not smoked)		
For 5 years	11	5.5
5-10 years	10	5
More than 10 years	21	10.6
Total	42	21.1
Continues to smoke	157	78.9
Exercise		
Regular	24	12.1
No	175	87.9
Obtaining method of water		
Purifier/fountain/glass	113	56.8
Plastic	85	42.7
Working condition		
Sedentary	139	69.8
Non-sedentary	51	25.6
Sleep duration		
Lower than 6 hours	41	20.6
6-8 hours	116	58.3
More than 8 hours	39	19.6
Sleep quality		
Interrupted	133	66.8
Never interrupted	62	31.2
Eating before sleeping		
Within 4 hours	126	63.3
After 4 hours	68	34.2
Habit before sleep		
Reading book	6	3
Mobile phone & tv & computer	146	73.4
None	40	20.1
Daily screen exposure time		
Less than 2 hours	52	26.1
More than 2 hours	137	68.8
Mood over the last few years		
Generally happy	54	27.1
Generally stressfully-upset	102	51.3
No dominant mood	38	19.1

the usage of radiation-emitting devices such as smartphones, wifi, and television in the university/college graduate group [n=87(82%)] was significantly higher than that in the less educated population (p<0.001)

In our study population, we found that 64 (70.3%) of patients in the advanced stage had sedentary working conditions. (p=0.148). Additionally, 79 (56.8%) of individuals with a sedentary lifestyle were college/university graduates, but this analysis did not reach statistical significance (p=0.35). Among the cancer patients participating in the study, a difference in daily sleep hours was observed between those with a screen time of ≤2 hours and those with >2 hours. Patients with shorter screen exposure (n=109, 64%) were able to sleep ≥6 hours per day (p<0.001). In addition, it was observed that the majority of cancer patients with sedentary working conditions [26 (65%)] could sleep less than 6 hours, and this difference was significant (p=0.005). It was observed that the rate of those who did not demonstrate sun protection behavior was significantly lower in the low-income group 48 (87%) than in the higher-income group of cancer patients (p=0.018).

Discussion

This study investigated the link between lifestyle behaviors and the risk of developing cancer. Additionally, this study addresses possible risk factors that were insufficiently studied previously, such as sleep duration.

Age is one of the major risk factors for cancer, more than 50 percent of cancer patients are >65 years of age in the world. Unfortunately, due to lifestyle changes, the age of cancer diagnosis has decreased significantly in the last few decades [12]. In our study population, which is consistent with the literature, the median age of patients was 60 years, with a range from 20 to 86; most patients were younger than 65 years of age.

Smoking is a well-established risk factor for several types of cancer, particularly lung cancer [13]. Approximately 80% of individuals diagnosed with lung cancer were current or former smokers [14]. Also, roughly 80% of lung cancer-related fatalities are observed in patients with a history of smoking [15,16]. Thirty-eight of the patients included in our study had lung cancer diagnoses, and a significant portion of them, 26 (69%), had smoked. Additionally, 98 (49.2%) of the cancer patients participating in the study were smokers. Strong evidence supports the idea that smoking increases the risk of not only lung cancer but also colorectal cancer by approximately 15% [17]. In our study, 57% of individuals diagnosed with colorectal cancer reported a history of smoking. The findings in our study support the relationship between smoking and cancer development.

In our study, the proportion of cancer patients using plastic bottles as a source of drinking water was 85 (42.7%), which is quite high. Studies in the literature investigating the potential carcinogenic effects of long-term plastic bottle usage show similarities with our findings [18-22]. This finding is also important and warrants further investigation regarding the relationship between plastic use and cancer development for public health.

According to Wang et al. [23], daily eating patterns are a significant factor in cancer development due to their impact on the endogenous circadian rhythm mechanism. In our study, 126 (63.3%) of cancer patients were found to consume food within four hours before sleep. This finding suggests that eating before sleep may disrupt the circadian rhythm, potentially playing a role in cancer development.

There is a substantial body of evidence indicating a relationship between regular physical activity, exercise timing, and cancer incidence [24,25]. Notably, a 2021 study conducted in Spain demonstrated that engaging in physical activities in the early morning and evening could reduce the risk of prostate and breast cancers [26]. Regular physical activity is a modifiable lifestyle factor that has been consistently associated with a significant reduction in cancer risk [27]. In our study, 174 (87.9%) of participants were found not to engage in regular exercise. This high percentage supports a strong link between physical inactivity and cancer development. Additionally, in our study, no statistically significant relationship was found between exercise time and cancer types (p=0.48).

Although alcohol is not as strongly associated with cancer as smoking, numerous studies have demonstrated that chronic alcohol consumption is a significant risk factor for various types of cancer [28,29]. Research conducted in Atlanta indicates that the combined use of alcohol and tobacco significantly increases the risk of cancer, particularly oropharyngeal, esophageal, laryngeal, and breast cancer, compared to the individual effects of either factor alone [30]. However, in this study, the relationship between combined alcohol-tobacco consumption and cancer types did not reach statistical significance (p=0.7).

The study by Sancar and Van Gelder [31] provided stronger evidence for the relationship between the circadian rhythm mechanism and cancer, allowing a better understanding of this connection. These findings highlight the need for further detailed investigation into the link between sleep patterns and cancer. As per Li et al. [32], the optimal duration of sleep for reducing cancer risk is 7-8 hours. Contrary to the literature, in our study, most cancer patients were found to sleep between 6-8 hours. Also consistent with our study, in a study conducted by Cai et al. [33], which examined the association between sleep duration and breast cancer, no statistically significant correlation was identified between the two variables.

Xiao et al. [34] demonstrated that nighttime exposure to light disrupts the circadian rhythm and may increase the risk of pancreatic cancer. In accordance with this finding, we found that 146 (73.4%) of participants reported using phones or computers before going to sleep. This suggests that blue light emitted from electronic devices may impact the circadian rhythm, potentially contributing to the development and progression of cancer.

Skin cancer is the most commonly diagnosed type of cancer in the United States, with millions of cases reported annually [35-38], of which 90% are non-melanoma. This finding emphasized that protecting against UV radiation plays a critical role in reducing the risk of skin cancer [39]. In our study, sun protection behavior was found to be significantly different

between patients in low and high-income groups. (p=0.018). Forty-eight (87%) individuals earning minimum wage or less reported not taking any precautions against sun exposure. Furthermore, these findings emphasize the importance of socioeconomic factors in playing a crucial role in influencing sun protection behaviors.

According to Stuebe [40], breastfeeding reduces the risk of breast and ovarian cancers, as well as several other types of cancer, in mothers. In our study, a significant relationship was found between the duration of breastfeeding and alcohol use in later years. 114 (82%) of patients who did not consume alcohol were found to have breastfed for more than six months (p=0.039). This finding suggests a strong potential relationship between alcohol consumption and breastfeeding, warranting further detailed investigation into this association.

The impact of working life on health is closely related to the environmental conditions. A study conducted in Japan in 2022 investigated the relationship between walking time, type of work, and posture during work and the incidence of colorectal cancer. The results indicated that increased physical activity during working hours, particularly walking, was associated with a reduced risk of colorectal cancer [41]. In our study, 27 (65%) of individuals who slept less than six hours per day were employed in sedentary jobs (p=0.005). This finding suggests that prolonged physical inactivity and irregular sleep habits may increase the risk of cancer.

The study by Wegrzyn et al. [42] indicated that long-term rotating night shift work, particularly among women working shifts during early adulthood, was associated with an increased risk of breast cancer. In our study, we obtained indirect data supporting this finding. There was a statistically significant association observed between night shift work and smoking. Among non-smokers, 77 (93%) had never worked night shifts (p<0.001), suggesting that smoking behavior may be linked to the sleep disturbances caused by night shift work. The literature also supports the notion that work-related factors may increase cancer risk [43].

Study Limitations

This study presents several limitations that should be taken into account when interpreting the findings. First, the absence of a control group and the cross-sectional nature of the study design limit the ability to establish causal relationships and reduce the etiological interpretability of the results. Second, the relatively small sample size may reduce statistical power, affecting the precision and reliability of the findings. Therefore, the generalizability of the results to broader populations remains limited. Third, reliance on self-reported data introduces the potential for bias, particularly recall and social desirability biases, which may compromise data accuracy. Finally, the study sample was drawn from a single geographic region, which may limit the representativeness of the findings and restrict their applicability to populations in different settings or healthcare systems. Taken together, these limitations highlight the need for cautious interpretation of the results and emphasize the importance of further studies with more rigorous designs and diverse, larger populations.

This study examined the impact of lifestyle behaviors on cancer development, highlighting both well-established and underexplored risk factors. Specifically, physical inactivity, prolonged exposure to blue light from electronic devices, irregular exercise habits, circadian rhythm disruption, and workplace conditions.

Conclusion

In conclusion, raising awareness about modifiable lifestyle-related risk factors such as physical activity, sun protection, working conditions, smoking, among others, and making these factors actionable for a wider population will play a critical role in reducing cancer incidence. More extensive, long-term, and prospective studies are needed to develop effective prevention strategies.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the Non-invasive Clinical Research Ethics Committee of Ankara City Hospital and was conducted in accordance with the Declaration of Helsinki (decision no: E2-22-2782, date: 09.11.2022).

Informed Consent: Personal permission was thoughtfully obtained from each individual, underscoring our commitment to transparency and respect throughout the research process.

Footnotes

Authorship Contributions

Surgical and Medical Practices: U.T.M., D.Ş.D., M.D.G., Concept: U.T.M., D.Ş.D., S.E., Design: U.T.M., D.Ş.D., M.D.G., S.E., Data Collection or Processing: U.T.M., D.Ş.D., S.E., Analysis or Interpretation: U.T.M., D.Ş.D., M.D.G., Literature Search: U.T.M., Writing: U.T.M., D.Ş.D., M.D.G.

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

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

References

1. Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. Carcinogenesis. 2010;31:100-110.
2. Van Cott C. Cancer genetics. Surg Clin North Am. 2020;100:483-498.
3. Hamilton AS, Mack TM. Puberty and genetic susceptibility to breast cancer in a case-control study in twins. N Engl J Med. 2003;348:2313-2322.
4. Anand P, Kunnumakkara AB, Sundaram C, et al. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res. 2008;25:2097-2116.
5. Newell GR. Nutrition and the etiology of cancer. Prim Care. 1982;9:573-580.
6. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol. 2006;71:1397-1421.

7. Food and Agriculture Organization of the United Nations. Projections of tobacco production, consumption and trade to the year 2010. Rome; FAO: 2003.
8. Loeb LA, Ernster VL, Warner KE, Abbotts J, Laszlo J. Smoking and lung cancer: an overview. Cancer Res. 1984;44:5940-5958.
9. Gebauer J, Higham C, Langer T, Denzer C, Brabant G. Long-term endocrine and metabolic consequences of cancer treatment: a systematic review. Endocr Rev. 2019;40:711-767.
10. Naughton MJ, Weaver KE. Physical and mental health among cancer survivors: considerations for long-term care and quality of life. N C Med J. 2014;75:283-286.
11. Fortin J, Leblanc M, Elgbeili G, Cordova MJ, Marin MF, Brunet A. The mental health impacts of receiving a breast cancer diagnosis: a meta-analysis. Br J Cancer. 2021;125:1582-1592.
12. NCI's Surveillance, Epidemiology, and End Results (SEER) Program. Incidence rates by age at diagnosis, all cancer types. SEER 21, 2013–2017. https://seer.cancer.gov/data/
13. Newcomb PA, Carbone PP. The health consequences of smoking. Cancer. Med Clin North Am. 1992;76:305-331.
14. Ozlü T, Bülbül Y. Smoking and lung cancer. Tuberk Toraks. 2005;53:200-209.
15. Lee PN, Forey BA, Coombs KJ. Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer. BMC Cancer. 2012;12:385.
16. O'Keeffe LM, Taylor G, Huxley RR, Mitchell P, Woodward M, Peters SAE. Smoking as a risk factor for lung cancer in women and men: a systematic review and meta-analysis. BMJ Open. 2018;8:e021611.
17. Matsuda T, Fujimoto A, Igarashi Y. Colorectal cancer: epidemiology, risk factors, and public health strategies. Digestion. 2025;106:91-99.
18. Baj J, Dring JC, Czeczelewski M, et al. Derivatives of plastics as potential carcinogenic factors: the current state of knowledge. Cancers (Basel). 2022;14:4637.
19. Li S, Keenan JI, Shaw IC, Frizelle FA. Could microplastics be a driver for early onset colorectal cancer? Cancers (Basel). 2023;15:3323.
20. Ungureanu EL, Soare AD, Mocanu AL, Iorga SC, Mustatea G, Popa ME. Occurrence of potentially toxic elements in bottled drinking water-carcinogenic and non-carcinogenic risks assessment in adults via ıngestion. Foods. 2022;11:1407.
21. Brandt-Rauf PW, Li Y, Long C, Monaco R, Kovvali G, Marion MJ. Plastics and carcinogenesis: the example of vinyl chloride. J Carcinog. 2012;11:5.
22. Kumar R, Manna C, Padha S, et al. Micro(nano)plastics pollution and human health: how plastics can induce carcinogenesis to humans? Chemosphere. 2022;298:134267.
23. Wang QJ, Guo Y, Yao CY, et al. Loss of diurnal behavioral rhythms and impaired lipid metabolism in growing pigs with mistimed feeding. FASEB J. 2021;35:e21972.
24. Booth FW, Chakravarthy MV, Gordon SE, Spangenburg EE. Waging war on physical inactivity: using modern molecular ammunition against an ancient enemy. J Appl Physiol (1985). 2002;93:3-30.
25. Jurdana M. Physical activity and cancer risk. Actual knowledge and possible biological mechanisms. Radiol Oncol. 2021;55:7-17.
26. Weitzer J, Castaño-Vinyals G, Aragonés N, et al. Effect of time of day of recreational and household physical activity on prostate and breast cancer risk (MCC-Spain study). Int J Cancer. 2021;148:1360-1371.
27. Feng Y, Feng X, Wan R, Luo Z, Qu L, Wang Q. Impact of exercise on cancer: mechanistic perspectives and new insights. Front Immunol. 2024;15:1474770.
28. Tuyns AJ. Epidemiology of alcohol and cancer. Cancer Res. 1979;39:2840-2843.
29. Hamajima N, Hirose K, Tajima K, et al. Alcohol, tobacco and breast cancer--collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer. 2002;87:1234-1245.
30. Calle J, Rodriguez C. Cancer facts and figures. Atlanta (GA): American Cancer Society; 2009.
31. Sancar A, Van Gelder RN. Clocks, cancer, and chronochemotherapy. Science. 2021;371:eabb0738.
32. Li Y, Cai S, Ling Y, et al. Association between total sleep time and all cancer mortality: non-linear dose-response meta-analysis of cohort studies. Sleep Med. 2019;60:211-218.
33. Cai Y, Zhaoxiong Y, Zhu W, Wang H. Association between sleep duration, depression and breast cancer in the United States: a national health and nutrition examination survey analysis 2009-2018. Ann Med. 2024;56:2314235.
34. Xiao Q, Jones RR, James P, Stolzenberg-Solomon RZ. Light at night and risk of pancreatic cancer in the nih-aarp diet and health study. Cancer Res. 2021;81:1616-1622.
35. Guy GP Jr, Thomas CC, Thompson T, et al. Vital signs: melanoma incidence and mortality trends and projections - United States, 1982-2030. MMWR Morb Mortal Wkly Rep. 2015;64:591-596.
36. Guy GP Jr, Machlin SR, Ekwueme DU, Yabroff KR. Prevalence and costs of skin cancer treatment in the U.S., 2002-2006 and 2007-2011. Am J Prev Med. 2015;48:183-187.
37. Rogers HW, Weinstock MA, Harris AR, et al. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. Arch Dermatol. 2010;146:283-287.
38. Kim Y, He YY. Ultraviolet radiation-induced non-melanoma skin cancer: regulation of DNA damage repair and inflammation. Genes Dis. 2014;1:188-198.
39. International Agency for Research on Cancer Working Group on artificial ultraviolet (UV) light and skin cancer. The association of use of sunbeds with cutaneous malignant melanoma and other skin cancers: a systematic review. Int J Cancer. 2007;120:1116-1122.
40. Stuebe A. The risks of not breastfeeding for mothers and infants. Rev Obstet Gynecol. 2009;2:222-231.
41. Eshak ES, Noda H, Tamakoshi A, Iso H. Walking time, sports activity, job type, and body posture during work in relation to incident colorectal cancer: the JACC prospective cohort study. Cancer Causes Control. 2022;33:473-481.
42. Wegrzyn LR, Tamimi RM, Rosner BA, et al. Rotating night-shift work and the risk of breast cancer in the nurses' health studies. Am J Epidemiol. 2017;186:532-540.
43. Y Yang T, Qiao Y, Xiang S, Li W, Gan Y, Chen Y. Work stress and the risk of cancer: a meta-analysis of observational studies. Int J Cancer. 2019;144:2390-2400.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):202-205

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-7-3

Comparison of Third-Line Treatment Options in Patients with Metastatic Renal Cell Carcinoma

Ömer Faruk Kuzu[1], Nebi Batuhan Kanat[2], Dilruba İlayda Özel Bozdağ[2], Alper Topal[3], Nuri Karadurmuş[4], İsmail Ertürk[4]

[1]*Çankırı State Hospital, Clinic of Oncology, Çankırı, Türkiye*
[2]*University of Health Sciences Türkiye, Gülhane Training and Research, Hospital, Department of Internal Medicine, Ankara, Türkiye*
[3]*Tokat Gaziosmanpaşa University Faculty of Medicine, Department of Oncology, Tokat, Türkiye*
[4]*University of Health Sciences Türkiye, Gülhane Training and Research, Department of Internal Medicine and Medical Oncology, Ankara, Türkiye*

ABSTRACT

Aim: Metastatic renal cell carcinoma (mRCC) is a challenging malignancy requiring multiple lines of therapy. While tyrosine kinase inhibitors remain the standard first-line treatment, resistance often necessitates further therapeutic options. Nivolumab, everolimus, axitinib, and cabozantinib are commonly used in later-line settings, but the optimal sequencing strategy remains unclear.

Methods: This retrospective study analyzed 32 patients with mRCC treated at University of Health Sciences Türkiye, Gülhane Training and Research Hospital between January 2015 and December 2022. All patients had received at least two prior systemic therapies. Survival outcomes were assessed using Kaplan-Meier analysis. Progression-free survival (PFS) and overall survival (OS) were compared among treatment groups using the Log-Rank test.

Results: Axitinib was the most frequently used third-line therapy (50.0%), followed by everolimus (25.0%), cabozantinib (12.5%), and nivolumab (12.5%). Nivolumab showed the longest median PFS (41.0 months, p=0.034) and OS (149.0 months), although OS differences were not statistically significant (p=0.154).

Conclusion: This study highlights variation in third-line treatment patterns and outcomes among mRCC patients. Nivolumab and axitinib demonstrated promising efficacy, suggesting their consideration as preferred options in this setting.

Keywords: Genitourinary cancer, metastatic renal cell carcinoma, third-line therapy

Introduction

Metastatic renal cell carcinoma (mRCC) remains a significant challenge in oncology, often requiring multiple lines of therapy to achieve sustained disease control. Tyrosine kinase inhibitors (TKIs), which target the vascular endothelial growth factor receptor (VEGFR) pathway, have long been the backbone of first-line treatment. However, the development of resistance is common, necessitating additional therapeutic options. Among patients who experience disease progression on immunotherapy, the role of titrated axitinib dosing as a subsequent therapy remains investigational, with only a single phase 2 study evaluating its efficacy [1].

The phase 3 METEOR trial compared cabozantinib and everolimus in 658 mRCC patients who had progressed after VEGF-TKI therapy, revealing that 69% had received one prior systemic therapy, while 31% had undergone two or more, underscoring the need for effective later-line treatment options [2-4]

Recent studies indicate that everolimus is increasingly being utilized in third-line and subsequent treatment lines for renal cell carcinoma, reflecting its evolving role in later treatment stages [5-8]. Additionally, immune checkpoint inhibitors (ICIs), such as nivolumab, have reshaped the treatment landscape, particularly in second-line and beyond settings. Nivolumab

Address for Correspondence: Ömer Faruk Kuzu MD, Çankırı State Hospital, Clinic of Oncology, Çankırı, Türkiye
E-mail: drfarukkuzu@gmail.com **ORCID ID:** orcid.org/0000-0003-3197-7861
Received: 09.07.2025 **Accepted:** 15.10.2025 **Epub:** 21.10.2025 **Publication Date:** 12.12.2025

Cite this article as: Kuzu ÖF, Kanat NB, Özel Bozdağ Dİ, Topal A, Karadurmuş N, Ertürk İ. Comparison of third-line treatment options in patients with metastatic renal cell carcinoma. Acta Haematol Oncol Turc. 2025;58(3):202-205

has demonstrated superior efficacy and tolerability compared to everolimus, leading to its widespread adoption in clinical practice [4].

Despite these advancements, the optimal sequencing of third-line therapies remains an area of active investigation, with treatment options including axitinib, everolimus, cabozantinib, and nivolumab.

This study aims to evaluate real-world treatment patterns and survival outcomes of mRCC patients receiving third-line therapy, focusing on the efficacy of available options including axitinib, everolimus, cabozantinib, and nivolumab.

Methods

We conducted an analysis of 32 patients diagnosed with mRCC who were managed at University of Health Sciences Türkiye, Gülhane Training and Research Hospital. The analyzed patients had previously received two lines of systemic therapy before initiating third-line treatment. The effectiveness of third-line therapies was assessed by comparing their impact on survival outcomes using the Kaplan-Meier analysis. Primary end points were time to progression-free survival (PFS) and overall survival (OS). OS is the duration of time from the start of treatment until death from any cause. PFS was defined as disease progression or death from any cause after third-line treatment.

Ethical approval for this study was obtained from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Ethics Committee, and the study was conducted in accordance with the principles of the Declaration of Helsinki (decision no: 2025/119, date: 12.06.2025). Due to the retrospective nature of the study, the Ethics Committee of University of Health Sciences Türkiye, Gülhane Training and Research Hospital waived the obligation to obtain informed consent.

Statistical Analysis

All statistical analyses were performed using the IBM Statistical Package for the Social Sciences Statistics 27.0 software package. Continuous variables were described as medians (interquartile range), and categorical variables were described as percentages. Survival curves and rates were estimated using the Kaplan-Meier method. The Log-Rank test was used to compare the survival outcomes between the groups. All reported p values were two-sided, and p values <0.05 were regarded as statistically significant

Results

A total of 32 patients were included in the study, with a median age of 55 years among them, 26 were male (81.25%) and most were diagnosed at stage 4 (68.8%). Clear cell carcinoma was the most prevalent histological subtype (84.4%), while papillary (9.4%) and chromophobe (6.3%) subtypes were less common. Additionally, sarcomatoid differentiation was identified in 9.4% of cases. Regarding metastatic involvement, lung metastases (78.1%) were the most frequently observed, followed by lymph node/soft tissue (68.8%), bone (50.0%), liver (15.6%), and brain (15.6%) metastases. The distribution of the International Metastatic RCC Database Consortium risk categories among the study cohort demonstrated that the majority of patients were classified as intermediate risk (78.1%), while 18.8% of patients were categorized as poor risk. Only 3.1% of patients were classified as having a good risk profile. In this study, all patients received TKI therapy as a first-line treatment. The most commonly used treatment among second-line treatments was nivolumab, which was administered to 27 patients (84%). Axitinib was used by 3 patients (9.4%), while sunitinib and everolimus were administered to 1 patient (3.1%). In the third-line treatment analysis, axitinib was the most frequently administered therapy, used by 16 patients (50.0%). Everolimus was the second most common option, given to 8 patients (25.0%), while cabozantinib and nivolumab were each used by 4 patients (12.5%) (Table 1).

Table 1. Clinical characteristic of patients

Variable	Category	Count (percentage)
Gender	Male	26 (81.3%)
	Female	6 (18.8%)
Diagnosis stage	Stage 1	2 (6.3%)
	Stage 2	6 (18.8%)
	Stage 3	2 (6.3%)
	Stage 4	22 (68.8%)
Histological type	Clear Cell	27 (84.4%)
	Papillary	3 (9.4%)
	Chromophobe	2 (6.3%)
Sarcomatoid features	No	29 (90.6%)
	Yes	3 (9.4%)
Metastasis status	Brain	5 (15.6%)
	Lung	25 (78.1%)
	Liver	5 (15.6%)
	Bone	16 (50.0%)
Lymph node/soft tissue	Absent	10 (31.3%)
IMDC risk	Poor	6 (18.8%)
	Intermediate	25 (78.1%)
	Good	1 (3.1%)
Second line treatments	Nivolumab	27 (84%)
	Axitinib	3 (9.4%)
	Sunitinib	1 (3.1%)
	Everolimus	1 (3.1%)
Third-line treatment	Aksitinib	16 (50.0%)
	Everolimus	8 (25.0%)
	Cabozantinib	4 (12.5%)
	Nivolumab	4 (12.5%)

IMDC: International Metastatic Database Consortium

Axitinib exhibited a median PFS of 13.0 months [95% confidence interval (CI): 5.03-20.97], whereas everolimus had a median PFS of 6.0 months (95% CI: 0.00-13.13). Cabozantinib demonstrated the shortest median PFS of 2.0 months (95% CI: 0.00-10.49). Nivolumab showed the longest median PFS of 41.0 months, though its CI could not be determined. Nivolumab provided a statistically significant PFS advantage compared to other treatment groups (p=0.034) (Figure 1).

Axitinib demonstrated a median OS of 59.0 (95% CI: 27.64-90.36), whereas everolimus had a median OS of 73.0 months (95% CI: 0.00-154.89). Cabozantinib showed a median OS of 22.0 months (95% CI: 0.00-49.44). Nivolumab exhibited the longest median OS of 149.0 months (95% CI: 106.93-358.58). No statistically significant difference in OS was observed among the treatment subgroups (p=0.154) (Figure 2).

Discussion

In this study, we evaluated the treatment patterns and outcomes of mRCC patients who received first, second, and third-line therapies. Our findings indicate that all patients received TKI therapy as a first-line treatment. While clinical trials have provided valuable insights into the efficacy of these agents, real-world data on third-line treatment choices and survival outcomes remain limited. Understanding the comparative effectiveness of these therapies in later-line settings is crucial for optimizing treatment strategies and improving patient prognosis.

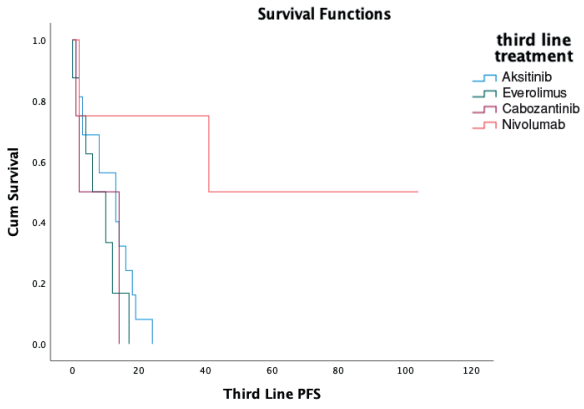


Figure 1. Third line progresyon-free survival

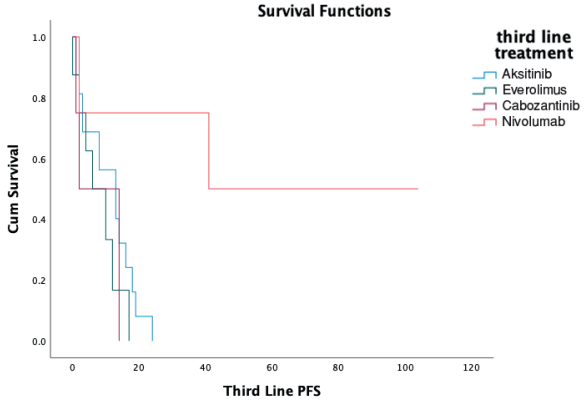


Figure 2. Overall survival

Among second-line treatments, nivolumab was the predominant choice, administered to 84% of patients, while other options such as axitinib, sunitinib, and everolimus were used less frequently. This suggests a preference for ICIs following TKI failure, consistent with the latest clinical guidelines favoring nivolumab-based regimens due to their superior efficacy and tolerability [4].

Our third-line treatment analysis revealed notable differences in PFS and OS among different therapeutic options. In our study axitinib was the most frequently administered therapy, used by 16 patients (50.0%). A study Tsironis et al. [8] demonstrated that axitinib is an effective therapeutic agent following second-line treatment. Similarly, Ishihara et al. [9] highlighted the promising efficacy of axitinib as a third-line treatment for mRCC, reporting 50.0% usage, median PFS of 12.8 months, and 1-year PFS rate of 51.3%. Additionaly, its objective response rate (29.4%) and disease control rate (94.1%) further support its effectiveness in this setting.

According to a study Rauthan et al. [10], nivolumab has demonstrated efficacy in the third-line or later setting, showing a 60% response rate and a median OS of 26 months. While median PFS was 5 months, some patients achieved long-term remission exceeding 40 months, reinforcing the treatment's role in later-line therapy for immunotherapy-sensitive cases. In our study nivolumab demonstrated the longest median PFS and a statistically significant advantage over other treatments (p=0.034), emphasizing its role as a durable treatment option. Furthermore, despite no statistically significant OS differences (p=0.15), nivolumab exhibited the longest median OS (149 months), suggesting potential long-term survival benefits. Meanwhile, the relatively limited efficacy of cabozantinib highlights the need for further investigation into optimal sequencing strategies in mRCC treatment.

Everolimus remains a third-line option for mRCC after VEGFR-TKI failure. In this study, a certain treatment was used in 25.0% of patients, showing better outcomes (PFS: 6, OS: 73 months) than cabozantinib but inferior to nivolumab (PFS: 41, OS: 149 months) and axitinib. A review conducted Buti et al. [11], which multiple studies on the efficacy of everolimus in the treatment of mRCC, demonstrated that the role of everolimus has shifted primarily to third-line and subsequent treatment options.

Study Limitations

This study has several limitations. First, the retrospective design introduces inherent risks of selection bias and incomplete data capture. Second, the relatively small sample size (n=32) limits the statistical power to detect differences in OS, and restricts the generalizability of the findings. Additionally, the single-center setting may not reflect variations in treatment practices across institutions. Finally, heterogeneity in prior lines of

therapy and patient characteristics could have influenced treatment outcomes.

Conclusion

In this retrospective single-center study evaluating third-line treatment options for mRCC, nivolumab demonstrated the longest PFS and OS among the evaluated therapies, although the difference in OS was not statistically significant. Axitinib also showed promising efficacy and remained the most frequently used third-line agent. These findings suggest that immunotherapy and VEGFR-targeted TKI continue to be relevant options in later-line settings. However, the optimal sequencing of therapies remains uncertain, underscoring the need for further prospective studies to establish individualized treatment strategies.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Ethics Committee, and the study was conducted in accordance with the principles of the Declaration of Helsinki (decision no: 2025/119, date: 12.06.2025).

Informed Consent: Due to the retrospective nature of the study, the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Ethics Committee waived the requirement to obtain informed consent.

Footnotes

Authorship Contributions

Concept: Ö.F.K., N.K., İ.E., Design: Ö.F.K., N.B.K., D.İ.Ö.B., N.K., Data Collection or Processing: Ö.F.K., N.B.K., D.İ.Ö.B., Analysis or Interpretation: Ö.F.K., A.T., Literature Search: Ö.F.K., D.İ.Ö.B., A.T., Writing: Ö.F.K., N.B.K.

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

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

References

1. Medline® Abstract for Reference 41 of "Antiangiogenic and molecularly targeted therapy for advanced or metastatic clear cell renal carcinoma" - UpToDate. Accessed March 20, 2025.https://www.uptodate.com/contents/antiangiogenic-and-molecularly-targeted-therapy-for-advanced-or-metastatic-clear-cell-renal-carcinoma/abstract/41
2. Choueiri TK, Escudier B, Powles T, et al. Cabozantinib versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373:1814-1823.
3. Donskov F, Motzer RJ, Voog E, et al. Outcomes based on age in the phase III METEOR trial of cabozantinib versus everolimus in patients with advanced renal cell carcinoma. Eur J Cancer. 2020;126:1-10.
4. Motzer RJ, Escudier B, George S, et al. Nivolumab versus everolimus in patients with advanced renal cell carcinoma: updated results with long-term follow-up of the randomized, open-label, phase 3 CheckMate 025 trial. Cancer. 2020;126:4156-4167.
5. Calvo E, Escudier B, Motzer RJ, et al. Everolimus in metastatic renal cell carcinoma: subgroup analysis of patients with 1 or 2 previous vascular endothelial growth factor receptor-tyrosine kinase inhibitor therapies enrolled in the phase III RECORD-1 study. Eur J Cancer. 2012;48:333-339.
6. Grünwald V, Karakiewicz PI, Bavbek SE, et al. An international expanded-access programme of everolimus: addressing safety and efficacy in patients with metastatic renal cell carcinoma who progress after initial vascular endothelial growth factor receptor-tyrosine kinase inhibitor therapy. Eur J Cancer. 2012;48:324-332.
7. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373:1803-1813.
8. Tsironis G, Liontos M, Kyriazoglou A, et al. Axitinib as a third or further line of treatment in renal cancer: a single institution experience. BMC Urol. 2020;20:60
9. Ishihara H, Takagi T, Kondo T, et al. Efficacy of axitinib after nivolumab failure in metastatic renal cell carcinoma. In Vivo. 2020;34:1541-1546.
10. Rauthan A, Murthy NY, Patil P, Nigade G, Somashekhar SP, Zaveri SS. Real-world experience with nivolumab in metastatic renal cell carcinoma patients who have progressed on prior therapies: a single-center study from India. South Asian J Cancer. 2022;11:133-139.
11. Buti S, Leonetti A, Dallatomasina A, Bersanelli M. Everolimus in the management of metastatic renal cell carcinoma: an evidence-based review of its place in therapy. Core Evid. 2016;11:23-36.

AHOT | Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):206-211

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-8-3

Use of a Collagen-Laminin-Based Dermal Matrix Combined with Resveratrol Microparticles (Dermalix) for Wound Complications After Breast Surgery

Simay Akyüz[1], Betülay Kılıç[2], Kemal İpek[3], Hikmet Erhan Güven[4], İbrahim Burak Bahçecioğlu[5], Müjdat Turan[6], Ebru Esen[7], Şevket Barış Morkavuk[5], Melih Akıncı[4], Kerim Bora Yılmaz[6], Mehmet Ali Gülçelik[5]

[1]*University of Health Sciences Türkiye, Gülhane Faculty of Nursing, Department of Oncology Nursing, Ankara, Türkiye*
[2]*University of Health Sciences Türkiye, Gülhane Faculty of Nursing, Department of Internal Medicine Nursing, Ankara, Türkiye*
[3]*Ankara Provincial Health Directorate Public Hospitals Services Directorate, Ankara, Türkiye*
[4]*University of Health Sciences Türkiye, Ankara Etlik City Hospital, Department of General Surgery, Ankara, Türkiye*
[5]*University of Health Sciences Türkiye, Gülhane Research and Training Hospital, Department of Surgical Oncology, Ankara, Türkiye*
[6]*University of Health Sciences Türkiye, Gülhane Research and Training Hospital, Department of General Surgery, Ankara, Türkiye.*
[7]*İstinye University Hospital, Bahçeşehir Liv Hospital, Department of General Surgery, İstanbul, Türkiye*

ABSTRACT

Aim: Wound complications after breast surgery may adversely affect the healing process and delay the initiation of adjuvant therapy. Despite appropriate surgical technique and postoperative care, wound complications can still occur following breast operations. To evaluate the role of a collagen-laminin-based dermal matrix combined with resveratrol microparticles in the management of wound complications after breast surgery.

Methods: This study was conducted in the Surgical Department of a Training and Research Hospital between March 2021 and March 2022, and included 15 patients who underwent breast surgery. Inclusion criteria were: (a) prior breast surgery; (b) postoperative wound complication; (c) presence of granulation tissue; (d) a microbiologically culture-negative wound; and (e) use of a collagen-laminin-based dermal matrix combined with resveratrol microparticles during wound care.

Results: The mean age of the patients was 52.67±8.61 years. All were female. Wound complications occurred after breast cancer surgery in 73.4% of patients, while in 26.6% they were related to other surgical indications. Consecutive applications resulted in a statistically significant mean reduction in wound dimensions (p=0.012). Collagen-laminin-based dermal matrix combined with resveratrol microparticles showed a maximal effect by day 8 (after two consecutive applications), producing a marked decrease in wound size. No studies have evaluated the role of a collagen-laminin-based dermal matrix combined with resveratrol microparticles in wound complications after breast surgery. This study is the first to evaluate the therapeutic role of a collagen-laminin-based dermal matrix combined with resveratrol microparticles in wound complications after breast surgery. A characteristic feature of our study is that the patient population predominantly comprises individuals with wound complications following breast cancer surgery.

Conclusion: Collagen-laminin-based dermal matrix combined with resveratrol microparticles may have a role in the ambulatory management of granulating wound complications with microbiologically clean wound beds following breast surgery. Larger multicenter randomized controlled trials are needed.

Keywords: Breast surgery, wound complication, dermalix

Address for Correspondence: Simay Akyüz PhD., RN., University of Health Sciences Türkiye, Gülhane Faculty of Nursing, Department of Oncology Nursing, Ankara, Türkiye

E-mail: simayakyuz@gmail.com **ORCID ID:** orcid.org/0000-0002-0464-8934

Received: 28.08.2025 **Accepted:** 05.11.2025 **Epub:** 12.11.2025 **Publication Date:** 12.12.2025

Cite this article as: Akyüz S, Kılıç B, İpek K, et al. Use of a collagen-laminin-based dermal matrix combined with resveratrol microparticles (Dermalix) for wound complications after breast surgery. Acta Haematol Oncol Turc. 2025;58(3):206-211

Introduction

Breast surgery is among the most commonly performed surgical procedures and is undertaken for a variety of indications. These procedures include breast-conserving surgery, modified radical mastectomy, and simple mastectomy [1].

Breast cancer is one of the principal indications for breast surgery; it is the most frequent malignancy in women, accounting for 23.8% of cancers in women [2]. Incidence rates of breast cancer in Türkiye are consistent with global statistics [3].

Although postoperative complication rates after breast surgery are generally low, morbidity rates reported in the literature vary widely, from 2% to 50%. The most commonly observed complications are infection, seroma formation, and hemorrhage [4]. Wound complications after breast surgery are of particular concern: the incidence of mastectomy-flap necrosis, nipple necrosis, wound dehiscence, and delayed wound healing has been reported to range from 6% to 30% [5].

Wound complications that develop after breast surgery may adversely affect the healing process and give rise to important clinical problems. Despite appropriate surgical technique and postoperative care, such complications can still occur. Failure to manage wound complications in accordance with established wound-care principles may increase the risk of infection, delay the initiation of adjuvant therapy, and ultimately impair patients' quality of life [6-8].

Consequently, effective management of wound complications requires alternative wound-care approaches capable of closing tissue defects and accelerating healing. Biomaterials that actively participate in the repair process are classified as bioactive wound dressings. Composed of natural extracellular matrix components, these materials provide structural support for tissue repair owing to their biocompatibility. Such biomaterials may include polymers such as collagen, hyaluronic acid, chitosan, and alginate.

A collagen-laminin-based dermal matrix loaded with resveratrol -containing microparticles is an innovative wound dressing consisting of dipalmitoylphosphatidylcholine (DPPC)-based microparticles incorporated into a three-dimensional porous collagen-laminin scaffold [9]. The composite formulation includes a glycosaminoglycan derivative (hyaluronic acid), collagen/gelatin to provide hydrophilicity and a three-dimensional porous architecture, laminin as a cell-binding protein, DPPC (a phospholipid abundant in cell membranes), and resveratrol, which has antioxidant properties [10].

The aim of this study was to evaluate the role of a collagen-laminin-based dermal matrix combined with resveratrol microparticles in the management of wound complications following breast surgery.

Methods

Study Setting and Design

This retrospective analysis was conducted in the Surgical Oncology Clinic of a Training and Research Hospital between March 2021 and March 2022. The study population comprised all patients who underwent breast surgery during the study period. The sample consisted of 15 patients who developed postoperative wound complications and were treated with a collagen-laminin-based dermal matrix combined with resveratrol collagen-laminin-based dermal matrix combined with resveratrol microparticles (Dermalix®).

Patient Selection Criteria

Inclusion criteria were:

- Underwent breast surgery,
- Developed a postoperative wound complication,
- Microbiologically culture-negative wound,
- Dermalix® used during the wound-care process.

Exclusion criteria were:

- Primary wound healing after breast surgery,
- Development of other postoperative complications that could affect wound management (e.g., Seroma, abscess).

Data Collection

Data were obtained from the hospital information system and wound-care records using a data collection form prepared by the investigators. For each patient, demographic information, type of surgical procedure, characteristics of the wound complication, and the healing course were recorded.

Collagen-Laminin-Based Dermal Matrix Combined with Resveratrol Microparticles Application and Wound-care Protocol

In patients with postoperative tissue defects or non-healing wounds, Dermalix® application was initiated after confirming negative culture results and the establishment of granulation tissue.

Application protocol:

1. Application of an antiseptic to the dressing area,
2. Gentle debridement to remove biofilm layers,
3. Wound cleansing followed by measurement of wound dimensions,
4. Placement of the Dermalix® dressing tailored to the wound size and positioned on the wound bed,
5. Placement of a secondary absorbent dressing or gauze over Dermalix® to control exudate and completion of the dressing.

Dressing changes were performed every four days. At each dressing change, the wound area was measured, photographed, and documented. Patients were followed on an outpatient basis until complete epithelialization of the wound.

Ethical Considerations

Institutional approval was obtained from the University of Health Sciences Türkiye, Gülhane Scientific Research Ethics Committee (decision no: 2025-283, date: 03.06.2025).

Statistical Analysis

Statistical analysis were performed using IBM Statistical Package for the Social Sciences statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA; released 2016). Descriptive statistics are presented as numbers (percentages), means, and standard deviations. The suitability of the data for a normal distribution was assessed by examining the skewness and kurtosis coefficients. Repeatedmeasures analysis of variance was used for comparisons across time points, and p values <0.05 p values <0.05 were considered statistically significant.

Results

The mean age of the patients included in the study was 52.67±8.61 years; all were female. An analysis of the surgical procedures performed on patients who developed wound complications revealed that 46.6% underwent oncoplastic breast surgery, 26.6% mastectomy, and 26.6% biopsy. Among the patients who had oncoplastic breast surgery, 42.8% underwent a subcutaneous mastectomy with implants, while 57.2% received level 2 glandular flaps. In the cohort of patients who underwent mastectomy, 75% had modified radical mastectomy and 25% had simple mastectomy. Additionally, localization of the wound typically occurred at the incision line and was accompanied by evisceration. Following wound bed preparation, the mean number of applications of a collagen laminin-based dermal matrix with resveratrol microparticles (Dermalix®) was 2.47±0.91, and the mean time to epithelialization was 15.33±5.43 days. Patient characteristics are summarized in Table 1.

Wound monitoring was performed on 15 samples. Because some patients improved during the study, the number of samples decreased to 12 on day 8, 6 on day 12, and 2 on day 16. The average wound size in 15 patients before application of Dermalix® was 7.87±9.21 cm^2; it decreased to 5.53±7.87 cm^2 (change -2.34±2.70) on day 4, 2.70±4.90 cm^2 (change -5.08±5.01) on day 8, 2.08±2.46 cm^2 (change -10.53±9.84) on day 12, and 1.45±1.76 cm^2 (change -21.30±16.87) on day 16.

Due to because of epithelialization-related inequalities imbalances in the data set, patients who were measured on days 4 and 8 were included in the analysis. Variance analysis of variance performed on repeated measurements showed that the wound healing process observed measured on days 4 and 8, based onrelative to initial wound sizes, exhibited a statistically significant improvement patternpattern of improvement (p=0.012). The mean reduction in wound size in sequential applicationswith sequential applications was found to be statistically significantwas statistically significant, and the cumulative effect of the wound healing process was observed to be maximalthe cumulative effect of wound healing peaked on day 8. In measurements taken on day 12, the difference in wound size compared to baseline reachedthe change in wound size from baseline was 10.53±9.84 cm^2 in six patients. In the final stage of treatment (day 16), the average wound size decreased

Table 1. Patient and wound characteristics following breast surgery

Characteristics	Mean ± SD	
Age	52.67±8.61	
Average number of dressing applications	2.47±0.91	
Wound measurement		
Pre-application measurement (n=15)	7.87±9.21	
First post-application measurement (n=15)	5.53±7.87	
Second post-application measurement (n=12)	2.70±4.90	
Third post-application measurement (n=6)	2.08±2.46	
Fourth post-application measurement (n=2)	1.45±1.76	
Time to epithelialization (days)	15.33±5.43	
	Number (n)	**Percentage (%)**
Diagnosis		
Breast cancer	11	73.4
Other	4	26.6
Surgical procedure		
Oncoplastic breast surgery	7	46.8
Mastectomy	4	26.6
Biopsy	4	26.6
Wound location		
Incision line	15	100.0
Number of wound dressing applications		
1	2	13.3
2	6	40.0
3	5	33.3
4	2	13.3

SD: Standard deviation

to 1.45±1.76 cm^2, and a total improvement of 21.30±16.87 cm^2 was achieved compared towith baseline. The characteristics of the wound healing process are presented in Table 2.

Discussion

This study found that the use of a collagen-laminin-based dermal matrix combined with resveratrol microparticles for the management of tissue defects following breast surgery may contribute to wound epithelialization. The findings show that, in wounds characterized by granulation tissue formation and a microbiologically negative wound bed, sequential use of the wound dressing produces maximal effect by day 8 (after two applications), resulting in a significant reduction in wound size.

The severity and prevalence of postoperative complications associated with breast surgery are variable and significantly related to the surgical procedure. Surgical complications may occur in the breast, at the lumpectomy or mastectomy site, or in the axillary region [4]. According to a meta-analysis investigating the effect of wound-related postoperative complications on oncological outcomes, which included 1,418 breast cancer patients, wound complications were occurred in 26.9% of patients, and the time to adjuvant therapy was statistically significantly longer in patients with wound complications, with delays ranging from an average of 1.18 to 16.21 days [6]. The importance of wound complications stems from their causing delays in adjuvant therapy among patients with breast cancer. The vast majority of patients who experienced complications also reported significant stress and anxiety [7,11]. This situation can also lead to prolonged hospital stays, increased risk of hospital-acquired infection, and higher healthcare costs. Considering these risks, effective wound management protocols are an absolute necessity. In our study sample, the reduction in wound size from the first to the last measurement, the average number of days to epithelialization, the ability to provide outpatient follow-up, and follow-ups performed by wound care professionals highlight the importance of effective wound management and wound-care product selection. Considering the negative effects on patients and healthcare services of complications following breast surgery, as reported in the literature, the correct selection of products can contribute to healing.

There is no single ideal product for the management of wound complications [12]. Given the wide range of wound-care products available, selecting a product suitable for the wound-bed characteristics and supportive of healing is critically important.

Wound care nurses and wound care specialists play an important role in this process. These professionals provide guidance on determining the type and cause of the wound, planning treatment and care goals, and managing difficult symptoms and complications. They also make important contributions to identifying factors that support wound healing through a holistic approach that includes consideration of the impacts of wounds on psychological well-being and quality of life [13]. To meet these needs, new-generation wound-care, enabled by technological developments, offer promising alternatives. One such alternative is a collagen-laminin-based dermal matrix combined with resveratrol microparticles, which we used in our research for wound management. Classified in the current literature as bioactive, these wound dressings can influence wound healing through various physiological mechanisms. These products, enriched with hyaluronic acid, honey, collagen, alginates, and polymers containing polyhexamethylene biguanide, chitin, and chitosan derivatives, create a suitable microenvironment for wound healing by providing pH regulation, moisture homeostasis, oxygen permeability, and exudate management [14]. Studies show that resveratrol stimulates collagen synthesis and fibroblast activation with its antioxidant properties, thereby supporting cellular proliferation [15]. Extracellular matrix components such as glycosaminoglycans and fibrous proteins are reported to facilitate cell migration, adhesion, and new tissue formation [9,16]. In this context, the developed collagen-laminin-based dermal matrix combined with a resveratrol microparticle formulation is reported to contain laminin, which plays a role in cell adhesion; collagen, which provides structural support; and hyaluronic acid, a glycosaminoglycan derivative; the lipid DPPC forms the microparticle structural component. In vitro studies have demonstrated that these components exhibit synergistic effects in tissue repair [10].

Clinical studies support these theoretical foundations. Gokce et al. [17] reported that a collagen-laminin dermal matrix impregnated with resveratrol-loaded hyaluronic acid-DPPC microparticles accelerated the wound healing process in a diabetic environment. In another study, Çetinkalp et al. [10] compared standard wound care with standard wound care combined with a collagen-laminin-based dermal matrix and resveratrol microparticles for 4 weeks in patients with diabetic foot wounds. At the end of the study, the wound closure rates were 57.82% in the dressing group and 26.63% in the standard care group. Statistical analyses showed that a collagen-laminin-based dermal matrix combined with resveratrol microparticles promoted rapid wound healing and reduced parameters of oxidative stress [10]. In line with

Table 2. Wound healing measurement results

	Initial wound size	Day 4 wound size	Day 8 wound size	Wilks' lambda distribution	F	p
Wound size	7.87±9.21	5.53±7.87	2.70±4.90	0.476	8.330	**0.012**
Difference compared to the beginning	-	-2.34±2.7	-5.08±5.01			

Analysis of variance in repeated measurements

these findings, the current literature supports the use of this wound dressing as a effective therapeutic agent in diabetic wound healing and in colonic anastomotic dehiscence [9,10,17]. No studies have evaluated the role of a collagen-laminin-based dermal matrix combined with resveratrol microparticles in wound complications following breast surgery. This study is the first to evaluate the therapeutic role of collagen laminin-based dermal matrix combined with resveratrol microparticles in wound complications after breast surgery. A characteristic feature of our study is that the patient population consists predominantly of cases with wound complications after breast cancer surgery.

The findings suggest that a collagen-laminin-based dermal matrix combined with resveratrol microparticles may have an alternative role in the management of wound complications after breast surgery, with clinical advantages such as shortening the time to start adjuvant therapy, enabling outpatient follow-up, and accelerating epithelialization. Furthermore, our study emphasizes the importance of specific wound-bed conditions to achieve the optimal effect of a collagen- and laminin-based dermal matrix combined with resveratrol microparticles. Specifically, this wound dressing was found to be effective in wound beds where granulation tissue was present and microbiological contamination was eliminated. Objective measurements showed that sequential applications of a collagen-laminin-based dermal matrix combined with resveratrol microparticles resulted in a statistically significant reduction in wound size on day 8 (after two consecutive applications). This finding suggests that the dressing has a cumulative effect on wound healing over time and that a clean, granulated wound bed is critical for optimal results. Similarly, the literature reports that wound bed preparation and control of microbial load increase the effectiveness of wound care [18].

Study Limitations

However, our study has some methodological limitations. The single-center design and relatively small sample size, along with substantial attrition over time, limit the generalizability of the results. Furthermore, the availability of the product and lack of coverage by reimbursement schemes may limit patient access in clinical practice and increase costs. These limitations indicate the need for future multicenter randomized controlled trials with larger sample sizes.

Conclusion

This study is the first to evaluate the role of a collagen-laminin-based dermal matrix combined with resveratrol microparticles in wound complications following breast surgery. sequential collagen-laminin-based dermal matrix combined with application of resveratrol microparticles in granulated, microbiologically clean wound beds showed the maximal effect on day 8, resulting in a significant reduction in wound size. With its clinical advantages such as minimizing delays to adjuvant treatment and enabling outpatient follow-up, this dressing may serve as an alternative in the management of wound complications following breast surgery. Due to the single-center design and limited sample size, multicenter randomized controlled trials are needed.

Ethics

Ethics Committee Approval: Institutional approval was obtained from the University of Health Sciences Türkiye, Gülhane Scientific Research Ethics Committee (decision no: 2025-283, date: 03.06.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

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

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

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

References

1. Özmen V, Özmen T, Doğru V. Türkiye›de meme kanseri analizi: 20000 hastanın analizi. 21. Ulusal Cerrahi Kongresi. Akademik Veri Yönetim Sistemi. 2018;ss1
2. International Agency for Research on Canser. Wold Health Organization. Dataviz. Accessed 15.08.2025. https://gco.iarc.fr/today/en/dataviz/
3. TC Sağlık Bakanlığı, Halk Sağlığı Genel Müdürlüğü, Türkiye Kanser İstatistikleri, 2020. https://hsgm.saglik.gov.tr/depo/birimler/kanser-db/Dokumanlar/Istatistikler/Turkiye_Kanser_Istatistikleri_2020.pdf. Accessed 10 Aug 2025.
4. Al-Hilli Z, Wilkerson A. Breast surgery: management of postoperative complications following operations for breast cancer. Surg Clin North Am. 2021;101:845-863.
5. Murthy BL, Thomson CS, Dodwell D, et al. Postoperative wound complications and systemic recurrence in breast cancer. Br J Cancer. 2007;97:1211-1217.
6. Balasubramanian I, Harding T, Boland MR, et al. The impact of postoperative wound complications on oncological outcomes following immediate breast reconstruction for breast cancer: a meta-analysis. Clin Breast Cancer. 2021;21:e377-e387.
7. Gopie JP, Timman R, Hilhorst MT, Hofer SO, Mureau MA, Tibben A. The short-term psychological impact of complications after breast reconstruction. Psychooncology. 2013;22:290-298.
8. Timman R, Gopie JP, Brinkman JN, et al. Most women recover from psychological distress after postoperative complications following implant or DIEP flap breast reconstruction: a prospective long-term follow-up study. PLoS One. 2017;12:e0174455.
9. Ersöz Ş, Konuk Y. The effect of Dermalix® on colon anastomosis leakage: experimental study. Ulus Travma Acil Cerrahi Derg. 2023;29:1212-1217.
10. Çetinkalp Ş, Gökçe EH, Şimşir I, et al. Comparative evaluation of clinical efficacy and safety of collagen laminin-based dermal matrix combined with resveratrol microparticles (dermalix) and standard wound care for diabetic foot ulcers. Int J Low Extrem Wounds. 2021;20:217-226.

11. Procházka V, Závadová A, Žatecký J, Martínek L. Incidence of postoperative complications in patients with breast cancer depending on the type of drain. Rozhl Chir. 2023;102:17-22.
12. Pallaske F, Pallaske A, Herklotz K, Boese-Landgraf J. The significance of collagen dressings in wound management: a review. J Wound Care. 2018;27:692-702.
13. J Clemett V. Wound assessment and documentation: rationale and guidance. Br J Nurs. 2025;34:S20-S26.
14. Polverino G, Russo F, D'Andrea F. Bioactive dressing: a new algorithm in wound healing. J Clin Med. 2024;13:2488.
15. Ndiaye M, Philippe C, Mukhtar H, Ahmad N. The grape antioxidant resveratrol for skin disorders: promise, prospects, and challenges. Arch Biochem Biophys. 2011;508:164-170.
16. Sangnim T, Puri V, Dheer D, Venkatesh DN, Huanbutta K, Sharma A. Nanomaterials in the wound healing process: new insights and advancements. Pharmaceutics. 2024;16:300.
17. Gokce EH, Tuncay Tanrıverdi S, Eroglu I, et al. Wound healing effects of collagen-laminin dermal matrix impregnated with resveratrol loaded hyaluronic acid-DPPC microparticles in diabetic rats. Eur J Pharm Biopharm. 2017;119:17-27.
18. De Francesco F, Ogawa R. From time to timer in wound healing through the regeneration. Adv Exp Med Biol. 2024;1470:1-18.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):212-217

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-7-4

Cladribine Treatment Outcomes in Hairy Cell Leukemia: A Single-center Experience

Sema Seçilmiş, Burcu Aslan Candır, Samet Yaman, Ersin Bozan, Bahar Uncu Ulu, Tuğçe Nur Yiğenoğlu, Dicle İskender, Merih Kızıl Çakar

University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Clinic of Hematology and Bone Marrow Transplantation Center, Ankara, Türkiye

ABSTRACT

Aim: Hairy cell leukemia (HCL) is a rare, indolent, chronic B-cell lymphoproliferative disorder. This study aimed to assess the clinical features, treatment responses, and outcomes of patients with HCL monitored at our institution.

Methods: We conducted a retrospective cohort study of 25 patients diagnosed with HCL who were followed at our center. Data on demographics, presenting symptoms, laboratory findings, immunophenotypic characteristics, treatment responses, and long-term follow-up were reviewed.

Results: The median age at diagnosis was 48 years, and most patients were male. Fatigue, abdominal fullness, and abdominal pain were the leading presenting symptoms. All patients had splenomegaly at diagnosis. All patients received cladribine as first-line therapy, achieving complete remission (CR) in 92.0% and partial remission in 8.0% of cases. During a median follow-up of 36.9 months after CR, three patients (12%) experienced relapse, but subsequently achieved CR following re-treatment with cladribine. No treatment-related deaths or secondary malignancies were recorded.

Conclusion: Cladribine continues to be a reliable and well-tolerated initial therapy for patients with HCL. In those who relapse, retreatment with cladribine is effective, reinforcing its established role in disease management.

Keywords: Hairy cell leukaemia, cladribine, prognosis, purine nucleoside analog

Introduction

Hairy cell leukemia (HCL) is a rare B-cell malignancy with an indolent course, first identified by Bouroncle et al. [1]. It is typically characterized by pancytopenia and splenomegaly and constitutes nearly 2% of all leukemia cases. The disease usually appears around the age of 55 and is 3-4 times more common in men than in women [2]. In many patients, long-lasting cytopenias are the first sign, but may remain undetected for years [2]. With more frequent peripheral blood testing, hairy cells can now be detected even in asymptomatic patients. In 5-10% of these cases, a watch-and-wait approach is used, with regular follow-up until treatment is needed [3,4].

Treatment becomes necessary when symptoms appear or blood counts continue to drop. While HCL usually progresses slowly, most patients eventually need therapy because of worsening cytopenias or an enlarged, symptomatic spleen [5]. A major advance in HCL treatment occurred in the early 1990's with the introduction of purine analogs such as cladribine [2-chlorodeoxyadenosine, (2-CdA)] [6] and pentostatin (2'-deoxycoformycin) [7,8]. These drugs are now the first-line treatment for suitable patients, leading to durable complete remissions (CR) and longer survival in those without active infections and with good performance status [9-12].

This study aimed to evaluate the clinical features and treatment outcomes of patients with HCL managed at our center.

Address for Correspondence: Sema Seçilmiş MD, University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Clinic of Hematology and Bone Marrow Transplantation Center, Ankara, Türkiye

E-mail: secilmissema1@gmail.com **ORCID ID:** orcid.org/0000-0003-2957-2339

Received: 09.07.2025 **Accepted:** 08.11.2025 **Epub:** 14.11.2025 **Publication Date:** 12.12.2025

Cite this article as: Seçilmiş S, Aslan Candır B, Yaman S, et al. Cladribine treatment outcomes in hairy cell leukemia: a single-center experience. Acta Haematol Oncol Turc. 2025;58(3):212-217

Methods

The study was approved by the Clinical Research Ethics Committee of the University of Health Sciences Türkiye, Dr. Abdurrahman Yurtarslan Ankara Oncology Health Application and Development Center of the (decision no: 2022-02/1651, date: 09.02.2022). All procedures were carried out in accordance with the ethical principles of the 1964 Helsinki Declaration.

This retrospective cohort study included 25 patients with classic HCL diagnosed between January 2010 and February 2022. Cases with variant HCL were excluded. Demographic characteristics, initial symptoms, physical examination findings, complete blood counts, biochemical test results, bone marrow biopsy reports, and peripheral blood smear evaluations were reviewed. Treatment responses, side effects, and relapses were also analyzed.

The diagnosis was made using peripheral blood smear, bone marrow morphology, and immunophenotypic and immunohistochemical findings [13,14].

Identifying typical "hairy" lymphoid cells under the microscope was key to the diagnosis. Imaging methods, such as computed tomography or ultrasound, were used to evaluate organ enlargement and lymph node involvement [5]. All patients underwent bone marrow aspiration and biopsy, and flow cytometry was used to confirm the diagnosis. This technique aided the identification of leukemic cells through detection of B-cell markers [Cluster of Differentiation 19 (CD19), CD20, CD22] and antigens such as CD11c, CD25, CD103, and CD123 [15,16].

Immunohistochemical staining of the bone marrow also supported the diagnosis [17,18]. Markers such as tartrate-resistant acid phosphatase (TRAP), annexin-1, and reticulin were used. Treatment was initiated in patients with cytopenias [Hemoglobin (Hb) <11 g/dL, white blood cell <1,000/µL, platelets <100,000/µL], symptomatic splenomegaly, systemic complaints, or frequent infections [5]. All patients had normal kidney and liver function and had no uncontrolled infections.

All patients were treated with the purine analog cladribine (2-CdA) as first-line therapy at a dose of 0.14 mg/kg/day subcutaneously for five days. Patients were monitored with daily temperature measurements and serial blood counts until the blood counts returned to normal.

Treatment response was assessed by physical examination, blood counts, and measurement of spleen size. Because bone marrow recovery after purine analog therapy can take several months, a repeat bone marrow biopsy was performed 4 to 6 months after treatment to confirm the clearance of leukemic cells [14]. CR was defined as normalized blood counts (Hb>11 g/dL without transfusion, platelets >100,000/µL, absolute neutrophil count>1500/µL), absence of organomegaly or lymphadenopathy, and absence of visible hairy cells in the blood or bone marrow. Partial remission (PR) was characterized by near-normalization of peripheral blood counts, accompanied by at least a 50% reduction in organomegaly and in bone marrow infiltration by hairy cells. Patients who did not meet the criteria for either CR or PR were classified as non-responders. Hematologic relapse refers to the recurrence of cytopenias. Morphologic relapse was defined as the reappearance of leukemic cells on smears or in biopsies without accompanying peripheral blood cytopenias. While morphologic relapse alone did not always require treatment, hematologic relapse was assessed based on symptoms and lab findings to decide on further management [14,19].

Overall survival (OS) was measured from diagnosis to death from any cause or to last follow-up. After treatment, patients were monitored for neutropenic fever and possible treatment-related side effects.

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous data were presented as medians with ranges, while categorical data were presented as percentages. OS and progression-free survival (PFS) were calculated using the Kaplan-Meier method.

Results

Patient Characteristics

This study analyzed 25 individuals diagnosed with HCL. Among them, 64% (n=16) were male and 36% (n=9) were female. The median age at diagnosis was 48 years, ranging from 27 to 68 years. The most frequent initial complaints included fatigue (84%), abdominal fullness (56%), and abdominal pain (48%). Only one patient (4%) had no symptoms at diagnosis and was referred to hematology following the detection of cytopenias during routine tests.

Splenomegaly was present in all patients at diagnosis, and 32% (n=8) had massive enlargement. Pancytopenia was observed in 72% (n=18) of patients. Hairy cells were identified in 88% (n=22) of peripheral blood smears, with a median infiltration rate of 30% (range: 0-80%). On bone marrow examination, the median infiltration by hairy cells was 70% (range, 5-99%).

All patients expressed CD19, CD20, and TRAP. CD103 was positive in 96% of cases, and CD11c and CD25 were positive in 92% of cases. Annexin A1 expression was detected in 88% of patients. The BRAF V600E mutation was tested for in four individuals, all of whom were positive. Due to technical constraints, this analysis could not be performed on the remainder of the cohort. A detailed overview of the demographic and clinical features is shown in Table 1.

Treatment Outcomes

All patients received cladribine as first-line therapy. Among them, 23 (92.0%) achieved CR, and 2 (8.0%) achieved PR. The median follow-up duration from CR was 36.9 months (range, 1.4-139.3 months). During follow-up, relapse occurred in three patients (12%), with a median time to relapse of 90 months (range 24-118 months) calculated from the date of CR. All relapsed cases were retreated with cladribine and achieved CR.

Table 1. Demographic and clinical characteristics of the patients (n=25)

Parameter	Value
Gender, n (%)	
Male	16 (64%)
Female	9 (36%)
Median age, years (range)	48 (27-68%)
Comorbidities, n (%)	11 (44%)
Type of HCL, n (%)	
De novo	24 (96%)
Therapy-related	1 (4%)
ECOG performance status, n (%)	
0	5 (20%)
1	18 (72%)
2	2 (8%)
Symptoms at diagnosis, n (%)	
Fatigue	21 (84%)
Weight loss	7 (28%)
Night sweats	2 (8%)
Fever	7 (28%)
Abdominal pain	12 (48%)
Abdominal fullness	14 (56%)
Infection	4 (16%)
Bone marrow fibrosis grade, n (%)	
Grade 1	10 (40%)
Grade 2	12 (48%)
Grade 3	3 (12%)
Bone marrow infiltration pattern	
Diffuse	17 (68%)
Interstitial	5 (20%)
Diffuse + interstitial	3 (12%)
Hairy cells in peripheral blood	22 (88%)
Peripheral LAP, n (%)	2 (8%)
Median hairy cells (%)	
In bone marrow	70% (5-99%)
In peripheral smear	30% (6-80%)
Splenic size by ultrasound (mm)	
Pre-treatment	170 (135-290)
Post-treatment	120 (106-160)
Laboratory findings at diagnosis	
Hemoglobin (g/dL)	9.6 (4.0-15.9)
WBC (×10^3/µL)	2.4 (0.7-18.1)
ANC (×10^3/µL)	0.51 (0.15-2.31)
Lymphocytes (×10^3/µL)	1.5 (0.1-13.6)
Monocytes (×10^3/µL)	0.09 (0-2.26)
Platelets (×10^3/µL)	58 (26-445)
Febrile neutropenia post-treatment	20 (80%)

HCL: Hairy cell leukemia, ECOG: Eastern Cooperative Oncology Group, LAP: Lymphadenopathy, WBC: White blood cell ANC: Absolute neutrophil count

Median OS and PFS were not reached. At 12 years, the estimated OS and PFS rates were 100% and 80% (95% confidence interval: 53-95%), respectively. Details of treatment outcomes are provided in Table 2, and PFS is illustrated by the Kaplan-Meier curve in Figure 1.

Toxicity and Safety

The most frequent side effect observed was hematologic toxicity, followed by infections and gastrointestinal complaints. Anemia and thrombocytopenia improved after treatment, and leukocyte counts normalized within three months. Febrile neutropenia developed in 80% (n=20) of patients during the neutropenic phase, representing grade 3 hematologic toxicity according to Common Terminology Criteria for Adverse Events v5.0. Due to the persistent risk of infection and potential treatment-related mortality, all patients received prophylactic measures and infection management as recommended in current guidelines [20,21]. Mild gastrointestinal symptoms (grade 1-2 nausea and vomiting) were reported by seven patients (28%). Transient elevations in liver enzymes (aspartate aminotransferase, alanine aminotransferase) or bilirubin (grade 1-2) occurred in 4 patients (16%). No grade 4 hematologic or grade ≥3 non-hematologic toxicities were recorded.

Discussion

Classic HCL is a rare and indolent B-cell malignancy, marked by the slow buildup of malignant lymphoid cells in the bone marrow and spleen. This process often leads to cytopenias and a higher risk of infections [22]. With improved treatment options, most patients diagnosed with HCL now have a life expectancy comparable to that of healthy individuals [23].

In line with previous reports [2], our study demonstrated a higher frequency of HCL in males. Only one patient (4%) was asymptomatic at diagnosis, but treatment was initiated because of significant cytopenias.

Fatigue and abdominal complaints—often associated with splenic enlargement—are frequent in HCL, occurring in up to 90% of cases [24-26]. Our findings were consistent with this pattern: fatigue was the leading symptom (84%), followed by abdominal fullness (56%), and abdominal pain (48%). Splenomegaly was identified in all patients and remained the most common physical sign.

Table 2. Treatment outcomes of patients with hairy cell leukemia

	n (%)
CR	23 (92%)
PR	2 (8%)
Relapsed disease	3 (12%)
Follow-up, months, median (min-max)	36.9 months (1.4-139.3)
PFS	Not reached
OS	Not reached

CR: Complete response, PR: Partial remission, PFS: Progression-free survival, OS: Overall survival, min-max: Minimum-maximum

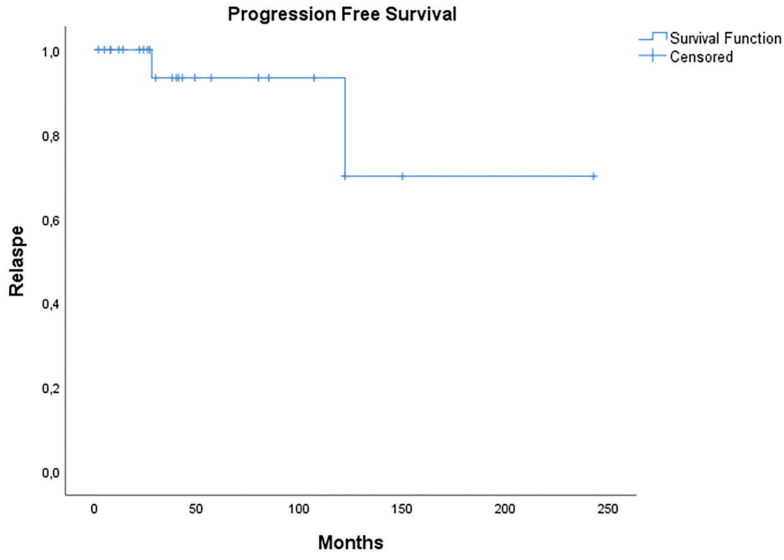


Figure 1. Progression-free survival (PFS)
Kaplan-Meier analysis showing PFS of the 25 patients. The median PFS was not reached. Censored observations are marked with a "+" symbol. At the 12-year follow-up, estimated PFS was 80% (95% confidence interval: 53-95%)

In our cohort, 72% of patients (n=18) had pancytopenia at diagnosis, reflecting baseline marrow suppression prior to therapy. Although no patient presented with febrile neutropenia at baseline, 80% developed it during cladribine treatment, which is consistent with the expected post-treatment cytopenic phase of purine analogs. This finding represents grade 3 hematologic toxicity, not an unexpected or life-threatening adverse event. Importantly, no grade 4 (life-threatening) hematologic toxicities were observed, and all episodes of febrile neutropenia resolved with appropriate antimicrobial and supportive management. Mild cytopenias and transient infections were observed; however, no grade ≥3 non-hematologic toxicities were observed in our cohort. Similar findings were reported in a Turkish series in which the rate of febrile neutropenia among patients treated with cladribine was 60% [27].

To minimize infection risk, prophylactic and supportive measures were systematically implemented. Patients with lymphopenia received prophylaxis with co-trimoxazole and aciclovir until lymphocyte recovery to >1×10^9/L [IV, B] [14]. Routine filgrastim use with cladribine is not recommended [IV, C] [20]; however, granulocyte colony-stimulating factor was administered selectively for severe infection-associated neutropenia. All patients were screened for hepatitis B, and those who tested positive received antiviral prophylaxis [14].

HCL typically causes suppression of at least two blood cell lineages, with leukopenia—particularly monocytopenia—being a hallmark feature. Monocyte depletion was observed in nearly all cases. Extranodal involvement, including lymphadenopathy and skeletal lesions, is uncommon at initial presentation but becomes more prominent in relapsed disease [28,29]. In our series, lymph node enlargement was detected in only two patients (8%).

Purine nucleoside analogs (PNAs), including cladribine and pentostatin, have achieved overall response rates exceeding 90%, with CR rates between 79% and 91%. No significant difference in efficacy has been noted between the two agents [3,9,30-32].

In our cohort, 23 patients (92%) achieved CR and 2 patients (8%) achieved PR following cladribine therapy. However, relapses can still occur despite the high efficacy of PNAs. A large cohort reported a relapse rate of only 3% after nearly 10 years of follow-up [30]. In another study, relapse rates for patients treated with pentostatin were 24% at 5 years and 42% at 10 years, whereas relapse rates for those receiving cladribine were 33% at 5 years and 48% at 10 years [33].

In our cohort, three patients (12%) experienced relapse after a median follow-up of 36.9 months. This relapse rate appears lower than those reported in large European series. In a study of 279 patients, Paillassa et al. [34] reported overall and CR rates of 97% and 78%, respectively, following first-line purine analog therapy. After a median follow-up of 10 years, the cumulative 10-year relapse incidence was 39%, and the median relapse-free survival was 11 years [34]. Despite the generally favorable long-term prognosis of HCL, relapses and secondary malignancies remain important concerns among long-term survivors.

The youngest individual in our cohort was 27 years old. Although prior studies have reported relapse rates as high as 58% among patients younger than 40 following cladribine therapy [35], both of our patients in this age group remained in long-term CR. Cladribine re-treatment has previously been associated with a 62% complete response rate and a 26% partial response rate [32]. In our series, all three patients who relapsed achieved CR again following a second course of cladribine.

Published data show OS rates of 96% at 4 years and 84% at 20 years for HCL patients [3,32]. By the fifth year after diagnosis, mortality risk becomes similar to that of peers in the general population [36]. In our cohort, no deaths were recorded over a median follow-up of 36.9 months.

Earlier reports indicate that 8-10 % of HCL survivors may develop secondary cancers over time [3,32]; nevertheless, no such events were detected in our series during follow-up.

In recent years, novel therapeutic strategies, including rituximab-based chemoimmunotherapy, targeted agents, such as BRAF and MEK inhibitors, and the BTK inhibitor ibrutinib, have demonstrated encouraging results in patients with relapsed or refractory HCL, providing new options beyond conventional purine-analog therapy [5].

The chief limitation of this work is the small number of patients, a consequence of the rarity of HCL. Moreover, assessment of the BRAF V600E mutation—found in nearly all classic HCL cases and viewed as a pivotal oncogenic driver [37]—was inconsistent because the study was retrospective and comprehensive molecular data were not always available.

In addition, molecular testing was limited to BRAF V600E and was performed in only four patients, while other potentially relevant mutations, such as MAP2K1 [38] and IGHV4-34 [24], were not assessed. These molecular aberrations are increasingly recognized as prognostic markers, and their absence restricts our ability to perform genotype-phenotype correlations and evaluate their impact on treatment response.

Unlike many other hematologic malignancies, HCL lacks a standardized risk stratification system. However, unfavorable outcomes have been linked to factors such as splenomegaly, elevated beta-2 microglobulin levels, leukocyte counts above 10×10^9/L, and levels of circulating hairy cells exceeding 5×10^9/L [29]. In our analysis, minimal residual disease assessment and extended molecular profiling were not performed, further limiting the evaluation of long-term prognostic factors.

Conclusion

Cladribine continues to serve as a highly effective and well-tolerated first-line therapy for HCL, providing strong response rates and lasting remissions. Its manageable toxicity profile supports its continued use in clinical practice. In relapsed cases, cladribine re-administration remains a viable and effective strategy. However, prospective studies in larger populations are needed to optimize treatment approaches and enhance long-term outcomes in HCL.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of the University of Health Sciences Türkiye, Dr. Abdurrahman Yurtarslan Ankara Oncology Health Application and Development Center of the (decision no: 2022-02/1651, date: 09.02.2022). All procedures were carried out in accordance with the ethical principles of the 1964 Helsinki Declaration.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.S., B.A.C., D.İ., M.K.Ç., Concept: S.S., B.A.C., T.N.Y., Design: S.S., D.İ., M.K.Ç., Data Collection or Processing: S.S., B.A.C., S.Y., E.B., B.U.U., T.N.Y., Analysis or Interpretation: S.S., B.A.C., E.B., Literature Search: S.S., B.A.C., S.Y., E.B., B.U.U., T.N.Y., Writing: S.S., B.A.C.

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

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

References

1. Bouroncle BA, Wiseman BK, Doan CA. Leukemic reticuloendotheliosis. Blood. 1958;13:609-630.
2. Tadmor T, Polliack A. Epidemiology and environmental risk in hairy cell leukemia. Best Pract Res Clin Haematol. 2015;28:175-179.
3. Cornet E, Tomowiak C, Tanguy-Schmidt A, et al. Long-term follow-up and second malignancies in 487 patients with hairy cell leukaemia. Br J Haematol. 2014;166:390-400.
4. Golomb HM. Hairy cell leukemia: lessons learned in twenty-five years. J Clin Oncol. 1983;1:652-656.
5. Maitre E, Cornet E, Troussard X. Hairy cell leukemia: 2020 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2019;94:1413-1422.
6. Piro LD, Carrera CJ, Carson DA, Beutler E. Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. N Engl J Med. 1990;322:1117-1121.
7. Kraut EH, Bouroncle BA, Grever MR. Pentostatin in the treatment of advanced hairy cell leukemia. J Clin Oncol. 1989;7:168-172.
8. Spiers AS, Moore D, Cassileth PA, et al. Remissions in hairy-cell leukemia with pentostatin (2'-deoxycoformycin). N Engl J Med. 1987;316:825-830.
9. Else M, Dearden CE, Matutes E, et al. Long-term follow-up of 233 patients with hairy cell leukaemia, treated initially with pentostatin or cladribine, at a median of 16 years from diagnosis. Br J Haematol. 2009;145:733-740.
10. Jehn U, Bartl R, Dietzfelbinger H, Haferlach T, Heinemann V. An update: 12-year follow-up of patients with hairy cell leukemia following treatment with 2-chlorodeoxyadenosine. Leukemia. 2004;18:1476-1481.
11. Andrasiak I, Rybka J, Wrobel T. Response to the therapy in hairy cell leukemia: systematic review and meta-analysis. Clin Lymphoma Myeloma Leuk. 2018;18:392-399.e3.
12. Goodman GR, Burian C, Koziol JA, Saven A. Extended follow-up of patients with hairy cell leukemia after treatment with cladribine. J Clin Oncol. 2003;21:891-896.
13. Parry-Jones N, Joshi A, Forconi F, Dearden C; BSH guidelines committee. Guideline for diagnosis and management of hairy cell leukaemia (HCL) and hairy cell variant (HCL-V). Br J Haematol. 2020;191:730-737.
14. Grever MR, Abdel-Wahab O, Andritsos LA, et al. Consensus guidelines for the diagnosis and management of patients with classic hairy cell leukemia. Blood. 2017;129:553-560.
15. Grever MR, Blachly JS, Andritsos LA. Hairy cell leukemia: update on molecular profiling and therapeutic advances. Blood Rev. 2014;28:197-203.
16. Venkataraman G, Aguhar C, Kreitman RJ, Yuan CM, Stetler-Stevenson M. Characteristic CD103 and CD123 expression pattern defines hairy cell leukemia: usefulness of CD123 and CD103 in the diagnosis of mature B-cell lymphoproliferative disorders. Am J Clin Pathol. 2011;136:625-630.
17. Sherman MJ, Hanson CA, Hoyer JD. An assessment of the usefulness of immunohistochemical stains in the diagnosis of hairy cell leukemia. Am J Clin Pathol. 2011;136:390-399.

18. Falini B, Tiacci E, Liso A, et al. Simple diagnostic assay for hairy cell leukaemia by immunocytochemical detection of annexin A1 (ANXA1). Lancet. 2004;363:1869-1870. Erratum in: Lancet. 2004;363:2194.
19. Jones G, Parry-Jones N, Wilkins B, Else M, Catovsky D; British Committee for Standards in Haematology. Revised guidelines for the diagnosis and management of hairy cell leukaemia and hairy cell leukaemia variant*. Br J Haematol. 2012;156:186-195.
20. Robak T, Matutes E, Catovsky D, Zinzani PL, Buske C; ESMO Guidelines Committee. Hairy cell leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26:v100-v107.
21. Cornet E, Delmer A, Feugier P, et al. Recommendations of the SFH (French Society of Haematology) for the diagnosis, treatment and follow-up of hairy cell leukaemia. Ann Hematol. 2014;93:1977-1983.
22. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117:5019-5032.
23. Dinmohamed AG, Posthuma EFM, Visser O, Kater AP, Raymakers RAP, Doorduijn JK. Relative survival reaches a plateau in hairy cell leukemia: a population-based analysis in The Netherlands. Blood. 2018;131:1380-1383.
24. Troussard X, Maître E, Cornet E. Hairy cell leukemia 2022: update on diagnosis, risk-stratification, and treatment. Am J Hematol. 2022;97:226-236.
25. Quest GR, Johnston JB. Clinical features and diagnosis of hairy cell leukemia. Best Pract Res Clin Haematol. 2015;28:180-192.
26. Kreitman RJ, Arons E. Diagnosis and treatment of hairy cell leukemia as the COVID-19 pandemic continues. Blood Rev. 2022;51:100888.
27. Maral S, Albayrak M, Afacan Öztürk HB, et al. Cladribine Treatment in Hairy Cell Leukemia. Medical Journal of Mugla Sitki Kocman University. 2020;7:131-134.
28. De Propris MS, Musiu P, Intoppa S, et al. Hairy cell leukaemia with low CD103 expression: a rare but important diagnostic pitfall. Br J Haematol. 2022;198:e28-e31.
29. Mendez-Hernandez A, Moturi K, Hanson V, Andritsos LA. Hairy cell leukemia: where are we in 2023? Curr Oncol Rep. 2023;25:833-840.
30. Chadha P, Rademaker AW, Mendiratta P, et al. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine (2-CdA): long-term follow-up of the Northwestern University experience. Blood. 2005;106:241-246.
31. Else M, Dearden CE, Matutes E, et al. Long-term follow-up of 233 patients with hairy cell leukaemia, treated initially with pentostatin or cladribine, at a median of 16 years from diagnosis. Br J Haematol. 2009;145:733-740.
32. Saven A, Burian C, Koziol JA, Piro LD. Long-term follow-up of patients with hairy cell leukemia after cladribine treatment. Blood. 1998;92:1918-1926.
33. Else M, Ruchlemer R, Osuji N, et al. Long remissions in hairy cell leukemia with purine analogs: a report of 219 patients with a median follow-up of 12.5 years. Cancer. 2005;104:2442-2248.
34. Paillassa J, Cornet E, Noel S, et al. Analysis of a cohort of 279 patients with hairy-cell leukemia (HCL): 10 years of follow-up. Blood Cancer J. 2020;10:62.
35. Rosenberg JD, Burian C, Waalen J, Saven A. Clinical characteristics and long-term outcome of young hairy cell leukemia patients treated with cladribine: a single-institution series. Blood. 2014;123:177-183.
36. Madanat YF, Rybicki L, Radivoyevitch T, et al. Long-term outcomes of hairy cell leukemia treated with purine analogs: a comparison with the general population. Clin Lymphoma Myeloma Leuk. 2017;17:857-862.
37. Tiacci E, Trifonov V, Schiavoni G, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med. 2011;364:2305-2315.
38. Maitre E, Tomowiak C, Lebecque B, et al. Deciphering genetic alterations of hairy cell leukemia and hairy cell leukemia-like disorders in 98 patients. Cancers (Basel). 2022;14:1904.

AHOT | Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):218-225

DOI: 10.4274/ahot.galenos.2025.2025-10-2

Original Article

Clinical Consequences of Cancer-related RAS Signaling Pathway Beyond Malignancy

Elifcan Taşdelen[1], Afife Büke[1], Abdullah Sezer[1], Abdulkerim Kolkıran[2], Ahmet Kablan[1], Ayşe Burcu Doğan Arı[3], Esra Kılıç[3], İbrahim Kaplan[4], Müzeyyen Gönül[5]

[1]*University of Health Sciences Türkiye, Ankara Etlik City Hospital, Clinic of Medical Genetics, Ankara, Türkiye*
[2]*University of Health Sciences Türkiye, Ankara Etlik City Hospital, Clinic of Pediatric Genetics, Ankara, Türkiye*
[3]*University of Health Sciences Türkiye, Ankara Bilkent City Children's Hospital, Clinic of Pediatric Genetics, Ankara, Türkiye*
[4]*University of Health Sciences Türkiye, Adana City Training and Research Hospital, Clinic of Medical Genetics, Adana, Türkiye*
[5]*University of Health Sciences Türkiye, Ankara Etlik City Hospital, Clinic of Dermatology, Ankara, Türkiye*

ABSTRACT

Aim: Mosaic variants in oncogenic signaling pathways, particularly the Rat Sarcoma/Mitogen-Activated Protein Kinase *(RAS/*MAPK) cascade, are increasingly recognized causes of non-malignant developmental disorders presenting with segmental cutaneous manifestations.

Methods: We evaluated patients carrying somatic mosaic variants in the Kirsten *RAS* Viral Oncogene Homolog, Neurofibromin 1, Fibroblast Growth Factor Receptor 3, and Neuroblastoma RAS Viral Oncogene Homolog genes. Molecular analyses were performed with emphasis on tissue-specific sequencing to detect low-level mosaicism.

Results: The reported cases demonstrate the broad phenotypic spectrum of mosaic *RAS/*MAPK-related disorders. Clinical severity was shown to depend on both the type of variant and the extent of mosaic distribution. Importantly, several low-frequency variants were detectable only in affected tissue, highlighting the diagnostic value of tissue-specific molecular testing.

Conclusion: Current American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines for germline and cancer-associated variants are insufficient to classify somatic mosaic variants underlying cutaneous disorders. Our findings emphasize the need to reshape diagnostic approaches and variant classification strategies for mosaic *RAS/*MAPK-related dermatologic conditions.

Keywords: Skin, somatic, RAS pathway, mosaic, postzygotic

Introduction

The Rat Sarcoma/Mitogen-activated Protein Kinase *(RAS/*MAPK) signaling pathway plays a central role in regulating fundamental biological processes such as cell growth, proliferation, and differentiation. Germline pathogenic variants in genes of this pathway are responsible for a group of neurocutaneous developmental disorders collectively known as *RASopathies*. In recent years, postzygotic (mosaic) activating variants in genes such as Harvey *RAS* Viral Oncogene Homolog (*HRAS),* Kirsten *RAS* viral oncogene homolog *(KRAS),* neuroblastoma *RAS* viral oncogene homolog *(NRAS)*, and Protein Tyrosine Phosphatase Non-receptor Type 11 (*PTPN11)* have also been increasingly recognized as causative of mosaic forms of *RASopathies*, which represent phenotypically distinct and increasingly well-defined clinical entities [1]. These disorders typically present in the neonatal or early childhood period with cutaneous, vascular, skeletal, and neurological anomalies, accompanied by segmental proliferative lesions and an increased risk of malignancy. Due to the mosaic nature of the variants, clinical findings are frequently asymmetric, segmental, or localized, and may be missed if genetic testing is limited to peripheral blood samples.

Address for Correspondence: Elifcan Taşdelen MD, University of Health Sciences Türkiye, Ankara Etlik City Hospital, Clinic of Medical Genetics, Ankara, Türkiye

E-mail: elifkarakaya2012@gmail.com **ORCID ID:** orcid.org/0000-0003-3917-9792

Received: 06.10.2025 **Accepted:** 08.11.2025 **Epub:** 27.11.2025 **Publication Date:** 12.12.2025

Cite this article as: Taşdelen E, Büke A, Sezer A, et al. Clinical consequences of cancer-related RAS signaling pathway beyond malignancy. Acta Haematol Oncol Turc. 2025;58(3):218-225

In this study, we present a series of patients harboring mosaic pathogenic variants in genes of the *RAS*/MAPK signaling pathway, and we emphasize the complementary role of genetic and dermatologic evaluation in the recognition of mosaic signaling disorders.

Methods

All procedures performed in this study were carried out in accordance with the ethical standards stated in the World Medical Association Declaration of Helsinki. Ethics committee approval for the study was received from the Scientific Research Evaluation and Ethics Committee of University of Health Sciences Türkiye, Ankara Etlik City Hospital on (decision no: AEŞH-BADEK-2024-757, date: 28.08.2024). This study is descriptive in nature, and no statistical analysis was performed because the dataset did not require comparative or inferential evaluation.

Five patients who presented to University of Health Sciences Türkiye, Ankara Etlik City Hospital were included in the study. Written informed consent was obtained from all patients or their legal guardians. In retrospectively evaluated clinical exome sequencing, the following kits were used. For G23-25265, G23-9663, and BSH1 patients' *genomic* deoxyribonucleic acid (DNA) extracted from affected skin tissue samples was used for library preparation with the Sophia Clinical Exome Solution V3 capture kit (SOPHiA Genetics SA, Switzerland) and was sequenced on the MiSeq platform (Illumina Inc., CA).

For patient G24-25268, DNA extracted from the patient's skin tissue sample was analyzed by next-generation sequencing (NGS) on the Seq Genomize V8.2.3 platform (Roche), and library preparation was performed using the KAPA HyperCap Custom kit. The corresponding Binary Alignment/Map file alignments, visualized with Integrative Genomics Viewer, are provided in the Supplementary Material.

Ribonucleic acid was isolated from the fibroblast tissue of patient G24-7460 and quantified using a Qubit fluorometer. Libraries were prepared with the Archer® Comprehensive Thyroid & Lung Kit, indexed, and sequenced on an NGS platform.

Results

Patients

G23-25265

An 8-year-old girl was referred for evaluation of congenital focal alopecia of the scalp and linear cutaneous hyperpigmentation. She was the fifth child of parents related at the third degree. Prenatal history was unremarkable, and neurodevelopment was age-appropriate. Vision and hearing assessments, echocardiography, brain magnetic resonance imaging (MRI), and abdominal ultrasound were within normal limits. Academic performance was reported as good. Anthropometric measurements were as follows: weight, 30 kg [standard deviation score (SDS): +0.48]; height, 121 cm (SDS: -1.57); and head circumference, 53 cm (SDS: +0.63). Physical examination revealed a relative nevus sebaceous on the scalp (Figure 1a), macrocephaly, sparse, lusterless hair with patchy alopecia, and dysmorphic features including coarse facial appearance, high forehead, mild synophrys, broad nasal root, full lips (Figure 1b), short neck, low posterior hairline, and hypopigmented macules along Blaschko's lines (Figure 1c). Conventional karyotyping and chromosomal microarray were normal. Because of pigmentary mosaicism and dysmorphic features, NGS of affected skin tissue was performed. A somatic *KRAS* (NM_004985.5) variant, c.26T>G; p.(Val9Gly), was detected [Variant Allele Frequency (VAF) 13; read depth 56]. According to American College of Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/AMP) guidelines, this variant was classified as likely pathogenic based on PM1, PM2, PP2, and PP3.

G24-25268

A 34-year-old woman was referred for evaluation of multiple neurofibromas localized to the back. Histopathological examination confirmed the diagnosis of neurofibroma, and the lesions were reported to have developed postnatally. Aside from cutaneous neurofibromas, she did not meet any other diagnostic criteria for Neurofibromin type 1 *(NF1)*. NGS of peripheral blood revealed no pathogenic variants in the *NF1* gene. However, because of a segmental distribution and the absence of systemic involvement, mosaic *NF1* was suspected. Targeted NGS of affected skin tissue identified a somatic *NF1* variant: NM_001042492.3:c.7797_7806del, p.(Glu2600PhefsTer21), with a VAF consistent with mosaicism (VAF 8.23%; read depth 243). According to ACMG/AMP guidelines, this frameshift deletion was classified as likely pathogenic based on pathogenic very strong 1 and PM2.

G23-9663

A 4-year-old boy was referred for evaluation of hyperpigmented skin lesions distributed in a linear pattern on the neck, trunk, and inguinal region, accompanied by pruritus. The lesions began to appear around the fourth month of life and progressively spread. Prenatal and perinatal histories were unremarkable; he was born at term by cesarean section, weighing 3350 g. There was no parental consanguinity, and neurodevelopmental milestones were normal. At presentation, anthropometric measurements were: weight 17 kg (SDS: -0.53), height 104 cm (SDS: -1.16), and head circumference 51 cm (SDS: -0.35). Physical examination revealed a widow's peak; wavy, woolly hair; downslanted palpebral fissures; a prominent lower lip; and papillomatous lesions around the perioral and periorbital areas. Additionally, hyperkeratotic verrucous plaques were noted in the cervical and axillary regions (Figures 1d, 1e), and linear and whorled hyperpigmented patches or plaques following Blaschko's lines were observed over the trunk (Figure 1f) and extremities (Figure 1g). Abdominal ultrasonography was unremarkable. Histopathological examination of a skin biopsy demonstrated basket-weave hyperkeratosis,

papillomatosis, and focal vacuolization in the basal layer of the epidermis. Targeted NGS of the affected tissue revealed a somatic Fibroblast Growth Factor Receptor 3 (*FGFR3)* variant: NM_000142.5:c.742C>T; p.(Arg248Cys) (VAF 9%; read depth 165). This variant is classified as pathogenic according to the ACMG guidelines (PS3, PS4, PM1, PM2, PP3, PP5).

G24-7460

A 4-year-old boy with a history of congenital giant nevus was referred for genetic evaluation. He was the child of parents described as non-consanguineous but with a known fourth-degree familial relationship. Prenatal and perinatal histories were unremarkable; he was born at 41+3 weeks' gestation via normal spontaneous vaginal delivery, with a birth weight of 3.600 g. Neurodevelopmental milestones were appropriate for age. At the time of examination, his weight, height, and head circumference were 13 kg, 94 cm, and 48 cm, respectively. Physical examination revealed lateral thinning of the eyebrowsand numerous melanocytic nevi with hairs of various lengths throughout the body, including a giant congenital nevus on the back (Figures 1h, 1i). In addition, bilateral pes planus and prominent heels were noted. Audiological and ophthalmological assessments, as well as cranial MRI and abdominal ultrasonography, were within normal limits. NGS of affected skin tissue identified a somatic variant in the *NRAS* gene (NM_002524.5:c.182A>G; p.Gln61Arg), consistent with a molecular diagnosis of congenital melanocytic nevus syndrome (CMNS) (VAF 25%, read depth: 300). This variant is classified as pathogenic according to ACMG guidelines (PS3, PS4, PM1, PM2, PP2, PP3).

BSH1

An 8-month-old female patient was born at 35 weeks' gestation with a birth weight of 2620 g. She was referred for dysmorphic facial features and skin lesions. Her parents were consanguineous. Physical examination revealed macrocephaly; hypertelorism; eyelid coloboma; bilateral eyelid hypoplasia (Figure 1j); protruding conjunctiva; sparse hair, eyebrows, and eyelashes; macroglossia; and hypopigmented linear verrucous plaques along Blaschko's lines on the chin and back (Figure 1k). Her weight was 4 kg [-5.9 standard deviation (SD)] and her length was 60 cm (-4.4 SD). Echocardiography demonstrated coarctation of the aorta and hypoplasia of the transverse aortic arch and isthmus. Ophthalmological examination of the left eye revealed a lid lipodermoid and aniridia. Abdominal ultrasonography and hearing screening were normal. Chromosome analysis revealed a 46,XX karyotype. A pathogenic *KRAS* variant, NM_004985.5:c.35G>A (p.Gly12Asp) (rs121913529) (VAF 31%, read depth 163), was identified by a *RASopathy* gene panel performed on a skin biopsy. This variant is classified as pathogenic according to ACMG guidelines (PS3, PS4, PM1, PM2, PP2, PP3). The patient died at 10 months of age due to respiratory distress and sepsis.

Table 1 presents the detected somatic mosaic variants in the study cohort, together with their tissue distribution, variant allele frequencies, and sequencing depths.

Discussion

The cases presented in this series illustrate the expanding spectrum and diagnostic complexity of mosaic disorders involving oncogenic signaling pathways, particularly the *RAS*/MAPK and *PI3K/AKT/mTOR* cascades. Although these pathways have traditionally been associated with cancer pathogenesis, dysregulation of these pathways due to postzygotic activating variants has increasingly been implicated in non-malignant developmental disorders with highly variable and often segmental phenotypic manifestations. Our findings underscore the diagnostic value of detailed dermatological assessment for the early recognition of mosaic signaling disorders, particularly in individuals with localized pigmentary or proliferative cutaneous anomalies. Furthermore, the detection of low-level somatic variants in affected tissue, undetectable in peripheral blood, highlights the necessity of tissue-specific molecular testing in the diagnostic evaluation of suspected mosaic phenotypes. These cases, which present both classical and atypical clinical features, contribute to the growing body of evidence bridging cancer biology and developmental genetics, and emphasize the importance of interdisciplinary collaboration in the management of such patients.

Our two patients with mosaic *KRAS* variants highlight the wide phenotypic spectrum of epidermal nevus syndromes. G23-25265, carrying the rare p.(Val9Gly) variant, presented with a scalp lesion that was clinically suggestive of nevus sebaceus, which dermatological evaluation described as more consistent with scarring alopecia, along with patchy alopecia, hypopigmented macules along Blaschko's lines, and dysmorphic features, without neurological or systemic involvement. Histopathological confirmation could not be performed; therefore, the exact classification of the lesion remains uncertain. The literature indicates that mosaic *KRAS* variants are not restricted to classical nevus sebaceus but can also present as linear or segmental keratinocytic epidermal nevi, pigmentary mosaicism, and occasionally mucosal involvement, supporting a broader phenotypic spectrum [2]. To the best of our knowledge, the p.(Val9Gly) variant has not previously been reported in association with this presentation, raising the possibility of a novel genotype-phenotype correlation at the milder end of the spectrum. In contrast, the BSH1 case supports previous reports of patients with Schimmelpenning-Feuerstein syndrome, in whom the p.Gly12Asp variant has been associated with multisystem involvement and a severe clinical course. Moreover, a relatively high mosaicism rate of 31% may account for the fatal outcome observed in this patient [2,3]. Taken together, these cases demonstrate that both the specific *KRAS* mutation and the extent of mosaic distribution are critical determinants of clinical severity, ranging from isolated cutaneous or dysmorphic findings to life-threatening multisystem disease.

The clinical and molecular findings of patient G24-25268 are consistent with a diagnosis of segmental neurofibromin (also referred to as mosaic *NF1*). According to current estimates, approximately 10% of patients with *NF1* have the mosaic form of the disease [2]. The patient presented with multiple

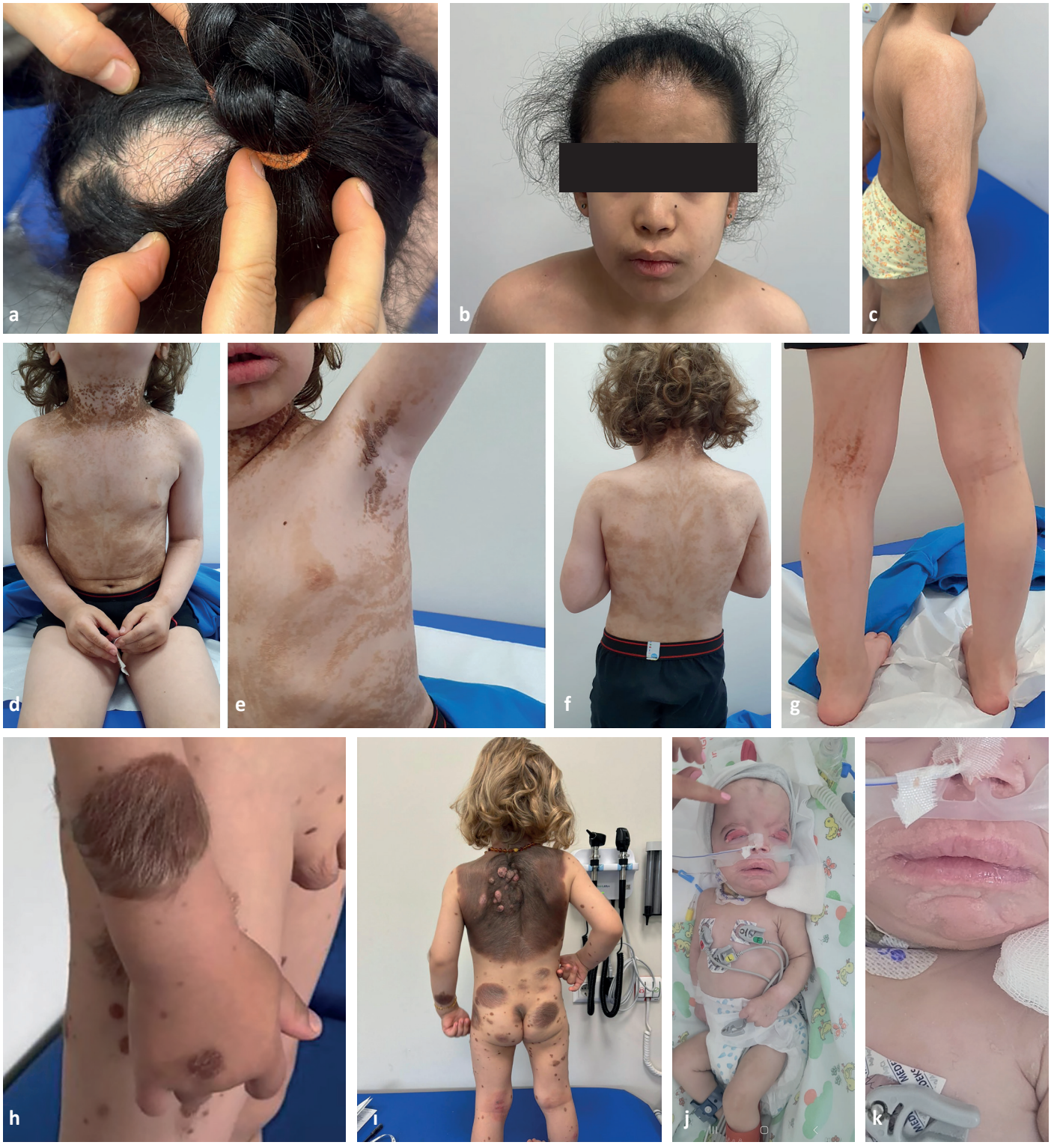


Figure 1. Patient photographs illustrating the phenotypic spectrum. **(a)** Patchy alopecia on the scalp **(b)** coarse facial features with a broad forehead, subtle synophrys, wide nasal bridge, and prominent lips **(c)** linear hypopigmented macules along Blaschko's lines on the right arm **(d)** linear blaskoid hyperpigmented verrucous plaques surrounding the neck and **(e)** axillary region and linear swirling hyperpigmented patches and plaques following Blaschko's lines on the anterior trunk, **(f)** back and **(g)** extremities **(h-i)** multiple melanocytic nevi of varying sizes with hypertrichosis on the right arm and back **(j)** macrocephaly, hypertelorism, eyelid coloboma, bilateral eyelid hypoplasia, sparse scalp hair, sparse eyebrows and eyelashes, macroglossia, and **(k)** hypopigmented linear verrucous plaques following Blaschko's lines on the chin, perioral region and around the nose

Table 1. Somatic mosaic variants identified in the study cohort, indicating the affected genes, variant type, tissue distribution, VAF, and coverage depth.

Patient no	*Gene*	Variant	Affected tissue	Blood	VAF	Depth
G23-25265	*KRAS*	c.26T>G; p.(Val9Gly)	+	-	13%	56
G24-25268	*NF1*	c.7797_7806del, p.(Glu2600PhefsTer21)	+	-	8.2%	243
G23-9663	*FGFR3*	c.742C>T; p.(Arg248Cys)	+	NA	9%	163
G24-7460	*NRAS*	c.182A>G; p.(Gln61Arg)	+	NA	25%	300
BSH1	*KRAS*	c.35G>A (p.Gly12Asp)	+	NA	31%	163

VAF: Variant allele frequency, *KRAS:* Kirsten Rat Sarcoma Viral Oncogene Homolog, *NF1*: Neurofibromin Type 1, *FGFR3*: Fibroblast Growth Factor Receptor 3, *NRAS*: Neuroblastoma Rat Sarcoma Viral Oncogene Homolog, NA: Not applicable

neurofibromas localized exclusively to the back and did not meet the National Institutes of Health diagnostic criteria for generalized *NF1*. The absence of systemic involvement and the postnatal onset of the lesions supported the suspicion of mosaicism. Targeted NGS of affected skin tissue revealed a somatic *NF1* frameshift variant—c.7797_7806del, p.(Glu2600PhefsTer21)—with a VAF of 8.23%, consistent with a mosaic pattern. This novel variant, not previously reported, was not detected in peripheral blood, further supporting its somatic origin. There are currently no specific follow-up guidelines for mosaic *NF1*. Based on previous reports indicating a 13% risk of malignancy in patients with mosaic *NF1* [2], the patient was counseled accordingly and scheduled for regular follow-up.

The *FGFR3* p.(Arg248Cys) hotspot mutation has been consistently reported as a pathogenic variant underlying mosaic epidermal nevus syndrome [4]. In some patients, this mutation extended beyond the epidermis into the oral mucosa or hematopoietic cells, suggesting an early embryonic mutational event [5]. In our patient, however, the mutation was confined to a nevus sebaceus on the scalp and to areas of hypopigmentation along Blaschko's lines, with no extracutaneous manifestations. The relatively low mutant allele frequency (~9-13%) further supports limited tissue involvement, which may account for the mild phenotype. While constitutional *Arg248Cys* mutations cause thanatophoric dysplasia, a typically lethal skeletal dysplasia [6], mosaic forms result in non-lethal presentations with variable expressivity. These observations highlight the wide phenotypic spectrum of *FGFR3* mosaicism and emphasize the need for careful dermatologic examination and tissue-specific molecular testing in suspected epidermal nevus syndromes. Even in the absence of systemic involvement, long-term surveillance is advisable, as extracutaneous features and rare complications have been described in previously reported cases [4,7].

In G24-7460, targeted sequencing of affected skin tissue identified the pathogenic hotspot *NRAS* p.(Gln61Arg), a recurrent postzygotic activating mutation well documented in CMNS and other mosaic *RASopathies* [8]. Together with p.Gln61Lys and p.Gly13Arg, this variant has been described as one of the most frequent drivers of large or multiple congenital melanocytic nevi and represents a prototypical example of somatic mosaicism [9]. The mutant allele frequency in our case (25%) was consistent with a mosaic state, as previously reported in similar patients [10]. Although extracutaneous features such as neurocutaneous melanosis, seizures, and structural central nervous system malformations have been described in association with CMNS [11], our patient exhibited only cutaneous findings, further highlighting the phenotypic variability of *NRAS*-driven mosaic disorders. The additional findings of pes planus and prominent heels may be incidental; however, given the pleiotropic effects of *RAS*/MAPK signaling, a contributory effect cannot be entirely excluded. Unlike some previously reported mosaic *RASopathy* patients in whom hypophosphatemic rickets has been documented [12], no biochemical evidence of hypophosphatemia was observed in our patient. However, because of the acute risk of developing rickets, he was placed under close clinical follow-up. Overall, our findings are consistent with previous reports and reinforce the concept that *NRAS* mosaicism underlies a clinically heterogeneous spectrum, emphasizing the importance of detailed dermatological assessment, tissue-specific molecular testing, and longitudinal surveillance in children presenting with extensive congenital melanocytic nevi.

There are currently no ACMG/AMP guidelines specifically developed for the classification of somatic mosaic variants associated with non-malignant cutaneous lesions, such as epidermal nevus syndromes or segmental neurofibromatosis. The widely adopted 2015 ACMG/AMP recommendations were primarily designed to evaluate germline variants, whereas the 2017 AMP/ASCO/CAP guideline was directed toward interpreting somatic variants in malignancies [13,14]. Consequently, the interpretation of variants identified in non-malignant cutaneous mosaic disorders generally relies on the application of germline ACMG/AMP criteria with appropriate modifications. However, the applicability and weight of these criteria can vary in the mosaic context. For example, evidence such as *de novo* occurrence or gene-specific loss-of-function can support pathogenicity, but interpretation must take into account the somatic mosaic nature and restricted tissue distribution of the variant. Collectively, our findings emphasize the need for tailored classification frameworks for somatic mosaic variants in dermatologic disorders, bridging the gap between germline guidelines and cancer-focused recommendations. Moreover, the genetic characterization of somatic mosaic skin disorders related to the *RAS*/MAPK pathway has advanced the understanding of disease pathogenesis and paved the way for the development of new

therapeutic targets. Indeed, targeted therapies originally developed for cancers with *RAS*/MAPK pathway alterations (such as MAPK inhibitors) may also hold therapeutic promise in cutaneous mosaic disorders driven by the same pathway, and their potential use in this context deserves further exploration. The increasing number of reported cases is expected to contribute to refining clinical approaches and to shaping treatment strategies with translational potential.

Study Limitations

This study is limited by the inability to demonstrate the absence of variants in blood samples from some patients (e.g., because of death), the lack of functional validation, and the absence of specific ACMG/AMP guidelines tailored for cutaneous mosaic variants. These factors may affect the generalizability and interpretation of our findings.

Conclusion

Mosaic disorders involving oncogenic signaling pathways represent a dynamic interface between developmental genetics and cancer biology. The cases presented herein highlight the wide phenotypic variability of *RAS*/MAPK-related mosaic syndromes and underscore the essential role of dermatologic evaluation and tissue-specific molecular testing in their diagnosis. The identification of novel and low-level mosaic variants further expands the genotypic and phenotypic spectrum of these disorders. Establishing standardized criteria for the interpretation of somatic mosaic variants in non-malignant settings and exploring the translational potential of pathway-targeted therapies remain important future goals.

Ethics

Ethics Committee Approval: Ethics committee approval for the study was received from the Scientific Research Evaluation and Ethics Committee of Ankara Etlik City Hospital on August 28, 2024 (decision no: Aeşh-BADEK-2024-757, date: 28.08.2024).

Informed Consent: Informed consent forms for publication were obtained from the adult patients and from the legal guardians of the pediatric patients.

Footnotes

Authorship Contributions

Concept: E.T., A.S., Design: E.T., A.S., M.G., Data Collection or Processing: E.T., A.B., A.K., A.B.D.A., E.K., E.K., İ.K., Analysis or Interpretation: E.T., A.S., A.B.D.A., E.K., M.G., Literature Search: E.T., A.B., M.G., Writing: E.T., M.G.

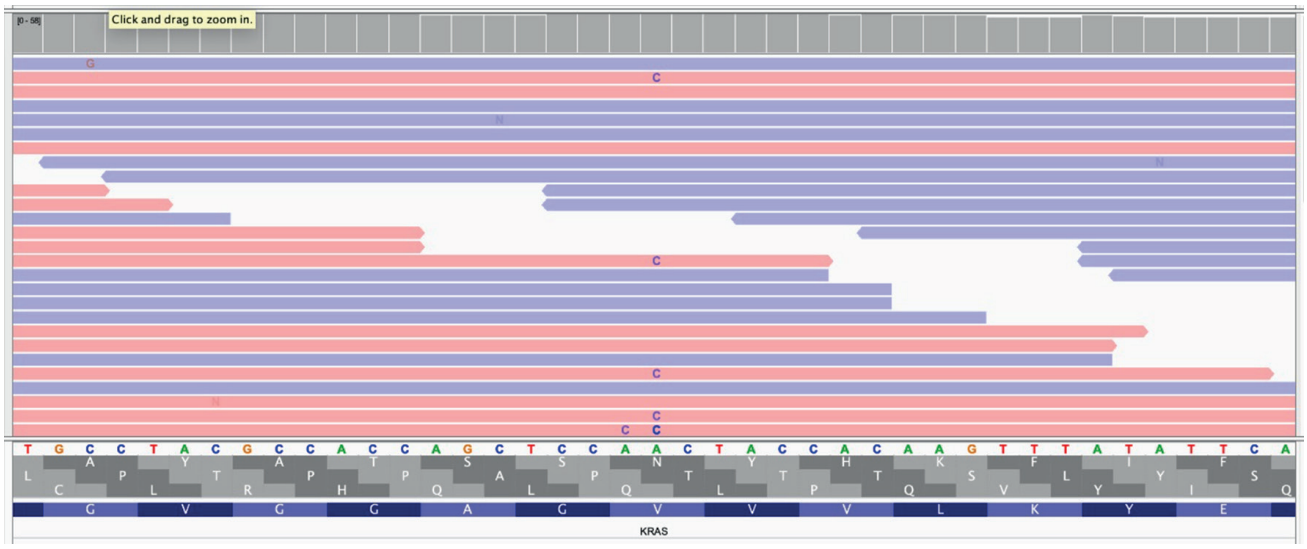
Conflict of Interest: No conflict of interest was declared by the authors.

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

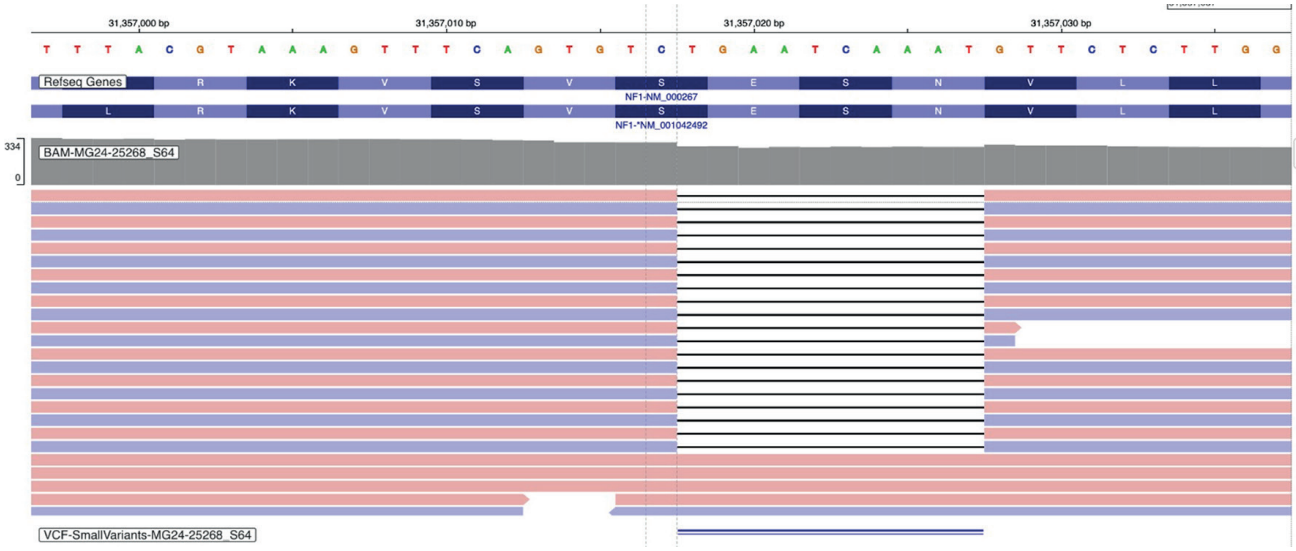
References

1. Carli D, Resta N, Ferrero GB, Ruggieri M, Mussa A. Mosaic *RASopathies*: a review of disorders caused by somatic pathogenic variants in the genes of the *RAS*/MAPK pathway. Am J Med Genet C Semin Med Genet. 2022;190:520-529.
2. Groesser L, Herschberger E, Ruetten A, et al. Postzygotic *HRAS* and *KRAS* mutations cause nevus sebaceous and Schimmelpenning syndrome. Nat Genet. 2012;44:783-787.
3. Hafner C, Toll A, Real FX. HRAS mutation mosaicism causing urothelial cancer and epidermal nevus. N Engl J Med. 2011;365:1940-1942.
4. Bygum A, Fagerberg CR, Clemmensen OJ, Fiebig B, Hafner C. Systemic epidermal nevus with involvement of the oral mucosa due to *FGFR3* mutation. BMC Med Genet. 2011;12:79.
5. García-Vargas A, Hafner C, Pérez-Rodríguez AG, et al. An epidermal nevus syndrome with cerebral involvement caused by a mosaic *FGFR3* mutation. Am J Med Genet A. 2008;146A:2275-2279.
6. Tavormina PL, Shiang R, Thompson LM, et al. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. Nat Genet. 1995;9:321-328.
7. Hafner C, van Oers JM, Vogt T, et al. Mosaicism of activating *FGFR3* mutations in human skin causes epidermal nevi. J Clin Invest. 2006;116:2201-2207.
8. Kinsler VA, Thomas AC, Ishida M, et al. Multiple congenital melanocytic nevi and neurocutaneous melanosis are caused by postzygotic mutations in codon 61 of *NRAS*. J Invest Dermatol. 2013;133:2229-2236.
9. Bauer J, Curtin JA, Pinkel D, Bastian BC. Congenital melanocytic nevi frequently harbor *NRAS* mutations but no *BRAF* mutations. J Invest Dermatol. 2007;127:179-182.
10. Charbel C, Fontaine RH, Malouf GG, et al. *NRAS* mutation is the sole recurrent somatic mutation in large congenital melanocytic nevi. J Invest Dermatol. 2014;134:1067-1074.
11. Kinsler VA, O'Hare P, Bulstrode N, et al. Melanoma in congenital melanocytic naevi. Br J Dermatol. 2017;176:1131-1143.
12. Carli D, Cardaropoli S, Tessaris D, et al. Successful treatment with MEK-inhibitor in a patient with NRAS-related cutaneous skeletal hypophosphatemia syndrome. Genes Chromosomes Cancer. 2022;61:740-746.
13. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-424.
14. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017;19:4-23.

Supplementary Material



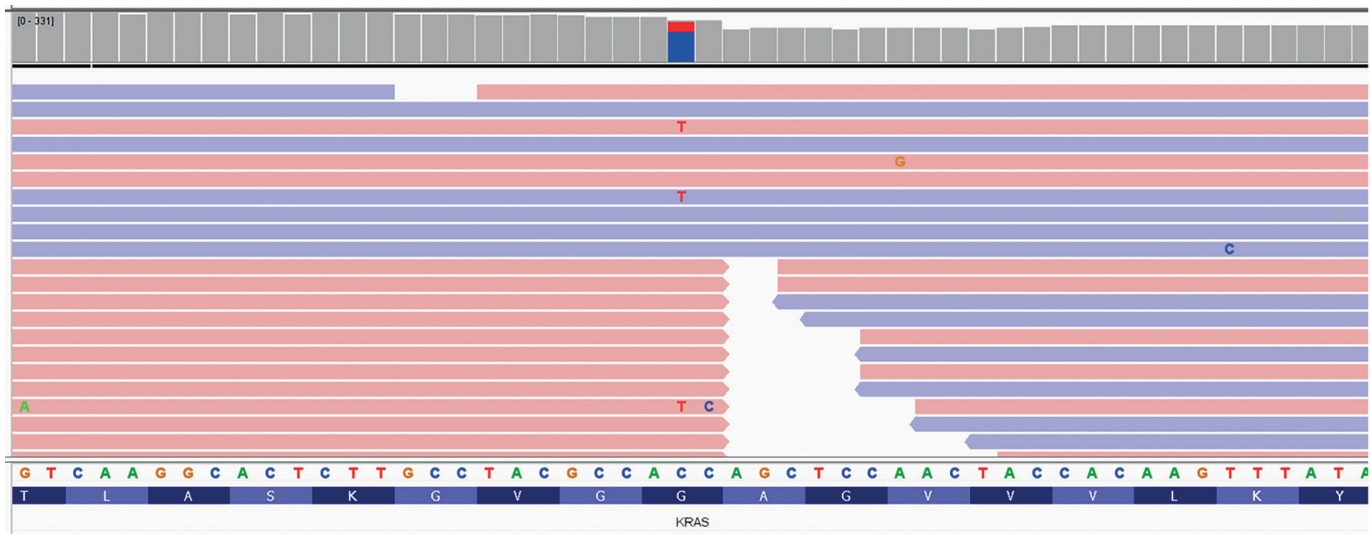
G23-25265



G24-25268

G23-9663

G24-7460



BSH1

Supplementary Material. Integrative Genomics Viewer (IGV) alignments showing the detected somatic variants, highlighted in red boxes: (a) *KRAS* c.26T>G; p.(Val9Gly), (b) *NF1* c.7797_7806del; p.(Glu2600PhefsTer21), (c) *FGFR3* c.742C>T; p.(Arg248Cys), (d) *NRAS* c.182A>G; p.(Gln61Arg), (e) *KRAS* c.35G>A; p.(Gly12Asp).

KRAS: Kirsten Rat Sarcoma Viral Oncogene Homolog, *NF1*: Neurofibromin Type 1, *FGFR3*: Fibroblast Growth Factor Receptor 3, *NRAS*: Neuroblastoma Rat Sarcoma Viral Oncogene Homolog

AHOT | Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):226-231

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-10-4

Clinical and Pathological Characteristics of Breast Cancer Cases with Germline Alterations in Homologous Recombination Defect-associated Genes

Murat Keser[1], Özge Özer Kaya[2], Taha Reşid Özdemir[3], Emel Ebru Pala[4], Olçun Ümit Ünal[5], Gözde Kubat[6]

[1]*Medicana International Hospital, Clinic of Medical Oncology, İzmir, Türkiye*
[2]*University of Health Sciences Türkiye, İzmir City Hospital, Clinic of Genetics, İzmir, Türkiye*
[3]*University of Health Sciences Türkiye, İzmir City Hospital, Clinic of Medical Genetics, İzmir, Türkiye*
[4]*University of Health Sciences Türkiye, İzmir Tepecik Education and Research Hospital, Clinic of Pathology, İzmir, Türkiye*
[5]*University of Health Sciences Türkiye, İzmir City Hospital, Clinic of Medical Oncology, İzmir, Türkiye*
[6]*Başkent University Faculty of Medicine, Ankara Hospital, Department of Biostatistics, Ankara, Türkiye*

ABSTRACT

Aim: Approximately 5-10% of breast cancer (BC) cases are hereditary, most frequently associated with germline variants in homologous recombination repair (*HRR*) genes such as *BRCA1/2*. However, non-*BRCA HRR* gene alterations, including *ATM* gene and *CHEK2*, may also influence tumor biology and clinical outcomes. This study aimed to evaluate the clinical relevance of germline homologous recombination deficiency (HRD)-related variants in BC and to compare the clinicopathological features and survival outcomes between *BRCA* and non-*BRCA* carriers.

Methods: A retrospective cohort of 148 BC patients with germline HRD-related variants identified by next-generation sequencing between 2018 and 2022 was analyzed. Variants were classified according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology 2015 criteria. Clinical, pathological, and survival data were assessed using descriptive statistics and Kaplan-Meier survival analysis with the Log-Rank test.

Results: Of 148 patients (mean age 45.2±10.1 years), 80 (54%) carried *BRCA* variants and 68 (46%) non-*BRCA* variants, most frequently *ATM* and *CHEK2*. Pathogenic or likely pathogenic variants were more frequent in *BRCA* carriers (77.5% vs. 58.8%, p=0.014). Disease-free survival (DFS) did not differ significantly between *BRCA* and non-*BRCA* groups (p=0.42). Prophylactic mastectomy and oophorectomy were performed significantly more often in *BRCA* carriers (p<0.05).

Conclusions: Although DFS was comparable between *BRCA* and non-*BRCA* carriers, relapse was more frequent in *BRCA1* and pathogenic variant carriers. These results emphasize the clinical importance of integrating germline *HRR* gene analysis into personalized surveillance and management strategies in BC.

Keywords: *BRCA*, breast cancer, disease free survival, germline mutations

Introduction

Breast cancer (BC) is the most frequent type of cancer among women, and approximately 5-10% of BC cases are attributable to hereditary factors. *BRCA1/2* are tumor suppressor genes that belong to the homologous recombination repair (HRR) family, which plays a crucial role in repairing deoxyribonucleic acid (DNA) double-strand breaks, which are harmful to cells. In addition to *BRCA1* and *BRCA2*, the HRR gene group includes genes such as *ATM*, *CHEK2*, *PALB2* (partner and localizer of *BRCA2*), and RAD51 (RAD51 recombinase). If a pathogenic variant is found in any of the HRR genes, the proteins encoded by these genes cannot perform their normal functions, resulting in homologous recombination deficiency (HRD) [1].

Women carrying pathogenic variants in *BRCA1* have a lifetime risk of BC of 50-80% and of ovarian cancer of 20-50%. For the *BRCA2* gene, the lifetime risks are 50% for BC and 20%

Address for Correspondence: Murat Keser MD, Medicana International Hospital, Clinic of Medical Oncology, İzmir, Türkiye
E-mail: drmksr35@gmail.com **ORCID ID:** orcid.org/0000-0003-3834-0863
Received: 25.11.2025 **Accepted:** 14.10.2025 **Publication Date:** 12.12.2025

Cite this article as: Keser M, Özer Kaya Ö, Özdemir TR, Pala EE, Ünal OÜ, Kubat G. Clinical and pathological characteristics of breast cancer cases with germline alterations in homologous recombination defect-associated genes. Acta Haematol Oncol Turc. 2025;58(3):226-231

for ovarian cancer [2]. In addition to *BRCA* germline variants, alterations can be detected in other genes associated with HRD, including those with high penetrance (*CDH1*, *TP53*, *PTEN*) and those with moderate penetrance (*PALB2*, *ATM*, *CHEK2*, *BRCA1*, *BRIP1*) [3,4]. These variants have been reported to increase BC risk by up to fourfold compared with the general population [5]. *ATM* and *CHEK2* variants, which, along with the PALB2 variant, play a role in DNA repair, are important in the context of hereditary BC [6,7]. In addition to known pathogenic variants, variants of uncertain clinical significance (VUS) may be detected [8]. Individuals with these variants may develop other malignancies alongside BC, presenting with substantially different pathological and clinical profiles [9]. Current guidelines emphasize that recommendations for treatment or preventive surgery based on these types of variants should not be made [10]. However, evaluating germline HRD-related genes is crucial for improving clinical outcomes through personalized therapies, such as targeted poly (ADP-ribose) polymerase (PARP) inhibitors for various cancers, particularly breast and ovarian cancers [11,12].

Although several studies have collected data on various malignancies associated with *BRCA* variants in our country [11,12], no study has clinically and pathologically evaluated *BRCA* and non-*BRCA* variants together, particularly among BC patients. This study aims to evaluate the clinical utility of germline variant analysis in BC for diagnosis and follow-up, and to determine its contribution to understanding the clinical significance of non-*BRCA* variants.

Methods

Study Design

This study had a retrospective design. All procedures and measurements in this study were performed according to the 1964 Helsinki Declaration or comparable ethical standards. Ethical board approval was granted by the Ethical Board for Clinical Studies at University of Health Sciences Türkiye, Tepecik Education and Research Hospital, with the following protocol number: approval number: 2021/05-37, date: 17.05.2021.

Patients

Data from patients diagnosed with BC, found to have germline HRD gene variants, and referred to the medical oncology clinic at University of Health Sciences, İzmir Tepecik Training and Research Faculty of Medicine Hospital were screened retrospectively between 2018 and 2022. The inclusion criteria were: (1) a confirmed diagnosis of BC, (2) age over 18 years, and (3) the presence of pathogenic germline variants. Patients whose clinical data were unavailable or whose germline variant analysis results were incomplete were excluded from the study. A total of 148 patients met the eligibility criteria and were included in the final analysis.

Genetic Testing

Peripheral blood samples were collected from patients for germline DNA extraction. DNA isolation was performed using standard commercial kits, following the manufacturer's instructions. The extracted DNA was analyzed with next-generation sequencing to identify germline variants in genes related to HRR and hereditary cancer predisposition. Only variants in HRR-associated genes were considered for analysis. Targeted sequencing included *BRCA1, BRCA2, APC, ATM, BARD1, BLM, BMPR1A, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MRE11, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PRSS1, PTEN, RAD50, RAD51C, RAD51D, SLX4, SMAD4, STK11, TP53,* and *VHL*. Sequence data were processed through a web-based bioinformatics pipeline (Genomize Seq Analysis v16.7.2) using the GRCh37 (hg19) human reference genome. All coding regions and exon-intron boundaries (±20 bp) were analyzed, ensuring at least 20× coverage depth across 100% of targeted areas. Detected variants were filtered based on a minor allele frequency<5% in population databases such as gnomAD, 1000 Genomes, ESP6500, and ExAC. Variant classification followed the ACMG/AMP 2015 guidelines [13]. Pathogenic and likely pathogenic variants were confirmed by Sanger sequencing before reporting. VUS were noted but not considered actionable for clinical decisions in accordance with current recommendations. The analysis excluded 3' and 5' untranslated regions, pseudogenes, and other homologous regions where accurate alignment was not feasible. Copy number variations, large deletions or duplications, repeat expansions, and mosaic variants were beyond the detection limits of this method.

Statistical Analysis

The data were expressed as mean and standard deviation for metric variables and as counts and percentages for nominal variables. Normality was assessed using Kolmogorov-Smirnov test and by examining Skewness and Kurtosis values. Univariate survival analysis was performed using the Kaplan-Meier method and the Log-Rank test for dichotomous data. Survival outcome was based on "death" for overall survival (OS) or "relapse" for disease-free survival (DFS) during the follow-up period, respectively. Data were analyzed at the 95% confidence level, and a p-value of 0.05 was considered significant. IBM Statistical Package for the Social Sciences version 27 (Chicago, IL, USA) was used for the analysis.

Results

This study included 148 patients with BC. Only one patient was male. The mean age of the patients was 45.18±10.11 years. The great majority of patients (n=104, 70.3%) were under the age of 40 at the time of diagnosis. 107 of 147 patients were premenopausal. The vast majority of patients (87.1%) had invasive ductal carcinoma. Nearly all patients had histological grades IIIII (97%). Thirteen of 148 patients had malignancies other than BC. By molecular subtype, 35 of 148 patients had triple-negative BC. Over half of the patients reported no HRD-related cancer (n=83, 56.5%). 23.4% and 19.7% of patients underwent prophylactic mastectomy and oophorectomy, respectively. The clinical and demographic characteristics of patients are shown in Table 1.

Table 1. Clinical and demographic characteristics of patients

	n	%
Sex		
Female	147	99.3
Male	1	0.7
Age groups		
>40	44	29.7
<40	104	70.3
Menapause		
Premenapause	108	73.0
Postmenapause	40	27.0
Histopathological Type		
Invasive ductal	130	87.8
Invasive lobular	10	6.7
Medullary	2	1.3
Solid papillary	1	0.67
Other	5	3.54
Grade		
I	4	2.7
II	76	51.3
III	68	46
Other malignities		
Yes	10	6.75
No	138	93.25
Molecular subtypes		
HR: +, Her2: -	79	53.3
HR: +, Her2: +	22	14.8
HR: -, Her2: +	12	8.1
TN	35	23.8
HRD related cancer		
Yes	64	43.3
No	84	56.7
RRM		
Yes	34	23.0
No	114	77.0
RRSO		
Yes	29	19.6
No	119	80.4
Pathogenity		
LP/P	9	6.1
P	93	62.8
VUS	46	31.1
Gene mutations		
BRCA	80	54.1
Non-*BRCA*	68	45.9

TN: Triple-negative, HR: Hormone receptor, Her2: Human epidermal growth factor receptor 2, RRM: Risk-reducing mastectomy, RRSO: Risk-reducing salpingo-oophorectomy, HRD: Homologous recombinant defect, LP/P: Likely pathogenic/pathogenic, VUS: Variants of uncertain clinical significance

The median follow-up time of patients was 50.46 months (interquartile range, 25-75: 33.80-81.10). During the follow-up period, 8 of 148 patients (5.4%) died of BC, while 26 (17.6%) experienced a relapse. 80 out of 148 patients had *BRCA*-related variants. The percentage distribution of *BRCA1* and *BRCA2* variants within the *BRCA*-related group was 46.8% and 53.2%, respectively. In the non-*BRCA* group, the most frequent types of variants were *ATM* (n=25, 38.5%) and *CHEK2* (n=24, 36.9%). Among variants from 148 patients, 102 (68.9%) were classified as likely pathogenic or pathogenic (LP/P), while the remainder were classified as VUS. Comparison of the distribution of variant pathogenicity between *BRCA* and non-*BRCA* groups revealed a significant difference (χ^2=5.985, p=0.014): 77.5% vs 58.8% LP/P in *BRCA* and non-*BRCA* groups, respectively. The frameshift variant was most frequent in the *BRCA* group (n=25, 31.6%), whereas the missense variant was most frequent in the non-*BRCA* group (n=40, 58.8%). 80.8% of patients who relapsed had an LP/P variant; however, there was no significant association between relapse and pathogenicity (χ^2=2.068, p=0.150), with relapse proportions of 80.8% versus 19.2% in the LP/P and VUS groups, respectively. There were also significant differences between *BRCA* and non-*BRCA* groups with respect to prophylactic mastectomy (χ^2=7.201, p=0.027; 30.8% vs 14.9% in the *BRCA* and non-*BRCA* groups, respectively) and prophylactic oophorectomy [χ^2(2)=9.904, p=0.007; 27.5% vs 10.4% in the *BRCA* and non-*BRCA* groups, respectively]. Among patients who relapsed, 54.2% had a *BRCA1* variant; nearly 70% of those patients had missense or frameshift variants. The Comparison of the clinical characteristics of patients with and without *BRCA* mutations is shown in Table 2.

[χ^2(1)=0.633, p=0.426], prophylactic mastectomy [χ^2(1)=1.678, p=0.190], prophylactic oophorectomy [χ^2(1)=1.506, p=0.223], histological grade [χ^2(2)=0.931, p=0.621], having HRD related cancer [χ^2(1)=0.103, p=0.747], menopausal state [χ^2(1)=0.048, p=0.822], age groups (<40<) [χ^2(1)=0.038, p=0.843] except for variant pathogenity [LP/P vs VUS, χ^2(1) =3.907, p=0.048]. Though the non-BRCA group showed higher median DFS time than the BRCA group (47.83 vs 37.13 months), the Log-Rank test was not significant [χ^2(1)=0.915, p=0.333]. The DFS also did not differ significantly between pathogenicity groups (LP/P vs P) [χ^2(1)=1.402, p=0.235]. Menopausal status, age groups, HRD-related cancer history, prophylactic mastectomy, and oophorectomy were also not significantly associated with DFS (p>0.050).

Discussion

In this retrospective cohort of 148 BC patients, with a median follow-up of more than four years, we observed no significant difference in DFS between *BRCA* carriers and non-*BRCA* carriers. Although the non-*BRCA* group showed a longer median DFS than the *BRCA* group, this difference did not reach statistical significance. Similarly, relapse was more frequently associated with *BRCA1* LP/P variants, but this association was not statistically significant. Prophylactic mastectomy and oophorectomy were significantly more common among *BRCA*

Table 2. Comparison of the clinical characteristics of patients with and without *BRCA* mutation

		BRCA		Non-*BRCA*		p
		n	%	n	%	
IHK_FISH	HR: +, Her2: -	35	44.3	44	55.7	0.001*
	HR: +, Her2: +	9	40.9	13	59.1	
	HR: -, Her2: +	3	50.0	3	50.0	
	TN	29	82.9	6	17.1	
Histological grade	I	1	25.0	3	75.0	0.286
	II	31	47.0	35	53.0	
	III	35	57.4	26	42.6	
Stage at the diagnosis	I	23	46.9	26	53.1	0.405
	II	20	57.1	15	42.9	
	III	30	58.8	21	41.2	
	IV	4	80.0	1	20.0	
Gene	*BRCA1*	37	97.4	1	2.6	0.000*
	BRCA2	42	100.0	0	0.0	
	ATM	0	0.0	25	100.0	
	BARD1	0	0.0	3	100.0	
	BRIP1	0	0.0	6	100.0	
	CHEK2	0	0.0	24	100.0	
	NBN	0	0.0	3	100.0	
	PALB2	0	0.0	3	100.0	
Pathogenity	LP/P	62	60.8	40	39.2	0.014*
	VUS	18	39.1	28	60.9	
Event	Censored	74	52.9	66	47.1	0.222
	Exitus	6	75.0	2	25.0	
Menopause	Menopause	21	52.5	19	47.5	0.775
	Premonapause	59	55.1	48	44.9	
Age	<40	30	68.2	14	31.8	0.025*
	>40	50	48.1	54	51.9	
Ki-67	<14	21	55.3	17	44.7	0.862
	>14	59	53.6	51	46.4	
Relapse	No	62	50.8	60	49.2	0.087
	Yes	18	69.2	8	30.8	
HRD associated cancer	No	42	50.6	41	49.4	0.402
	Yes	38	59.4	26	40.6	

*: Statistically significant at the level of p<0.05.
IHK_FISH: Immunohistochemistry+fluorescence *in situ* hybridization, VUS: Variants of uncertain clinical significance, HR: Hormone receptor, Her2: Human epidermal growth factor receptor 2

carriers, reflecting current clinical practice guidelines [14,15]. The distribution of variants in our cohort was consistent with previous studies, showing a higher proportion of *BRCA*-related variants and a predominance of invasive ductal carcinoma and luminal molecular subtypes [16]. In addition, the most frequently observed variants in the non-*BRCA* group were *ATM* and *CHEK2* [2,7]. Age, menopausal status, and histological grade were not significantly associated with DFS in our cohort, a finding that may be explained by the predominance of advanced-stage disease and the relatively small sample size.

Our findings are consistent with several prior studies reporting comparable survival outcomes between *BRCA* and non-*BRCA* carriers [17]. Liu et al. [18] reported that patients with *BRCA* variants have lower OS rates based on analysis of data from nearly 36,000 patients. While some reports suggest that *BRCA1* variants may be linked to a worse prognosis due to their association with aggressive tumor biology [17,19], others have found no significant differences in long-term survival [20,21]. Nevertheless, it should be noted that *BRCA* variant carriers have a lifetime risk of BC exceeding 50% [22]. The lack of statistical significance in our study might

have originated from limited sample size, heterogeneity in treatment modalities, or the impact of novel therapeutic approaches, including the increasing use of PARP inhibitors and platinum-based chemotherapy, which may mitigate the adverse prognostic impact of *BRCA* variants. Moreover, data from the literature [23] suggest that many confounding factors beyond *BRCA* status may influence OS. In our study, nearly half of the patients who experienced relapse were *BRCA1* carriers, a finding consistent with previous reports [17,19].

The distribution of variant pathogenicity in our cohort is also noteworthy. A higher proportion of LP/P variants was detected in *BRCA* carriers than in non-*BRCA* patients, suggesting potential clinical implications for genetic counseling and risk-adapted management [14,15]. On the other hand, the most common variant types differed between groups: frameshift variants predominated in *BRCA* carriers, whereas missense variants predominated in non-*BRCA* carriers. Similarly, Yazıcı et al. [24] reported that frameshift variants were the most frequent type, accounting for 63.5% of *BRCA1/2* carriers in their study. From a clinical perspective, our results support the notion that genetic testing should not only focus on *BRCA* variants but also encompass non-*BRCA* variants such as *ATM* and *CHEK2*, which were frequent in our cohort. Toss et al. [25] also reported that these two variants are associated with a higher risk of developing BC. In our study, nearly 40% of patients within groups with *ATM* and *CHEK2* variants had positive family history, which is comparable yet lower than the rates of over 60% reported by Toss et al. [25]. Given the increased risk of BC in *ATM* and *CHEK2* variant carriers, the National Comprehensive Cancer Network recommends annual mammography beginning at age 40 [10]. Moreover, since those variants are also reported to be associated with other malignancies, such as ovarian, colorectal, and kidney cancers, individuals with a positive family history should be thoroughly investigated [26]. Consistent with this observation, six of our patients with other malignancies—most commonly ovarian cancer—carried *BRCA* variants, while four carried *CHEK2* variants. Although the prognostic implications of these variants remain uncertain, their detection has potential value for individualized surveillance strategies and familial risk assessment [25]. Metcalfe et al. [15] reported that nearly 50% of *BRCA* carriers in their study underwent risk-reducing mastectomy (RRM), and that the risk of death from BC was approximately 1% within 15 years following RRM. The higher rate of prophylactic surgery among *BRCA* carriers in our study underscores adherence to guideline-based preventive strategies, which may contribute to long-term outcomes regardless of differences in DFS.

In conclusion, our study demonstrated no significant DFS differences between *BRCA* carriers and non-*BRCA* carriers in BC, though relapse events were more frequent among carriers of *BRCA1* and LP/P variants. These findings highlight the complexity of interpreting genetic variants in BC and the importance of integrating molecular, clinical, and therapeutic factors into prognostic assessment. Larger, multicenter studies with longer follow-up are warranted to validate these observations and to clarify the prognostic impact of *BRCA* and non-*BRCA* variants. These findings emphasize the need for broader genetic testing panels in routine clinical practice, particularly in young BC patients without *BRCA1/2* variants. Future studies integrating germline and somatic HRD profiles with treatment outcomes could help define molecular prognostic models for BC.

Study Limitations

The strengths of this study include a relatively long follow-up period and a detailed molecular characterization of variants. Several limitations should be acknowledged in this study. The single-center design, relatively small sample size, and inclusion of only one male patient limit the generalizability of our results. Moreover, subgroup analyses (e.g., *BRCA1* vs *BRCA2*, LP/P vs VUS) may have been underpowered to detect small but clinically relevant differences. In addition, since nearly all patients in our study were white women, the generalizability of our results to other ethnic groups may be limited.

Conclusion

Our findings suggest that while DFS did not significantly differ between *BRCA* and non-*BRCA* carriers, relapse was more common among *BRCA1* pathogenic variant carriers. This highlights the biological and clinical heterogeneity within HRR gene alterations. Incorporating germline HRR gene testing into the diagnostic and follow-up process can support more individualized risk assessment, surveillance, and preventive strategies for BC patients.

Ethics

Ethics Committee Approval: Ethical board approval was granted by the Ethical Board for Clinical Studies at University of Health Sciences Türkiye, Tepecik Education and Research Hospital, with the following protocol number: approval number: 2021/05-37, date: 17.05.2021.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.K., Concept: M.K., Ö.Ö.K., E.E.P., O.Ü.Ü., Design: M.K., T.R.Ö., Data Collection or Processing: M.K., Ö.Ö.K., T.R.Ö., Analysis or Interpretation: M.K., T.R.Ö., E.E.K., O.Ü.Ü., G.K., Literature Search: M.K., Ö.Ö.K, E.E.P., Writing: M.K.

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

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

References

1. Balmaña J, Díez O, Castiglione M; ESMO Guidelines Working Group. BRCA in breast cancer: ESMO clinical recommendations. Ann Oncol. 2009;20:19-20.

2. Tung N, Lin NU, Kidd J, et al. Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. J Clin Oncol. 2016;34:1460-1468.
3. Khatcheressian JL, Wolff AC, Smith TJ, et al. American Society of Clinical Oncology 2006 update of the breast cancer follow-up and management guidelines in the adjuvant setting. J Clin Oncol. 2006;24:5091-5097.
4. Han MR, Zheng W, Cai Q, et al. Evaluating genetic variants associated with breast cancer risk in high and moderate-penetrance genes in Asians. Carcinogenesis. 2017;38:511-518.
5. Hollestelle A, Wasielewski M, Martens JW, Schutte M. Discovering moderate-risk breast cancer susceptibility genes. Curr Opin Genet Dev. 2010;20:268-276.
6. Reiner AS, Sisti J, John EM, et al. Breast cancer family history and contralateral breast cancer risk in young women: an update from the women's environmental cancer and radiation epidemiology study. J Clin Oncol. 2018;36:1513-1520.
7. Fan Z, Ouyang T, Li J, et al Identification and analysis of CHEK2 germline mutations in Chinese BRCA1/2-negative breast cancer patients. Breast Cancer Res Treat. 2018;169:59-67.
8. Taylor A, Brady AF, Frayling IM, et al. Consensus for genes to be included on cancer panel tests offered by UK genetics services: guidelines of the UK Cancer Genetics Group. J Med Genet. 2018;55:372-377.
9. Kwong A, Ho CYS, Shin VY, Au CH, Chan TL, Ma ESK. How does re-classification of variants of unknown significance (VUS) impact the management of patients at risk for hereditary breast cancer? BMC Med Genomics. 2022;15:122.
10. Daly MB, Pal T, Berry MP, et al. Genetic/familial high-risk assessment: breast, ovarian, and pancreatic, version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19:77-102.
11. Demir S, Tozkir H, Gurkan H, et al. Genetic screening results of individuals with high risk BRCA-related breast/ovarian cancer in Trakya region of Turkey. J BUON. 2020;25:1337-1347.
12. Manguoğlu E, Güran S, Yamaç D, et al. Germline mutations of BRCA1 and BRCA2 genes in Turkish breast, ovarian, and prostate cancer patients. Cancer Genet Cytogenet. 2010;203:230-237.
13. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-424.
14. Jacobson M, Coakley N, Bernardini M, et al. Risk reduction strategies for BRCA1/2 hereditary ovarian cancer syndromes: a clinical practice guideline. Hered Cancer Clin Pract. 2021;19:39.
15. Metcalfe K, Huzarski T, Gronwald J, et al. Risk-reducing mastectomy and breast cancer mortality in women with a BRCA1 or BRCA2 pathogenic variant: an international analysis. Br J Cancer. 2024;130:269-274.
16. Celik Demirbas B, Kilic Erciyas S, Sukruoglu Erdogan O, et al. Genetic insights into BRCA1/2 associated breast cancer in Türkiye: focus on early-onset and aggressive subtypes. Discov Oncol. 2025;16:746.
17. Zhu Y, Wu J, Zhang C, et al. BRCA mutations and survival in breast cancer: an updated systematic review and meta-analysis. Oncotarget. 2016;7:70113-70127.
18. Liu M, Xie F, Liu M, Zhang Y, Wang S. Association between BRCA mutational status and survival in patients with breast cancer: a systematic review and meta-analysis. Breast Cancer Res Treat. 2021;186:591-605.
19. Lee EH, Park SK, Park B, et al. Effect of BRCA1/2 mutation on short-term and long-term breast cancer survival: a systematic review and meta-analysis. Breast Cancer Res Treat. 2010;122:11-25.
20. Sambiasi D, Lambo R, Pilato B, et al. BRCA1/2 and clinical outcome in a monoinstitutional cohort of women with hereditary breast cancer. Oncol Rep. 2014;31:365-369.
21. Huzarski T, Byrski T, Gronwald J, et al. Ten-year survival in patients with BRCA1-negative and BRCA1-positive breast cancer. J Clin Oncol. 2013;31:3191-196.
22. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol. 2007;25:1329-1333.
23. Dwornik R, Białkowska K. Insights into genetic modifiers of breast cancer risk in carriers of BRCA1 and BRCA2 pathogenic variants. Hered Cancer Clin Pract. 2025;23:15.
24. Yazıcı H, Kılıç S, Akdeniz D, et al. Frequency of rearrangements versus small indels mutations in *BRCA1* and *BRCA2* genes in Turkish patients with high risk breast and ovarian cancer. Eur J Breast Health. 2018;14:93-99.
25. Toss A, Tenedini E, Piombino C, et al. Clinicopathologic profile of breast cancer in germline ATM and CHEK2 mutation carriers. Genes (Basel). 2021;12:616.
26. Cybulski C, Górski B, Huzarski T, et al. CHEK2 is a multiorgan cancer susceptibility gene. Am J Hum Genet. 2004;75:1131-1135.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):232-239

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-10-6

Broadening the Clinical and Molecular Spectrum of *BAP1* Tumor Predisposition Syndrome: Findings from the First Reported Turkish Cohort

Ceren Damla Durmaz[1], Elifcan Taşdelen[2], Erdem Kındış[1], Hande Nur Cesur Baltacı[3], İrem Koç[4], Nesibe Saliha Bulut[1], Eren Kılınç[5], Özge Özalp[3], Munis Dündar[5], Naz Güleray Lafcı[1], Hayyam Kıratlı[4]

[1]*Hacettepe University Faculty of Medicine, Department of Medical Genetics, Ankara, Türkiye*

[2]*University of Health Sciences Türkiye, Ankara Etlik City Hospital, Clinic of Medical Genetics, Ankara, Türkiye*

[3]*University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Clinic of Medical Genetics Türkiye, Ankara, Türkiye*

[4]*Hacettepe University Faculty of Medicine, Department of Ophthalmology, Ankara, Türkiye*

[5]*Erciyes University Faculty of Medicine Hospitals, Department of Medical Genetics, Kayseri, Türkiye*

ABSTRACT

Aim: *BAP1*-tumor predisposition syndrome (*BAP1*-TPDS) is a rare autosomal dominant condition predisposing to multiple malignancies, most notably uveal melanoma and mesothelioma. The full phenotypic and genotypic spectrum remains incompletely defined, particularly in underrepresented populations.

Methods: Six unrelated Turkish probands carrying germline pathogenic or likely pathogenic *BAP1* variants were identified through multigene hereditary cancer panel testing. Clinical data, family histories, and segregation analyses were evaluated, and variant classification followed American College of Medical Genetics and Genomics guidelines.

Results: All six affected individuals were female, with cancer onset between 38 and 57 years of age. Breast carcinoma was the most common diagnosis (n=4), followed by uveal melanoma (n=2). Three novel *BAP1* variants were identified, expanding the mutational landscape of *BAP1*-TPDS. Pedigree analysis revealed extensive familial clustering of malignancies, including uveal melanoma, colon carcinoma, hepatocellular carcinoma, and mesothelioma. None of the breast cancer patients carried additional pathogenic variants in known susceptibility genes.

Conclusion: This study describes the first Turkish cohort of germline *BAP1* carriers and broadens the clinical and genetic spectrum of *BAP1*-TPDS. The predominance of breast carcinoma highlights the need to consider *BAP1* testing in patients with early-onset or familial breast cancer. Integrating *BAP1* analysis into hereditary cancer panels will enhance recognition, risk stratification, and surveillance across diverse populations.

Keywords: *BAP1*, uveal melanoma, *BAP1*-tumor predisposition syndrome, breast cancer, hereditary cancer syndromes

Introduction

BAP1-tumor predisposition syndrome (*BAP1*-TPDS) is a rare autosomal dominant hereditary cancer syndrome caused by heterozygous germline pathogenic or likely pathogenic variants in the BRCA1-associated protein 1 (*BAP1*) gene [1,2]. *BAP1*-TPDS was first reported in 2011 in families with uveal melanoma and mesothelioma. Since then, it has been recognized as involving a broader spectrum of cancers, most commonly uveal melanoma, malignant mesothelioma, cutaneous melanoma, and renal cell carcinoma, which are regarded as the core tumors of the syndrome [1-3]. These cancers often arise at younger ages than their sporadic counterparts, and multiple tumor types may develop within the same individual or within a family [4,5].

Address for Correspondence: Ceren Damla Durmaz MD, Hacettepe University Faculty of Medicine, Department of Medical Genetics, Ankara, Türkiye
E-mail: ceren.damla.durmaz@hacettepe.edu.tr **ORCID ID:** orcid.org/0000-0002-6054-0709

Received: 27.10.2025 **Accepted:** 02.12.2025 **Publication Date:** 12.12.2025

Cite this article as: Durmaz CD, Taşdelen E, Kındış E, Cesur Baltacı HN, Koç İ, Bulut NS, Kılınç E, Özalp Ö, Dündar M, Lafcı NG, Kıratlı H. Broadening the clinical and molecular spectrum of *BAP1* tumor predisposition syndrome: findings from the first reported Turkish cohort. Acta Haematol Oncol Turc. 2025;58(3):232-239

In addition to malignant tumors, *BAP1* mutation carriers frequently develop *BAP1*-inactivated melanocytic tumors (MBAITs). These are benign dermal lesions with distinctive histopathologic and immunohistochemical features, including loss of nuclear *BAP1* expression [6]. Although they are non-malignant, MBAITs are increasingly regarded as cutaneous indicators of germline *BAP1* mutations and may precede malignant transformation [2]. Over the past decade, additional tumor types, including meningioma, cholangiocarcinoma, hepatocellular carcinoma, basal cell carcinoma, and breast carcinoma, have been proposed as part of the *BAP1*-TPDS spectrum, although the strength of these associations remains under investigation [5,7,8]. Louie and Kurzrock [9] summarized over 200 germline carriers, highlighting this heterogeneity and underscoring the growing relevance of *BAP1* in diverse cancer contexts.

Recent consensus guidelines recommend annual dermatologic and ophthalmologic surveillance, periodic imaging for mesothelioma and renal cancer, and cascade testing in families with confirmed pathogenic variants [10]. However, surveillance strategies for other, less established tumor types are not yet defined because their associations remain uncertain. Therefore, the publication of new clinical and genotypic data from diverse cohorts is essential to refine management recommendations and delineate the full phenotypic spectrum of the syndrome.

BAP1 is a tumor suppressor gene located on chromosome 3p21.1 that encodes a ubiquitin carboxyl-terminal hydrolase involved in chromatin remodeling, histone modification, the deoxyribonucleic acid (DNA) damage response, cell-cycle regulation, and apoptosis [11]. As the catalytic subunit of the polycomb repressive deubiquitinase complex, *BAP1* removes monoubiquitin from histone H2A (H2AK119ub) and thereby helps maintain transcriptional control and genomic integrity [12]. Similar to many hereditary cancer syndromes, tumor development in *BAP1*-TPDS is thought to follow a two-hit mechanism involving germline loss of one allele and somatic inactivation of the other [13].

Despite growing awareness of *BAP1*-TPDS, its true prevalence and associated cancer risks remain uncertain. Current estimates suggest lifetime risks of approximately 20-25% for uveal melanoma, mesothelioma, and cutaneous melanoma, with an overall risk of any *BAP1*-associated malignancy approaching 80-85% [5]. However, these figures are likely influenced by ascertainment bias, as most published series focus on index cases with multiple tumors or strong family histories, while unaffected carriers are underrepresented. In several studies, only probands underwent genetic testing, further limiting accurate penetrance estimates [7].

Most germline *BAP1* carriers reported to date are of European ancestry, while considerably fewer cases have been described in other ethnic groups, resulting in the underrepresentation of some populations in current knowledge of the syndrome. To date, no germline *BAP1*-positive families have been reported in Türkiye, highlighting a major gap in global data.

In this context, the present study reports the first Turkish cohort of individuals carrying germline *BAP1* variants, including those with uveal melanoma and breast carcinoma. The series also expands the genotypic landscape by identifying novel *BAP1* variants and integrates clinical and molecular data, enhancing understanding of *BAP1*-TPDS across populations.

Methods

This multicenter study included six unrelated probands from different families who were found to carry germline *BAP1* pathogenic or likely pathogenic variants. These variants were detected among individuals undergoing multigene hereditary cancer panel testing for various clinical indications. All participants were evaluated at the Department of Medical Genetics because of a clinical suspicion of hereditary cancer predisposition.

Clinical information, including tumor type, age at diagnosis, histopathologic findings, and family history of malignancy, was obtained through review of medical records and patient interviews.

The study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval number: AEŞH-BADEK-2024-845, date: 25.09.2024) and conducted in accordance with the ethical standards of the Declaration of Helsinki. Written informed consent for genetic testing and publication of anonymized results was obtained from all participants before inclusion.

Peripheral blood samples were collected in ethylenediaminetetraacetic acid tubes, and genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Next-generation sequencing was performed with a custom hereditary cancer panel targeting the coding exons and exon-intron boundaries of at least forty tumor-predisposition genes, including *APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, FANCC, FLCN, GALNT12, HOXB13, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS2, POLD1, POLE, PTCH1, PTEN, RAD51C, RAD51D, RB1, RET, SMAD4, STK11, TP53,* and *VHL*.

Variant filtering and interpretation were conducted using the Seq Genomize Variant Analysis Platform and, in some cases, the Sophia DDM software, in accordance with each laboratory's standard workflow, following the recommendations of the American College of Medical Genetics and Genomics (ACMG) [14]. Only variants classified as pathogenic or likely pathogenic were reported. Each variant was manually reviewed in the Integrative Genomics Viewer (IGV) to confirm accuracy. Segregation analysis by Sanger sequencing was performed in available relatives who provided informed consent; this was feasible in only one family, in which the *BAP1* variant was tested in three unaffected daughters of the proband (P6).

Statistical Analysis

Descriptive statistics were applied to summarize demographic and clinical variables. Categorical data were expressed as frequencies and percentages, and continuous variables as medians with corresponding ranges. Owing to the descriptive nature of this cohort, no comparative or inferential statistical analyses were conducted. All calculations were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA; accessed October 2025).

Results

Clinical Characteristics of Affected Individuals

This study included six affected individuals from six unrelated families, each carrying germline *BAP1* variants. Detailed demographic, clinical, and molecular characteristics are summarized in Table 1. All patients were female and had a confirmed diagnosis of cancer. The ages at diagnosis ranged from 38 to 57 years, with a median of 47.5 years.

Breast carcinoma was the most frequent malignancy, occurring in four patients (66.7%). All tumors were unilateral; three were diagnosed as invasive ductal carcinoma and one as an invasive pleomorphic lobular carcinoma. Uveal melanoma was identified in the remaining two patients (33.3%), both of whom had unilateral involvement. One of these patients underwent enucleation and later developed distant metastases to the liver and iliac bone, while the other remained disease-free under regular ophthalmologic surveillance.

Pedigree analysis showed that four of the six families had at least two relatives affected by cancer, whereas the remaining families had only one or no additional affected individuals. In Family 6, the proband's brother was diagnosed with uveal melanoma and died of the disease; one of his sons developed a central nervous system tumor. Segregation analysis confirmed the presence of the familial *BAP1* variant in three unaffected daughters of the proband (P6). They are currently under follow-up in the dermatology, ophthalmology, and urology departments for surveillance of potential *BAP1*-associated manifestations. Family 5 also exhibited a substantial cancer burden, with close relatives diagnosed with colon carcinoma, mesothelioma, hepatocellular carcinoma, and primary brain tumors. Several other families had recurrent gastrointestinal or breast cancers across generations.

Genetic Findings

Six distinct *BAP1* variants, classified as pathogenic or likely pathogenic, were identified in the six affected individuals (NM_004656.4). These included three splice-site variants, two frameshift variants, and one non-sense variant (Figure 1). Three of these alterations [c.659+1G>A, c.784-2A>G, and c.1294_1295insGAA (p.Ser432Ter)] were novel and not previously reported in population or disease databases.

All affected individuals were heterozygous for their respective *BAP1* variants. The splice-site variant c.659+1G>A is located within the ubiquitin carboxy-terminal hydrolase (UCH) domain, which mediates the deubiquitinase activity of the *BAP1* protein [9]. The other two splice-site variants (c.783+1G>A and c.784-2A>G) are located in the *BARD1*-binding region, which interacts with the *BRCA1/BARD1* E3 ubiquitin ligase complex and participates in homologous recombination-mediated DNA repair (Figure 1) [9].

The frameshift variant c.1766_1770del (p.Ile589ArgfsTer52) and the nonsense variant c.1294_1295insGAA (p.Ser432Ter)

Table 1. Clinical and molecular characteristics of six affected individuals with germline *BAP1* variants

ID	Gender	Diagnosis	Age at cancer diagnosis (y)	Family history	*BAP1* (NM_004656.4) variant	Concurrent variant
P1	F	Breast cancer	38	-	c.1934del (p.Asn645Thr*fs*Ter10)	*BRCA2* (NM_000059.4) c.8524C>T p.(Arg2842Cys)
P2	F	Breast cancer	49	Father pancreas ca	c.659+1G>A	-
P3	F	Breast cancer	55	Father gastric ca, sister breast cancer, maternal cousin gastric ca	c.783+1G>A	-
P4	F	Breast cancer	42	Father prostate and lung ca, maternal uncle colon ca, maternal cousin leukemia, another maternal cousin breast ca	c.784-2A>G	-
P5	F	Uveal melanoma	44	Sister colon ca, another sister mesothelioma, maternal uncle HCC; one of his daughter CNS tumor, another maternal uncle dermatological ca, maternal cousin CNS tumor, paternal cousin colon ca	c.1766_1770del (p.Ile589Arg*fs*Ter52)	-
P6	F	Uveal melanoma	57	Brother uveal melanoma, one of his son CNS	c.1294_1295insGAA (p.Ser432Ter)	-

F: Female, HCC: Hepatocellular carcinoma, CNS: Central nervous system, ca: Carcinoma, –: not detected

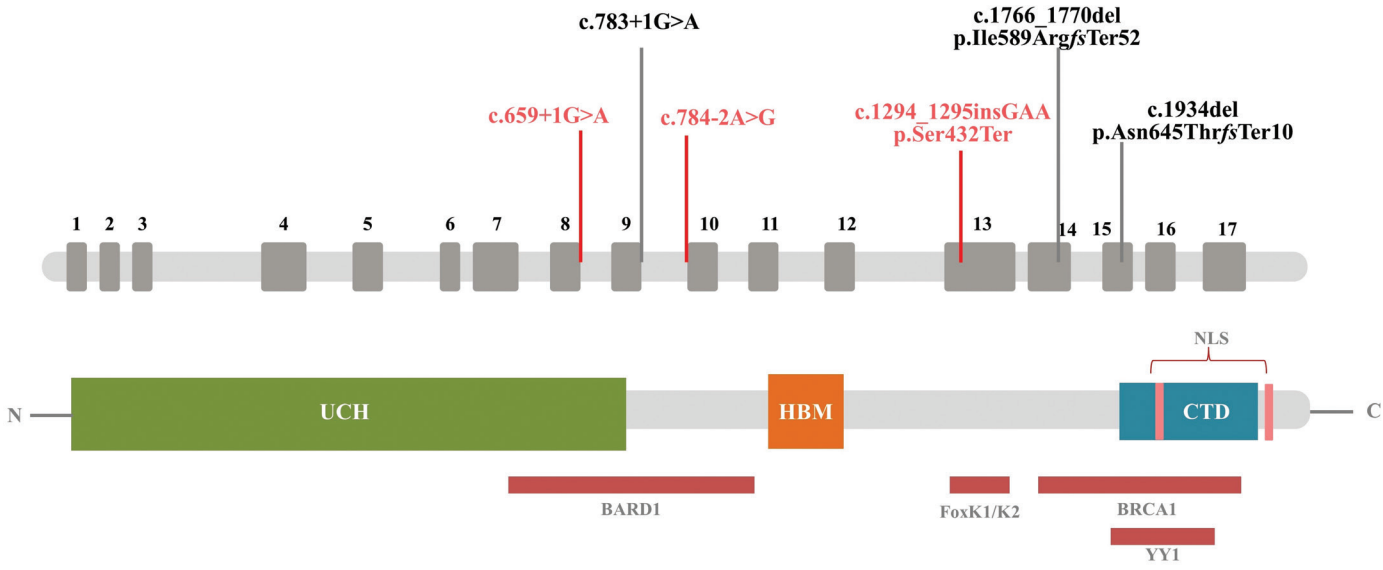


Figure 1. Schematic representation of *BAP1* protein domains and distribution of germline mutations
The upper bar represents the exons of the *BAP1* gene, while the lower bar depicts the corresponding functional protein domains. Intermittent red bars indicate known binding regions. Pathogenic variants identified in our cohort are mapped along the *BAP1* gene with the corresponding functional domains; novel variants are highlighted in red

UCH: Ubiquitin carboxyl hydrolase domain, *BARD1*: *BRCA1*-associated RING domain protein 1 (*BARD1*) binding region, HBM: host cell factor 1 (HCF1) binding domain, FoxK1/K2: Forkhead Box Protein K1/2 binding region, *BRCA1*: Breast cancer type 1 (*BRCA1*)-binding region, CTD: C-terminal domain, YY1: Yin Yang 1 (YY1) binding domain, NLS: Nuclear localization signals

both affect the C-terminal region of the protein, which is predicted to be intrinsically flexible and involved in dynamic interactions with chromatin-associated and transcriptional regulators [15,16]. The remaining frameshift variant c.1934del (p.Asn645ThrfsTer10) is located in the BRCA1-binding region, which is essential for the tumor-suppressive cooperation between *BAP1* and *BRCA1* in maintaining genomic stability [9]. Read-level IGV and Sanger views for all six germline *BAP1* variants are presented in Figure 2. In patients with breast cancer, no pathogenic or likely pathogenic variants were detected in other well-established breast cancer susceptibility genes. A heterozygous *BRCA1* variant (NM_000059.4: c.8524C>T, p.Arg2842Cys) identified in one patient was classified as a variant of uncertain significance according to ACMG guidelines [14].

Discussion

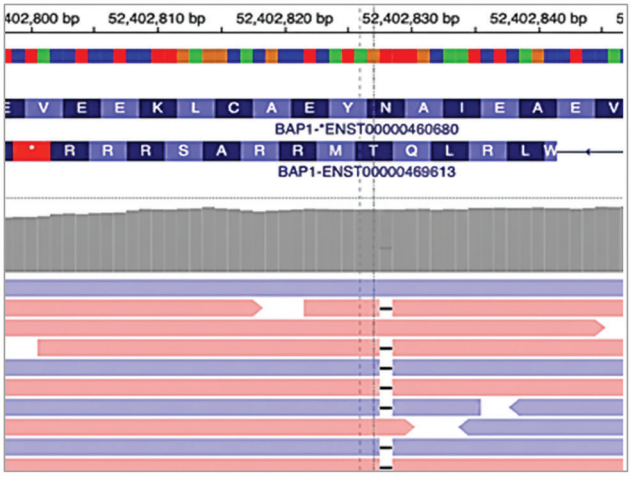
This study is the first report from Türkiye of a small cohort of six unrelated probands with germline *BAP1*-related cancers. The cohort included two patients with uveal melanoma and four with breast cancer, highlighting both classical and non-classical manifestations of *BAP1*-TPDS.

All six probands in our study carried heterozygous loss-of-function *BAP1* variants, comprising three splice-site and three frameshift mutations. Three of the six variants were novel, thus contributing to the expanding genotypic spectrum of *BAP1*-TPDS. All identified alterations are predicted to result in premature truncation or aberrant splicing, leading to complete loss of functional *BAP1* protein. Saturation genome-editing data from Waters et al. [17] show that variants impacting the catalytic and UCH domains, as identified in our patients, are strongly depleted, which is consistent with a loss of deubiquitinase function and supports their pathogenic role. These findings reinforce the concept that pathogenic *BAP1* variants act as tumor suppressors, with tumorigenesis following biallelic inactivation via a somatic second hit.

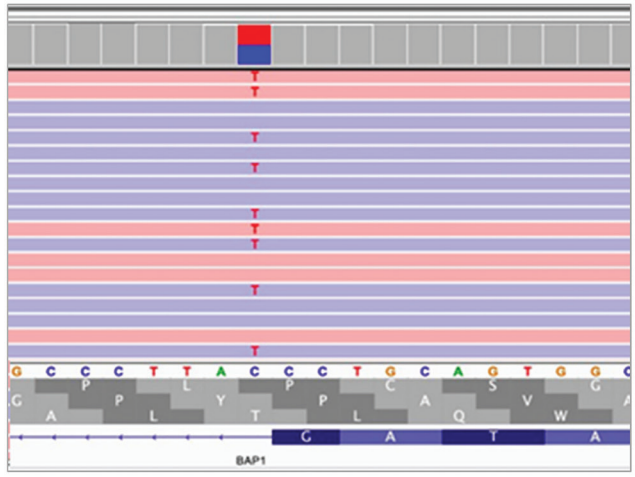
The clinical characteristics of our cohort illustrate the diversity of tumor presentation in *BAP1*-TPDS. All six probands were female, with cancer onset between 38 and 57 years of age. Four patients presented with breast carcinoma, three of whom were diagnosed before the age of 50, supporting the trend toward early tumor onset in *BAP1*-associated cancers, which is consistent with prior studies. The breast tumors were mainly invasive ductal carcinomas; one was of the lobular subtype, and several showed hormone receptor positivity and a high proliferative index.

The predominance of breast carcinoma in our cohort adds to the growing discussion about the potential inclusion of breast carcinoma within the *BAP1*-associated tumor spectrum. While these tumors have not yet been incorporated into formal diagnostic criteria, several case reports and small series have described this association [5,8]. Non-etheless, the predominance of breast cancer in our series may partly reflect selection bias, as individuals with suspected hereditary breast cancer represent the largest group referred for multigene panel testing in our clinical practice. Functionally, the link is biologically plausible: *BAP1* interacts with BRCA1 and other homologous recombination (HR) repair proteins, and its loss impairs double-strand break repair, resulting

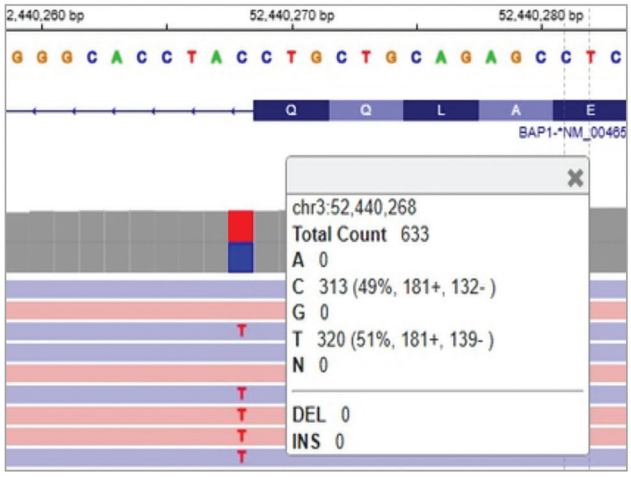
P1 *BAP1*: c.1934del (p.Asn645Thr*fs*Ter10)



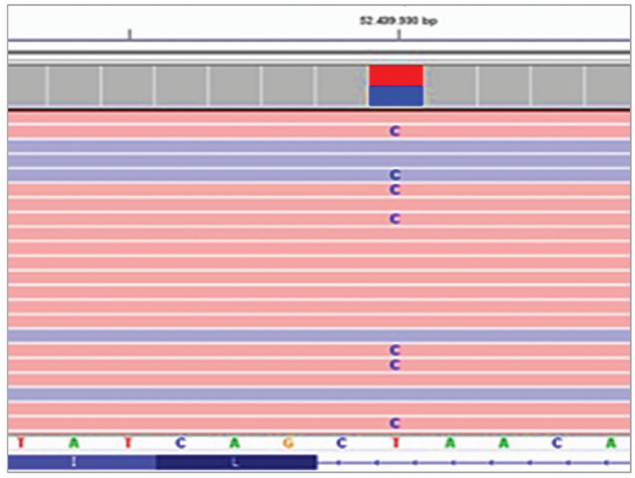
P2 *BAP1*: c.659+1G>A



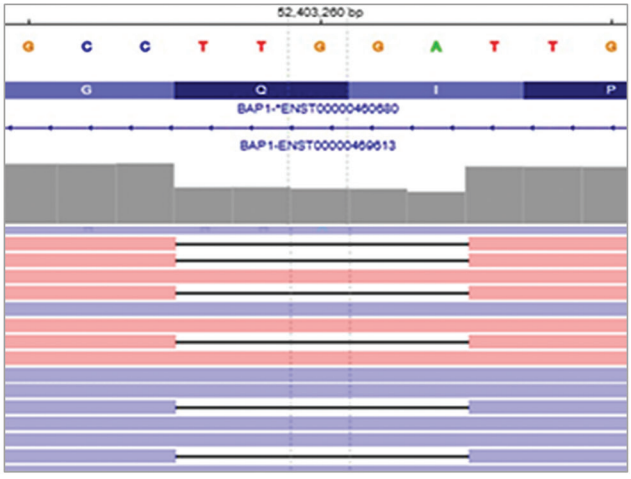
P3 *BAP1*: c.783+1G>A



P4 *BAP1*: c.784-2A>G



P5 *BAP1*: c.1766_1770del (p.Ile589ArgfsTer52)



P6 *BAP1*: c.1294_1295insGAA (p.Ser432Ter)

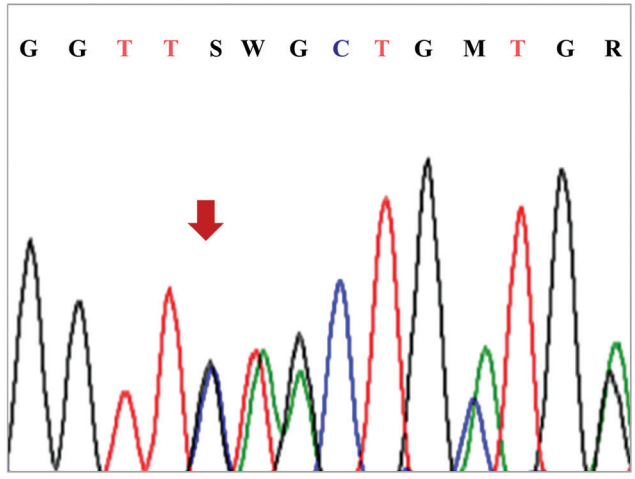


Figure 2. IGV and Sanger representations of the germline *BAP1* variants in probands P1-P6
Integrative genomics viewer (IGV) screenshots and Sanger sequencing chromatograms demonstrating the six germline *BAP1* variants detected in probands P1-P6

in genomic instability [18]. This HR-deficient phenotype resembles that seen in *BRCA1/2*-mutated tumors, suggesting potential therapeutic overlap. As such, confirming the role of *BAP1* in breast cancer predisposition could have clinical implications, including the application of poly (adenosine diphosphateribose) polymerase inhibitors in selected patients. Evaluating homologous recombination deficiency (HRD) in *BAP1*-mutant breast cancers may help identify those who could benefit from such therapies, representing an important direction for future studies.

The two probands with uveal melanoma were diagnosed at ages 44 and 57, respectively, consistent with the typical tumor spectrum of *BAP1*-TPDS. Their family histories were remarkable for additional malignancies: the 44-year-old proband had a sister with malignant mesothelioma, and the 57-year-old proband had a brother who died from uveal melanoma. In our cohort, family histories also included a wide range of other cancers—such as breast, colorectal, bladder, pancreatic, and hematologic malignancies—among relatives who were not genetically tested. The occurrence of these diverse tumors within *BAP1*-positive families may indicate that the phenotypic spectrum of *BAP1*-TPDS is broader than currently appreciated.

Beyond its role in inherited cancer risk, *BAP1* status influences tumor aggressiveness and treatment response, making it a potential biomarker for prognosis and therapy selection. *BAP1*-mutant uveal melanoma is associated with aggressive clinical behavior and early metastasis [19], whereas *BAP1*-deficient mesothelioma paradoxically exhibits improved outcomes and longer survival compared with sporadic forms of mesothelioma, likely due to increased sensitivity to platinum-based chemotherapy [20]. Thus, *BAP1* testing can inform both prognosis and therapy, identifying patients who may benefit from DNA-damaging agents or, conversely, those who require intensified surveillance.

Clinicians should maintain a high level of suspicion for *BAP1*-TPDS in specific clinical contexts. Testing should be considered in patients with (i) early-onset or multifocal uveal melanoma, (ii) patients with malignant mesothelioma diagnosed before age 50 or in the absence of significant asbestos exposure, (iii) patients with atypical melanocytic lesions with *BAP1* loss on immunohistochemistry, and (iv) individuals or families with multiple primary cancers, including uveal melanoma, mesothelioma, renal cancer, or early-onset breast carcinoma [7,10] (Table 2). From a clinical management perspective, our findings emphasize the importance of including *BAP1* in hereditary cancer panels. Several of our patients were identified through broad multigene testing rather than clinical suspicion alone, demonstrating that single-gene approaches may overlook this syndrome. The recognition of *BAP1*-TPDS in patients with early-onset or multiple primary cancers is crucial, especially when *BRCA1/2* testing is negative. Panel inclusion not only improves detection but also enables family-based risk assessment and cascade testing.

Surveillance and prevention remain the cornerstones of managing *BAP1* carriers. Current consensus guidelines recommend initiating follow-up in late adolescence, with annual dermatologic and ophthalmologic examinations, periodic chest and abdominal imaging, and avoidance of exposure to ultraviolet radiation and asbestos [7,10]. Predictive genetic testing should be offered once a familial pathogenic variant is confirmed. Identifying carriers before tumor onset enables early intervention and risk-adapted monitoring.

Although our study includes a small number of cases, it provides valuable insight from an underrepresented population. The predominance of breast carcinoma, a history of multifocal tumors, and strong familial clustering observed in our cohort highlight the importance of considering *BAP1* alterations in patients with suggestive clinical features. Further functional and clinical studies are needed to better define the prevalence and biological behavior of *BAP1*-mutant breast cancers and to identify potential therapeutic vulnerabilities related to HRD.

While *BAP1* loss impairs several DNA damage-response pathways and contributes to genomic instability, environmental factors may further modify cancer risk and phenotype in certain populations. Cappadocia, in central Türkiye, has a high incidence of mesothelioma owing to long-standing environmental exposure to erionite and asbestos [21]. Previous investigations in these villages did not identify germline *BAP1* mutations, suggesting that environmental carcinogenesis alone was sufficient to explain the observed clustering of cases [22]. Nevertheless, given the established synergistic effect of *BAP1* deficiency and mineral fiber exposure in experimental models, continued genetic surveillance in this region remains warranted. Identifying potential germline carriers among individuals with early-onset or familial mesothelioma could provide valuable insight into the interplay between environmental and hereditary risk factors.

Study Limitations

The main limitation of this study is the small cohort size, which restricts the generalizability of the findings. A second limitation is the inability to perform functional assays to validate the pathogenicity of the novel *BAP1* variants. In addition, segregation analysis in cancer-affected relatives could not be completed, as several affected family members were not available for testing. Despite these constraints, the study provides valuable insight into the clinical and genetic features of *BAP1*-TPDS in an underrepresented population.

Conclusion

In conclusion, our findings demonstrate that *BAP1*-TPDS is a clinically actionable hereditary cancer syndrome. It should be considered in patients with early-onset cancers or multiple primary malignancies, particularly when the family history includes diverse cancer types. Recognizing and testing for *BAP1* is crucial for accurate risk assessment, prognostic evaluation, and treatment planning. Incorporating *BAP1* analysis into hereditary cancer panels will facilitate the timely identification of carriers, the implementation of structured surveillance, and

Table 2. Clinical situations in which germline *BAP1* testing may be considered

Clinical context	Rationale
Early-onset uveal melanoma (<50 years) or multifocal uveal melanoma	Classical *BAP1*-associated tumor; early onset increases suspicion
Malignant mesothelioma <50 years or without significant asbestos exposure	Suggests hereditary etiology rather than environmental cause
Atypical melanocytic lesions with loss of *BAP1* expression on IHC (MBAITs)	Recognized dermatological marker of germline *BAP1* variants
Individuals with multiple primary tumors, including any core *BAP1*-associated tumors	Fits the typical pattern of *BAP1*-TPDS
Families with ≥2 relatives affected by uveal melanoma, mesothelioma, renal cell carcinoma, or related malignancies	Suggestive familial clustering
Early-onset or familial breast cancer with additional diverse tumors in the family	Emerging association; not a formal criterion, but may raise suspicion

Core *BAP1*-associated tumors: uveal melanoma, malignant mesothelioma, cutaneous melanoma, and renal cell carcinoma. IHC: Immunohistochemistry, MBAITs: BAP1-inactivated melanocytic tumors, BAP1-TPDS: *BAP1*-tumor predisposition syndrome

the consideration of targeted therapeutic strategies, such as platinum-based therapies. Altogether, this study reinforces that *BAP1* predisposition extends beyond its classical tumor spectrum and carries significant implications for precision oncology and the prevention of familial cancer.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval number: AEŞH-BADEK-2024-845, date: 25.09.2024) and conducted in accordance with the ethical standards of the Declaration of Helsinki.

Informed Consent: Written informed consent for genetic testing and publication of anonymized results was obtained from all participants before inclusion.

Declaration Regarding the Use of AI and AI-Assisted Technologies

During manuscript preparation, the authors used OpenAI's ChatGPT (GPT-5, San Francisco, CA, USA) as a language-support tool to improve the clarity, grammar, and fluency of the text. The tool was not used for data analysis, figure preparation, or the generation of scientific content. All outputs produced by the AI tool were carefully reviewed, edited, and verified by the authors. The authors take full responsibility for the integrity and accuracy of the entire manuscript.

Footnotes

Authorship Contributions

Surgical and Medical Practices: C.D.D., E.T., E.K., H.N.C.B., İ.K., Ö.Ö., M.D., N.G.L., H.K., Concept: C.D.D., H.K., Design: C.D.D., N.S.B., N.G.L., H.K., Data Collection or Processing: C.D.D., E.T., E.K., H.N.C.B., İ.K., Ö.Ö., M.D., N.G.L., H.K., Analysis or Interpretation: C.D.D., E.T., E.K., H.N.C.B., İ.K., Ö.Ö., N.G.L., Literature Search: C.D.D., N.G.L., Writing: C.D.D., E.T., E.K., H.N.C.B., İ.K., Ö.Ö., M.D., N.G.L., H.K.

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

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

References

1. Testa JR, Cheung M, Pei J, et al. Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet. 2011;43:1022-1025.
2. Wiesner T, Obenauf AC, Murali R, et al. Germline mutations in BAP1 predispose to melanocytic tumors. Nat Genet. 2011;43:1018-1021.
3. Popova T, Hebert L, Jacquemin V, et al. Germline BAP1 mutations predispose to renal cell carcinomas. Am J Hum Genet. 2013;92:974-980.
4. Rai K, Pilarski R, Cebulla CM, Abdel-Rahman MH. Comprehensive review of BAP1 tumor predisposition syndrome with report of two new cases. Clin Genet. 2016;89:285-294.
5. Walpole S, Pritchard AL, Cebulla CM, et al. Comprehensive study of the clinical phenotype of germline BAP1 variant-carrying families worldwide. J Natl Cancer Inst. 2018;110:1328-1341.
6. Carbone M, Ferris LK, Baumann F, et al. BAP1 cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MBAITs. J Transl Med. 2012;10:179.
7. Pilarski R, Byrne L, Carlo MI, et al. BAP1 Tumor Predisposition Syndrome. 2016 Oct 13 [Updated 2024 Dec 5]. In: Adam MP, Bick S, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2025.
8. Abdel-Rahman MH, Pilarski R, Cebulla CM, al. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. J Med Genet. 2011;48:856-859.
9. Louie BH, Kurzrock R. BAP1: not just a BRCA1-associated protein. Cancer Treat Rev. 2020;90:102091.
10. Lalloo F, Kulkarni A, Chau C, et al. Clinical practice guidelines for the diagnosis and surveillance of BAP1 tumour predisposition syndrome. Eur J Hum Genet. 2023;31:1261-1269.
11. Wang A, Papneja A, Hyrcza M, Al-Habeeb A, Ghazarian D. Gene of the month: BAP1. J Clin Pathol. 2016;69:750-753.
12. Ventii KH, Devi NS, Friedrich KL, et al. BRCA1-associated protein-1 is a tumor suppressor that requires deubiquitinating activity and nuclear localization. Cancer Res. 2008;68:6953-6962.
13. Harbour JW, Onken MD, Roberson ED, et al Frequent mutation of BAP1 in metastasizing uveal melanomas. Science. 2010;330:1410-1413.
14. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-424.
15. Daou S, Hammond-Martel I, Mashtalir N, et al. The BAP1/ASXL2 histone H2A deubiquitinase complex regulates cell proliferation and is disrupted in cancer. J Biol Chem. 2015;290:28643-28663.

16. Sharma A, Biswas A, Liu H, et al. Mutational landscape of the BAP1 locus reveals an intrinsic control to regulate the miRNA network and the binding of protein complexes in uveal melanoma. Cancers (Basel). 2019;11:1600.
17. Waters AJ, Brendler-Spaeth T, Smith D, et al. Saturation genome editing of BAP1 functionally classifies somatic and germline variants. Nat Genet. 2024;56:1434-1445.
18. Ismail IH, Davidson R, Gagné JP, Xu ZZ, Poirier GG, Hendzel MJ. Germline mutations in BAP1 impair its function in DNA double-strand break repair. Cancer Res. 2014;74:4282-4294.
19. Robertson AG, Shih J, Yau C, et al. Integrative analysis identifies four molecular and clinical subsets in uveal melanoma. Cancer Cell. 2017;32:204-220.e15.
20. Baumann F, Flores E, Napolitano A, et al. Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. Carcinogenesis. 2015;36:76-81.
21. Carbone M, Emri S, Dogan AU, et al. A mesothelioma epidemic in Cappadocia: scientific developments and unexpected social outcomes. Nat Rev Cancer. 2007;7:147-154.
22. Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. BAP1 and cancer. Nat Rev Cancer. 2013;13:153-159.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):240-244

Original Article

DOI: 10.4274/ahot.galenos.2025.2025-11-5

Treatment Outcomes and Prognostic Factors Affecting Survival in Thymic Carcinoma

Abdülkadir Koçanoğlu[1], Yakup Düzköprü[2], Esra Zeynelgil[3], Nesrin Gürçay[4], Serdar Karakaya[1], Doğan Yazılıtaş[3]

[1]*Health Sciences University Türkiye, Ankara Atatürk Sanatoryum Training and Research Hospital, Clinic of Medical Oncology, Ankara, Türkiye*
[2]*Aksaray Training and Research Hospital, Clinic of Medical Oncology, Aksaray, Türkiye*
[3]*Health Sciences University Türkiye, Etlik City Hospital, Clinic of Medical Oncology, Ankara, Türkiye*
[4]*Health Sciences University Türkiye, Ankara Atatürk Sanatoryum Training and Research Hospital, Clinic of Pathology, Ankara, Türkiye*

ABSTRACT

Aim: Treatment guidelines for thymic carcinoma, a rare cancer involving multiple clinical subgroups, are primarily derived from retrospective studies. We aimed to evaluate the frequency of histopathological subgroups, treatment modalities, clinicopathological characteristics, and to assess their correlation with the odds of survival of patients with thymic cancer.

Methods: A retrospective analysis was performed on data from 19 individuals monitored in outpatient oncology clinics following diagnosis of thymic carcinoma. Surgical history, margin status, sites of recurrence or metastasis, date of final follow-up, and date of death were among the clinical and demographic factors recorded and examined.

Results: For the whole group, the median overall survival (OS) was 20 months [95% confidence interval (CI): 2.25-37.75]. The median OS was ten months for individuals who were not operated on (95% CI: 7.43-12.56), compared with 68 months for operated patients (95% CI: 16.67-119.32) (p=0.010). The median OS for patients receiving mediastinal radiation was 68 months (95% CI: 15.67-120.32), compared with 8 months (95% CI: 3.19-12.80) for those not receiving RT. Although the difference did not reach statistical significance (p=0.064), patients with stage IV disease had a median OS of 14 months (95% CI: 6.45-21.55), whereas patients without stage IV disease had a median OS of 68 months (95% CI: 22.18-113.83).

Conclusion: Consistent with our findings, postoperative radiotherapy and surgical excision seem to be the most significant prognostic factors. Surgical options-including upfront surgery-and postoperative radiotherapy should be seriously considered in the treatment of thymic carcinoma.

Keywords: Thymic carcinoma, adjuvant radiotherapy, prognostic factors

Introduction

According to data from the World Health Organisation, thymic malignancies are uncommon cancers, with an incidence of 0.15-0.25 per 100,000 people. Histologically, they are classified into two major groups: thymomas and thymic carcinomas [1]. Roughly 20% of all thymic epithelial malignancies are thymic carcinomas, which have been reported to be more prevalent among Asia-Pacific [2]. In the United States, they are more common in men, and previous studies have reported a median age at diagnosis ranging from 54 to 65.5 years [3,4]. Squamous cell carcinoma (SCC) is the most frequently encountered histological subtype. Low-grade papillary adenocarcinoma, lymphoepithelial carcinoma, clear cell carcinoma, and basaloid carcinoma are further subtypes [5]. Thymomas are often diagnosed early and remain confined, in contrast to thymic carcinomas, which usually present with metastases. Given that total resection yields the most favorable survival outcomes, surgical management continues to be the preferred therapeutic option [6,7]. However, recommendations for metastatic disease are limited and mainly derived from small retrospective studies [8,9].

Several studies have demonstrated a survival benefit from postoperative radiotherapy (RT) in patients with thymic cancer

Address for Correspondence: Abdülkadir Koçanoğlu MD, Health Sciences University Türkiye, Ankara Atatürk Sanatoryum Training and Research Hospital, Clinic of Medical Oncology, Ankara, Türkiye
E-mail: kadirkocanoglu@hotmail.com **ORCID ID:** orcid.org/0000-0002-5579-2135
Received: 14.11.2025 **Accepted:** 04.12.2025 **Publication Date:** 12.12.2025

Cite this article as: Koçanoğlu A, Düzköprü Y, Zeynelgil E, Gürçay N, Karakaya S, Yazılıtaş D. Treatment outcomes and prognostic factors affecting survival in thymic carcinoma. Acta Haematol Oncol Turc. 2025;58(3):240-244

who had positive surgical margins or received a total resection [10-12]. A multicenter European analysis reported that the most pronounced survival improvement with postoperative RT was observed in disease stages III and IV, and that it also conferred a statistically significant benefit among individuals with positive surgical borders [12].

For unresectable thymic carcinoma, platinum-based chemotherapy (CT) regimens combined with agents such as paclitaxel, anthracyclines, or etoposide may be used; these combinations have shown survival benefits [13,14]. In locally advanced cases that respond to CT, surgery is an feasible option for further treatment [15]. For metastatic thymic carcinoma, carboplatin plus paclitaxel is the preferred first-line regimen [16]. One study reported that this combination resulted in a 36% objective response rate and a median progression-free survival of 7.5 months [17]. Other platinum-based regimens are also used in clinical practice. Thymic carcinoma is a rare malignancy with multiple pathological subtypes, and treatment recommendations are largely based on retrospective data. We aimed to evaluate the frequencies of histopathological subgroups, treatment modalities, and clinicopathological characteristics, and their associations with survival in individuals with thymic carcinoma.

Methods

Patient Population and Data Collection

Information about individuals with thymic carcinoma who attended the outpatient cancer clinics of our hospital between January 1, 2012, and October 1, 2022, was examined retrospectively. Participants were included if they were at least 18 years old, had thymic carcinoma confirmed by histopathology, had an Eastern Cooperative Oncology Group performance status of 0-1 at diagnosis, and had comprehensive follow-up information available in clinical and electronic records. Patients with a history of another primary malignancy were not considered eligible. Nineteen of the twenty-four thymic cancer patients who were screened during the study period met the inclusion criteria and were included in the analysis.

Data were obtained from hospital files and electronic medical records. Collected variables included diagnostic date, disease location, histological subtype, disease stage, receipt of RT, CT regimens, comorbidities, smoking and alcohol history, surgical history, margin status, date and site of recurrence or metastasis, treatments received after recurrence or metastasis, date of death and date of most recent follow-up.

The Ethics Committee of Health Sciences University Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital approved the study (approval number: 141/13; date: 04.07.2022). The tenets of the 1964 Declaration of Helsinki were adhered to when carrying out the research protocol.

Statistical Analysis

IBM SPSS statistics (version 22.0; IBM Corp., Armonk, NY, USA) was used to perform statistical analyses. Utilising descriptive statistics to summarise clinical and demographic data. Non-normally distributed variables were expressed as median (range). Counts and percentages were used to present numerical and categorical variables (n, %). To compare categorical variables, the chi-square test was used. Overall survival (OS) was defined as the time from diagnosis to death or to last follow-up. The Log-Rank test was utilised to compare groups, and the Kaplan-Meier method was employed for survival analysis. For all analyses, a p value of less than 0.05 was deemed statistically significant.

Results

Three women and sixteen men comprised the study's 19 patients. The median age at diagnosis was 55 years (range: 20-80 years). Fifteen patients (78.9%) had a history of smoking. Stage IV disease was identified in 57.9% of the individuals. Thirteen patients (68.4%) underwent surgical resection. Among them, 9 (47.4%) had R0 resection and 4 (21.1%) had R1 resection. Regarding histopathological subtypes, the most prevalent type was SCC, observed in 15 cases (78.9%). Of the 13 patients who received curative treatment, 7 (53.8%) experienced recurrence. Six individuals (31.6%) had metastases in their lymph nodes, which were the most prevalent site. Thirteen patients received mediastinal RT; of these, 12 (63.2%) received it as adjuvant therapy and one for palliative purposes. Among them, 5 were stage I-II, 2 were stage III, and 6 were stage IV. Six patients (31.6%) underwent combined CT and RT after R0 resection, whereas four patients (21.1%) received the combination following R1 resection. All 8 patients who received adjuvant CT were treated with carboplatin plus paclitaxel. In first-line treatment for metastatic disease, the most commonly used regimen was carboplatin plus paclitaxel (n=7, 36.8%), followed by the cisplatin, doxorubicin, and cyclophosphamide (PAC) regimen (n=5, 26.3%). For second-line treatment, PAC was again the most commonly administered regimen (n=5, 26.3%). Table 1 summarizes the patients' clinicopathological and demographic characteristics.

In terms of OS, the cohort's median OS was 20 months [95% confidence interval (CI): 2.25-37.75]. The median OS was 10 months (95% CI: 7.43-12.56) in non-operated patients, whereas it was 68 months (95% CI: 16.67-119.32) in those who received surgery (p=0.010). Patients who did not receive mediastinal RT had a median OS of 8 months (95% CI: 3.19-12.80), while those who got RT had an OS median of whereas those who received RT had a median OS of 68 months (95% CI: 15.67-120.32). According to tumor, node, metastasis staging staging at diagnosis, the median OS for patients with stage IV disease was 14 months (95% CI: 6.45-21.55), while the median OS for those without stage IV disease was 68 months (95% CI: 22.18-113.83). This difference was not statistically significant (p=0.064). Table 2 summarises, and Figure 1 depicts, the relationship between clinicopathological features and OS.

Table 1. Clinicopathological and demographic characteristics of the patients

Features	n (%)
Age (median, minimum-maximum)	55 (20-80)
Gender	
Female	3 (15.8%)
Male	16 (84.2%)
Smoking status	
Yes	15 (78.9%)
No	4 (21.1%)
ECOG Performance Status	
0	5 (26.3%)
1	14 (73.7%)
Stage at diagnosis (TNM)	
I	4 (21.1%)
II	1 (5.3%)
III	3 (15.8%)
IV	11 (57.9%)
Surgery	
Performed	13 (68.4%)
Not performed	6 (31.6%)
Type of resection	
R0	9 (47.4%)
R1	4 (21.1%)
Histopathological subtype	
Squamous cell carcinoma	15 (78.9%)
Adenocarcinoma	2 (10.5%)
Basaloid type	1 (5.3%)
Adenosquamous carcinoma	1 (5.3%)
Mediastinal RT	
Yes	13 (68.4%)
No	6 (31.6%)
Site of metastasis	
Lymph nodes	6 (31.6%)
Lung	1 (5.3%)
Multiple sites	4 (21.1%)
First-line CT regimen for metastatic disease	
Carboplatin+paclitaxel	7 (36.8%)
PAC	5 (26.3%)
Cisplatin+gemcitabine	1 (5.3%)
Second-line CT regimen for metastatic disease	
PAC	5 (26.3%)
Carboplatin+paclitaxel	1 (5.3%)
Gemcitabine	1 (5.3%)

ECOG: *Eastern Cooperative Oncology Group,* CT: *Chemotherapy,* RT: *Radiotherapy,* PAC: *Cisplatin, doxorubicin, and cyclophosphamide, TNM: T*umor, node, metastasis staging

Discussion

Thymic carcinoma is an uncommon and serious cancer that develops from the epithelial cells of the thymus, characterized by its heterogeneous histopathological features and a generally unfavorable prognosis [18,19]. Despite improvements in diagnostic imaging, surgical techniques, and multimodal treatment strategies, the optimal management of thymic carcinoma remains a subject of ongoing debate. In the present investigation, our goal was to evaluate the clinicopathological characteristics, treatment preferences, survival rates, and prognostic variables in individuals with thymic carcinoma.

In our study, of all patients, 84.2% of patients were male; however, male and female patients did not differ significantly in OS. Overwhelmingly predominant pathologic subtype was SCC (78.9%), with adenocarcinoma, basaloid thymic carcinoma, and adenosquamous carcinoma following closely behind. No significant difference in OS was detected among histological subtypes. Similarly, Petat et al. [9] reported SCC as the predominant histological type (67%), followed by undifferentiated carcinoma, basaloid carcinoma, sarcomatoid carcinoma, and adenocarcinoma, in their cohort.

In the same study, 50% of the patients had stage IV cancer, including 15% with stage IVa; the presence or absence of surgery was identified as the most important prognostic factor, even among those who underwent upfront surgery. The PAC regimen was preferred by 62% of patients and the carboplatin-paclitaxel regimen by 22%, with PAC being linked to better progression-free survival (p=0.001) [9]. In our cohort, 57.9% of patients presented with stage IV disease, and 31.5% had stage IVa disease at diagnosis. Median OS was 68 months for surgically treated patients versus 10 months for those not operated on (p=0.010), underscoring the prognostic importance of surgical resection in thymic carcinoma. Among patients receiving CT as a first-line treatment for metastases, 36.8% received carboplatin-paclitaxel and 26.3% received the PAC regimen; there was no discernible variation in survival between regimens.

In a large analysis of 462 patients with thymic carcinoma, mediastinal RT after surgery did not significantly improve OS among stage I-II patients who underwent complete tumor resection (p=0.14). In contrast, OS was significantly better

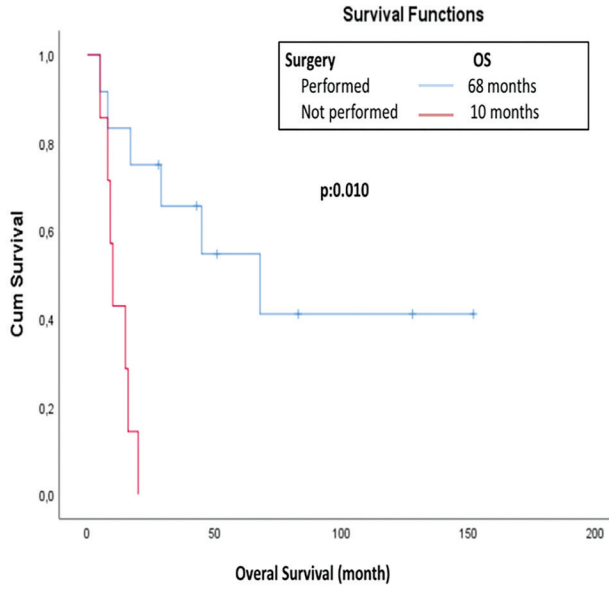


Figure 1. Kaplan-Meier curves of clinically significant prognostic factors (surgery and mediastinal RT)
RT: Radiotherapy, OS: Overal survival

Table 2. Univariate and multivariate analysis of prognostic factors for overall survival

Features	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p value	HR (95% CI)	p value
Gender Female Male	0.64 (0.17-2.33)	0.49		
Histopathological subtype Squamous cell carcinoma Adenocarcinoma Basaloid type Adenosquamous carcinoma	2.09 (0.44-9.92)	0.144		
Smoking status Yes No	2.64 (0.57-12.16)	0.213		
ECOG PS 0 1	4.18 (0.90-19.50)	0.069		
At diagnosis, stage IV? Yes No	3.21 (0.94-10.97)	0.064		
Surgery Performed Not performed	5.56 (1.5-20.64)	0.010	Ref 0.872 (0.41-18.75)	0.930
Type of resection R0 R1	2.53 (0.41-15.62)	0.317		
Treatment modalities Total excision+CT+RT Total excision+RT No total excision, CT+RT No total excision, CT only No surgery, palliative CT		0.054		
Mediastinal RT Yes No	0.15 (0.04-0.54)	0.004	Ref 6.01 (0.27-132.12)	0.255
Adjuvant CT Yes No	0.66 (0.19-2.33)	0.522		
First-line CT regimen for metastatic disease PAC Carboplatin+Paklitaksel Sisplatin+Gemsitabin		0.321		

CI: Confidence interval, ECOG PS: Eastern Cooperative Oncology Group Performance Status, CT: Chemotherapy, RT: Radiotherapy, PAC: Cisplatin, doxorubicin, and cyclophosphamide, HR: Hazard ratio

in stage III-IV patients with R0 resection (p=0.043) and in patients with residual disease after surgery (R1-2) (p=0.001). The authors reported that pathological stage, postoperative RT, and complete resection were independent predictors of survival [12]. Similarly, Nazzal et al. [20] analyzed data from patients with stage II thymic carcinoma and reported that adjuvant RT did not confer a survival benefit in early-stage R0-resected cases. In our study, the median OS was 8 months for those without mediastinal RT and 68 months for those who did receive mediastinal RT (p=0.004), suggesting a survival benefit associated with postoperative mediastinal RT. However, OS did not differ significantly by surgical margin status, likely because of the limited sample size and the relatively small proportion (21.1%) of R1-resected patients.

Previous studies have shown that disease stage at diagnosis is a crucial predictor of survival outcomes [12,21]. In our study, patients with stage IV disease had a median OS of 14 months, compared with 68 months for patients without stage IV disease. Although this difference did not reach statistical significance (p=0.064), which is likely due to the relatively small sample size, the numerical trend suggests a clinically meaningful impact of advanced stage on survival.

Study Limitations

Our study's retrospective design and relatively small patient population are its primary limitations. Because thymic carcinoma is rare, larger patient cohorts in multicentre prospective studies are needed to better define optimal treatment strategies and prognostic variables.

Conclusion

Thymic carcinoma is an infrequent malignancy with a challenging treatment course, and recommended treatment strategies are largely based on retrospective studies. As demonstrated in our study, postoperative RT and the extent of surgical resection are the most influential prognostic factors. Accordingly, management decisions should include a thorough evaluation of surgical strategies, including upfront surgery and postoperative RT.

Ethics

Ethics Committee Approval: The Ethics Committee of Health Sciences University Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital approved the study (approval number: 141/13; date: 04.07.2022).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

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

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

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

Referances

1. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. Introduction to the 2015 World Health Organization classification of tumors of the lung, pleura, thymus, and heart. J Thorac Oncol. 2015;10:1240-1242.
2. Hsu CH, Chan JK, Yin CH, Lee CC, Chern CU, Liao CI. Trends in the incidence of thymoma, thymic carcinoma, and thymic neuroendocrine tumor in the United States. PLoS One. 2019;14:e0227197.
3. Roden AC, Yi ES, Cassivi SD, Jenkins SM, Garces YI, Aubry MC. Clinicopathological features of thymic carcinomas and the impact of histopathological agreement on prognostical studies. Eur J Cardiothorac Surg. 2013;43:1131-1139.
4. Bakhos CT, Salami AC, Kaiser LR, Petrov RV, Abbas AE. Thymic neuroendocrine tumors and thymic carcinoma: demographics, treatment, and survival. Innovations (Phila). 2020;15:468-474.
5. Singh G, Singh A, Dave R. An update on WHO classification of thoracic tumours 2021-newly described entities and terminologies. Journal of Clinical and Diagnostic Research. 2023;17:EE01-EE05.
6. Wei Y, Gu Z, Shen Y, et al. Preoperative induction therapy for locally advanced thymic tumors: a retrospective analysis using the ChART database. J Thorac Dis. 2016;8:665-672.
7. Ahmad U, Yao X, Detterbeck F, et al. Thymic carcinoma outcomes and prognosis: results of an international analysis. J Thorac Cardiovasc Surg. 2015;149:95-100, 101.e1-2.
8. Ko R, Shukuya T, Okuma Y, et al. Prognostic factors and efficacy of first-line chemotherapy in patients with advanced thymic carcinoma: a retrospective analysis of 286 patients from NEJ023 study. Oncologist. 2018;23:1210-1217.
9. Petat A, Dansin E, Calcagno F, et al. Treatment strategies for thymic carcinoma in a real-life setting. Insights from the RYTHMIC network. Eur J Cancer. 2022;162:118-127.
10. Omasa M, Date H, Sozu T, et al. Postoperative radiotherapy is effective for thymic carcinoma but not for thymoma in stage II and III thymic epithelial tumors: the Japanese association for research on the thymus database study. Cancer. 2015;121:1008-1016.
11. Shepherd A, Riely G, Detterbeck F, et al. Thymic carcinoma management patterns among international thymic malignancy interest group (ITMIG) physicians with consensus from the thymic carcinoma working group. J Thorac Oncol. 2017;12:745-751.
12. Rimner A, Ahmad U, Lobaugh SM, et al. Postoperative radiation therapy for thymic carcinoma: an analysis of the international thymic malignancy interest group/european society of thoracic surgeons database. J Thorac Oncol. 2024;19:626-635.
13. Fornasiero A, Daniele O, Ghiotto C, et al. Chemotherapy for invasive thymoma. A 13-year experience. Cancer. 1991;68:30-33.
14. Giaccone G, Ardizzoni A, Kirkpatrick A, Clerico M, Sahmoud T, van Zandwijk N. Cisplatin and etoposide combination chemotherapy for locally advanced or metastatic thymoma. A phase II study of the European organization for research and treatment of cancer lung cancer cooperative group. J Clin Oncol. 1996;14:814-820.
15. Ma WL, Lin CC, Hsu FM, et al. Clinical outcomes for patients with thymoma and thymic carcinoma after undergoing different front-line chemotherapy regimens. Cancer Med. 2022;11:3445-3456.
16. Lemma GL, Lee JW, Aisner SC, et al. Phase II study of carboplatin and paclitaxel in advanced thymoma and thymic carcinoma. J Clin Oncol. 2011;29:2060-2065.
17. Hirai F, Yamanaka T, Taguchi K, et al. A multicenter phase II study of carboplatin and paclitaxel for advanced thymic carcinoma: WJOG4207L. Ann Oncol. 2015;26:363-368.
18. Travis WD, Brambilla E, Nicholson AG, et al. The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol. 2015;10:1243-1260.
19. Engels EA. Epidemiology of thymoma and associated malignancies. J Thorac Oncol. 2010;5:S260-S265.
20. Nazzal A, Yayan J, Biancosino C, Tabatabaei SV, Hekmat K. Effectiveness of adjuvant chemo- and radiotherapy in thymic carcinoma stage II: a systematic review and meta-analysis. Cancer Control. 2024;31:10732748241292781.
21. Gao Y, Fan X, Hua C, et al. Failure patterns for thymic carcinoma with completed resection and postoperative radiotherapy. Radiother Oncol. 2023;178:109438.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):245-250

DOI: 10.4274/ahot.galenos.2025.2025-10-1

Original Article

Nodular Lymphocyte Predominant Hodgkin Lymphoma: A Single Center Experience

Kübra Oral, Ayşe Uysal

Fırat University Faculty of Medicine, Department of Hematology, Elazığ, Türkiye

ABSTRACT

Aim: Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare subtype of lymphoma classified within Hodgkin lymphomas. Often the diagnosis is made at an early stage. Treatment varies according to the stage of the disease and the underlying prognostic factors. Because of cluster of differentiation 20 expression, rituximab-based agents can be used for treatment. In this study, we aimed to present the demographic, treatment, and survival data of patients with NLPHL in the context of the existing literature.

Methods: In our study, demographic characteristics, laboratory findings, disease stage, and treatments administered to patients diagnosed with NLPHL between 2012 and 2024 were evaluated.

Results: Of the 13 patients enrolled in the study, seven were male, and the median age was 44 years. Of the patients, eight were in the early stage and five were in the advanced stage. One patient had liver involvement, three had splenic involvement, seven had subdiaphragmatic involvement, and four had a bulky mass. Rituximab was added to the treatment regimens of six patients. Progression was observed in two patients. One patient died from Coronavirus disease 2019-related pneumonia. While the 2-year and 5-year overall survival of our patients were both 92%, progression-free survival was 100% at 2 years and 45.5% at 5 years.

Conclusion: NLPHL is a rare condition that, despite its generally favorable prognosis, requires effective treatment because of the risk of recurrence and transformation into diffuse large B-cell lymphoma (DLBCL). Although data on the transformation of subdiaphragmatic involvement into DLBCL exist in the literature, information on disease progression is lacking.

Keywords: Nodular lymphocyte-predominant hodgkin lymphoma, overall survival, progression free survival

Introduction

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare subtype, accounting for 5%-13% of all patients with Hodgkin lymphoma (HL) [1]. NLPHL occurs predominantly in males, accounting for 75% of patients. NLPHL usually occurs between the ages of 30 and 40 [2,3].

In NLPHL, the malignant cells are called lymphocyte-predominant (LP) cells. Although Reed-Sternberg cells and LP cells seen in classical HL originate from germinal centre cells, they show morphological differences [4]. Unlike Reed Sternberg cells, LP cells express cluster of differentiation 20 (CD20) from B cell markers, while they do not express CD15 and CD30 [5].

Most patients have localized disease with a slowly progressive course. It usually involves peripheral lymph nodes. Central lymph node involvement and extranodal involvement are rarely observed. Bone marrow involvement is observed in 1-2%, liver involvement in 2-3%, and spleen involvement in 5% [1].

Because the pathological and clinical features of the disease differ from classical HL, the treatment approach also differs. The ideal treatment for NLPHL has not yet been determined. Depending on disease stage and patient risk factors, treatment options include monotherapy, anti-CD20 antibody therapy, radiotherapy (RT) alone, or combination therapy involving anti-CD20 antibody with or without RT plus chemotherapy regimens such as doxorubicin, bleomycin, vinblastine, and dacarbazine

Address for Correspondence: Kübra Oral MD, Fırat University Faculty of Medicine, Department of Hematology, Elazığ, Türkiye
E-mail: kubraaltun88@gmail.com **ORCID ID:** orcid.org/0000-0001-6461-5811
Received: 06.10.2025 **Accepted:** 04.12.2025 **Publication Date:** 12.12.2025

Cite this article as: Oral K, Uysal A. Nodular lymphocyte predominant hodgkin lymphoma: a single center experience. Acta Haematol Oncol Turc. 2025;58(3):245-250

(ABVD) or cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). Treatment selection should primarily take into account effectiveness and toxicity, since the disease progresses slowly and has a good prognosis [6]. Although this disease is associated with longer survival than lymphoma, it also has higher rates of secondary malignancies and treatment-related deaths; therefore, patient-specific treatment selection is important [7].

In this study, we aimed to contribute survival data to the literature by evaluating treatments given, demographic characteristics, clinical and laboratory values, stage, and prognostic risk factors of patients we followed with an NLPHL diagnosis.

Methods

The study included 13 patients diagnosed with NLPHL who were followed at the Fırat University Faculty of Medicine Adult Hematology Clinic between December 2012 and December 2024. Ethics committee approval, was obtained from the Fırat University Rectorate Non-Interventional Scientific Research Ethics Committee (approval no: 2025/06-48, date: 24/04/2025). Since the data were collected from medical records without revealing the identities of the participants and the study was retrospective, consent was not obtained from the patients.

The patients' data were obtained from the patient files and the hospital information system. The following variables were evaluated: Age; gender; survival status; mean follow-up time; recurrence status; stage; international prognostic index (IPI) score in advanced-stage (stage III-IV) disease; presence of adverse risk factors in early-stage (stage I-II) disease; subdiaphragmatic involvement; liver and spleen involvement; presence of bulky disease; treatment response; haemogram and biochemical parameters; overall survival (OS); and progression-free survival (PFS) were evaluated.

For early-stage patients, mediastinal mass, extranodal disease, involvement of ≥ three nodes, and elevated sedimentation rate (>30 mm/h in the presence of B symptom, >50 mm/h in the absence of B symptom) were considered adverse risk factors, and lymphocyte counts below 1,000/mm³ were considered indicative of lymphopenia.

Patients in the early stage received only RT, and patients in both early and advanced stages received chemotherapy ± Rituximab ± RT, according to the follow-up physician's decision. The time from diagnosis to death or last follow-up was defined as OS, and the time from remission after treatment to relapse or death was defined as PFS. Complete response (CR) was defined as the absence of disease symptoms, a positive positron emission tomography (PET) at baseline or a negative PET in those with any residual mass, or absence of involvement on subsequent biopsies when initial bone marrow involvement was present.

Patients' responses were evaluated according to the Lugano response criteria [8]. Recurrences occurring within 12 months after treatment were considered early recurrences.

Statistical Analysis

Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 25.0 (SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as n and % for categorical variables and median (minimum-maximum) for continuous variables. The Kaplan-Meier method was used to determine survival times; $p<0.05$ was considered statistically significant.

Results

The median age of patients is 44 years (range 18-73 years); 53% are male and 46% are female. Of the patients, five were diagnosed at stage I and three at stage II; in total, eight were diagnosed at an early stage. Among patients with early-stage disease, two had a negative risk factor, whereas six had none. At diagnosis, five patients had advanced disease. According to IPI scores for patients with advanced-stage disease, two patients were classified as low risk and three were classified as intermediate risk. Liver involvement was present in only one patient, splenic involvement in three, subdiaphragmatic involvement in seven, and bulky disease in four (Table 1).

Table 2 shows the patients' laboratory results at the time of diagnosis; three patients had anemia and one patient had lymphopenia.

As first-line treatment, three patients received RT only, two received ABVD, two received ABVD+RT, three received R-ABVD, one received R-ABVD+RT, and two received R-CHOP therapy. Progressive disease was present in two patients. In one of these patients, an early relapse occurred after first-line treatment despite an initial CR; autologous Hematopoietic stem cell transplantation was performed after Rituximab, cisplatin, cytarabine, dexamethasone, and the patient is still being followed in remission. The other patient was treated with rituximab-bendamustine; rituximab, gemcitabine, dexamethasone, cisplatin; and R2 (rituximab, lenalidomide), due to refractory disease. Eleven patients achieved CR after first-line treatment, while two patients experienced progression. One of the two patients with progression was stage II with a bulky lesion, and the other was stage IV without a bulky lesion; subdiaphragmatic involvement was detected in both patients. Table 3 presents information on the clinical course of the patients.

During a median follow-up of 60 months (range, 8-144 months), median OS was not reached, and the 2- and 5-year OS rates were both 92.5%. Median PFS was 54.0 months (95% confidence interval: 0.0-112.26), and 100% 5-year PFS with 2- and 5-year PFS being 45.5%. Twelve of the patients are still alive, while one patient is deceased. An ex-patient died of Coronavirus disease (COVID)-related pneumonia while in CR after first-line treatment.

Discussion

NLPHL is a rare subtype of HL with a slow course, with a good prognosis. Patients diagnosed with NLPHL have been shown

Table 1. Characteristic features of patients

Variables	n=13	%
Age		
Median (min-max)	44.0 (18-73)	
Gender		
Male	7	53.8
Female	6	46.2
Stage		
Stage-1	5	38.5
Stage-2	3	23.1
Stage-3	2	15.4
Stage-4	3	23.1
Advanced stage IPI score		
1	1	20.0
2	1	20,0
3	3	60.0
Early stage negative risk factor		
No	6	75.0
Yes	2	25.0
L involvement		
No	12	92.3
Yes	1	7.7
Spleen involvement		
No	10	76.9
Yes	3	23.1
Subdiaphic involvement		
No	6	46.2
Yes	7	53.8
Bulky disease		
No	9	69.2
Yes	4	30.8
1st line treatment		
RT only	3	23.1
ABVD	2	15.4
ABVD+RT	2	15.4
R-ABVD	3	23.1
R-ABVD+RT	1	7.7
R-CHOP	2	15.4
Relapse		
No	11	84.6
Yes	2	15.4
Mortality		
Alive	12	92.3
Ex	1	7.7

RT: Radiotherapy, ABVD: Doxorubicin, bleomycin, vinblastine, dacarbazine, R-CHOP: Rituximab, cyclophosphamide, adriamycin, vincristine, prednisone, Li: Liver, IPI: International prognostic index

to account for 5-13% of HL cases [9,10]. Among our patients, those diagnosed with NLPHL constitute 9% of all patients with HL. From this perspective, it appears consistent with the literature. In this subtype of lymphoma, which is more common in males aged 30-40 years, 53% of our patients were male, and the median age in our study was 44 years [2]. Although our study is compatible with the literature in terms of sex distribution, with a higher prevalence in males, the patients' mean age was slightly higher than that reported in the literature.

The clinical course of NLPHL is indolent, and patients are usually in an early stage at initial diagnosis. In our study, 61% of our patients were early-stage, which is consistent with the literature [11]. Despite a slow course and good prognosis, transformation to diffuse large B-cell lymphoma (DLBCL) is observed during patient follow-up. The risk of transformation to DLBCL is higher in patients with a bulky mass, subdiaphragmatic disease, and splenic involvement [12]. In our study, a bulky mass was observed in 30% (n=4) of patients, subdiaphragmatic involvement in 53% (n=7) of patients, and spleen involvement in 23% (n=3) of patients; no transformation to DLBCL was observed in any of our patients. Although no transformation was observed in our

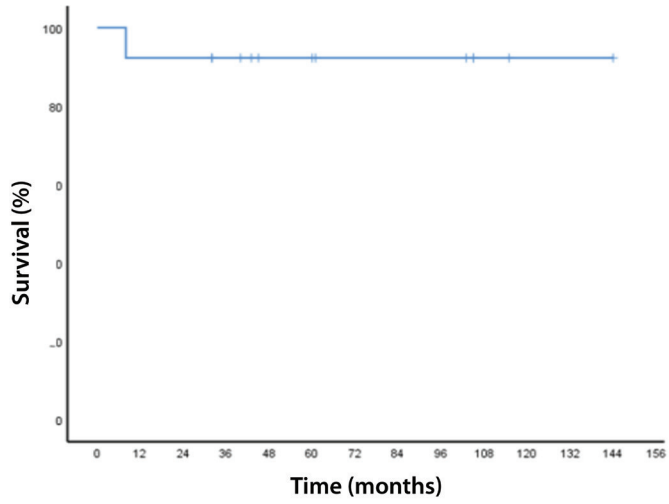


Figure 1. Overall Survival Curve

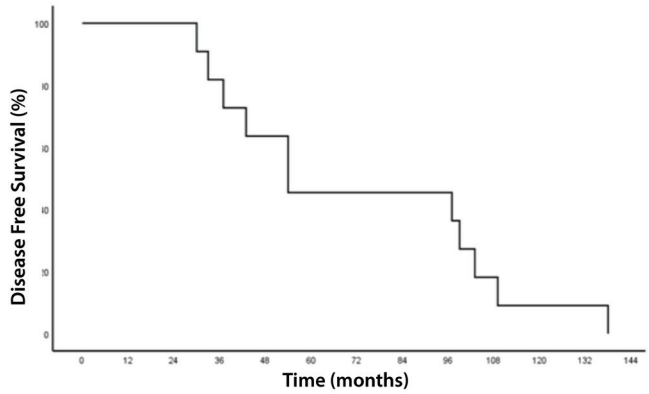


Figure 2. Progression-free Survival Curve

Table 2. Laboratory results at the time of diagnosis of patients

Patient	WBC	HB	PLT	LYM	NEU	ALB	ALT	CR	LDH	SEDIM	CRP
1	4050	13.1	283000	1440	1660	4.3	42	1.2	220	11	11
2	6680	10.1	406000	1460	4430	4.8	11	0.54	167	11	3
3	4230	14.3	199000	1020	2270	4.2	50	0.6	234	7	3
4	8920	15.4	339000	1190	6990	4.8	38	0.82	283	20	3
5	5180	15.9	254000	1210	3110	3.9	28	0.78	226	7	3
6	5990	15.4	230000	1330	3920	4.4	26	0.49	287	7	3
7	8950	11.6	210000	1100	6930	4.3	19	0.5	204	35	3
8	8240	12.6	219000	1690	5530	3.8	13	0.65	254	66	32
9	5370	13.8	210000	990	3370	4.6	15	0.84	276	18	8
10	6990	14	189000	1240	3490	4	19	0.61	218	11	3
11	8260	12.6	330000	2930	4700	4.6	18	0.44	245	21	4
12	6740	13.9	256000	1370	4660	4.5	28	0.7	159	11	3
13	4160	10.5	329000	1380	2500	4.4	17	0.47	192	8	3

WBC: White blood cell, HB: Haemoglobin, PLT: Platelet, LYM: Lymphocyte, NEU: Neutrophil, ALB: Albumin, ALT: Alanine aminotransferase, CR: Creatinine, LDH: Lactate dehydrogenase, SEDIM: Sedimentation rate, CRP: C-reactive protein

Table 3. Clinical course of patients

Patient	Age	Gender	Stage	Liver	Spleen	Subdiaphragmatic invol	Bulky disease	1. Line treatment	Mortality
1	45	Male	3	No	No	Yes	No	R-ABVD	alive
2	29	Female	3	No	No	Yes	No	R-ABVD+RT	alive
3	51	Male	2	No	No	Yes	Yes	ABVD	alive
4	38	Male	2	No	No	No	Yes	R-ABVD	alive
5	29	Male	4	Yes	Yes	Yes	Yes	R-ABVD	Ex
6	32	Male	1	No	No	No	No	RT only	alive
7	18	Female	2	No	No	No	No	ABVD	alive
8	68	Female	4	No	Yes	Yes	No	R-CHOP	alive
9	73	Male	4	No	Yes	Yes	Yes	R-CHOP	alive
10	56	Female	1	No	No	No	No	ABVD+RT	alive
11	37	Female	1	No	No	No	No	RT only	alive
12	66	Male	1	No	No	Yes	No	RT only	alive
13	44	Female	1	No	No	No	No	ABVD+RT	alive

RT: Radiotherapy, ABVD: Doxorubicin, bleomycin, vinblastine, dacarbazine, R-CHOP: Rituximab, cyclophosphamide, adriamycin, vincristine, prednisone

patients, subdiaphragmatic involvement in two patients who progressed drew our attention.

In the literature, splenic involvement is observed in 5% of patients, whereas hepatic involvement is observed in 2-3% [1]. In our patient group, splenic involvement was observed in 23% (n=3) of patients, and hepatic involvement in 7% (n=1). We attribute the discordance between our data and the literature to the small number of patients.

In this rare subtype of lymphoma, the prognosis is usually good and survival rates are high. Eichenauer et al. [13] evaluated 85 early-stage NLPHL patients and found 5-year PFS and OS to be 90% and 100%, respectively. According to the study conducted by Xing et al. [14] involving 42 patients with advanced-stage NLPHL, 5-year OS and 10-year OS were 89% and 86%, respectively; 5-year PFS and 10-year PFS were 72% and 63%, respectively. In the study by Lazarovici et al. [15] involving 314 patients, 10-year PFS was 44% and 10-year OS was 94%. Although results for survival in studies of NLPHL differ in the literature, we believe these discrepancies are attributable to differences in disease stage (some studies included early-stage patients, others late-stage), sample size, and adverse risk factors. In our study, the median 5-year OS was 92% during the 60-month follow-up period, while 2-year PFS was 100% and 5-year PFS was 45%.

Due to the rarity of NLPHL, there is no standardized method of treatment, as studies to guide treatment selection are retrospective. Defining the optimal treatment method remains a significant challenge given that studies are generally small and single-center. When we examine studies in the literature on early-stage disease, Chen et al. [16], in a study of 113 patients, found that the addition of chemotherapy to RT did not improve PFS or OS compared with RT alone. Eichenauer et al. [17] compared combined modality therapy (CMT), extended field radiotherapy (EF-RT), involved field (IF)-RT, four-week standard-dose rituximab treatment, and found that rituximab treatment alone increased the risk of relapse, whereas CMT was equivalent to EF-RT and IF-RT in disease control. They emphasized that IF-RT would be the appropriate treatment for Stage IA patients because of concerns regarding toxicity [17]. Among other studies of treatment for early-stage NLPHL, Savage et al. [18] conducted a study of 88 patients, comparing 32 who received RT alone with 56 who received ABVD, found that both OS (93% vs. 85%) and PFS (93% vs. 66%) rates were higher in the ABVD arm during the 10-year follow-up period. Despite these data, it is difficult to interpret differences among treatment methods and regimens because adverse risk factors were not assessed in these early-stage patient populations treated with each approach. In our study, three patients with Stage I disease received only RT. In Stage I, two patients also received ABVD+RT. In stage 2, two patients were given ABVD and one patient was given R-ABVD. Only two of our early-stage patients had adverse risk factors, and no transformation to DLBC, relapse, or death occurred during follow-up.

Data on the optimal treatment of advanced-stage NLPHL are also limited, as this rare subtype of lymphoma accounts for only 20% of advanced-stage cases. Xing et al. [14] compared advanced-stage NLPHL with classical HL; both groups received ABVD-like chemotherapy and reported that, although OS was similar in both groups, NLPHL patients had a higher recurrence rate than patients with classical HL. Similarly, in their study evaluating treatment outcomes of classical HL and NLPHL treated with ABVD, Ames et al. [19] reported that, among eight patients with stage III-IV NLPHL treated with ABVD, the 5-year recurrence rate was over 50%, and they had the highest recurrence rate compared with all groups (early-advanced-stage HL, early-stage NLPHL). The PFS of patients with advanced NLPHL was lower than that of patients with early-stage NLPHL and of patients with classical HL at early and advanced stages (47% vs. 97%, 85%, and 74%) [19]. According to the results of R-CHOP treatment in NLPHL conducted by Fanale et al. [20] with 59 patients in both early and advanced stages, 27 patients received R-CHOP and the estimated 5 and 10-year PFS during a median follow-up period of 6.7 years was found to be 88.5% and 59.3%, respectively, and among the patients who received systemic treatment, the PFS of those who received R-CHOP was shown to be better compared to all other regimens. Consistent with these studies, R-CHOP therapy is more effective than ABVD-based therapies for patients with advanced-stage disease. In our study, two of the patients with advanced disease received R-ABVD, two received R-CHOP, and one received R-ABVD+RT. While our patient who received R-ABVD achieved CR after treatment, another of our patients died from COVID pneumonia, and one of our patients who received R-CHOP therapy was switched because of refractoriness to treatment.

Study Limitations

The limitations of our study are that it is based on a single center; that data on the regions in which patients received RT were not available; and that survival data by stage could not be calculated because of the small number of patients.

Conclusion

As a result, demographic information, treatment methods, and survival data of this extremely rare group of patients were reported in the literature. Although data on the transformation of subdiaphragmatic involvement into DLBCL exist in the literature, we believe that it will lead to further studies in this regard, since information on disease progression is lacking.

Ethics

Ethics Committee Approval: Ethics committee approval, was obtained from the Fırat University Rectorate Non-Interventional Scientific Research Ethics Committee (approval no: 2025/06-48, date: 24/04/2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: K.O., A.U., Concept: K.O., Design: K.O., Data Collection or Processing: K.O., A.U., Analysis or Interpretation: K.O., Literature Search: K.O., A.U., Writing: K.O., A.U.

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

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

References

1. Salvaris RT, Allanson BM, Collins G, Cheah C. Nodular lymphocyte-predominant Hodgkin lymphoma: advances in disease biology, risk stratification, and treatment. Haematologica. 2024;109:3476-3487.
2. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. Blood. 2006;107:265-276.
3. Berber İ, Sarıcı A. Epidemiology and etiology in hodgkin lymphoma. 1st edition. Kaya E. Ankara: Türkiye Klinikleri; 2020. p.1-5.
4. Brune V, Tiacci E, Pfeil I, et al. Origin and pathogenesis of nodular lymphocyte-predominant Hodgkin lymphoma as revealed by global gene expression analysis. J Exp Med. 2008;205:2251-2268.
5. Al-Mansour M, Connors JM, Gascoyne RD, Skinnider B, Savage KJ. Transformation to aggressive lymphoma in nodular lymphocyte-predominant Hodgkin's lymphoma. J Clin Oncol. 2010;28:793-799.
6. Eichenauer DA, Engert A. How I treat nodular lymphocyte-predominant Hodgkin lymphoma. Blood. 2020;136:2987-2993.

7. Eichenauer DA, Plütschow A, Fuchs M, et al. Long-term follow-up of patients with nodular lymphocyte-predominant hodgkin lymphoma treated in the HD7 to HD15 trials: a report from the German Hodgkin Study Group. J Clin Oncol. 2020;38:698-705.
8. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol. 2014;32:3059-3068.
9. Swerdlow SH, Campo E, Harris NL, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: International agency for research on cancer; 2008.
10. Juntikka T, Malila N, Ylöstalo T, Merikivi M, Jyrkkiö S. Epidemiology of classic and nodular lymphocyte predominant hodgkin lymphoma in Finland in 1996-2015. Acta Oncol. 2020;59:574-581.
11. Binkley MS, Flerlage JE, Savage KJ, et al. International prognostic score for nodular lymphocyte-predominant hodgkin lymphoma. J Clin Oncol. 2024;42:2271-2280.
12. Fan Z, Natkunam Y, Bair E, Tibshirani R, Warnke RA. Characterization of variant patterns of nodular lymphocyte predominant hodgkin lymphoma with immunohistologic and clinical correlation. Am J Surg Pathol. 2003;27:1346-1356.
13. Eichenauer DA, Kreissl S, Bühnen I, et al. PET-2-guided escalated BEACOPP for advanced nodular lymphocyte-predominant Hodgkin lymphoma: a subgroup analysis of the randomized German Hodgkin Study Group HD18 study. Ann Oncol. 2021;32:807-810.
14. Xing KH, Connors JM, Lai A, et al. Advanced-stage nodular lymphocyte predominant Hodgkin lymphoma compared with classical Hodgkin lymphoma: a matched pair outcome analysis. Blood. 2014;123:3567-3573.
15. Lazarovici J, Dartigues P, Brice P, et al. Nodular lymphocyte predominant Hodgkin lymphoma: a Lymphoma Study Association retrospective study. Haematologica. 2015;100:1579-1586.
16. Chen RC, Chin MS, Ng AK, et al. Early-stage, lymphocyte-predominant Hodgkin's lymphoma: patient outcomes from a large, single-institution series with long follow-up. J Clin Oncol. 2010;28:136-141.
17. Eichenauer DA, Plütschow A, Fuchs M, et al. Long-term course of patients with stage IA nodular lymphocyte-predominant Hodgkin lymphoma: a report from the German Hodgkin study group. J Clin Oncol. 2015;33:2857-2862.
18. Savage KJ, Skinnider B, Al-Mansour M, Sehn LH, Gascoyne RD, Connors JM. Treating limited-stage nodular lymphocyte predominant Hodgkin lymphoma similarly to classical Hodgkin lymphoma with ABVD may improve outcome. Blood. 2011;118:4585-4590.
19. Ames J, Maganti M, Monteith BE, et al. Outcomes of nodular lymphocyte predominant hodgkin lymphoma (NLPHL) vs. classical hodgkin lymphoma (cHL) at princess margaret cancer centre. Blood. 2015;126:3863-3863.
20. Fanale MA, Cheah CY, Rich A, et al. Encouraging activity for R-CHOP in advanced stage nodular lymphocyte-predominant Hodgkin lymphoma. Blood. 2017;130:472-477.

AHOT Acta Haematologica Oncologica Turcica

Acta Haematol Oncol Turc 2025;58(3):251-255

Case Report

DOI: 10.4274/ahot.galenos.2025.2025-4-2

The Role of Radiotherapy in Myeloid Sarcoma: A Case Report and Review of the Literature

Senay Mutaf[1], Zeynep Güral[2], Ant Uzay[3], Çağla Şafak Karaoğlan Yıldırım[4], Fulya Ağaoğlu[2]

[1]*Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Department of Radiation Oncology, İstanbul, Türkiye*
[2]*Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Atakent Hospital, Department of Radiation Oncology, İstanbul, Türkiye*
[3]*Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Atakent Hospital, Department of Hematology, İstanbul, Türkiye*
[4]*University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of Pathology, İstanbul, Türkiye*

ABSTRACT

Myeloid sarcoma (MS) is a rare tumor characterized by the extramedullary proliferation of immature granulocytic cells. It can occur as a primary disease or as a manifestation of acute myeloid leukemia, with an incidence of three to five percent in patients diagnosed with the condition. This report presents a thirty-seven-year-old female patient with acute monoblastic leukemia (French-American-British classification, subtype M5) who achieved complete remission after induction therapy, and allogeneic bone marrow transplantation. Six-teen months after achieving remission, the patient experienced a relapse in the pituitary gland, which was initially misdiagnosed as a pituitary adenoma. Following gross total surgical resection, the diagnosis of MS was confirmed. Adjuvant craniospinal radiotherapy and systemic therapy with azacitidine and venetoclax led to sustained remission without further complications. This case highlights the diagnostic and therapeutic challenges of MS, particularly in its rare presentation involving the pituitary gland. Radiotherapy, in combination with systemic treatment, played a crucial role in the management of this relapse. The findings emphasize the importance of a multidisciplinary approach in managing rare cases of MS to optimize patient outcomes.

Keywords: Acute myeloid leukemia, myeloid sarcoma, craniospinal radiotherapy, pituitary relapse

Introduction

Myeloid sarcoma (MS) is a rare hematologic malignancy characterized by the uncontrolled proliferation of immature granulocytic cells in extramedullary tissues. It may occur *de novo*, represent an extramedullary manifestation of acute myeloid leukemia (AML), or develop following hematopoietic stem cell transplantation. In the literature, it is also referred to as granulocytic sarcoma, chloroma, or extramedullary myeloid tumor.

The reported incidence of isolated *de novo* MS is approximately 2 per 100,000 in adults and 0.7 per 1,000,000 in children. When presenting concurrently with AML, the frequency ranges between 2-9% in adults and 10.9-23.3% in pediatric patients. In the post-transplant remission setting, its incidence has been reported between 5% and 12%.

MS most commonly involves the skin, lymph nodes, soft tissues, and bones, while central nervous system (CNS) localization is exceedingly rare and typically does not cause parenchymal damage. Østgaard et al. [1] found that MS was located in the craniospinal system in only 0.4% of AML patients. Pituitary involvement represents only a very small fraction of CNS cases, with fewer than 10 cases of pituitary or sellar region MS described in the literature to date (10, 12, 20).

In this report, we present a rare case of pituitary relapse of MS occurring after allogeneic hematopoietic stem cell transplantation in a female patient with AML and discuss its diagnostic and therapeutic aspects in the context of current literature.

Address for Correspondence: Senay Mutaf MD, Acıbadem Mehmet Ali Aydınlar University Faculty of Medicine, Department of Radiation Oncology, İstanbul, Türkiye
E-mail: sny.mutaf@gmail.com **ORCID ID:** orcid.org/0000-0003-4921-554X
Received: 25.04.2025 **Accepted:** 07.10.2025 **Epub:** 30.10.2025 **Publication Date:** 12.12.2025

Cite this article as: Mutaf S, Güral Z, Uzay A, Karaoğlan Yıldırım ÇŞ, Ağaoğlu F. The role of radiotherapy in myeloid sarcoma: a case report and review of the literature. Acta Haematol Oncol Turc. 2025;58(3):251-255

Case Report

A 37-year-old female patient presented with gingival bleeding, fatigue, and skin rash. Hematological analysis revealed a hemoglobin level of 9 g/dL, leukocyte count of 300,000/µL, and platelet count of 34 k/µL. Due to gingival hypertrophy, AML-M5 was suspected, and subsequent bone marrow biopsy confirmed acute monoblastic leukemia with the feline mcdonough sarcoma-like tyrosine kinase 3/internal tandem duplication (FLT3/ITD) mutation. The patient underwent induction therapy consisting of continuous administration of cytarabine for seven days, in combination with idarubicin during the first three days, followed by midostaurin administered from days 8 to 21. Following confirmation of the diagnosis with bone marrow biopsy results, consolidation therapy was initiated with high-dose cytarabine, complemented by midostaurin administered during days 8 to 21 of each cycle. The patient achieved complete remission with minimal residual disease (MRD) negativity. After one month, the patient underwent myeloablative conditioning and received an allogeneic bone marrow transplant from her sibling. At 1, 3, 6, and 12 months post-transplantation, the patient exhibited 100% chimerism and maintained MRD-negative status. No graft-versus-host disease, cytomegalovirus infection, or other complications were observed. Six-teen months after treatment completion, the patient presented with somnolence, visual impairment, polydipsia, and fatigue. Hematological, hormonal, and biochemical analyses revealed normal results. However, magnetic resonance imaging (MRI) of the pituitary gland revealed a 19×17 mm diffuse contrast-enhancing lesion extending from the stalk level to the superior optic chiasm, initially diagnosed as a pituitary adenoma (Figure 1). In March 2023, gross total resection was performed, and the diagnosis was confirmed of MS (Figure 2). At this juncture, a bone marrow biopsy demonstrated remission, 100% chimerism, and a negative FLT3/ITD mutation. Postoperative cranial MRI

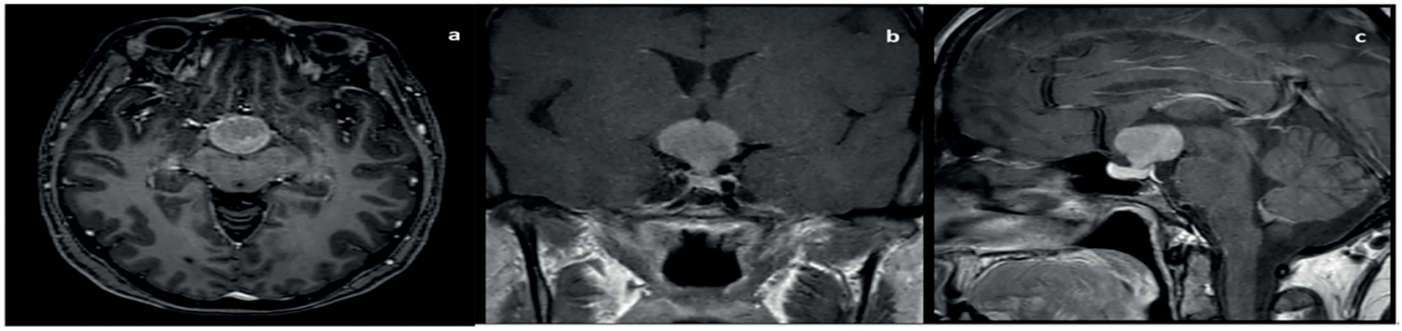


Figure 1. Preoperative contrast-enhanced brain magnetic resonance imaging (MRI) images, (a) axial section, (b) coronal section, (c) sagittal section. MRI of the pituitary gland revealed a 19×17 mm diffuse contrast-enhancing lesion extending from the stalk level to the superior optic chiasm

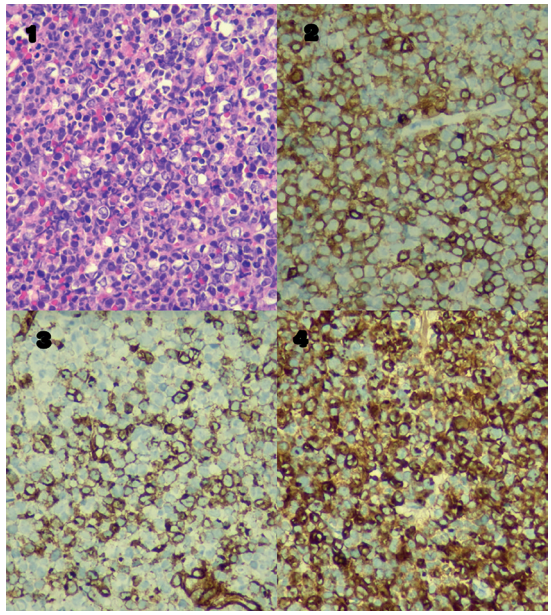


Figure 2. Pathology specimen images
1. The nucleus is irregularly elongated with occasional indentations, containing fine vesicular chromatin, a prominent nucleolus, and discohesive cells (hematoxylin and eosin, x400)
2. Positive reaction with cluster of differentiation 117 (CD117) (x400)
3. Positive reaction with CD34 (x400)
4. Positive reaction with myeloperoxidase (x400)

revealed an approximately 3 cm hemorrhage in the suprasellar region and postoperative changes in the right temporal area (Figure 3). One month after surgery, the patient received adjuvant craniospinal radiotherapy with 18 Gy in 10 fractions and an additional dose of 23.4 Gy in 13 fractions to the tumor bed (Figure 4). The patient continued to receive systemic treatment. At the third month after radiotherapy, the patient experienced restricted eye movements with limited upward gaze in the right eye, and limited outward gaze in the left eye. However, MRIs showed changes consistent with the post-surgical effects. The patient received 11 cycles of azacitidine and venetoclax for maintenance therapy, and remained in remission with negative bone marrow, and extramedullary involvement and 100% chimerism. The patient was monitored without complications and received supportive treatment for the pituitary insufficiency. The patient's data used in this study have been fully anonymized, and no identifiable information is included. Therefore, consent to publish is not required.

Discussion

MS is a rare tumor characterized by extramedullary localization of immature granulocytic cells. It is also known as a myeloblastoma, chloroma, or granulocytic sarcoma. The term chloroma is derived from the green staining of cells due to myeloperoxidase content [2]. This condition was first described in the orbit by Allan [3]. Regarding its pathogenesis, it is hypothesized that there is an aberrant expression of homing signals of leukemic blasts in the extramedullary region compared to the bone marrow [4]. Avni and Koren-Michowitz [5] reported that AML blast cells express chemokine receptors that are absent in normal bone marrow and peripheral blood blasts.

MS occurs most commonly with AML, followed by chronic myeloid leukemia. It can present *de novo* or as an initial symptom in patients who have not yet been diagnosed with AML. In addition, it may appear as an early relapse manifestation after bone marrow transplantation in patients with known AML. The incidence of AML in patients ranges from 3% to 5%. It can also develop as a transformation of myeloproliferative diseases or myelodysplastic syndromes [4]. Our patient presented with an MS relapse while in remission from AML after allogeneic bone marrow transplantation. MS can manifest at any age without a sex predilection [6]. A meta-analysis encompassing CNS MS cases reported a median age of 35 years, with a higher incidence observed in males [7]. MS occurs more frequently in cases of French-American-British classification M4/M5 types, cytogenetic abnormalities such as t, (8; 21), inversion 16, infant leukemias, chromosomal 11q abnormalities, cellular immunodeficiency, and following allogeneic stem cell transplantation [2]. Although the patient in this study did not exhibit any of these chromosomal abnormalities, the FLT-3/ITD mutation, which is associated with a poor prognosis, was present. Furthermore, allogeneic bone marrow transplantation, which increases the frequency of MS, constitutes a risk factor for patients [8]. A review conducted by Paydas et al. [8] determined that the interval between previous hematological diseases and MS diagnosis ranged from 5 to 60 months [9]. A meta-analysis by Lee et al. [6] reported a mean duration of 25.5 months between AML remission and MS diagnosis. This meta-analysis did not demonstrate the impact of the duration between AML and MS on patient mortality. The patient in this study experienced an MS relapse 17 months after AML remission. The most common sites for MS are the subperiosteal regions of the skull, paranasal sinuses, sternum, ribs, vertebrae, pelvis, lymph nodes, and the skin. CNS involvement is infrequent and typically manifests as leptomeningeal or extra-axial cranial bone-based masses. Parenchymal involvement rarely occurs subsequent to meningeal involvement [10]. Due to its rarity, MS presents diagnostic challenges, with an overall misdiagnosis rate of up to 40%. Biopsy remains the preferred diagnostic method [11].

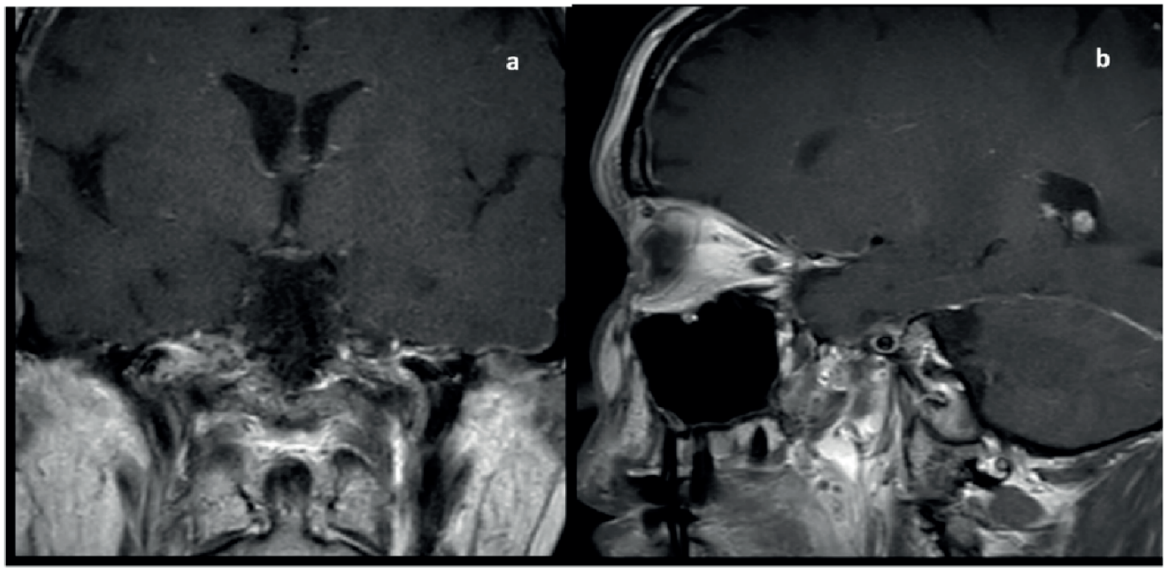


Figure 3. Postoperative cranial magnetic resonance imaging images, (a) coronal section, (b) sagittal section. An approximately 3 cm hemorrhage in the suprasellar region and postoperative changes in the right temporal area

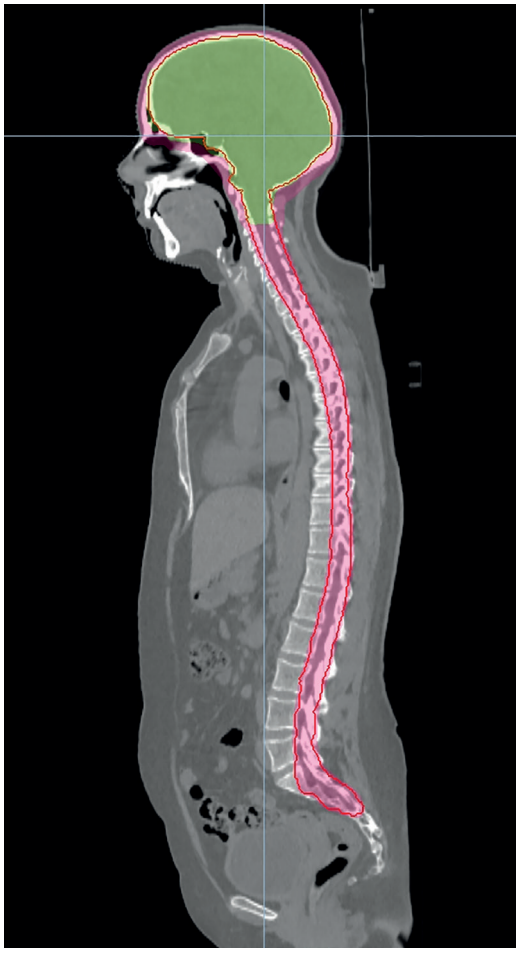


Figure 4. Treatment field of a patient undergoing craniospinal radiotherapy with helical intensity-modulated radiation therapy technique on a radixact device

MS infrequently presents as intracranial MS (IMS) of the skull [12]. The most prevalent locations for IMS are the temporal lobes, cerebellum, and falx or parasagittal regions, accounting for approximately 30.9% of IMS cases [12]. In the present case, the pituitary mass was initially diagnosed as pituitary adenoma. Differential diagnosis for pituitary masses includes adenoma, sarcoidosis, optic nerve glioma, aneurysm, craniopharyngioma, Rathke cleft cyst, and teratoma [13]. Based on our current knowledge and literature review, this case is considered one of the exceedingly rare reported instances of isolated pituitary MS. Clinical symptoms of MS vary according to anatomical location. Symptoms arise from the tumor's mass effect or organ dysfunction, due to infiltration. In instances of pituitary involvement, symptoms can include visual disturbances, diabetes insipidus, and panhypopituitarism [14]. In the present case, the patient exhibited symptoms of decreased vision, somnolence, and polydipsia due to the mass effect of the pituitary tumor. The treatment of MS remains a subject of debate, with a lack of prospective randomized controlled trials [15]. Chemotherapy regimens employed for AML are applicable to both isolated MS and MS occurring concurrently with AML. Patients with isolated multiple sclerosis MS who undergo local treatment exhibit a higher rate of progression compared to those receiving systemic treatment [16,17]. Imrie et al. [17] observed that chemotherapy in isolated MS patients was associated with improved overall survival rates. While the efficacy of combining radiotherapy with chemotherapy is not fully elucidated, radiotherapy is frequently administered in conjunction with chemotherapy. Radiotherapy has been demonstrated to enhance progression-free survival in MS patients, although its impact on overall survival remains uncertain [18]. Lan et al. [15] reported no significant difference in overall survival between groups receiving chemotherapy with radiotherapy and those receiving chemotherapy without radiotherapy in MS patients. The treatment of IMS primarily comprises radiotherapy and chemotherapy. A meta-analysis conducted by Lee et al. [6] demonstrated that the addition of chemotherapy or radiotherapy to IMS treatment significantly reduces mortality. No correlation was identified between surgical resection and its extent and mortality. Although surgical resection is essential for histological diagnosis, its role remains uncertain. It is observed that surgical resection may enhance quality of life, in patients experiencing neurological symptoms or mass effect [7]. In the case reported, the patient initially received systemic treatment for AML and subsequently presented with symptoms related to the pituitary mass while in remission. Post-surgical radiotherapy was administered with the intention of preventing local recurrence, and subsequent chemotherapy resulted in remission.

Conclusion

In conclusion, MS is a neoplasm that may arise from the myeloid lineage in any part of the body, while sellar involvement is exceedingly rare. Nonetheless, it should be considered in the differential diagnosis of pituitary and parasellar masses, and it is important to note that no consensus currently exists regarding its optimal management. Radiotherapy provides rapid local control and symptomatic relief when administered in combination with systemic therapy, may further improve clinical outcomes. Reporting such cases is of great importance, as it contributes to the accumulation of collective experience and guides the development of treatment strategies in these rare clinical scenarios.

Ethics

Informed Consent: The patient's data used in this study have been fully anonymized, and no identifiable information is included. Therefore, consent to publish is not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.M., Z.G., A.U., Concept: S.M., Z.G., F.A., Design: S.M., Z.G., Ç.Ş.K.Y., F.A., Data Collection or Processing: S.M., A.U., Analysis or Interpretation: S.M., Ç.Ş.K.Y., Literature Search: S.M., Writing: S.M., Z.G., F.A.

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

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

References

1. Østgaard LSG, Sengeløv H, Holm MS, et al. Extramedullary leukemia and myeloid sarcoma in aml: new clues to an old issue. Results of a retrospective population-based registry-study of 2261 patients. Accessed; 2021.
2. Frietsch JJ, Hunstig F, Wittke C, et al. Extra-medullary recurrence of myeloid leukemia as myeloid sarcoma after allogeneic stem cell transplantation: impact of conditioning intensity. Bone Marrow Transplant. 2021;56:101-109.
3. Allan B. Observations of surgical anatomy head and neck. USA: Baltimore; 1823. p.364-366.
4. Siraj F, Kaur M, Dalal V, Khanna A, Khan AA. Myeloid sarcoma: a report of four cases at unusual sites. Ger Med Sci. 2017;15:Doc03.
5. Avni B, Koren-Michowitz M. Myeloid sarcoma: current approach and therapeutic options. Therapeutic Advances in Hematology. 2011;2:309-316.
6. Lee D, Omofoye OA, Nuño MA, Riestenberg RA, Shahlaie K. Treatment outcomes of intracranial myeloid sarcomas: a meta-analysis. World Neurosurg. 2021;148:29-37.
7. Ahn JY, Kwon SO, Shin MS, Kim YR. Meningeal chloroma (granulocytic sarcoma) in acute lymphoblastic leukemia mimicking a falx meningioma. J Neurooncol. 2002;60:31-35.
8. Paydas S, Zorludemir S, Ergin M. Granulocytic sarcoma: 32 cases and review of the literature. Leukemia & Lymphoma. 2006;47:2527-2541.
9. Novello M, Coli A, Della Pepa GM, et al. Myeloid sarcoma in the sellar region. Neuropathology. 2014;34:179-184.
10. Pileri S, Ascani S, Cox M. et al. Myeloid sarcoma: clinico-pathologic, phenotypic and cytogenetic analysis of 92 adult patients. Leukemia. 2007;21:340-350.
11. Athukuri P, Khan AB, Gadot R, et al. Myeloid sarcoma of the skull base: a case report and systematic literature review. Surg Neurol Int. 2022;13:220.
12. Vannelli S, Avataneo T, Benso L, et al. Magnetic resonance and the diagnosis of short stature of hypothalamic-hypophyseal origin. Acta paediatrica. 1993;2:155-61.
13. Magdy M, Abdel Karim N, Eldessouki I, Gaber O, Rahouma M, Ghareeb, M. Myeloid sarcoma. Oncol Res Treat. 2019;42:224-229.
14. Yamauchi K, Yasuda M. Comparison in treatments of nonleukemic granulocytic sarcoma: report of two cases and a review of 72 cases in the literature. Cancer. 2002;94:1739-1746.
15. Lan TY, Lin DT, Tien HF, Yang RS, Chen CY, Wu K. Prognostic factors of treatment outcomes in patients with granulocytic sarcoma. Acta Haematologica. 2009;122:238-246.
16. Tsimberidou AM, Kantarjian HM, Wen S, et al. Myeloid sarcoma is associated with superior event-free survival and overall survival compared with acute myeloid leukemia. Cancer. 2008;113:1370-1378.
17. Imrie KR, Kovacs MJ, Selby D, et al. Isolated chloroma: the effect of early antileukemic therapy. Ann Intern Med. 1995;123:351-353.
18. Tsimberidou AM, Kantarjian HM, Estey E, et al. Outcome in patients with nonleukemic granulocytic sarcoma treated with chemotherapy with or without radiotherapy. Leukemia. 2003;17:1100-1103.

2025 Referee Index